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National Center for Environmental Assessment-RTP Division
Office of Research and Development
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Acronyms and Abbreviations

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
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
AF	absorbed fraction; absorption fraction
A/G	albumin/globulin
Ag	silver
A-horizon	topsoil horizon (surface soil)
AKI	acute kidney injury
Al	aluminum
ALA	aminolevulinic acid
ALAD	aminolevulinic acid dehydratase; aminolevulinic acid dehydrogenase; aminolevulinate dehydratase; δ-aminolevulinic acid dehydratase
ALAD 1-1	aminolevulinate delta-dehydratase 1-1
ALAD-2	aminolevulinate delta-dehydratase-2
ALD	aldehyde dehydrogenase
ALM	Adult Lead Methodology
ALP	alkaline phosphatase
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
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)
As	arsenic

AST	aspartate aminotransferase
ASV	anode stripping voltammetry
ATLD	ataxia-telangiectasia-like disorder
ATOFMS	aerosol time-of-flight mass spectrometry
ATP	adenosine-triphosphate
ATPase	adenosine triphosphatase; adenosine triphosphate synthase
ATSDR	Agency for Toxic Substances and Disease Research
Au	gold
avg	average
AVS	acid-volitile 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
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	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 20 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
cAMP	cyclic adenosine monophosphate
CASAC	Clean Air Scientific Advisory Committee
CASM	Comprehensive Aquatic Systems Model
$CaSO_4$	calcium sulfate
$CaSO_4.2H_2O$	gypsum
CAT	catalase
CBLI	cumulative blood lead index
CBSA	core based statistical area
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	contanst flux layer
CFR	Code of Federal Regulations
cGMP	cyclic guanosine monophosphate
ChAT	chlorine acetyltranferase
CHD	coronary heart disease
CHL	Chinese hamster lung
CHO	Chinese hamster ovary cell line
C-horizon	soil horizon under A- and B- horizons, may contain lumps or shelves of rock and parent material
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 phopholipidase A_2
CPRS-R	Conners' Parent Rating Scale-Revised
Cr	chromium; creatine
Cr III	chromium III
CRAC	Ca^{2+} release activated calcium
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	cardiovasicular 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-hyrdoxylase
DBP	diastolic blood pressure
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; techetium-diethylenetriamine-pentaacetic acid
E	east; expression for exposure
E2	estradiol
e	exponential function
EC	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
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	soil horizon with eluviated or leached of mineral and/or organic content
EI-MS	electron impact ionization mass spectrometry
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
EU	European Union

EURO	European emission standard
eV	electronvolts
EXAFS	X-ray absorption fine structure spectroscopy
F_0	filial 0 generation
F_1	first offspring generation
F_2	second offspring generation
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 triodothyronine
FT4	free thyroxine
G	pregnancy; guanine
G2	gap 2 Phase
g, mg, kg, μg, ng, pg	gram(s), milligram(s), microgram(s), kilogram(s), nanogram(s), picogram(s)
G93A	mouse model
GABA	γ-aminobutyric acid; gamma aminobutyric acid
GABAergic	gamma aminobutyric acid-ergic
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
GI	gastrointestinal
GIS	Geographic Information System
G+L	pregnancy plus lactation
GLE	gestationally-lead exposed
GM	geometric mean
GMR	geometric mean blood lead 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	hydroxyalkenals
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
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
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 lead
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
i.p.	intraperitoneal (route)
IQ	intelligence quotient
IQR	interquartile range
IRE1	inositol-requiring enzyme-1
ISA	Integrated Science Assessment
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; potassium; resupsension 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 protooncogene
Λ	lambda; resuspension rate
L	length
L, mL, dL	liter(s), milliliter(s), deciliter(s)
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
LOEC	lowest-observed-effect concentration
log	logarithm
LPb	low lead
LPS	lipopolysaccharide
LSO	lateral superior olive
M	metal
M, mM, μM, nM	Molar, milliMolar, microMolar, nanoMolar
m, cm, mm, μm, nm, km	meter(s), centimeter(s), millimeter(s), micrometer(s), nanometer(s), kilometer(s)
MAP	mean arterial pressure
MAPK	mitogen-activated protein kinase(s), MAP kinase
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
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
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)
MOUDI	multi-orifice uniform deposit impactor
MPb, m-Pb	moderate lead
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
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	Normative Aging Study
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; nitrogen monoxide
NO_2	nitrogen dioxide
No.	number
NOAA	National Oceanic and Atmospheric Administration
NOAEL	no observed adverse effect level
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 + NO2)
NP	nanoparticle
NPSH	nonprotein sulfhydryl
NQO1	NAD(P)H-quinone oxidoreductase (genotype)
NRC	National Research Council

NRCS	Natural Resouces Conservation Service
Nrf2	nuclear factor erythroid 2-related factor 2
NS	not specified
NTPDase	nucleoside triphosphate diphosphohydrolase
NW	northwest
NYC	New York City
NZ	New Zealand
O_2	molecular oxygen
O_2^-	superoxide
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
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 paried hourly observations; statistical significance
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
$PbCO_3$	cerrusite; 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
$PbFe_6(SO_4)_4(OH)_{12}$	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
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 PM_X 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.
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 $PM_{10-2.5}$ concentration of $PM_{10-2.5}$ measured by the 40 CFR Part 50 Appendix O reference method which consists of currently operated, collocated low-volume (16.7 Lpm) PM_{10} and $PM_{2.5}$ 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
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
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
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)
SEM	scanning electron microscopy; simultaneously extracted metal; standard error of the mean
SES	socioeconomic status
Sess.	session
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
SHM	Stockholm humic model

siRNA	small interfering RNA
SJW	silver jewelry workers
SLAMS	State and Local Air Monitoring Stations
SMC	smooth muscle cells
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
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 radation 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
Syb	synaptobrevin
Syn	synaptophysin
Syt	synaptotagmin
SZn	supplemental zinc
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
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-ovalabumin
TPR	total peripheral vascular resistance
TS	transferrin saturation
TSH	thyroid stimulating hormone; total sulfhydryl
TSP	total suspended particles
TSS	total suspended solids
TXB_2	thromboxane
UA	urbanized area
UBL	urban boundary layer
UCL	urban canopy layer
UDPGT	uridine diphosphate (UDP)-glucuronosyltransferase(s)
U.K.	United Kingdom
U.S.	United States of America
USC	U.S. Code
U.S. EPA	United States Environmental Protection Agency
USF	uptake slope factor
USGS	United States Geological Survey
USL	urban surface layer
UUDS	urban dynamic driving schedule
UV	ultraviolet radiation
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
VGLUT1	vesicular glutamate transporter 1
VIQ	verbal intelligence quotient (IQ)
VLPb	very low lead
VMAT2	vesicular monoamine transporter-2
VO_4^{3-}	vanadate ion
VOC(s)	volatile organic compound(s)
vs., v.	versus
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
WISC	Weschler Intelligence Scale for Children
WISC-R	Weschler Intelligence Scale for Children-Revised

wk	week(s)
WML	white matter lesions
WPPSI-III	Wechsler Preschool and Primary Scales of Intelligence-III
WPPSI-R	Weschler 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
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

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Chapter 1. Introduction

This first external review draft Integrated Science Assessment (ISA) is a concise review, synthesis, and evaluation of the most policy-relevant evidence, and communicates critical science judgments relevant to the National Ambient Air Quality Standards (NAAQS) review. As such, the ISA forms the scientific foundation for the review of the primary (health-based) and secondary (welfare-based) NAAQS for lead (Pb). The ISA accurately reflects "the latest scientific knowledge useful in indicating the kind and extent of identifiable effects on public health which may be expected from the presence of [a] pollutant in ambient air" (42 U.S.C. 7408). Key information and judgments formerly contained in an Air Quality Criteria Document (AQCD) for Pb are incorporated in this assessment. This ISA thus serves to update and revise the evaluation of the scientific evidence available at the time of the previous review of the NAAQS for Pb that was concluded in 2008.

The draft *Integrated Review Plan for the National Ambient Air Quality Standards for Lead* (U.S. EPA, 2011) identifies a series of policy-relevant questions that provide a framework for this assessment of the scientific evidence. These questions 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 any risk analyses and policy considerations to help the U.S. Environmental Protection Agency (EPA) address these questions during the NAAQS review for Pb. In evaluating the health evidence, the focus of this assessment is on scientific evidence that is most relevant to the following questions taken directly from the Integrated Review Plan:

- What new evidence is available on exposure to Pb through air-related pathways? Can air-related pathways be disentangled from water- and soil-related pathways using available data?
- What new evidence is available regarding observational studies of Pb exposure? How do these studies inform the assessment of exposure to air-related pathways?
- What new evidence is available on biological and other factors that could affect the distribution and accumulation of Pb into blood and bone (e.g., age, diet, gender, race)?

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

- How and to what extent does previous or concurrent Pb exposure, including duration (e.g., acute, subchronic, chronic) and pattern (e.g., continuous low, extreme peak) impact blood and bone Pb?
- What new evidence is available on the relationship between air Pb and blood Pb levels and uncertainties in that relationship? What new knowledge exists regarding the characterization of changes in this relationship when accounting for the multiple pathways of Pb exposure and body burden associated with Pb exposure? What does the current evidence indicate regarding variation in the relationship with variation in blood Pb levels or air Pb levels?
- To what extent does new scientific evidence increase our understanding of the contributions of Pb from different sources and exposure pathways to blood Pb levels or to other indicators of Pb body burden (e.g., contributions from various air-related pathways, including diet and indoor dust pathways)?
- How do results of recent epidemiologic studies and current or new interpretations of previous findings expand our understanding of the relationship between body burdens of Pb and neurological effects in children and adults, including deficits in IQ, behavior, learning, and motor skills, as well as risk of neurodegenerative diseases? What new evidence is available on the potential clinical relevance of these effects? Do recent studies expand the current understanding of concentration-response relationships pertinent to the range of Pb exposures currently experienced by the U.S. population?
- For what Pb-induced health effects, is there sufficient evidence in multiple species to support a quantitative comparison of exposures that induce the effects?
- To what extent are the health effects observed in epidemiological studies attributable to exposure to Pb rather than co-exposures to other toxic metals or environmental contaminants?
- In epidemiologic studies, what are the uncertainties in Pb effect estimates due to potential confounding factors (e.g., demographic and lifestyle attributes, socioeconomic status [SES], genetic susceptibility factors, occupational exposure, and access to medical care)?
- Based on the new body of evidence, what uncertainties remain regarding the nature and shape of concentration-response relationships (e.g., threshold, linear, nonlinear)? What evidence is newly available on the uncertainties related to other aspects of statistical model specification and how can it be used to assess the influence of these uncertainties

on the results of epidemiologic studies? What evidence is available from toxicological studies of dose-response relationships?

- To what extent is key evidence now available regarding mechanisms for neurological effects associated with "lower" (<10 µg/dL or <5 µg/dL) blood Pb levels (e.g., oxidative stress)? What toxicological evidence is available on mechanisms and dose-response relationships for other health outcomes (e.g., cardiovascular, renal, or immunological effects), and is there coherence between this and epidemiologic findings for these endpoints?
- To what extent is key new evidence available that could inform the understanding of populations that are particularly susceptible to Pb exposures? What is known about genetic traits, pre-existing conditions (obesity), or other factors that affect susceptibility (sex)? To what extent is the strength of epidemiologic or toxicological evidence driven by effects observed in populations with increased susceptibility?
- To what extent is key evidence now available to inform our understanding of developmental lifestages that are particularly susceptible to Pb exposures? What is known about critical windows of exposure for Pb with regard to their impact on concentration-response relationships and/or effects elicited?
- What do the currently available studies indicate regarding the relationship between exposures to Pb and health effects in those with preexisting diseases (e.g., renal diseases) compared to healthy individuals? What medical conditions are identified as increasing susceptibility to Pb effects? What is the nature and time-course of the development of effects in previously healthy persons and in persons with pre-existing disease (e.g., cardiovascular disease)? What are the pathways and mechanisms through which Pb may be acting for these groups?

In evaluating evidence on welfare effects of Pb, the focus will be on evidence that can help inform these questions from the Integrated Review Plan:

- What new information is available about the nature of the effects of Pb on terrestrial ecosystems, especially Pb that is relevant to air-related pathways? Is there new evidence of effects at current ecosystem loads? Is there new evidence that, in combination with the previously existing evidence, supports the development of critical loads for terrestrial ecosystems?
- Is there new information available for establishing specific exposure levels at which terrestrial ecological receptors are expected to experience effects?

- Are there new empirical data or modeling results that would improve our understanding of the movement of Pb in or through terrestrial systems, or would improve our understanding of Pb bioavailability and pathways of exposure for terrestrial organisms?
- Is there new evidence that contributes to a better understanding of the nature and magnitude of the potential effects of Pb on terrestrial ecosystem services?
- What new information is available about the nature of the effects of Pb on aquatic ecosystems, especially Pb that is relevant to air-related pathways? Is there new evidence of effects at current ecosystem loads? Is there new evidence that, in combination with the previously existing evidence, supports the definition of critical loads for aquatic ecosystems?
- Is there new information available for establishing specific exposure levels at which aquatic ecological receptors are expected to experience effects?
- Are there new empirical data or modeling results that would improve our understanding of the movement of Pb in or through aquatic systems or would improve our understanding of Pb bioavailability and pathways of exposure for aquatic organisms?
- Is there new evidence that contributes to a better understanding of the nature and magnitude of the potential effects of Pb on aquatic ecosystem services?

This introductory chapter (Chapter 1) of the Pb ISA presents: (1) background information on pertinent Clean Air Act legislative requirements, the air quality criteria and NAAQS review process, and the history of previous Pb NAAQS reviews; (2) an overview of the ISA development process and an orientation to the general organizational structure and content of this Pb ISA; and (3) the framework for causal determination used to evaluate the causal nature of air pollution-induced health and environmental effects in NAAQS reviews.

1.1. Legislative Requirements

Two sections of the Clean Air Act (CAA) govern the establishment and revision of the NAAQS. Section 108 (42 USC §7408) directs the Administrator to identify and list certain air pollutants and then 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 and welfare;" "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 identifiable effects on public health or welfare which may be expected from the presence of [a] pollutant in ambient air...." 42 USC §7408(b). Section 109 (42 USC §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."[1] 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."[2]

The requirement that primary standards include 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. See *Lead Industries Association v. EPA*, 647 F.2d 1130, 1154 (D.C. Cir 1980), *cert. denied*, 449 U.S. 1042 (1980); *American Petroleum Institute v. Costle*, 665 F.2d 1176, 1186 (D.C. Cir. 1981), *cert. denied*, 455 U.S. 1034 (1982); *American Farm Bureau v. EPA*, 559 F. 3d 512, 533 (D.C. Cir. 2009); *Coalition of Battery Recyclers Association v. EPA*, 604 F. 3d 613, 617-18 (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 include 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, see *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

[1] 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).

[2] 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."

Administrator's judgment. See *Lead Industries Association v. EPA*, 647 F.2d at 1161-62; *Whitman v. American Trucking Associations*, 531 U.S. 457, 495 (2001).

In setting primary and secondary standards that are "requisite" to protect public health and welfare, respectively, 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, 475-76 (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 at 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 1980s, this independent review function has been performed by the Clean Air Scientific Advisory Committee (CASAC).[1]

1.2. History of Reviews of the NAAQS for Lead

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 µg micrograms per cubic meter (µg/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. This standard was based on the 1977 AQCD for Pb (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 Lead AQCD (U.S. EPA, 1986a), the associated Addendum (U.S. EPA, 1986b), 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, 1990b). 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, 1990b). After consideration of the documents

[1] Lists of CASAC members and of members of the CASAC Pb Review Panel are available at: http://yosemite.epa.gov/sab/sabproduct.nsf/WebCASAC/CommitteesandMembership?OpenDocument

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 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, 2005b) and *Plan for Review of the National Ambient Air Quality Standards for Lead (*U.S. EPA, 2006c*).*[1] 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; amended 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 AQCD for Pb(U.S. EPA, 2006a), 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, 2006b). 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, 2007). 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, 2006d), 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 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

[1] In the current review, these two documents have been combined into an integrated plan (this document).

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 Report. 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). In addition to public comments on the proposal received during the public comment period, both written and oral at two public hearings, the CASAC Pb Panel 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 revisions to the primary and secondary NAAQS for Pb. In consideration of the much-expanded health effects evidence on neurocognitive effects of Pb in children, EPA substantially revised the primary standard 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 standard was based on the weight of the scientific evidence and guided by an evidence-based framework that integrates evidence for relationships between Pb in air and Pb in children's blood and Pb in children's blood and IQ loss. The level of 0.15 $\mu g/m^3$ was estimated to protect against air Pb-related IQ loss 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 3-month period with a maximum (not-to-be-exceeded) form, evaluated over a 3-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 in total suspended particles (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 reporting requirements, as well as emissions inventory reporting requirements.[3] One aspect of the new data handling requirements is

[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.

[3] 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).

the allowance for the use of Pb-PM_{10} monitoring for Pb NAAQS attainment purposes in certain limited circumstances at non-source oriented sites. Subsequent to the 2008 rulemaking, additional revisions were made to the monitoring network requirements.

1.3. ISA Development

EPA announced the initiation of the current periodic review of the air quality criteria for Pb and the Pb NAAQS in April 2010 and issued a call for information from the public (75 FR 20843). In addition to the call for information, publications were identified through an ongoing literature search process that includes extensive computer database mining on specific topics in a variety of disciplines. Literature searches were conducted to identify studies published since the last review, focusing on publications from January 2006 to March 2011. Search strategies were iteratively modified in an effort to optimize the identification of pertinent publications. Additional papers were identified for inclusion in several ways: review of pre-publication tables of contents for journals in which relevant papers may be published and independent identification of relevant literature by expert authors and peer reviewers. It is anticipated that further identification of studies will occur during the external review process by the public and CASAC. Publications considered for inclusion in the ISA were added to the Health and Environmental Research Online (HERO) database recently developed by EPA (http://hero.epa.gov); note that all citations in the ISA are electronically linked to the database. Typically, only information that underwent scientific peer review and was published or accepted for publication was considered. All relevant epidemiologic, animal toxicological, and ecological and welfare effects studies published since the last review were considered, including those related to exposure-response relationships, mode(s) of action (MOA), and susceptible populations. Additionally, air quality and emissions data, studies on atmospheric chemistry, environmental fate and transport, as well as issues related to Pb toxicokinetics and exposure were considered for inclusion in the document. The process for identifying studies for consideration and inclusion in the Pb ISA is provided in Figure 1-1. All references that were considered for inclusion can be found within the HERO website (http://hero.epa.gov/lead). This site contains HERO links to lists of references that are cited in the ISA, as well as those that were considered for inclusion, but not cited in the ISA, with bibliographic information and abstracts.

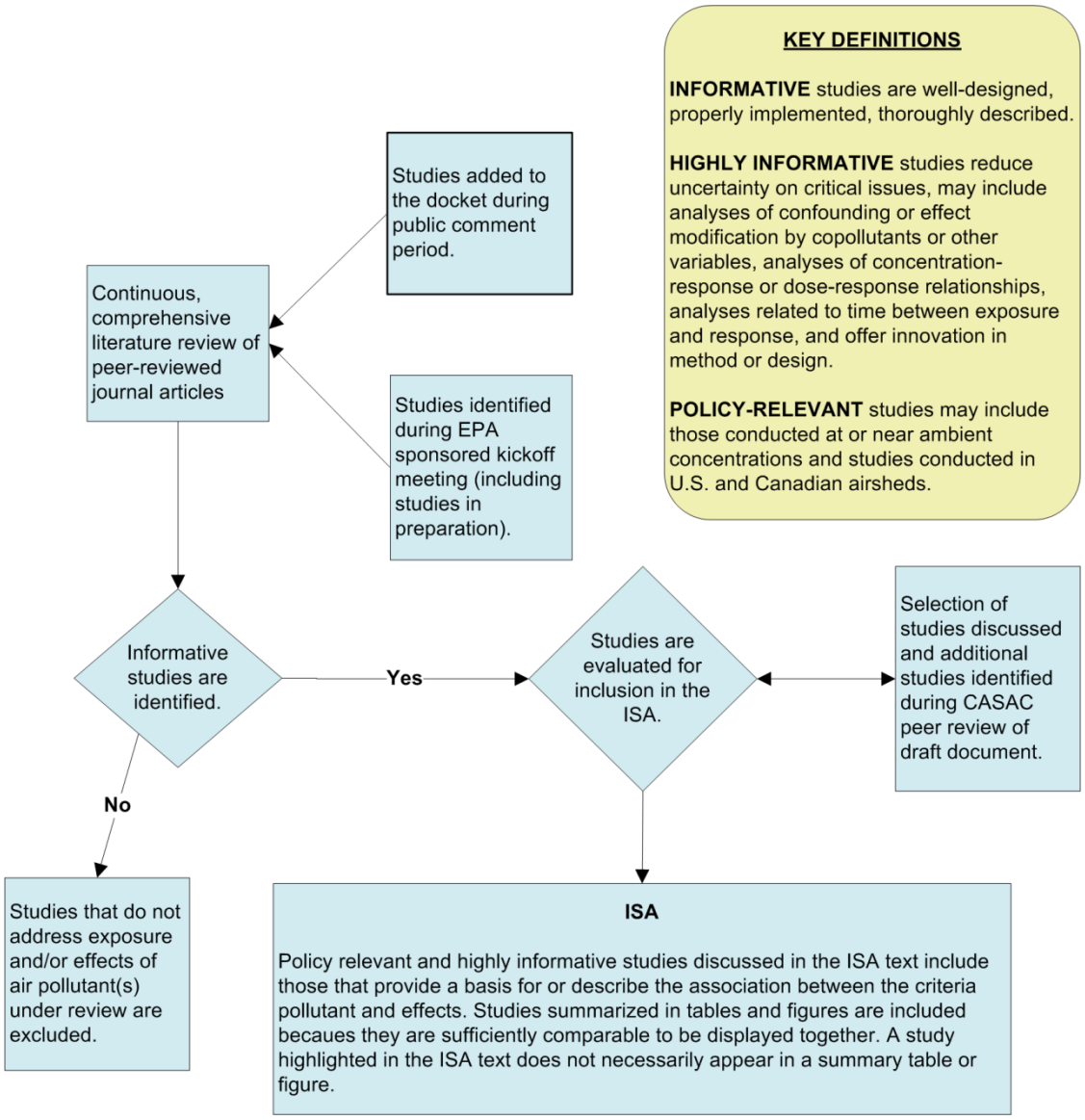

Figure 1-1. Identification of studies for inclusion in the ISA.

The ISA builds upon the conclusions of the previous review of the air quality criteria for Pb, presented in the 2006 Pb AQCD (U.S. EPA, 2006a), and focuses on peer reviewed literature published thereafter and on any new interpretations of previous literature. The 2006 Pb AQCD (U.S. EPA, 2006a) evaluated literature published through December 2005. In subsequent chapters, the results of recent scientific studies are integrated with previous findings. Important older studies may be discussed in detail to reinforce key concepts and conclusions or if they are open to reinterpretation in light of newer data. Older studies also are the primary focus in some areas of the document where research efforts have subsided, and these older studies remain the definitive works available in the literature. Emphasis is placed on studies that examine effects associated with Pb

concentrations relevant to current population and ecosystem exposures, and particularly those pertaining to Pb 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.

Discussions in the ISA primarily focus on scientific evaluations that can inform the key policy questions described in the Integrated Review Plan (U.S. EPA, 2011). Although emphasis is placed on discussion of health and welfare effects information, other scientific information is also presented and evaluated in order to provide a better understanding of the sources of Pb to ambient air, measurement and concentrations of Pb in ambient air, its subsequent fate and transport in the environment, pathways of human and ecological exposure, and toxicokinetic characteristics of Pb in the human body, as well as the measurement of population exposure to Pb.

In general, in assessing the scientific quality and relevance of health and environmental effects studies, the following considerations were 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?
- Are the health or welfare effect measurements meaningful and reliable?

Studies published since the 2006 Pb AQCD are emphasized; however, evidence from studies described in previous assessment that are needed to characterize the current state of the science as well as new interpretations of older evidence is also considered. Among the studies EPA included in the ISA, particular focus is for the following areas:

- New studies with adequate blood Pb data at the low end of the distribution (e.g., <10 µg/dL);
- New studies that provide quantitative effect estimates for populations or lifestages and concentrations of interest;
- Lead exposure or effects in susceptible populations and lifestages;
- Issues related to the potential for confounding of study effects/responses by non-Pb exposure-related factors or variables, and to the modification of Pb-related effects;

- The timing (e.g., across/within specific lifestages) and duration of exposure associated with specific responses;
- Concentration-response relationships for specific Pb-related effects;
- The interpretation of Pb biomarkers in epidemiological studies; and air-to-blood Pb or air-to-bone Pb relationships;
- Studies that evaluate Pb as a component of a complex mixtures of pollutants.

In selecting epidemiologic studies for inclusion in the present assessment, EPA has considered studies containing information on: (1) recent or cumulative exposures relevant to current population exposure levels of Pb; (2) health endpoints that repeat or extend findings from earlier assessments as well as those not previously extensively researched; (3) populations and lifestages that are susceptible to Pb exposures; (4) issues related to potential confounding, and modification of effects; and/or (5) important methodological issues (e.g., timing and duration of exposure, concentration-response relationships, interpretation of biomarkers in epidemiological studies, and air-to-blood/bone relationships) related to Pb exposure effects. In selecting the most informative and policy-relevant epidemiologic studies on which to give particular focus in the Pb ISA, emphasis is placed on those most relevant to standard setting in the United States. Informative studies conducted in other countries are discussed, as appropriate (e.g., studies for which the mean blood Pb level in the population studied is comparable to the current mean blood Pb level in the corresponding U.S. population).

In reviewing new studies that evaluated the response of laboratory animals to Pb exposure, focus in on studies that reveal the effects of Pb exposure within the previously identified target biological systems (e.g., neurological, cardiovascular, renal, immune). Additionally, particular focus is on those studies that involve doses or blood Pb/bone Pb levels that approximate human doses or blood Pb/bone Pb levels relevant to current U.S. populations. Studies at higher exposure doses that result in body burdens above what is found in the current U.S. population are included when the study can provide information relevant to potential MOA, information on exposure-response relationships, or otherwise improve our understanding of susceptible populations. Studies of the efficacy of chelation as a treatment for Pb poisoning in humans and laboratory animals are excluded, except where they provide evidence for the reversibility of a given health effect.

In selecting informative studies of welfare effects, emphasis is placed on recent studies that: (1) evaluate the occurrence of effects associated with Pb exposure at current ambient concentrations, with a particular focus on ambient concentrations resulting from ambient air Pb; and/or (2) investigate the effects of Pb on ecosystems at any scale. Studies conducted in geographical areas outside the U.S. are included in the assessment if they contribute to the general knowledge of the

effects of Pb irrespective of species or locality. As in the selection of health-related scientific studies, welfare-related studies were selected that advance our understanding of MOA by which Pb directly affects terrestrial and aquatic biota. These MOAs, as they pertain to Pb exposures of short or longer duration, informs our understanding of indirect effects that Pb may exert more broadly on ecosystem structure, function and services. Key studies identified for welfare effects are integrated into the discussion to inform our interpretation of the ecological literature and our characterization of uncertainties.

The criteria described here provide generalized benchmarks to guide the inclusion in the ISA of the highest quality and most policy-relevant studies. Of most relevance for evaluation of studies is whether they provide useful qualitative or quantitative information on exposure-effect or exposure-response relationships for effects associated with current blood Pb or bone Pb levels likely to be encountered in the U.S. population. Detailed critical analysis of all studies of the effects of Pb on health and welfare, especially in relation to the above considerations, is beyond the scope of this document. Since the last AQCD was completed in 2006, a considerable portion of the current ISA is devoted to summarizing previously available evidence that contributed to the basis for the last rulemaking.

As discussed previously, studies included in the text of the ISA are those deemed informative to the NAAQS review process (e.g., policy relevant) and of adequate quality. The ISA text, tables and figures highlight and summarize key study details that are needed to understand and interpret the results of a study. This information, which was described in the text as well as reiterated in the annex tables of previous documents, includes the air quality system (AQS) data; studies of fate and transport in air, water, and soil; human exposure and dosimetry studies; blood or tissue Pb levels corresponding to adverse health effects and dose and duration of exposure in toxicological studies; and, effect estimates, study location, population, exposure metric and time window, as well as the characteristics of the exposure/dose distribution for epidemiologic studies. In addition, supplementary materials are provided in the form of output from the HERO database. A key function of the HERO output is to document the base of evidence containing publications evaluated for the Pb review, including any publications considered but not included in the ISA. This information is presented as links to lists of references in the HERO database, which include bibliographic information and abstracts and can be found at http://hero.epa.gov/lead. In addition, certain study characteristics of epidemiologic studies, including location, ages investigated, outcomes, and health endpoints, are summarized in tables included in Chapter 5.

In developing the Pb ISA, EPA began by reviewing and summarizing the evidence on: (1) atmospheric sciences and exposure; (2) the health effects evidence from in vivo and in vitro animal toxicological and epidemiologic studies; and (3) the welfare effects of Pb, including ecological effects. In December 2010, EPA held a peer-review input workshop to obtain review of the scientific

content of initial draft materials or sections for the ISA. The purpose of the peer-review input workshop was to ensure that the ISA was up to date and focused on the most policy-relevant findings, and to assist EPA with integration of evidence within and across disciplines. Subsequently, EPA addressed comments from the peer-review input workshop and completed the initial integration and synthesis of the evidence.

The integration of evidence on health or welfare effects involves collaboration between scientists from various disciplines. As described in the section below, the ISA organization is based on health and welfare effect categories. As an example, an evaluation of health effects evidence would include summaries of findings from epidemiologic and toxicological studies, and integration of the results to draw conclusions based on the causal framework described below. Using the causal framework described in Section 1.6, EPA scientists consider aspects such as strength, consistency, coherence and biological plausibility of the evidence, and develop draft causality judgments on the nature of the relationships. The draft integrative synthesis sections and conclusions are reviewed by EPA internal experts and, as appropriate, by outside expert authors. In practice, causality determinations often entail an iterative process of review and evaluation of the evidence. The draft ISA is released for review by the CASAC and the public. Comments on the characterization of the science as well as the implementation of the causal framework are carefully considered in revising and completing the ISA.

1.4. Document Organization

This ISA is composed of seven chapters. This introductory chapter presents background information, and provides an overview of EPA's framework for making causal judgments. Chapter 2 is an integrated summary of key findings and conclusions regarding the source-to-dose paradigm, toxicokinetics, MOA, important health effects of Pb, including neurological, cardiovascular, renal, immunological, reproductive and developmental, and cancer outcomes, and welfare effects of Pb, including terrestrial and aquatic ecosystem impacts. More detailed summaries, evaluations, and integration of the evidence are included in Chapters 3 through 7. Chapter 3 highlights key concepts or issues relevant to understanding the sources, ambient concentrations, atmospheric behavior, and fate of Pb in the environment. Chapter 4 summarizes key concepts and recent findings on exposures to Pb using a conceptual model that reflects the multimedia nature of Pb exposure, toxicokinetics, biomarkers of Pb exposure and body burden, and models. Chapter 5 presents a discussion of the MOA of Pb and evaluates and integrates epidemiologic and animal toxicological information on health effects related to Pb exposures, including neurological, cardiovascular, renal, immunological, reproductive and developmental, and cancer outcomes. Chapter 6 summarizes the evidence on

potentially susceptible populations for health effects of Pb exposure. Chapter 7 evaluates welfare effects evidence that is relevant to the review of the secondary NAAQS for Pb, including ecological effects. The chapter also presents key conclusions and scientific judgments regarding causality for welfare effects of Pb.

1.5. Document Scope

For the current NAAQS review of the primary Pb standard, relevant scientific information on human exposures and health effects associated with exposure to ambient Pb has been assessed. Previous reviews have included an extensive body of evidence from the major health disciplines of toxicology and epidemiology on the health effects of Pb exposure (U.S. EPA, 1986a, 2006a). In this review, the conclusions from previous reviews are summarized at the beginning of each health outcome discussion to provide the foundation for consideration of evidence from recent studies. In some cases where no new information is available, the summary of key findings and conclusions from the previous Pb AQCDs serve as the basis for current key conclusions. Results of key studies from previous reviews are included in ISA 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 review also assesses scientific information associated with known or anticipated ecological and public welfare effects that is relevant to the review of the secondary Pb standard. Research on the ecological effects of Pb, including impacts on vegetation, has been discussed extensively in previous AQCDs. This review incorporates and discusses findings of recent studies, building upon previous evaluations and conclusions.

1.6. EPA Framework for Causal Determination

The EPA has developed a consistent and transparent basis to evaluate the causal nature of air pollution-induced health or environmental effects. The framework described below establishes uniform language concerning causality and brings more specificity to the findings. This standardized language was drawn from across the federal government and wider scientific community, especially from the National Academy of Sciences (NAS) Institute of Medicine (IOM) document, *Improving the Presumptive Disability Decision-Making Process for Veterans*, (2008) the most recent comprehensive work on evaluating causality.

This introductory section focuses on the evaluation of health effects evidence. While focusing on human health outcomes, the concepts are also generally relevant to causality determination for welfare effects. This section:

- describes the kinds of scientific evidence used in establishing a general causal relationship between exposure and health effects;
- defines causation, in contrast to statistical association;
- discusses the sources of evidence necessary to reach a conclusion about the existence of a causal relationship;
- highlights the issue of multifactorial causation;
- 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 (2006), *EPA Guidelines for Carcinogen Risk Assessment* (2005a), Centers for Disease Control and Prevention (2004), and National Acid Precipitation Assessment Program (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. Moreover, these frameworks have supported decision-making under conditions of uncertainty.

1.6.1. Scientific Evidence Used in Establishing Causality

Causality determinations are based on the evaluation and synthesis of evidence from across scientific disciplines; the type of evidence that is most important for such determinations will vary by assessment. The most direct evidence of a causal relationship between pollutant exposures and human health effects comes from controlled human exposure studies. This type of study experimentally evaluates the health effects of administered exposures in human volunteers under highly-controlled laboratory conditions. Controlled human exposure studies are not done for Pb, and thus, are unavailable for consideration.

In most epidemiologic or observational studies of humans, the investigator does not control exposures or intervene with the study population. Broadly, observational studies can describe associations between exposures and effects. In the case of Pb, most observational studies use

biomarkers of Pb (i.e., blood or bone Pb) rather than exposures to relate to effects. These studies fall into several categories: cross-sectional and longitudinal studies. "Natural experiments" offer the opportunity to investigate changes in health with a change in exposure; these include comparisons of health effects before and after a change in population exposures, such as the closure of a pollution source.

Experimental animal data can help characterize effects of concern, exposure-response relationships, susceptible populations, MOAs and enhance understanding of biological plausibility of observed effects. In the absence of human data, animal data alone may be sufficient to support a likely causal determination, assuming that similar responses are expected in humans.

1.6.2. Association and Causation

"Causation" is a significant, effectual relationship between an agent and an effect on health or welfare. "Association" is the statistical dependence among events, characteristics, or other variables. An association is *prima facie* evidence for causation; alone, however, it is insufficient proof of a causal relationship between exposure and disease or health effect. Determining whether an observed association is causal rather than spurious involves consideration of a number of factors, as described below. Much of the newly available health information evaluated in this ISA comes from epidemiologic studies that report a statistical association between ambient exposure and health outcomes.

Many of the health and environmental outcomes reported in these studies have complex etiologies. Diseases such as asthma, cardiovascular disease, Parkinson's disease or cancer are typically initiated by a web of multiple agents. Outcomes depend on a variety of factors, such as age, genetic susceptibility, nutritional status, immune competence, and social factors (Gee & Payne-Sturges, 2004; Samet & C. C. Bodurow, 2008). Effects on ecosystems are 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 represents the net effect of many actions and counteractions.

1.6.3. Evaluating Evidence for Inferring Causation

Moving from association to causation involves the elimination of alternative explanations for the association. In estimating the causal influence of an exposure on health or environmental effects, it is recognized that scientific findings incorporate uncertainty. "Uncertainty" can be defined as a state of having limited knowledge where it is impossible 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 characterization and uncertainty assessment are two activities that lead to

different degrees of sophistication in describing uncertainty. Uncertainty characterization generally involves a qualitative discussion of the thought processes that lead to the selection and rejection of specific data, estimates, scenarios, etc. Uncertainty assessment is more quantitative. The process begins with simpler measures (e.g., ranges) and simpler analytical techniques and progresses, to the extent needed to support the decision for which the assessment is conducted, to more complex measures and techniques. Data will not be available for all aspects of an assessment and those data that are available may be of questionable or unknown quality. In these situations, evaluation of uncertainty can include professional judgment or inferences based on analogy with similar situations. The net result is that the assessment will be based on a number of assumptions with varying degrees of uncertainty. Uncertainties commonly encountered in evaluating health evidence for the criteria air pollutants are outlined below for epidemiologic and experimental studies. Various approaches to evaluating uncertainty include classical statistical methods, sensitivity analysis, or probabilistic uncertainty analysis, in order of increasing complexity and data requirements. 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.

Meta-analysis may be a valuable tool for evaluating evidence by combining results from a body of studies. Blair et al. (1995) observe that meta-analysis can enhance understanding of associations between exposures and effects that are not readily apparent in examination of individual study results and can be particularly useful for formally examining sources of heterogeneity. However, these authors note that meta-analysis may not be useful when the relationship between the exposure and outcome is obvious, when only a few studies are available for a particular exposure-outcome relationship, where there is limited access to data of sufficient quality, or where there is substantial variation in study design or population. In addition, important differences in effect estimates, exposure metrics, or other factors may limit or even preclude quantitative statistical combination of multiple studies.

Epidemiologic studies provide important information on the associations between health effects and exposure of human populations to ambient air pollution. 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 & Greenland, 1998). One approach to remove spurious associations from possible confounders is to control for characteristics that may differ between exposed and unexposed persons; this is frequently termed "adjustment." Appropriate statistical adjustment for confounders requires identifying and measuring all reasonably expected confounders. Deciding which variables to control for in a statistical analysis of the association between exposure and disease or health outcome depends on knowledge about possible mechanisms and the distributions of these factors in

the population under study. In addition, scientific judgment is needed regarding likely sources and magnitude of confounding, together with consideration of how well the existing constellation of study designs, results, and analyses address this potential threat to inferential validity.

Another important consideration in the evaluation of epidemiologic evidence is effect modification. "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" (Rothman & Greenland, 1998). Examples of effect modifiers in some of the studies evaluated in this ISA include environmental variables, such as temperature or humidity, individual risk factors, such as education, cigarette smoking status, age in a prospective cohort study, and community factors, such as percent of population >65 years old. It is often possible to stratify the relationship between health outcome and exposure or biomarker by one or more of these risk factor variables. 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. Effect modifiers may be encountered (1) within single-city time-series studies; or (2) across cities in a two-stage hierarchical model or meta-analysis.

Several statistical methods are available to detect and control for potential confounders, with none of them being 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 pollutant likely responsible for the health outcome 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, more than one pollutant may exert similar health effects, resulting in independently observed associations for multiple pollutants. Further, the correlation between the air pollutant of interest and various copollutants may make it difficult to discern associations between different pollutant exposures and health effects. Thus, results of models that attempt to distinguish gaseous and particle effects must be interpreted with caution. 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.

Another way to adjust for potential confounding is through stratified analysis, i.e., examining the association within homogeneous groups with respect to the confounding variable. The use of stratified analyses has an additional benefit: it allows examination of effect modification through comparison of the effect estimates across different groups. If investigators successfully measured characteristics that distort the results, adjustment of these factors help separate a spurious from a true causal association. Appropriate statistical adjustment for confounders requires identifying and measuring all reasonably expected confounders. Deciding which variables to control for in a statistical analysis of the association between exposure and disease or health outcome depends on knowledge about possible mechanisms and the distributions of these factors in the population under study. Identifying these mechanisms makes it possible to control for potential sources that may result in a spurious association.

Adjustment for potential confounders can be influenced by differential exposure measurement error. There are several components that contribute to exposure measurement error in 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. Previous AQCDs have examined the role of measurement error in time-series epidemiologic studies using simulated data and mathematical analyses and suggested that "transfer of effects" would only occur under unusual circumstances (i.e., "true" predictors having high positive or negative correlation; substantial measurement error; or extremely negatively correlated measurement errors) (U.S. EPA, 2004).

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. Thus, multicity studies which use a consistent method to analyze data from across locations with different levels of covariates can provide insight on potential confounding in associations. Intervention studies, because of their quasi-experimental nature, can be particularly useful in characterizing causation.

In addition to controlled human exposure and epidemiologic studies, the tools of experimental biology have been valuable for developing insights into human physiology and pathology. Laboratory tools have been extended to explore the effects of putative toxicants on human health, especially through the study of model systems in other species. These studies evaluate the effects of exposures to a variety of pollutants in a highly controlled laboratory setting and allow exploration of MOAs or mechanisms by which a pollutant may cause effects. Background knowledge of the biological mechanisms by which an exposure might or might not cause disease can prove crucial in establishing or negating a causal claim. There are, however, uncertainties associated with quantitative extrapolations between laboratory animals and humans on the pathophysiological effects

of any pollutant. Animal species can differ from each other in fundamental aspects of physiology and anatomy (e.g., metabolism, airway branching, hormonal regulation) that may limit extrapolation.

Interpretations of experimental studies of pollutant effects in laboratory animals, as in the case of environmental comparative toxicology studies, are affected by limitations associated with extrapolation models. The differences between humans and rodents with regard to pollutant absorption and distribution profiles based on metabolism, hormonal regulation, exposure dose, and differences in target organ structure and anatomy, all have to be taken into consideration. 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 molecular differences, at this time there are uncertainties associated with quantitative extrapolations between laboratory animals and humans of observed pollutant-induced pathophysiological alterations under the control of widely varying biochemical, endocrine, and neuronal factors.

1.6.4. Application of Framework for Causal Determination

EPA uses a two-step approach to evaluate the scientific evidence on health or environmental effects of criteria pollutants. The first step determines the weight of evidence in support of causation and characterizes the strength of any resulting causal classification. The second step includes further evaluation of the quantitative evidence regarding the concentration-response relationships and the loads or levels, 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 most widely cited aspects of causality in epidemiology, and public health, in general, were articulated by Sir Austin Bradford Hill (1965) and have been widely used (CDC, 2004; IARC, 2006; NRC, 2004; Samet & C. C. Bodurow, 2008; U.S. EPA, 2005a). Several adaptations of the Hill aspects have been used in aiding causality judgments in the ecological sciences (Adams, 2003; Collier, 2003; Fox, 1991; Gerritsen et al., 1998). These aspects (Hill, 1965) have been modified (Table 1-1) for use in causal determinations specific to health and welfare effects or pollutant exposures.[2] Some aspects are more likely than others to be relevant for evaluating evidence on the health or environmental effects of criteria air pollutants. For example, the analogy aspect does not always apply and specificity would not be expected for multi-etiologic health outcomes such as asthma or cardiovascular disease, or ecological effects related to acidification. Aspects that usually

[1] The "aspects" described by 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, and animal toxicological studies, as well as in vitro data, and to be more consistent with EPA's Guidelines for Carcinogen Risk Assessment.

play a larger role in determination of causality are consistency of results across studies, coherence of effects observed in different study types or disciplines, biological plausibility, exposure-response relationship, and evidence from "natural" experiments.

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 important considerations are taken into account with the goal of producing an objective appraisal of the 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 1-1 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 (CDC, 2004).

Table 1-1. Aspects to aid in judging causality

Aspect	Decription
Consistency of the observed association	An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies, conducted in multiple locations by multiple investigators. 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 epidemiologic associations may be strengthened by other lines of evidence (e.g., controlled human exposure and animal toxicological studies) that support a cause-and-effect interpretation of the association. Causality is also supported when epidemiologic associations are reported across study designs and across related health outcomes. 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 paleological/ historical reconstructions). The coherence of evidence from various fields greatly adds to the strength of an inference of causality. The absence of other lines of evidence, however, is not a reason to reject causality
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. A lack of biological understanding, however, is not a reason to reject causality.
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). There are, however, many possible reasons that a study may fail to detect an exposure-response relationship. Thus, although the presence of a biological gradient may support causality, the absence of an exposure-response relationship does not exclude a causal relationship.
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, given a truly causal agent, a small magnitude in the effect could follow from a lower level of exposure, a lower potency, or the prevalence of other agents causing similar effects. While large effects support causality, modest effects therefore do not preclude it.
Experimental evidence	The strongest evidence for causality can be provided when a change in exposure brings about 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	As originally intended, this refers to increased inference of causality if one cause is associated with a single effect or disease (Hill, 1965). Based on the current understanding this is now considered one of the weaker guidelines for causality; for example, many agents cause respiratory disease and respiratory disease has multiple causes. At the scale of ecosystems, as in epidemiology, complexity is such that single agents causing single effects, and single effects following single causes, are extremely unlikely. The ability to demonstrate specificity under certain conditions remains, however, a powerful attribute of experimental studies. Thus, although the presence of specificity may support causality, its absence does not exclude it.
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.

1.6.5. Determination of Causality

In the ISA, EPA assesses the results of recent relevant publications, building upon evidence available during the previous NAAQS review, to draw conclusions on the causal relationships between relevant exposures or body burden, as measured by blood or tissue Pb levels, and health

effects and relevant Pb concentrations and environmental effects. This ISA uses a five-level hierarchy that classifies the weight of evidence for causation, not just association[1]; that is, whether the weight of scientific evidence makes causation at least as likely as not, in the judgment of the reviewing group. 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* (2008), EPA's Guidelines for Carcinogen Risk Assessment (2005a), and the U.S. Surgeon General's smoking reports (CDC, 2004). In the ISA, EPA uses a series of five descriptors to characterize the weight of evidence for causality. This weight of evidence evaluation is based on 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 1-2.

For the Pb ISA, determination of causality involved the evaluation of evidence for different types of health effects associated with Pb biomarkers of exposure and body burden (i.e., blood and tissue). In making determinations of causality for Pb, evidence was evaluated for health outcome categories, such as neurological effects, and then conclusions were drawn based upon the integration of evidence from across disciplines (e.g., epidemiology and toxicology) and also across the suite of related individual health outcomes. To accomplish this integration, evidence from multiple and various types of studies was considered. Response was evaluated over a range of observations which was determined by the type of study and methods of exposure or dose and response measurements. Results from different protocols were compared and contrasted.

[1] It should be noted that the 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 (U.S. EPA, 2005a) and to provide a more nuanced set of categories.

Table 1-2. Weight of evidence for causal determination

Determination	Health Effects	Ecological and Welfare Effects
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant blood or tissue Pb levels. That is, blood or tissue Pb levels have 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 replicated and consistent high-quality studies by multiple investigators.	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures. 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 blood or tissue Pb levels, but important uncertainties remain. That is, blood or tissue Pb levels have 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 confounding factors 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 replicated and high-quality studies by multiple investigators.	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 blood or tissue Pb levels, but is limited because chance, bias and confounding cannot be ruled out. For example, at least one high-quality epidemiologic study shows an association with a given health outcome but the results of other studies are inconsistent.	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 blood or tissue Pb levels. 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 blood or tissue Pb levels. Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter and considering susceptible 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.

In drawing judgments regarding causality for the criteria air pollutants, EPA typically focuses on evidence of effects at relevant pollutant exposures. For making causality judgments for Pb health effects, the focus is on evidence of exposure or body burden as indicated by relevant (within one order of magnitude) blood or tissue Pb levels of the current U.S. population (median blood Pb level

= 1.3; 95th percentile = 4.1; 99th percentile = 7.2). Studies of the efficacy of chelation therapy in Pb-poisoned children and toxicological studies in which Pb levels were sufficiently high to induce an overtly toxic response in the animals (e.g., mortality) were specifically excluded. Studies of workers exposed to Pb in occupational settings were generally considered in the causal determinations. Building upon the determination of causality are questions relevant to quantifying health or environmental risks based on our understanding of the quantitative relationships between pollutant exposures or biomarkers and health or welfare effects. While the causality determination is based primarily on evaluation of health or environmental effects evidence, EPA also evaluates evidence related to the doses or biomarker levels at which effects are observed. Considerations relevant to evaluation of quantitative relationships for health and environmental effects are summarized below.

1.6.5.1. 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 appear to be differentially affected (i.e., more susceptible to effects)?

To address these questions, the entirety of policy-relevant quantitative evidence is evaluated to best quantify those concentration-response relationships that exist. For Pb, evaluation of blood or tissue Pb concentrations at which effects were observed for exposed populations, including potentially susceptible populations, has been an important element of this process. The integration of evidence resulted in identification of a study or set of studies that best approximated the concentration-response relationships between blood Pb and various health outcomes, given the current state of knowledge and the uncertainties that surrounded these estimates. To accomplish this, evidence is considered from multiple and diverse types of studies. To the extent available, the ISA evaluates results from across epidemiologic studies that use various methods to evaluate the form of relationships between blood or tissue Pb concentrations and health outcomes and draws conclusions on the most well-supported shape of these relationships. Animal data may also inform evaluation of concentration-response relationships, particularly relative to MOAs and characteristics of susceptible populations. Chapter 2 presents the integrated findings informative for evaluation of population risks.

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 full concentration range encountered or if nonlinear relationships exist along any part of this range. In general, the shape of the concentration-response curve varies, depending on the type of health outcome, underlying MOA and dose. At the human population level, however, various sources of variability and uncertainty, such as the low data density at the lowest blood Pb levels, possible influence of exposure measurement error, and individual differences in susceptibility to Pb health effects, tend to smooth and "linearize" the concentration-response function. In addition, many chemicals and agents may act by perturbing naturally occurring background processes that lead to disease, which also linearizes population concentration-response relationships (Clewell & Crump, 2005; Crump et al., 1976; Hoel, 1980). These attributes of population dose-response may explain why the available human data at ambient concentrations for some environmental pollutants (e.g., Pb, PM, O_3, environmental tobacco smoke [ETS], radiation) do not exhibit evident thresholds for cancer or noncancer health effects, even though likely mechanisms include nonlinear processes for some key events. These attributes of human population dose-response relationships have been extensively discussed in the broader epidemiologic literature (Rothman & Greenland, 1998). Of particular interest for Pb is the shape of the concentration- response curve at the low end (<10 μg/dL) of current blood Pb concentrations observed in the U.S. population.

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, and publication bias can also 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 (Anderson et al., 2005).

Finally, identification of the susceptible population groups contributes to an understanding of the public health impact of pollutant exposures. In this ISA, the term "susceptible population" will be used as an overarching concept to encompass populations variously described as susceptible, vulnerable, or sensitive. "Susceptible populations" is defined here as those populations that have a greater likelihood of experiencing health effects related to exposure to an air pollutant (e.g., Pb) due to a variety of factors including but not limited to: genetic or developmental factors, race, gender, lifestage, lifestyle (e.g., smoking status and nutrition) or preexisting disease; as well as population-level factors that can increase an individual's exposure to an air pollutant (e.g., Pb) such as socioeconomic status [SES], which encompasses reduced access to health care, low educational attainment, residential location, and other factors. Epidemiologic studies can help identify susceptible populations by evaluating health responses in the study population. Examples include stratified analyses for subsets of the population under study or 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. Further discussion of these groups is presented in Chapter 6.

1.6.5.2. 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?
- Under what exposure conditions (amount deposited or concentration, duration and pattern) are effects observed?
- 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 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 available site by site. For example, an ecological response to deposition of a given pollutant can differ greatly between ecosystems. Where results from greenhouse or animal ecotoxicological studies are available, they may be used to aid in characterizing exposure-response relations, particularly relative to mechanisms of action, and characteristics of sensitive biota.

1.6.6. Concepts in Evaluating Adversity of Health Effects

In evaluating the health evidence, a number of factors can be considered in determining the extent to which health effects are "adverse" for health outcomes such as changes in lung function or in cardiovascular health measures. Some health outcome events, such as hospitalization for respiratory or cardiovascular diseases, are clearly considered adverse; what is more difficult is determining the extent of change in the more subtle health measures that is adverse. What constitutes an adverse health effect may vary between populations. Some changes in healthy individuals may not be considered adverse while those of a similar type and magnitude are potentially adverse in more susceptible individuals.

For example, 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?* (2000). This statement updated the guidance for defining adverse respiratory health effects that had been published 15 years earlier (ATS, 1985), taking into account

new investigative approaches used to identify the effects of air pollution and reflecting concern for impacts of air pollution on specific susceptible groups. In the 2000 update, there was an increased focus on quality of life measures as indicators of adversity and a more specific consideration of population risk. Exposure to air pollution that increases the risk of an adverse effect to the entire population is viewed as adverse, even though it may not increase the risk of any identifiable 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.

1.7. Summary

This draft ISA is a concise evaluation and synthesis of the most policy-relevant science for reviewing the NAAQS for Pb, and it is the chief means for communicating the critical science judgments relevant to that NAAQS review. It reviews the most policy-relevant evidence from atmospheric science, exposure, and health and environmental effects studies and includes mechanistic evidence from basic biological science. A framework for making critical judgments concerning causality was presented in this chapter. It relies on a widely accepted set of principles and standardized language to express evaluation of the evidence. This approach can bring rigor and clarity to current and future assessments. Once complete, the ISA should assist EPA and others, now and in the future, to accurately represent what is presently known and what remains unknown concerning the effects of Pb on human health and public welfare.

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Chapter 2. Integrative Health and Ecological Effects Overview

The subsequent chapters of this ISA will present the most policy relevant information related to this review of the science supporting the NAAQS for Pb. This chapter integrates the key findings from the disciplines evaluated in this current assessment of the Pb scientific literature, which includes studies of Pb sources, fate and transport of Pb, ambient air concentrations, exposure assessments, toxicokinetics, biomarkers and models of Pb burden, health (e.g., both toxicology and epidemiology), and ecological effects of Pb. The EPA framework for causal determinations described in Chapter 1 has been applied to the body of scientific evidence in order to collectively examine the health and ecological effects attributed to Pb exposure in a two-step process. The first step is to establish causal relationships followed by identification of concentration-response relationships

As described in Chapter 1, EPA assesses the results of recent relevant publications, building upon evidence available during the previous NAAQS reviews, to draw conclusions on the causal relationships between relevant pollutant exposures and health or environmental effects. 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

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 ecological 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?

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

- What exposure conditions (dose or exposure, exposure pathways, duration and pattern) are important?
- What populations and lifestages appear to be differentially affected i.e., more susceptible to 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?

To address these questions, in the second step of the EPA framework, the entirety of quantitative evidence is evaluated to identify and characterize potential concentration-response relationships. This requires the evaluation of the levels of the pollutant and the exposure durations at which effects were observed for exposed populations including potentially susceptible populations.

This chapter summarizes and integrates the newly available scientific evidence that best informs consideration of the policy-relevant questions that frame this assessment, presented in Chapter 1. Section 2.1 discusses the trends in ambient Pb sources and concentrations, including the fate and transport of Pb in the environment, and provides a brief summary of the topics covered. Section 2.2 presents the evidence regarding exposure to ambient Pb and describes air-related Pb exposure pathways. Section 2.3 provides a discussion of the toxicokinetics of Pb and Pb biomarkers are discussed in Section 2.4. Section 2.5 summarizes effects of Pb on specific health endpoints and Section 2.6 summarizes the evidence relating to the ecological effects of Pb. Section 2.7 integrates the scientific evidence across various health and ecological endpoints highlighting common modes of action where applicable. Finally, Section 2.8 provides a discussion of policy relevant considerations including air-to-blood relationships, concentration-response relationships, timing and duration of exposure and susceptible populations. Section 2.9 is a summary of the health and ecological effects of Pb.

2.1. Ambient Lead: Source to Concentration

2.1.1. Sources, Fate and Transport of Ambient Lead

The findings of this review with respect to sources of atmospheric Pb build upon those from the 2006 Pb AQCD (U.S. EPA, 2006), which 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 1990 and then by an additional 77% from 1990 to 2008, at which time emissions were 1,200 tons per year. Data from the 2008 National Emissions Inventory (NEI) (U.S. EPA, 2011) illustrate that piston engine aircraft emissions now comprise the largest share (~49%) of total atmospheric Pb emissions; the 2008 NEI estimated that 590 tons of Pb were emitted from this source. Other sources of ambient air Pb, beginning with the largest, include metals processing, fossil fuel combustion, other industrial sources, roadway

related sources, and historic Pb. Chemical speciation of Pb had also been fairly well characterized in the 2006 Pb AQCD (2006). Estimates from the 1986 Pb AQCD (U.S. EPA, 1986, 2006) for organic automotive Pb emissions provides an upper bound for organic vapor emissions of 20% of total Pb dibromide and Pb bromide emissions from piston engine aircraft. Recent speciation studies of smelting and battery-recycling operations have shown that Pb sulfide and Pb sulfates are abundant within the emissions mixture for such industrial operations.

The atmosphere is the main environmental transport pathway for Pb, and on a global scale atmospheric Pb is primarily associated with fine particulate matter (PM). Global atmospheric Pb deposition peaked in the 1970s, followed by a more recent decline. On a local scale, Pb concentrations in soils (including urban areas where historic use was widespread) can be substantial, and coarse Pb-bearing PM experiences cycles of deposition and resuspension that serve to distribute it. Both wet and dry deposition are important removal mechanisms for atmospheric Pb. Because Pb in fine particles is typically fairly soluble, wet deposition is more important for fine Pb. In contrast, Pb associated with coarse particles is usually insoluble, and removed by dry deposition. However, local deposition fluxes are much higher near local industrial sources and a substantial amount of emitted Pb is deposited near sources, leading to high soil Pb concentrations. Resuspension by wind and traffic can be an important source of airborne Pb near sources where Pb occurs in substantial amounts in surface dust.

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, leading to high soil concentrations, while fine Pb-bearing PM can be transported long distances, leading to contamination of remote areas. Surface waters act as an important reservoir, with Pb lifetimes largely controlled by deposition and resuspension of Pb in sediments. Substantial amounts of Pb from vehicle wear and building materials can also be transported by runoff waters without becoming airborne. Pb containing sediment particles can be remobilized into the water column, and sediment concentrations tend to follow those in overlying waters.

2.1.2. Monitoring and Concentrations of Ambient Air Lead

In recognition of the role of all PM sizes in ambient air Pb exposures, including the ingestion of particles deposited onto surfaces, the indicator for the Pb NAAQS is Pb in total suspended particulate (Pb-TSP). Although there is a lower rate of error in estimating ambient Pb from Pb-PM_{10} monitoring than from Pb-TSP monitoring, the Pb-TSP indicator was retained in 2008 because ingestion after deposition in the upper respiratory tract was considered an important component of Pb exposure. A new federal reference method (FRM) for Pb-PM_{10} has been implemented in which ambient air is drawn through an inertial particle size separator for collection on a polytetrafluoroethylene (PTFE) filter. Several FEMs

have also been approved. The FRM is based on flame AAS. ICPMS is under consideration as a new FRM for Pb-TSP.

Monitoring for ambient Pb levels is required for all areas where Pb levels have been shown or are expected to contribute to maximum concentrations of 0.10 μg/m3 or greater over a 3-year time period. Pb is monitored routinely at state and local air monitoring stations (SLAMS) that report data used for NAAQS compliance to the air quality system (AQS) database. Pb monitoring requirements have experienced several changes since publication of the 2006 Pb AQCD (U.S. EPA, 2006). 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, and is planned for the national core multipollutant monitoring network (NCore) network. While monitoring in multiple networks provides extensive geographic coverage, measurements between networks are not directly comparable in all cases because different particle size ranges are sampled in different networks. Depending on monitoring network, Pb is monitored in TSP, PM_{10}, or $PM_{2.5}$. Monitors reporting to the AQS were considered for the purpose of this ISA to be source oriented if they were designated in AQS as source oriented, or they were located within 1 mile of a 0.5 ton per year or greater source, as noted in the 2005 NEI (U.S. EPA, 2008). Non-source oriented monitors were those monitors not considered to be source oriented.

Ambient air Pb concentrations have declined drastically over the period 1980-2009. The median annual concentrations have dropped by 97% from 0.87 $\mu g/m^3$ in 1980 to 0.025 $\mu g/m^3$ in 2009. While the sharpest drop in Pb concentration occurred during 1980-1990, a declining trend was observed between 1990 and 2009. Compared to 1980-1990, a smaller reduction was observable among source oriented Pb concentration (56%) and non-source oriented Pb data (51%) for 2000-2009.

AQS data for source oriented and non-source oriented monitoring were analyzed for 2007-2009. For source oriented monitoring, the three-month rolling average was measured to be above the level of the NAAQS in fourteen counties across the U.S. Pb concentrations, seasonal variations, inter-monitor correlations, and wind data were analyzed for six counties: Los Angeles County, CA; Hillsborough/Pinellas Counties, FL; Cook County, IL; Jefferson County, MO; Cuyahoga County, OH; and Sullivan County, TN. Spatial and temporal variability of Pb concentrations in each county were commonly high. Meteorology, 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. PM size distribution also influenced how far the particle will travel upon initial emission or resuspension before being deposited. Additionally, resuspension and urban background levels of Pb were uncertain influential factors of ambient Pb concentrations. Given variability in these conditions, it was very difficult to predict how Pb concentration varies over time and space. This was consistent with field studies to characterize Pb concentrations that were described in the literature.

Size distribution of Pb-bearing PM was demonstrated to vary substantially for several studies presented, depending on the nature of Pb sources and proximity of the monitors to the Pb sources. Variation in the correlation of size fractionated Pb samples among different land use types may be explained by differences in sources across land use types. Additionally, Pb concentrations exhibited varying degrees of association with other criteria pollutant concentrations. Overall, Pb was moderately associated with $PM_{2.5}$, PM_{10} and NO_2. Pb was moderately associated with CO in fall and winter only. The poorest associations were observed between Pb and O_3. Among trace metals, the strongest association was with Zn. Br, Cu, and K concentrations also exhibited moderate associations with Pb concentrations. Such correlations may suggest some common sources affecting the concentrations of various pollutants.

2.1.3. Ambient Lead 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. After the 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 have also been observed. In contrast, Pb is retained in soils and sediments, where it provides 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 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. Although 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 emissions reductions from other sources.

2.2. Exposure to Ambient Lead

Exposure data considered in this assessment build upon the conclusions of the 2006 Pb AQCD (2006), which found air Pb concentrations in the U.S. and associated biomarkers of exposure to have decreased substantially following the ban on Pb in gasoline as well as earlier bans on Pb in 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 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, and a fraction of that suspended Pb may originate from waters used to irrigate the soil. Air-related pathways of Pb exposure are the focus of this assessment. In general, air-related pathways include those pathways where Pb passes through ambient air on its path from a source to human exposure. 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). Non-air-related exposures include occupational exposures, hand-to-mouth 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 non-air exposures are reviewed in this section because they can also contribute to Pb body burden.

Section 4.1 presents data illustrating potential exposure mechanisms. Several studies suggested that soil can act as a reservoir for historically deposited and contemporaneous Pb emissions from industrial or other activities. Exposure to soil contaminated with deposited Pb can occur through resuspended PM as well as shoe tracking and hand-to-mouth contact. In general, soil Pb concentrations tended to be higher within inner-city communities compared with suburban neighborhoods. Infiltration of Pb dust has been demonstrated, and Pb dust has been shown to persist in indoor environments even after repeated cleanings. Measurements of particle-bound Pb exposures reported in this assessment have shown that personal exposure to Pb is typically higher than indoor or outdoor ambient Pb concentrations. These findings regarding personal exposure may be related resuspension of Pb that occurs with body movement.

Observational studies using biomarkers of Pb as exposure metrics are also included in Section 4.1. The median blood Pb level for the entire U.S. population is 1.2 μg/dL and the 95th percentile blood Pb level is 3.7 μg/dL, based on the 2007-2008 NHANES data (NCHS, 2010). Among children aged 1-5 years, the median and 95th percentiles were slightly higher at 1.4 μg/dL and 4.1 μg/dL, respectively. Overall, trends in blood Pb levels have been decreasing among U.S. children and adults over the past 20 years. Concurrent changes in isotopic ratios of blood Pb samples reflect changes in source composition over the past several decades. Several studies have regressed blood Pb as a function of environmental Pb samples such as air Pb or Pb dust fall. Recent studies have observed a relationship between blood Pb and soil Pb concentration. Studies have suggested that blood Pb is associated with exposure to Pb paints in older homes, Pb released into drinking water, and occupational work with materials containing Pb. Studies that examine blood Pb as a function of ambient air Pb measurements are discussed in Section 2.8.1 that follows.

Sequential extraction has been used to estimate the gastric bioavailability of particle bound Pb after exposure occurs. Findings from these studies have been mixed, ranging from 13 to 86%, but such

variation is likely a function of the particle sizes from which the Pb was extracted as well as the acid mixture used to simulate gastric juices. Estimates of bioavailability of inhaled organic Pb to the lungs are available only from older studies in the literature and suggest a that it is possible for all inhaled organic Pb to enter the blood stream (Chamberlain et al., 1975).

2.3. 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. Saturable binding to RBC proteins contributes to an increase in the plasma/blood Pb ratio with increasing blood Pb level concentration and curvature to the blood Pb-plasma Pb relationship. As blood Pb level increases and the higher affinity binding sites for Pb in RBCs become saturated at approximately 40 µg/dL blood, 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 compartment (bone) and a smaller fast compartment (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 & Wittmers, 1992). 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.

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 to 20. 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 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 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). Due to the more rapid turnover of bone mineral in children, changes in blood Pb concentration are thought to more closely parallel changes in total body burden. However, some Pb accumulated in bone during childhood does persist into later life. Potential

mobilization of Pb from the skeleton could occur in adults at times of physiological stress associated with enhanced bone remodeling such as during pregnancy and lactation, menopause or in older adulthood, extended bed rest, hyperparathyroidism, and weightlessness. Regardless of age, however, similar blood Pb concentrations in two individuals (or populations) do not necessarily translate to similar body burdens or similar exposure histories.

The kinetics of elimination of Pb from the body reflects the existence of fast and slow pools of Pb in the body. The dominant phase of Pb kinetics in the blood, exhibited shortly after a change in exposure occurs, has an elimination 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 may become evident with longer observation periods following a decrease in exposure due to the gradual redistribution of Pb among other compartments via the blood. Therefore, a single blood Pb concentration may reflect the near-term or longer-term history of the individual to varying degrees, depending on the relative contributions of internal (e.g., bone) and external sources of Pb to blood Pb, which in turn will depend on the exposure history and possibly age-related and individual-specific (e.g., pregnancy, lactation) characteristics of bone turnover. In general, higher blood Pb concentrations can be interpreted as indicating higher exposures (or Pb uptakes); however, they do not necessarily predict higher body burdens, especially in adults.

2.4. Lead Biomarkers

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 variables that may affect bone remodeling (e.g., nutritional status, pregnancy, and menopause). Blood Pb in adults is typically more an index of recent exposures than body burden, whereas bone Pb is an index of cumulative exposure and body burden. In children, 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 some physiological circumstances (e.g., osteoporosis), bone Pb may contribute to blood Pb in adults. The disparity between blood Pb and body burden may have important implications for the interpretation of blood Pb measurements in some epidemiology studies. Conceptually, measurement of long-term Pb body burden (i.e., based on tibia Pb) may be the appropriate metric if the effects of Pb on a particular outcome are lasting and cumulative. However, if the effects of Pb on the outcome represent the acute effects of current exposure, then blood Pb may be the preferred metric. In the absence of clear evidence as to whether a particular outcome is an acute effect of recent Pb dose or a chronic effect of cumulative Pb exposure, both blood and bone metrics should be considered.

Cross-sectional studies that sample blood Pb once generally provide an index of recent exposures. In contrast, cross-sectional studies of bone Pb and longitudinal samples of blood Pb concentrations over time provide 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.

The concentration of Pb in urine is a function of the urinary Pb excretion and the urine flow rate. Urine flow rate requires collection of a timed urine sample, which is often problematic in epidemiologic studies. Collection of un-timed ("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). Urinary Pb concentration reflects, mainly, the exposure history of the previous few months; thus, a single urinary Pb measurement cannot distinguish between a long-term low level of exposure or a higher acute exposure. 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 better reflect body burden.

2.5. Health Effects

This section evaluates the evidence from toxicological and epidemiologic studies that examined the health effects associated with exposure to Pb. The results from the health studies evaluated in combination with the evidence from other disciplines (e.g., fate and transport, exposure sciences, toxicokinetics) contribute to the causal determinations (Section 1.6.4) made for the health outcomes discussed in this assessment. In the following sections a discussion of the causal determinations will be presented for the health effects for which sufficient evidence was available to conclude a causal or likely to be causal relationship (Table 2-1). Although not presented in depth in this chapter, a detailed discussion of the underlying evidence used to formulate each causal determination can be found in Chapter 5 of this document.

Table 2-1. Summary of causal determinations between exposure to Pb and health outcomes

Outcome	Causality Determination
Neurological Effects	Causal Relationship
Cardiovascular Effects	Causal Relationship
Renal Effects	Causal Relationship
Immune System Effects	Causal Relationship
Effects on Heme Synthesis and Red Blood Cell Function	Causal Relationship
Reproductive Effects and Birth Outcomes	Causal Relationship
Cancer	Likely Causal Relationship

2.5.1. Neurological Effects

The 2006 Pb AQCD concluded that the collective body of epidemiologic studies provides clear and consistent evidence for the effects of Pb exposure on neurocognitive function in children. This conclusion was substantiated by findings in diverse populations that blood Pb levels were associated with a broad spectrum of cognitive and behavioral endpoints, including IQ, higher-order processes such as language and memory, academic achievement, behavior and conduct, sensory acuities, and changes in brain structure and activity as assessed by magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS). Toxicological studies not only provided coherence with similarly consistent findings for Pb-induced impairments in learning, behavior, and sensory acuities, but also provided biological plausibility by characterizing mechanisms for Pb-induced neurotoxicity. These mechanisms included Pb-induced inhibition of neurotransmitter release and decreases in synaptic plasticity, neuronal differentiation, and blood-brain-barrier integrity. Both epidemiologic studies (in children) and toxicological studies, demonstrated neurocognitive deficits in association with blood Pb levels at and below 10 μg/dL, and evidence from both disciplines supported a nonlinear exposure-response relationship, with greater effects estimated for lower blood Pb levels. Among environmentally-exposed adults, the most consistent findings were associations between cumulative Pb exposure, as assessed by serial blood Pb or bone Pb measurements, and cognitive deficits.

Building on this strong body of extant evidence, recent studies continue to demonstrate associations between Pb exposure and neurological effects. While recent epidemiologic studies in children continued to demonstrate associations with IQ, most evidence emphasized associations of blood Pb levels (as low as 2 μg/dL) with specific indices of neurocognitive function such as reading and verbal skills, memory, visuospatial processing, and academic achievement. Nonetheless, these newer findings are concordant with the previous body of evidence given that IQ is a global measure of cognitive function that reflects the integration of several neurocognitive domains. Additional coherence for findings in

children is provided by evidence in animals that blood Pb levels of 1.8 µg/dL and higher are associated with decrements in learning and memory. New findings in animals emphasized the role of stress in potentiating the low dose effects of Pb on behavior and memory. In animals, the developmental period is the most sensitive window for Pb-dependent neurotoxicity, whereas in children, concurrent blood Pb was generally found to be the best predictor of cognitive decrements.

Recent studies in children continue to support associations of Pb exposure (blood Pb levels 3-11 µg/dL) with a range of behavioral problems from anxiety and distractibility to conduct disorder and delinquent behavior. Whereas previous evidence was not compelling, new evidence indicates associations comparing the lowest quartiles of blood Pb level (0.8-1 µg/dL versus <0.8 µg/dL) and ADHD. These findings for ADHD are well supported by observations in animals of Pb-induced increased response rates and impulsivity. Both epidemiologic studies in children and toxicological studies demonstrate associations of Pb exposure with deficits in visual acuity and hearing and auditory processing. New evidence from toxicological studies demonstrates these effects at lower exposure levels (blood Pb levels <15 µg/dL). Combined evidence for Pb-associated neurocognitive deficits (e.g., inattention, conduct disorder, and effects on sensory function) provides plausible mechanisms by which Pb exposure may contribute to academic underachievement and to more serious problems of delinquent behavior.

Studies of adults without occupational Pb exposure have not provided consistent evidence for associations between blood Pb and the range of neurological effects. One explanation for the weaker evidence may be that cognitive reserve may compensate for the effects of Pb exposure on learning new information. Compensatory mechanisms may become less effective with increasing age, explaining the consistent associations between measures of cumulative Pb exposure and neurocognitive deficits. Among recent studies of adults, blood Pb and bone Pb are associated with essential tremor and Parkinson's disease, respectively. Consistent with these findings, toxicological studies demonstrate Pb-induced decreased dopaminergic cell activity in the substantia nigra, which contributes to the primary symptoms of Parkinson's disease. Biological plausibility also is provided by observations of developmental Pb exposures of monkeys and rats inducing neurodegeneration in the aged brain. A recent epidemiological study indicated that early-life ALAD activity, a biomarker of Pb exposure, may be associated with schizophrenia later in adulthood. Consistent with these findings, toxicological studies have observed Pb-induced emotional changes in males and depression in females. It is not surprising that Pb exposure may increase the risk of different neurological endpoints in children and adults given the predominance of age-dependent neurological processes, in particular, neurogenesis and brain development in children and neurodegeneration in adults.

Extensive evidence from toxicological studies, as well as evidence in some aquatic and terrestrial animal taxa (Section 2.6.9) clearly substantiates the biological plausibility for epidemiologic findings by characterizing mechanisms underlying neurological effects. Pb induces complex neurochemical changes in the brain that differ by region of the brain, neurotransmitter type, age, and sex of the organism. These

changes remain aberrant over time but are dynamic in nature. Pb exposure of animals induces changes in the transmission of dopamine, which plays a key role in cognitive functions mediated by the prefrontal cortex and in motor functions mediated by the substantia nigra. Current toxicological research has been expanded to document that early-life Pb exposure can contribute to neurodegeneration and neurofibrillary tangle formation in the aged brain. Pb exposure can affect NMDA receptors, which can contribute to mood disorders. Synapse formation, adhesion molecules, and nitrosative stress continue to be areas in which research is being conducted related to Pb-associated neurotoxicity. Finally, the new area of epigenetics shows that Pb exposure affects methylation patterns in rodent brains. These toxicological data provide coherence with epidemiologic observations, in particular, associations of Pb exposure with cognitive deficits, Parkinson's disease, and mood disorders.

In summary, recent evidence substantiates and expands upon the established epidemiologic and toxicological literature demonstrating the neurological effects of Pb exposure. Both the consistency of evidence across toxicological and epidemiologic studies and the coherence of findings across the full spectrum of neurological endpoints, from mechanistic changes to impairments in cognitive function and behavior and to poorer academic achievement and delinquency, are illustrated in Figure 2-1. In epidemiologic studies of children, consistently positive associations of blood Pb levels with deficits in neurocognitive function, attention, and sensory acuities support observed associations with academic underachievement, which in turn, may explain associations with delinquent and criminal behavior. In particular, observations of cognitive and behavioral deficits in association with blood Pb levels in the range of 1-2 µg/dL indicate that a threshold may not exist for the neurological effects of Pb in children. Epidemiologic findings are strengthened by their biological plausibility in light of toxicological study findings and their coherence with toxicological findings for similar or parallel endpoints and for the mechanisms underlying the neurological effects. The collective body of evidence integrated across epidemiologic and toxicological studies and across the spectrum of neurological endpoints is sufficient to conclude that there is a **causal relationship between Pb exposures and neurological effects**.

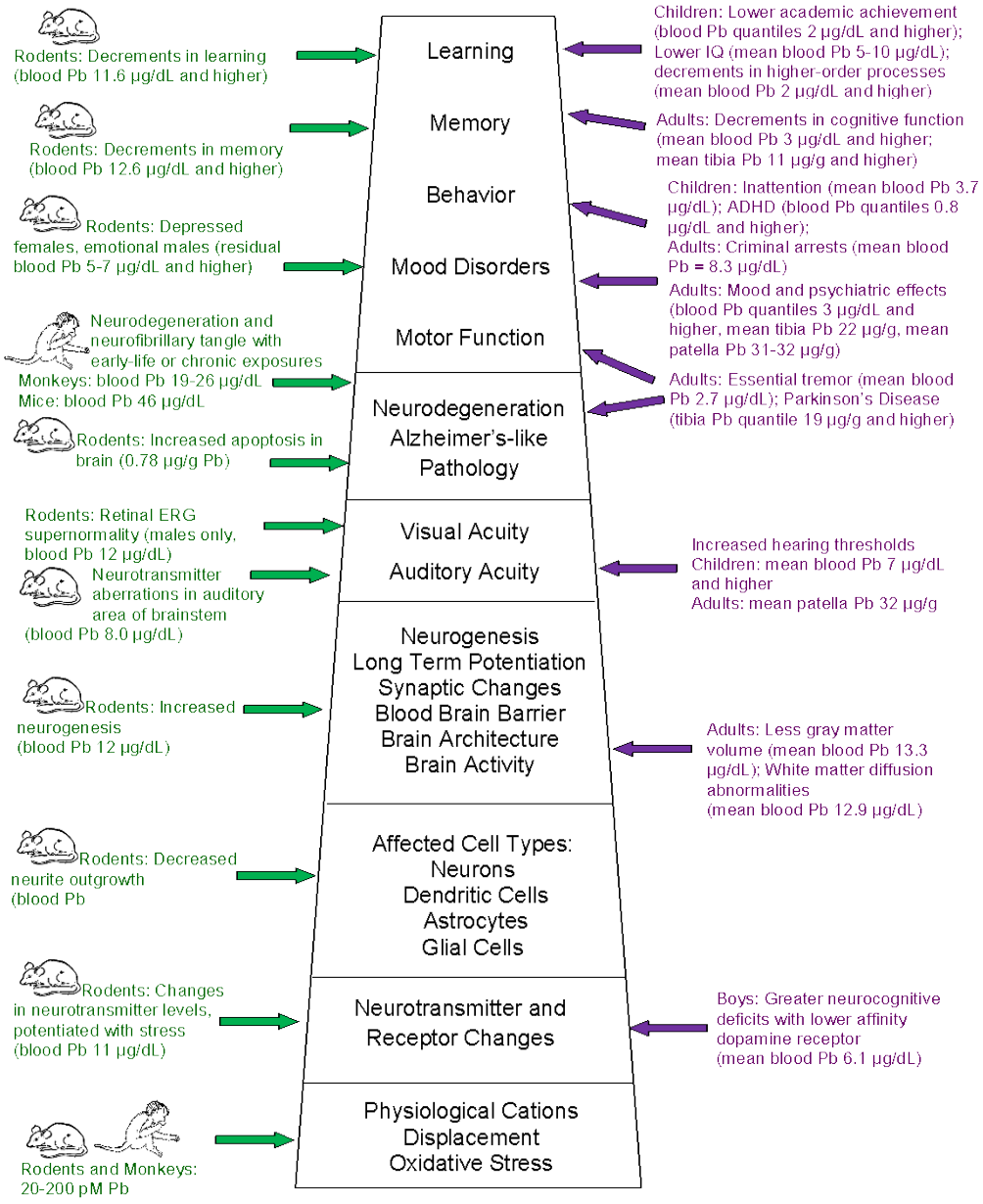


Figure 2-1. Snapshot of evidence for the spectrum of effects to the nervous system associated with Pb exposure. Green=animal toxicological studies (left side); purple=epidemiological studies (right side).

2.5.2. Cardiovascular Effects

The 2006 Pb AQCD concluded that there was a relationship between increased Pb exposure and increased adverse cardiovascular outcomes, including increased blood pressure (BP) and increased incidence of hypertension (U.S. EPA, 2006). Meta-analysis of these studies found that each doubling of 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. In addition, most of the reviewed studies using cumulative Pb exposure measured by adult bone Pb levels showed increased BP. Toxicological studies provided evidence for exposure to low levels of Pb (e.g., 2 µg/dL) resulting in increased BP in experimental animals that persists long after the cessation of Pb exposure and also provided mechanistic evidence to support the biological plausibility of Pb-induced hypertension, including oxidative stress, altered sympathetic activity, and vasomodulator imbalance. Finally, limited evidence suggested a connection between Pb exposure and the development of IHD, cerebrovascular disease, peripheral vascular disease (PVD) and mortality.

Building on the strong body of evidence presented in the 2006 Pb AQCD, recent studies continue to support associations between Pb exposure and exposure biomarkers and cardiovascular effects with recent epidemiologic studies informing past uncertainties (e.g., confounding, low Pb exposures). A recent study suggested that Pb has an acute effect on BP as a function of recent dose measured by blood Pb and a chronic effect on hypertension risk as a function of cumulative exposure measured by tibia Pb (Martin et al., 2006). This study also verified the magnitude of change in BP observed in the past meta-analysis. Additionally, recent epidemiologic studies provided evidence for associations between blood Pb and hypertension in adults with relatively low blood Pb levels; a positive relationship was found in the NHANES (1999-2002) data set at a geometric mean blood Pb level of 1.64 µg/dL (Muntner et al., 2005). Animal toxicological studies also provide support for effects of low blood Pb level on increased BP with statistically significant increases shown in animals with blood Pb levels as low as 2 µg/dL. New studies also demonstrate a partial reversibility of Pb-induced increased BP following Pb exposure cessation or chelation.

Epidemiologic studies continue to investigate the relationship between bone Pb and increased BP. Recent epidemiologic studies also emphasize the interaction between cumulative Pb exposure and factors that moderate or modify the Pb effect on BP and hypertension (e.g., chronic stress and metabolic syndrome). Further, recent epidemiologic studies found that the effects of Pb on cardiovascular endpoints (including BP, pulse pressure [PP], and QT interval) were modified by genotypes (including ALAD and genes involved in hemochromatosis or Fe metabolism). Epidemiologic and toxicological studies also provided evidence for Pb exposure to contribute to increased development of atherosclerosis, thrombosis, ischemic heart disease, peripheral artery disease, arrhythmia, and cardiac contractility. Animal toxicological evidence continues to build on the evidence supporting the biological plausibility leading to these cardiovascular alterations. New evidence extends the potential continuum of Pb-related cardiovascular effects in adults by demonstrating associations of Pb concentrations in blood and bone with both cardiovascular and all-cause mortality.

In summary, new studies evaluated in the current review support or expand upon the strong body of evidence presented in the 2006 Pb AQCD that Pb exposure is causally associated with cardiovascular health effects. Both epidemiologic and toxicological studies continue to demonstrate a consistently

positive relationship between Pb exposure and increased BP or hypertension development in adults and this relationship is observed in more recent studies of adults with blood Pb levels (mean: 2 μg/dL) lower than those reported in the 2006 Pb AQCD. While some studies evaluate concentration-response relationships of blood Pb with BP or mortality, the information is inconclusive (Section 2.8.2). Recent studies investigating measures of cumulative Pb exposure measures and suggest that bone Pb related strongly to hypertension risk. Evidence of Pb increasing the risk of development of other cardiovascular diseases also is shown. By demonstrating Pb-induced oxidative stress including ˙NO inactivation, endothelial dysfunction leading to altered vascular reactivity, activation of the RAAS, and vasomodulator imbalance, toxicological studies have characterized the mode of action of Pb and provided biological plausibility for the consistently positive associations observed in epidemiologic studies between blood and bone Pb and cardiovascular effects. These observed associations between Pb exposure and cardiovascular morbidity are supported by recent reports of increased cardiovascular mortality. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of cardiovascular health endpoints is sufficient to conclude that there is a **causal relationship between Pb exposures and cardiovascular health effects**.

2.5.3. Renal Effects

The 2006 Pb AQCD stated 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 (U.S. EPA, 2006). These findings were substantiated by the coherence of effects observed across epidemiologic and toxicological studies. Toxicological studies provided mechanistic evidence to support the biological plausibility of Pb-induced renal effects, including oxidative stress leading to ˙NO inactivation. Uncertainty remained on the implications of effects in children, confounding, hyperfiltration, and reverse causality.

Recent epidemiologic studies in general and patient populations of adults have, with few exceptions, been consistent in observing associations between bone or blood Pb levels and worse kidney function; and provide important evidence that nephrotoxicity occurs at current population Pb biomarker levels. Further, current evidence does not allow for the identification of a threshold for Pb-related nephrotoxicity. The odds of reduced eGFR increased by 36% (95% CI: 0.99, 1.85) at blood Pb levels as low as 1.6-2.4 μg/dL and by 56% (95% CI: 1.17, 2.08) at blood Pb >2.4 μg/dL. These studies benefit from a number of strengths that vary by study but include: comprehensive assessment of Pb dose (using bone Pb [as a measure of cumulative body burden], and chelatable Pb [as a measure of bioavailable Pb]); prospective study design; and statistical approaches that utilize a range of exposure and outcome measures, while adjusting for numerous kidney and Pb risk factors. General population studies also benefit from large populations in both Europe and the U.S. At blood Pb levels that are common in the

general U.S. population, Pb increases the risk for clinically relevant effects particularly in susceptible populations such as those with underlying chronic medical diseases that increase chronic kidney disease (CKD) risk such as diabetes mellitus and hypertension and co-exposure to other environmental nephrotoxicants. The uncertainty around the role of reverse causality, which attributes increases in blood Pb levels to compromised kidney excretion rather than as a causative factor for CKD, was reduced by evidence that the association between blood Pb and serum creatinine occurred over the entire serum creatinine range, including the normal range where reverse causality would not be expected. Further, recent studies have extended the limited body of evidence for effects of Pb on the kidney in children. Toxicological studies contribute support to effects of Pb in the early life window of exposure adding to the strength of the association between Pb and altered renal function in children.

CKD results in substantial morbidity and mortality and is an important risk factor for cardiac disease. As kidney dysfunction can increase BP and increased BP can lead to further damage to the kidneys, Pb-induced damage to either or both the renal and cardiovascular systems may result in a cycle of increased severity of disease. Pb exposure has been causally linked to both increased BP and other cardiovascular effects (Section 5.4) and renal dysfunction and, it is possible that the cardiovascular and renal effects of Pb observed are mechanistically linked and are contributing to the progression of the diseases. Recently available animal toxicological studies strengthen the evidence regarding the mechanisms leading to these renal alterations including oxidative stress, which is also related to CVD, infiltration of lymphocytes and macrophages associated with increased expression of NF-κB in proximal tubules and infiltrating cells, mitochondrial dysfunction, renal cell apoptosis, and glomerular hypertrophy.

In summary, new studies evaluated in the current review support or expand upon the strong body of evidence presented in the 2006 Pb AQCD that Pb exposure is associated with renal health effects. Epidemiologic studies continue to demonstrate a consistently positive relationship between blood Pb level and kidney dysfunction at blood Pb levels comparable to those occurring in the current U.S. population with no evidence for a threshold across the range of levels studied. Uncertainty regarding effects in children, confounding, hyperfiltration, and reverse causality have been reduced through consideration of the recent evidence. By demonstrating Pb-induced oxidative stress and describing mechanisms of acute changes following Pb exposure, toxicological studies provide biological plausibility for the associations observed in epidemiologic studies between Pb and kidney dysfunction. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of kidney health endpoints is sufficient to conclude that there is a **causal relationship between Pb exposures and renal health effects**.

2.5.4. Immune System Effects

The collective body of evidence integrated across epidemiologic and toxicological study findings consistently demonstrates that Pb exposure is associated with changes in a spectrum of immune mediators and functions. The majority of results from animal studies indicates that immune changes are observable at blood Pb levels in the range of 2 to 8 µg/dL. Likewise, in the newly expanded body of epidemiologic studies in environmentally-exposed children and adults, changes in immune function are demonstrated in association with mean blood Pb levels in the range of 1 to 10 µg/dL.

The strength of evidence for Pb-associated immune effects is derived not only from the consistency of associations but also from the coherence of findings between toxicological and epidemiologic studies and coherence of findings across the spectrum of related immune changes. Toxicological and epidemiologic evidence links higher Pb exposures with decreases in various T cell subtypes. These changes can affect cell-to-cell interactions that mediate acquired immunity required in subsequent memory responses to antigen exposures; however, it is unclear what effect the observed magnitudes of changes may have in attenuating acquired immunity.

The key immunomodulatory effect of Pb exposure, in terms of coherence across immune endpoints and implications for developing immune-based diseases, is the skewing of immune function away from a Th1 phenotype towards a Th2 phenotype. In toxicological studies and epidemiologic studies, this shift is well demonstrated by suppressed production of Th1 cytokines (e.g., IFN-γ) and increased production of Th2 cytokines (e.g., IL-4). A recent in vitro study indicates that Pb may promote Th2 responses by acting directly on dendritic cells, the major effector in antigen response. An increase in IL-4 from activated Th2 cells induces differentiation of B cells into Ab producing cells, thereby promoting the secretion of IgE, IgA, and IgG. In support of this well-established mechanism, toxicological studies describe Pb-induced changes in IgA, IgG, and IgM. Additionally, epidemiologic studies in children consistently link higher Pb exposures with increases in B cell abundance and increases in IgE. Observations of Pb-associated increases in Th2 responses and circulating IgE levels provide biological plausibility for epidemiologic observations in children of associations of blood Pb with asthma and allergic conditions. Such epidemiologic data are sparse, and additional studies with more rigorous methodology (e.g., longitudinal design and adjustment for potential confounders such as smoking, SES, and exposures to other metals) are needed to substantiate the findings.

Further evidence of Pb-associated suppressed Th1 activity is provided by toxicological and epidemiologic observations that Pb exposure is associated with impaired killing capacity of macrophages and neutrophils. There is toxicological and epidemiologic evidence of suppressed Th1 activity and effects on macrophage and neutrophil functional activities. This evidence provides biological plausibility for observations in animals of the Pb-induced suppression of the DTH response and the observations in both animals and humans that Pb exposure and increases the risk of infection.

Toxicological studies and a limited set of epidemiologic studies demonstrate that Pb induces macrophages into a hyperinflammatory state as characterized by suppressed production of NO and enhanced production of ROS, TNF-α, and the immunosuppressive PGE_2. Specialized macrophages residing in airways, reproductive organs, and in the nervous system indicate that immunomodulation may underlie the documented associations of Pb exposure with effects in these organ systems. Although limited mostly to toxicological studies, Pb has been shown to induce the generation of auto-antibodies, suggesting that Pb exposure may increase the risk of autoimmune conditions.

In summary, recent toxicological and epidemiologic studies support the strong body of evidence presented in the 2006 Pb AQCD that Pb exposure is associated with a broad spectrum of changes in both cell-mediated and humoral immunity to promote a Th2 phenotype and inflammation. The consistency and coherence of findings among these related immune effects, in turn, establish the biological plausibility for Pb exposure being associated with increased susceptibility to infection, autoimmunity, allergy, and effects in other organ systems. Animal studies and to a limited extent, epidemiologic studies, demonstrate increased susceptibility of prenatal exposures and enhanced responses with co-exposures to other metals. The consistency of findings and the coherence between toxicological and epidemiologic findings across the continuum of related immune responses are sufficient to conclude that there is a **causal relationship between Pb exposures and immune effects.**

2.5.5. Heme Synthesis and RBC Function

Consistent with conclusions of the 2006 Pb AQCD as well as previous assessments, recent evidence in the toxicological and epidemiologic literature supports the longstanding relationship between Pb exposure and effects on hematological endpoints, including altered heme synthesis, decreased RBC survival and function, and increased RBC oxidative stress.

Multiple occupational epidemiologic studies have shown that Pb affects several hematological parameters such as Hb, PCV, MCV, MCH, and MCHC. Although the majority of occupationally- exposed adults had blood Pb levels in excess of 20 µg/dL, decreases in Hb and PCV were also observed in an occupational cohort with a mean blood Pb level of 7 µg/dL. In addition, Pb exposure was shown to reduce Ca-ATPase and Ca-Mg-ATPase activity in RBC membranes at cord blood Pb levels of 3.54 µg/dL. Decreases in Ca-ATPase and Ca-Mg-ATPase activity leads to an increase in RBC $[Ca^{2+}]i$, increased membrane fragility, and abnormal morphological changes. Studies in children are less consistent than those investigating occupationally-exposed adults; this may due to the comparatively shorter duration of and magnitude of exposure experienced by children. Toxicological studies have also observed decreases in hematocrit and hemoglobin and increases in hemolysis and reticulocyte density in rats and mice with blood Pb levels as low as 6.6-7.1 µg/dL. Pb exposure has also been observed to increase PS expression on RBC membranes, leading to cell shrinkage, eryptosis, and destruction of the RBCs by macrophages.

Suggestive evidence of disrupted hematopoiesis evidenced by decreased serum erythropoietin was observed in occupationally exposed adults with blood Pb levels of 6.4 µg/dL; toxicological studies in rats also indicate that Pb is cytotoxic to RBC-progenitor cells after chronic exposure. Taken together, these studies provide consistent evidence that exposure to Pb adversely effects RBC function and survival, and leads to the reduction of RBCs in circulation. Although this decrease in RBCs may be explained by both decreased cell survival and/or disruption of hematopoiesis, the observation of increased reticulocytes seems to represent compensation for decreased RBC survival due to Pb exposure.

Pb has been found to inhibit several enzymes involved in heme synthesis, namely ALAD (cytoplasmic enzyme catalyzing the second, rate-limiting, step of the heme biosynthesis pathway), coporphyrinogen oxidase (catalyses the 6th step in heme biosynthesis converting coporphyrinogen III into protoporphyrinogen IX), and ferrochelatase (catalyses the terminal step in heme synthesis converting protoporphyrin IX into heme). Recently, numerous epidemiologic studies have confirmed that decreases in RBC ALAD levels and activity are strongly associated with blood Pb levels in as low as 7.1 µg/dL in children and blood Pb levels as low as 6.4 µg/dL in adults. Decreases in blood ALAD activity were also seen in rats with blood Pb levels of 6.5 µg/dL. There is also a considerable body of evidence for a negative correlation between ALAD activity and Pb concentration in various invertebrate and vertebrate taxa (Section 2.6.7). In addition to ALAD, recent studies have shown that Pb exposure inhibits the activity of ferrochelatase, leading to increased RBC ZPP in humans and animals. Pb has also been shown to inhibit the activities of other enzymes in RBCs, including those involved in nucleotide scavenging, energy metabolism, and acid-base homeostasis.

Lastly, Pb exposure induces lipid peroxidation and oxidative stress in RBCs. Epidemiologic studies have observed increases in MDA in occupationally-exposed adults with blood Pb levels as low as 7.9 µg/dL. Other measures of oxidative stress observed included lowered activities of SOD, GR, and CAT, and increased CRP. Indices of RBC oxidative stress were also seen in adolescents and children exposed to Pb. In vitro and in vivo studies have also demonstrated that prior, con-current, or subsequent treatment with various antioxidants has been shown to at least partially ameliorate Pb-induced oxidative stress in RBCs.

In conclusion, the recent epidemiologic and toxicological literature provides strong evidence that exposure to Pb is associated with numerous deleterious effects on the hematological system, including altered heme synthesis mediated through decreased ALAD and ferrochelatase activities, decreased RBC survival and function, decreased hematopoiesis, and increased oxidative stress and lipid peroxidation. The consistency of findings in the epidemiologic and toxicological literature and coherence across the disciplines is sufficient to conclude that there is a **causal relationship between Pb exposure and heme synthesis and RBC function.**

2.5.6. Reproductive Effects and Birth Outcomes

Epidemiologic and toxicological studies of the effects of Pb on reproductive outcomes have covered outcomes such as female and male reproductive function, birth defects, spontaneous abortions, infant mortality, preterm birth, low birth weight, and developmental effects.

Many of the Pb-induced effects in toxicological studies have been observed at maternal blood Pb levels that do not result in overt clinical toxicity in the dams. Recent toxicological studies have shown the effects of Pb exposure during early development to include disruption of endocrine function; delay in the onset of puberty and alteration in reproductive function later in life; and changes in morphology or histology in sex organs and placenta. Additionally, epidemiologic studies of reproductive factors among males and females investigated whether Pb levels were associated with hormone levels, fertility, and onset of puberty. Epidemiologic studies showed associations between blood Pb and hormone levels for females. Studies of Pb and fertility are limited and inconsistent for females and males. Strong and consistent associations were observed between Pb levels in adult males exposed to Pb in occupational settings with blood Pb as low as 20-45 µg/dL and sperm count and quality. Decreased sperm viability and altered morphology in sperm is also observed in invertebrate species (Sections 7.2.4 and 7.3.4) Multiple studies of Pb and puberty have shown inverse associations between blood Pb levels and delayed pubertal development for girls and boys. These associations are consistently observed in multiple epidemiologic studies and demonstrate effects on pubertal development at blood Pb levels <10µg/dL.

Pb-mediated changes in levels or function of reproductive and growth hormones have been demonstrated in past and more recent toxicological studies; however the findings are inconsistent. More data are needed to determine whether Pb exerts its toxic effects on the reproductive system by affecting the responsiveness of the hypothalamic-pituitary-gonad axis or by suppressing circulating hormone levels. More recent toxicological studies suggested that oxidative stress is a major contributor to the toxic effects of Pb on male and female reproductive systems. Several recent studies showed an association between increased generation of ROS and germ cell injury as evidenced by destruction of 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 demonstrated increased oxidative stress also reported increased apoptosis, which is likely a critical underlying mechanism in Pb-induced germ cell DNA damage and dysfunction.

Overall, results of pregnancy outcomes were similar to those of the 2006 Pb AQCD; inconsistent evidence of a relationship with Pb was available for preterm birth and little evidence was available to study the associations with spontaneous abortions. The previous Pb AQCD included a few studies that reported possible associations between Pb and neural tube defects, but the recent epidemiologic studies found no association. Possible associations were observed between Pb and low birth weight when epidemiologic studies used measures of maternal bone Pb or air exposures, but the associations were less

consistent when using maternal blood Pb or umbilical cord and placenta Pb. Effects of Pb exposure during early development in toxicological studies included reduction in litter size, implantation, birth weight and postnatal growth.

Additional evidence for Pb exposure negatively affecting development is provided by toxicological studies demonstrating developmental Pb exposures leading to impaired development of the retina, skin, teeth and altered development of the hematopoietic and hepatic systems. In summary, the recent toxicological and epidemiologic literature provides strong evidence that Pb exposure is related to delayed onset of puberty in both males and females. Additionally, Pb exposure has been shown to have detrimental effects on sperm (at higher blood Pb levels in epidemiologic studies and lower doses in the toxicological literature). Furthermore, evidence from invertebrate and vertebrate taxa in both terrestrial and aquatic ecosystems provide additional support for reproductive and developmental effects associated with Pb exposure (Sections 2.6.8, 7.2.4, and 7.3.4). The data on preterm birth, low birth weight, spontaneous abortions, birth defects, hormonal influences, and fecundity are less consistent between the toxicological and epidemiologic literature. Despite some inconsistencies for particular endpoints, the evidence for Pb-related reproductive effects is strengthened by the coherence with similar findings in invertebrate species. The collective body of evidence integrated across epidemiologic and toxicological studies, with a focus on the strong relationship observed with negative effects on sperm and delayed pubertal onset, is sufficient to conclude that there is a **causal relationship between Pb exposures and reproductive effects and birth outcomes**.

2.5.7. Effects on Other Organ Systems

In the 2006 Pb AQCD, exposure to Pb was shown to exert effects in organ systems not yet explicitly covered in the preceding sections of this document. These organ systems included the liver, gastrointestinal tract, endocrine system, bone and teeth. In the current document, effects on these organ systems, as well as effects on the respiratory system, have been organized in one section because the amount of new evidence appearing since the 2006 Pb AQCD is limited. The few recent studies, however, find that Pb may negatively affect the function of these systems at lower blood Pb levels than previously described.

There is evidence from recent epidemiologic and toxicological studies that exposure to Pb results in altered liver function and hepatic toxicity, including the observation of altered serum protein levels, increased serum enzyme activities, and altered hepatic lipid metabolism. Multiple studies in humans and animals have observed hepatic oxidative stress (generally indicated by an increase in lipid peroxidation, along with a decrease in GSH levels and CAT, SOD, and GPx activities) following exposure to Pb. Effects observed in occupational cohorts of painters, battery- and jewelry-workers, as well as animal

toxicological studies (applying a wide range of exposure regimens), occurred at blood Pb levels >20 µg/dL.

Relatively few human studies have been conducted on gastrointestinal toxicity of Pb since the completion of the 2006 Pb AQCD. GI symptoms were observed in battery workers and painters exposed to Pb in India (mean blood Pb level = 42.40 ± 25.53 and 8.04 ± 5.04 µg/dL, respectively). Toxicological evidence for Pb-induced GI health effects in rats includes altered muscle relaxations and markers of oxidative stress in the gastric fundus and mucosa. The observation of oxidative stress was accompanied gastric mucosal damage following short-term, sub-chronic and chronic exposures. The anterior intestine of fish has also been identified as a target of Pb (Section 2.6.2).

The endocrine processes most impacted by exposure to Pb include changes in thyroid function, as well as alteration in sex and stress hormone profiles. TSH was negatively correlated with blood Pb in women that ate fish contaminated with Pb as well as other chemicals (median blood Pb level = 1.7 µg/dL, less than the detection limit for the study), and FT4, but not FT3, was decreased in adolescent male auto repair workers (blood Pb level = 7.3 ± 2.92 µg/dL). Significant differences in the levels of sex hormones, including total and free testosterone, estradiol, aromatase, and luteinizing hormone, were observed in Belgian adolescents residing in areas with different levels of industrial pollution including Pb (mean blood Pb levels of 2.2 µg/dL.) Toxicological evidence for similar effects was observed in adults cow reared in an environment containing Pb and other contaminants: positive correlations were reported between blood Pb and plasma T3, T4, and estradiol levels. In study of children (mean age 9.5 years) challenged with an acute stressor, increasing blood Pb was associated with significant increases in salivary cortisol responses comparing blood Pb levels of 1.1-1.4 µg/dL to blood Pb levels <1 µg/dL.

Multiple epidemiologic studies investigated the association between Pb exposure and bone and tooth health in adults. High blood Pb has been observed to be associated with decreased BMD in non-Hispanic white males (blood Pb level = 4.9 µg/dL). In elderly women, blood Pb levels (≥ 8 µg/dL) were associated with an increased risk of falls and fractures, including osteoporosis-related falls. Linear skeletal growth in children (7-17 years of age [mean blood Pb level = 7.7 µg/dL)]), was negatively correlated with increasing blood Pb levels. Epidemiologic studies (investigating Pb exposure and tooth loss) reported that long-term, cumulative exposure to Pb is associated with increased odds of tooth loss, periodontitis in men and women, and that periodontitis is associated with oxidative stress/damage in individuals exposed in an occupational setting.

New toxicology studies have reported ocular effects (i.e., retinal progenitor cell proliferation) at blood Pb levels as low as <10 µg/dL (Section 5.3.4.3), and one human study reported an association between heavy smoking, increased blood Pb, and cataracts. Investigation of the respiratory effects of Pb exposure has been limited; however, cross-sectional studies have indicated an association of increasing blood Pb with increased prevalence of respiratory tract illnesses (Section 5.6.4.1) and asthma in children

(Section 5.6.4.2). Additionally, Pb-induced production of ROS is implicated in increased BR and decrements in lung function (Section 5.6.4.3).

2.5.8. Cancer

Toxicological literature on the genotoxic, mutagenic, and carcinogenic potential of Pb includes strong evidence of effects in laboratory animals. Both the International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP) have examined the role of Pb in cancer. IARC classified inorganic Pb compounds as probable human carcinogens and organic Pb compounds as not classifiable (IARC, 2006; Rousseau et al., 2005). The NTP reported Pb and Pb compounds to be 'reasonably anticipated to be human carcinogens'(NTP, 2004).

In laboratory studies, high-dose Pb has been demonstrated to be an animal carcinogen. Pb is likely to be a human carcinogen based on strong evidence from animal toxicology data (IARC, 2006; Waalkes et al., 1995)and less definitive epidemiological data. Mechanistic understanding of the carcinogenicity of Pb is expanding with work on the antioxidant selenium and metallothionein, a protein that binds Pb and reduces its bioavailability. Pb is clastogenic and mutagenic in some but not all models. Clastogenicity and mutagenicity may be possible mechanisms contributing to cancer, but are not necessarily associated with the induction of cancer. Due to the disruption of metal cofactors binding to Zn-finger proteins, Pb has the potential to induce indirect effects that can contribute to carcinogenicity via interactions at hormone receptors, at cell-cycle regulatory proteins, with tumor suppressor genes like p53, with DNA repair enzymes, and with histones. These indirect effects may act at a post-translational level to alter protein structure and DNA repair. In addition, some evidence of epigenetic changes associated with Pb exposure is available in the recent literature. Epigenetic changes may further alter DNA repair or change the expression of a tumor suppressor gene or oncogene. Thus, the animal toxicology literature provides a strong base for understanding the potential contribution of Pb exposure to cancer in laboratory animals.

Multiple epidemiologic studies have been performed examining the association with cancer incidence and mortality with Pb exposure assessed using biological measures and exposure databases. Mixed results have been reported for cancer mortality studies; one strong epidemiologic study of US adults (Schober et al., 2006) demonstrated a positive association between blood Pb and cancer mortality, but the other studies reported null results (Menke et al., 2006). Although the previous Pb AQCD reported that some studies were suggestive of an association between Pb exposure and lung cancer, current studies, which mostly examined occupational exposure 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 genotypes. A limited amount of research on other types of cancer has been performed. The previous Pb AQCD reported evidence that suggested an association between Pb exposure

and stomach cancer, but recent studies of this association are lacking, with only one study published since the last Pb AQCD (U.S. EPA, 2006), which reported mixed results.

Among epidemiologic studies on genotoxicity, positive associations were observed between high Pb blood levels and sister chromatid exchange (SCE) among adults but not children. Other epidemiologic studies of DNA damage reported inconsistent results. Consistent with previous toxicological findings, Pb does appear to have genotoxic activity inducing SCE, MN and DNA strand breaks. Only $PbCrO_4$ produces chromosomal aberrations but this effect is likely due to chromate. Pb does not appear to be very mutagenic unless a cell signaling pathway was disturbed.

Epigenetic effects, particularly with respect to methylation and effects on DNA repair were observed consistently. In humans, epigenetic studies examining Pb and LINE-1 and Alu consistently demonstrated an inverse association between patella Pb and global DNA LINE-1 methylation (Pilsner et al., 2009; R. O. Wright et al., 2010). Toxicological studies show that Pb can activate or interfere with a number of signaling and repair pathways, though it is unclear whether these are in response to epigenetics or genotoxicity. Thus, an underlying mechanism is still unknown, but likely involves either genomic instability or epigenetic modifications or both.

Overall, there is some epidemiologic evidence supporting associations between Pb and cancer. Strong evidence from toxicological studies demonstrates an association between Pb and cancer, genotoxicity/clastogenicity or epigenetic modification. The collective body of evidence integrated across epidemiologic and toxicological studies is sufficient to conclude that there is a **likely causal relationship between Pb exposures and cancer.**

2.5.9. Human Health Effects and Corresponding Blood Pb Levels

Tables 2-2 and 2-3 summarize the health effects in children (and adults and the lowest blood Pb level at which the weight of the evidence substantiates a causal relationship. The 2006 Pb AQCD did not identify a safe level of exposure for Pb and concluded that any threshold for Pb neurotoxicity would have to exist at levels distinctly lower than the lowest exposures examined in the epidemiologic studies included in the assessment. Recent studies continue to find associations between a wide range of health endpoints and increasingly lower levels of blood Pb. The lack of a reference population with blood Pb levels reflecting pre-industrial Pb exposures continues to limit the ability to identify a threshold. Estimates of “background” blood Pb levels have been measured in ancient bones from pre-industrialized societies. These studies suggest that the level of lead in blood in pre-industrial humans was approximately 0.016 µg/dL (Flegal & Smith, 1992), approximately 65-fold lower than that currently measured in U.S. populations. In this context, a blood Pb level of 1 µg/dL is not relatively low. Further, if a threshold did

exist, in order to demonstrate it, the scale at which blood Pb level is measured will likely have to be adjusted to parts per million (µg/L) instead of parts per hundred thousand (µg/dL).

Table 2-2. Summary of Pb-induced health effects in children and the lowest mean blood Pb level in the population(s) studied

Blood Pb Level	Neurological Effects	Renal Effects	Immune Effects	Effects on Heme Synthesis and RBC Function
20 µg/dL			• Macrophage hyper-inflammation[i]	
15 µg/dL			• Lymphocyte activation[j]	• Increased Zn protoporphyrin[p] • Lipid peroxidation[q]
10 µg/dL	• Delinquent behavior[a] • Increased hearing threshold[b]			• Altered antioxidant enzyme activities[r] • Decreased ALAD activities[s] • Altered hematological parameters (i.e., decreased hemoglobin)[t]
5 µg/dL	• Inattention[c] • Decrements in full scale IQ[d] • Decrements in specific neurocognitive domains[e] • Poorer school performance[f] • ADHD[g]	Decreased eGFR[h]	• Increased B cell abundance[k] • Increased IgE[l] • Increased risk of infection[m] • Decreased T cell abundance[n] • Allergic sensitization[o]	• Decreased Ca-Mg ATPase activity[u]
1 µg/dL				

Note: Endpoints where the weight of the evidence, overall, substantiates the causal association with blood Pb levels in the range noted on the figure. Since no evident threshold has yet been clearly established for most effects, the existence of such effects at still lower blood Pb levels cannot be ruled out based on available information.

Supporting references: Wright et al. (2008)[a]; Hwang et al. (2009) and Schwartz and Otto (1991)[b]; Nicolescu et al. (2010)[c]; Kim et al. (2009)[d]; Krieg et al. (2010)[e]; Miranda et al. (2009)[f]; Braun et al. (2006) and Braun et al. (2008)[g]; Fadrowski et al. (2010)[h]; Pineda-Zavaleta et al. (2004)[i]; Lutz et al. (1999)[j]; Sarasua et al. (2000)[k]; Karmaus et al. (2005)[l]; Karmaus et al. (2005)[m]; Karmaus et al. (2005)[n]; Jedrychowski et al. (2011)[o]; Wang et al. (2010)[p]; Ahamed et al. (2006)[q]; Ahamed et al. (2005)[r]; Wang et al.(2010)[s]; Riddell et al. (2007)[t]; Huel et al. (2008)[u]

Table 2-3. Summary of Pb-induced health effects in adults and the lowest mean blood Pb level in the population(s) studied

Blood Pb Level	Neurological Effects	Cardiovascular Effects	Renal Effects	Immune Effects	Reproductive Effects and Birth Outcomes	Effects on Heme Synthesis and RBC Function
30 µg/dL		• Arrhythmia[g]		• Decreased neutrophil function[p]	• Sperm abnormalities[u]	• Decreased hematocrit[w] • Decreased Ca-Mg ATPase activity[x]
20 µg/dL			• Impaired renal tubular function[n]	• Increased auto-antibodies[q]		
15 µg/dL	• Brain MRI changes[a] • Increased hearing threshold[b]					
10 µg/dL	• Criminal arrest[c]					• Lipid peroxidation[y] • Decreased antioxidant enzyme activities[y] • Altered hematological parameters (e.g., decreased hemoglobin, packed cell volume)[z] • Decreased serum erythropoietin[aa] • Increased Zn protoporphyrin[bb] • Altered ALAD activity[bb]
5 µg/dL 1 µg/dL	• Decrements in cognitive function[d] • Essential tremor[e] • Mood disorders[f]	• Mortality[n] • Decreased HRV[i] • Ischemic heart disease[j] • Peripheral Artery Disease[k] • Hypertension[l] • Increased BP[m]	• Elevated serum creatinine[o] (↓ creatinine clearance, GFR)	• Shift to Th2 cytokines[r] • Increased bronchial reactivity[s] • Increased inflammation[t]	• Delayed puberty[v]	

Note: Endpoints where the weight of the evidence, overall, substantiates the causal association with blood Pb levels in the range noted on the figure. Since no evident threshold has yet been clearly established for most effects, the existence of such effects at still lower blood Pb levels cannot be ruled out based on available information.

Supporting references: Brubaker et al. (2010; 2009)[a]; Hwang et al. (2009) and Chuang et al. (2007)[b]; Wright et al. (2008)[c]; Krieg et al. (2009)[d]; Dogu et al. (2007)[e]; Bouchard et al. (2009)[f]; Reza et al. (2008)[g]; Menke et al. (2006)[h]; Park et al. (2009)[i]; Jain et al. (2007)[j]; Muntner et al. (2005)[k]; Scinicariello et al. (2010) and Park et al. (2009)[l]; Scinicariello et al. (2010) and Martin et al. (2006)[m]; Sun et al. (2008), Lin and Tai-yi (2007) and Wang et al. (2010)[n]; Tsaih et al. (2004), Akesson et al. (2005) and Yu et al. (2004)[o]; Valentino et al. (1991)[p]; El-Fawal et al. (1999)[q]; Kim et al. (2007)[r]; Min et al. (2008)[s]; Songdej et al. (2010)[t]; Telisman et al. (2007) and Hsu et al. (2009)[u]; Hauser et al. (2008), Williams et al. (2010), Denham et al. (2005), Selevan et al. (2003) and Wu et al. (2003)[v]; Karita et al. (2005)[w]; Abam et al. (2008)[x]; Ergurhan-Ilhan et al. (2008)[y]; Ukaejiofo et al. (2009)[z]; Sakata et al. (2007)[aa]; Wang (2010)[bb]

2.6. Ecological Effects

This section evaluates the evidence from studies of ecological effects associated with exposure to Pb. The results from the studies evaluated in combination with the evidence from other disciplines (e.g., fate and transport) contribute to the causal determinations for the ecological outcomes discussed in this assessment. In the following sub-sections, a discussion of the causal determinations is presented for the

ecological effects. Effects determined to be causal at the species level contribute to the body of evidence for causal effects at the community and ecosystem scale. Where the causal determination varies substantially between types of organisms (typically between plants and other organisms), the divergence is noted.

The evidence used to formulate each causal determination is summarized here, and the corresponding detailed discussion can be found in Chapter 7 of this document. In Chapter 7, the effects on terrestrial (Section 7.2) and aquatic (Section 7.3) ecosystems are presented separately, and each of the sections first discusses effects at the species level, followed by community and ecosystem levels. In each of the two main sections, biogeochemistry and chemical effects of Pb that influence bioavailability are considered first, as Pb must first move from the environmental media (soil, water, sediment etc.) into biota. Next, uptake of Pb from soil and water are discussed, then new information on biological effects of Pb on plants, invertebrates and vertebrates, followed by data on exposure-response relationships. Ecosystem-scale responses to Pb exposure are considered along with critical loads, characterization of sensitivity and vulnerability, and the effect of Pb on ecosystem services. Finally, in Section 7.4, a synthesis of the effects of Pb observed across terrestrial and aquatic habitats is presented along with causal determinations for those effects, which are also summarized in Table 2-4 below. In this chapter, Pb effects on terrestrial and aquatic systems from Chapter 7 are summarized (Sections 2.6.1 and 2.6.2); followed by a summary of the evidence for the causal determinations (Sections 2.6.3 to 2.6.10) and consideration of atmospheric deposition of Pb as related to ecological effects (Section 2.6.11).

Table 2-4. Summary of causal determinations for Pb in plants, vertebrates and invertebrates

Effect	Causality Determination
Bioaccumulation – All Organisms	Causal Relationship
Mortality - Plants	Inadequate to Infer Causal Relationship
Mortality - Vertebrates and Invertebrates	Causal Relationship
Growth - Plants	Causal Relationship
Growth - Invertebrates	Causal Relationship
Growth - Vertebrates	Suggestive of a Causal Relationship
Physiological Stress – All Organisms	Causal Relationship
Hematological Effects – Invertebrates and Vertebrates	Causal Relationship
Development and Reproduction- Invertebrates and Vertebrates	Causal Relationship
Development and Reproduction-Plants	Inadequate to Infer Causal Relationship
Neurobehavior – Invertebrates and Vertebrates	Causal Relationship
Community and Ecosystem Level Effects	Causal Relationship

2.6.1. Summary of Terrestrial Ecosystem Effects

Section 7.2 focuses on the effects of Pb in terrestrial systems. Pb in terrestrial ecosystems is either deposited directly onto plant surfaces, or incorporated into 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 variables: (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) CEC; and (6) aging. Since 2006, further details have been contributed to the understanding of the role of pH, cation exchange capacity (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 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, 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.

There is evidence over several decades of research previously reviewed in Pb AQCDs and in recent studies reviewed in this ISA that Pb bioaccumulates in plants, invertebrates and vertebrates in terrestrial systems. 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. Multiple new studies showed that in trees, the latter 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. 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. Studies with trees found that soil Pb is generally translocated from the roots.

Since the 2006 Pb AQCD, various species of terrestrial snails have been found to accumulate Pb from both diet and soil. New 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 may result from layer differences in interacting factors such as pH and CEC. 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 point sources.

There were a few new 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 point 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. New 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.

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. 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, reduced growth was observed in some experiments, as well as genotoxicity, decreased germination, and pollen sterility.

In terrestrial invertebrates, evidence for Pb effects have included neurological and reproductive endpoints. Recently published studies have shown neuronal damage in nematodes exposed to low concentrations of Pb (2.5 μM), 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 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. Evidence suggested that aging and pH are important modifiers.

Effects on amphibian 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. 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.

Evidence reviewed in Sections 7.2.3 and 7.2.4 demonstrates clearly that increased 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, a few studies of exposure-response have been conducted with earthworms, and results have been inconsistent.

New evidence of effects of Pb at the community and ecosystem scale 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. Most recently published research on community and ecosystem scale 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. 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 have been found in plants, birds, and invertebrates.

2.6.2. Summary of Aquatic Ecosystem Effects

Section 7.3 focuses on the effects of Pb in aquatic systems. Once atmospherically-derived Pb enters surface waters, its fate and bioavailability are influenced by Ca^{2+} concentration, pH, alkalinity, total

suspended solids, and dissolved organic carbon (DOC, including humic acids). In sediments, Pb bioavailability may be influenced by the presence of other metals, sulfides, Fe and Mn oxides and physical disturbance. In many, but not all aquatic organisms, Pb dissolved in the water can be the primary exposure route to gills or other biotic ligands. As recognized in the 2006 Pb AQCD and further supported in this review, chronic exposures to Pb may also include dietary uptake, and there is an increasing body of evidence showing that differences in uptake and elimination of Pb vary with species. 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.

According to the 2006 Pb AQCD, and further supported in this review, Pb adsorption, complexation, chelation, etc., are processes that alter bioavailability to aquatic biota. Given the low solubility of Pb in water, bioaccumulation 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.

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 and closely related species can vary greatly in bioaccumulation of Pb and other non-essential metals. Recent studies on uptake of Pb by aquatic plants and algae support the findings of previous Pb AQCDs that all 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 in responses to Pb in water and sediments. In invertebrates, Pb can be bioaccumulated from multiple sources, including the water column, sediment, and dietary exposure. Since the last review, new 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. 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 several species.

The conclusions of the 2006 Pb AQCD 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 are supported in this review. The anterior intestine has been newly identified as a site of uptake of Pb through dietary exposure studies. There are few new studies on Pb uptake by amphibians and mammals. 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 higher trophic-level consumers, 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 (biodilution).

Evidence in this ISA further supports the findings of the previous Pb AQCDs that waterborne Pb is highly toxic to aquatic organisms, with toxicity varying with species and lifestage, duration of exposure, form of Pb, and water quality characteristics. Effects of Pb on algae reported in the 2006 Pb AQCD are further supported by evidence from additional species in this review. They include decreased growth, deformation and disintegration of cells, and blocking of the pathways that lead to pigment synthesis, thus affecting photosynthesis. Effects on plants supported by additional evidence in this review include oxidative damage, decreased photosynthesis and reduced growth. The mechanism of Pb toxicity in plants is likely mediated by damage to photosystem II through alteration of chlorophyll structure. Elevated levels of antioxidant enzymes are commonly observed in aquatic plant, algae, and moss species exposed to Pb.

Since the 2006 Pb AQCD, there is additional evidence for Pb effects on antioxidant enzymes, lipid peroxidation, stress response and osmoregulation in aquatic invertebrates. Studies of reproductive and developmental effects of Pb in this review provide further support for findings in the 2006 Pb AQCD. These new studies include reproductive endpoints for rotifers and freshwater snails as well as multigenerational effects of Pb in mosquito larvae. Growth effects are observed at lower concentrations in some aquatic invertebrates since in the 2006 Pb AQCD, including juveniles of the freshwater snail *Lymnaea stagnalis* where growth is affected at <4 µg Pb/L (Grosell et al., 2006). Behavioral effects of Pb in aquatic invertebrates reviewed in this ISA include decreased valve closing speed in scallops and slower feeding rate in blackworms.

Evidence in this ISA supports the findings of reproductive, behavioral, and growth effects in previous Pb AQCDs, as well as effects on blood parameters in vertebrates. Additional mechanisms of Pb toxicity have been elucidated in the gill and the renal system of fish since the 2006 Pb AQCD. Furthermore, the mitogen-activated protein kinases, ERK1/2 and $p38^{MAPK}$ were identified for the first time as possible molecular targets for Pb neurotoxicity in a teleost (Leal et al., 2006). 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 tadpole endpoints including growth, deformity, and swimming ability.

Concentration-response data from plants, invertebrates and vertebrates is consistent with findings in previous AQCDs of species differences in sensitivity to Pb in aquatic systems. In this ISA (and previous AQCDs), aquatic plant growth was shown to be adversely affected by Pb exposure. The lowest EC_{50} for growth observed in marine microalgae and freshwater microalgae was in the range of 100 µg Pb/L. In the 2006 Pb AQCD, concentrations at which effects were observed in aquatic invertebrates ranged from 5 to 8,000 µg/L. Several studies in this review have provided evidence of effects at lower

concentrations. Among the most sensitive species, growth of juvenile freshwater snails (*L. stagnalis*) was inhibited at an EC_{20} of <4 µg Pb/L. (Grosell & Brix, 2009; Grosell et al., 2006). A chronic value of 10 µg Pb/L obtained in 28-day exposures of 2-month-old *Lampsilis siliquoidea* juveniles was the lowest genus mean chronic value ever reported for Pb (N. Wang et al., 2010). 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. However, adverse effects in fish observed in recent studies fall within the range of concentrations observed in the previous Pb AQCD.

Since the 2006 Pb AQCD, additional evidence for community and ecosystem level effects of Pb have been observed primarily in microcosm studies or field studies with other metals present. Ecological effects associated with Pb, reported in previous Pb AQCDs, include alteration of predator-prey dynamics, species richness, species composition, and biodiversity. New studies in this ISA provide evidence in additional habitats for these community and ecological-scale 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 the species sensitivity, lifestage, or seasonally-affected physiological state.

2.6.3. Bioaccumulation of Lead in Terrestrial and Aquatic Biota as it Affects Ecosystem Services

Pb deposited on the surface of, or taken up by organisms has the potential to alter the services provided by terrestrial and aquatic biota to humans. Ecosystem services are the benefits people obtain from ecosystems. They include supporting, provisioning, regulating and cultural services that are vital for the functioning of the biosphere and provide the basis for the delivery of tangible benefits to human society. There is compelling evidence over several decades of research and in recent studies reviewed in this ISA (Sections 7.2.3 and 7.3.3.) that Pb bioaccumulates in plants, invertebrates and vertebrates in terrestrial and aquatic systems. Generally, there are considerable differences between species in the amount of Pb taken up from the environment, and in the amounts of Pb retained in the organism. In order for Pb to reach a biological receptor, the metal must first cross the membranes of organisms to reach the target organ or site of storage. This process varies between plants, invertebrates and vertebrates, and furthermore, uptake and sequestration are at times similar in unrelated species, while substantially different between related ones. Uptake of Pb from environmental media is dependent upon the bioaccessibility of Pb (reviewed in Chapter 3) which is influenced by many factors including, but not limited to, temperature, pH, presence of humic acid and dissolved organic matter, presence of other metals, and speciation of Pb.

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. 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). 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. Overall, based on the consistency of findings across taxa, the evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and bioaccumulation of Pb that affects ecosystem services associated with terrestrial and aquatic biota.**

2.6.4. Mortality

The relationship between Pb exposure and mortality has been well demonstrated in terrestrial and aquatic species as presented in Sections 7.2.5 and 7.3.5 of this ISA and in the 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. However, the LC_{50} is usually much higher than current environmental levels of Pb, even though physiological dysfunction that adversely impacts the fitness of an organism often occurs well below concentrations that result in mortality.

Pb is generally not phytotoxic to plants at concentrations found in the environment away from point-sources, 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. Invertebrates are generally more sensitive to Pb exposure than other taxa, with survival adversely impacted in a few species at concentrations occurring near point-sources, or at concentrations near ambient levels. These impacted species may include candidate or endangered species. The freshwater mussel *Lampsilis 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. Other invertebrates such as odonates may be tolerant of Pb concentrations that greatly exceed environmental levels.

Thirty day LC_{50} values for larval fathead minnows ranged from 39 to 1,903 µg/L in varying concentrations of DOC, $CaSO_4$ and pH. (Grosell et al., 2006). 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 $CaCO_3$, a value much lower than in testing with older fish (Mebane et al., 2008).

The evidence is inadequate to conclude that there is a **causal relationship between Pb and mortality in plants.**

The evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and mortality in sensitive terrestrial and aquatic animal taxa.**

2.6.5. Growth Effects

Evidence for Pb effects on growth is strongest in plants, with limited information in invertebrates and vertebrates. There is evidence over several decades of research that Pb inhibits photosynthesis and respiration in plants, both of which reduce growth (U.S. EPA, 1977, 2006). Many laboratory and greenhouse toxicity studies have reported effects on plants, and there are few field toxicity studies. Pb has been shown to affect photosystem II with the hypothesized mechanism being that Pb may replace either Mg or Ca in chlorophyll, 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. The lowest 72-hour EC_{50} for growth inhibition reported for algae was 105 µg Pb/L in Chaetoceros sp. Most primary producers experience EC_{50} values for growth in the range of 1,000 to 100,000 µg Pb/L (U.S. EPA, 2006).

In previous Pb AQCDs, growth effects of Pb have been reported in fish (growth inhibition), birds (changes in juvenile weight gain), and frogs (delayed metamorphosis, smaller larvae). Growth effects observed in invertebrates and vertebrates underscore the importance of lifestage to overall Pb susceptibility. In general, juvenile organisms are more sensitive than adults. Several studies since the last review have demonstrated effects of Pb on growth at lower concentrations than in previous literature. Among the animal taxa tested, aquatic invertebrates were the most sensitive to the effect of Pb, with adverse effects being reported as low as 4 µg Pb/L. Growth of juvenile freshwater snails *L. stagnalis* was inhibited at EC_{20} <4 µg Pb/L (Grosell & Brix, 2009; Grosell et al., 2006). In the freshwater mussel, fatmucket (*L. siliquoidea*) juveniles were the most sensitive lifestage (N. Wang et al., 2010). 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.

Evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and growth effects in plants and invertebrates.** Evidence is **suggestive of a causal relationship between Pb exposures and growth effects in vertebrates.**

2.6.6. Physiological Stress

In this ISA and previous Pb AQCDs, there is consistent and coherent 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. There is considerable evidence for antioxidant activity in invertebrates, including gastropods, mussels, and crustaceans, in response to Pb exposure. Upregulation of antioxidant enzymes are also observed in fish. 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 aquatic 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. Glycogen levels in the freshwater snail *Biomphalaria glabrata* were significantly decreased at near environmentally-relevant concentrations (50 µg Pb/L and higher) (Ansaldo et al., 2006).

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.

Evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and physiological stress in plants, invertebrates and vertebrates.**

2.6.7. Hematological Effects

Hematological responses are commonly reported effects of Pb exposure in invertebrates and vertebrates in both aquatic and terrestrial systems. In environmental assessments of metal-impacted habitats, ALAD is a recognized biomarker of Pb exposure (U.S. EPA, 2006). ALAD activity is negatively correlated with total Pb concentration in bivalves. Lower ALAD activity has been significantly correlated with elevated blood Pb levels in fish and mammals as well. In the 1986 Pb AQCD, decreases in RBC ALAD activity following Pb exposure were well documented in birds and mammals. Further evidence from the 2006 Pb AQCD and this review for Pb effects on ALAD enzymatic activity including effects in bacteria, amphibians and additional field and laboratory studies on fish suggest this enzyme is an indicator for Pb exposure across a wide range of taxa. Limited evidence of Pb effects on other blood parameters including altered serum profiles and changes in white blood cell counts in fish and amphibians support the finding of the hematological system as a target for Pb in natural systems. This evidence is strongly coherent with observations from human epidemiologic and animal toxicology studies where a causal relationship was identified between exposure to Pb and hematological effects in humans and laboratory animals (Sections 2.5.5 and 5.7). Based on observations in both terrestrial and aquatic 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 invertebrates and vertebrates.**

2.6.8. Developmental and Reproductive Effects

Evaluation of the literature on Pb effects in aquatic and terrestrial species indicates that exposure to Pb is associated with adverse effects on development and reproduction. Evidence in this review and the previous Pb AQCDs from invertebrate and vertebrate studies indicate that Pb is adversely affecting reproductive performance in multiple species. In plants, few studies are available that specifically address reproductive effects of Pb exposure.

Several new studies of snails, clams and rotifers support previous findings of adverse impacts to embryonic development. In addition to affecting the embryo, Pb can alter developmental timing, 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.

Reproductive effects have also been observed in multi-generational studies. Larval settlement rate and rate of population increase was adversely impacted in rotifers. Rotifers have decreased fertilization rate associated with Pb exposure that appeared to be due to decreased viability of sperm and eggs. 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.

In 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 subtle skeletal malformations. Previous Pb AQCDs have reported developmental effects in fish, specifically spinal deformities in larvae. 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 fish, there is new evidence in this ISA of Pb effects on steroid profiles. 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. Additional reproductive parameters in fish observed to be impacted by Pb include decreased oocyte diameter and density in toadfish associated with elevated Pb levels in gonad.

In terrestrial vertebrates, evidence from Chapter 7 and in previous Pb AQCDs indicates an association between observed adverse reproductive effects and Pb exposure. Decreased testis weight was observed in lizards. In mammals, few studies in the field have addressed Pb specifically, due to most available data in wild or grazing animals being from near smelters, where animals are co-exposed to other metals. 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. New toxicological data support trans-generational effects, a

finding that is also an area of emerging interest in the ecology. The evidence presented in Section 5.8 is sufficient to conclude that there is a causal relationship between Pb exposure and reproductive effects in humans and laboratory animals. The strongest and most consistent evidence, which was coherent across epidemiologic and toxicological studies, was for effects of Pb on sperm and the onset of puberty in males and females.

Adverse effects of Pb on reproduction in invertebrates and vertebrates indicate that Pb is likely affecting fecundity of Pb-exposed organisms in both aquatic and terrestrial habitats, and the evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and reproductive effects in terrestrial and aquatic invertebrates and vertebrates.** In plants, the evidence is **inadequate to conclude a causal relationship between Pb exposures and plant reproductive effects.**

2.6.9. Neurobehavioral Effects

Evidence from laboratory studies and limited data from field studies reviewed in Chapter 7 have shown adverse effects of Pb on neurological endpoints in both aquatic and terrestrial animal taxa. These include changes in behaviors that may decrease the overall fitness of the organism. There is also evidence from both invertebrate and vertebrate studies that Pb adversely affects behaviors that may decrease the ability of an organism to escape predators or capture prey.

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). Additionally, there is new evidence for neurotoxic action of Pb in invertebrates with exposure to Pb observed to cause changes in the morphology of GABA motor neurons in nematodes (*C. elegans*) (Du & Wang, 2009).

Decreased food consumption of Pb-contaminated diet has been demonstrated in some invertebrates (snails) and vertebrates (lizards, pigs). 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 & Xing, 2008). 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 & Gochfeld, 2005). Lizards exposed to Pb through diet in the laboratory exhibited abnormal coloration and posturing behaviors. Other behavioral effects affected by Pb exposure include increased hyperactivity in fish and hypoxia-like behavior in frogs.

These findings are coherent with findings from studies in laboratory animals described in Sections 2.5.1 and 5.3 of the ISA that show that Pb induces changes in attention, increased response rates and motor function. The evidence presented in those sections is sufficient to conclude that there is a causal relationship between Pb exposure and neurobehavioral effects (Section 5.3). These data from laboratory

toxicology studies, especially neurobehavioral findings and structural changes are highly coherent with data from ecological studies. Overall, the evidence from aquatic and terrestrial systems is sufficient to conclude that there is a **causal relationship between Pb exposures and neurobehavioral effects in invertebrates and vertebrates.**

2.6.10. Community and Ecosystem Level Effects

Uptake of Pb into aquatic and terrestrial organisms and subsequent effects on survival, reproduction, growth, behavior and other physiological variables at the species scale are likely to result in effects at the population, community and ecosystem scale. The effects may include alteration of predator-prey dynamics, species richness, species composition, and biodiversity. There are few field studies that directly consider effects of Pb on these measures of ecosystem health. Ecosystem-level studies are complicated by the confounding of Pb exposure with other factors such as trace metals and acidic deposition. In natural systems, Pb is often found co-existing with other stressors, and observed effects may be due to cumulative toxicity.

Most direct evidence of community and ecosystem level effects is from near point sources. 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 aquatic systems, the literature focuses on evaluating ecological stress from Pb originating from urban and mining effluents rather than atmospheric deposition. 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. Effects of Pb on mortality, growth, physiological stress, blood, neurobehavioral and developmental and reproductive endpoints at the individual level are expected to have ecosystem level consequences, and thus provide consistency and plausibility for causality in ecosystem level effects.

Avoidance response to Pb exposure varies widely in different species and this could affect community composition. For example, frogs and toads lack avoidance response while snails and fish avoid higher concentrations of Pb. New evidence, published since the 2006 Pb AQCD indicates that some species of worms will avoid Pb-contaminated soils (Langdon et al., 2005).

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.

Since the 2006 Pb AQCD, there is further evidence for effects of Pb in sediment-associated communities. Exposure to three levels of sediment Pb contamination (322, 1,225, and 1,465 µg Pb/g dry weight) in a microcosm experiment significantly reduced nematode diversity and resulted in profound restructuring of the community structure (Mahmoudi et al., 2007). Sediment-bound Pb contamination appears to differentially affect members of the benthic invertebrate community, potentially altering ecosystems dynamics in small urban streams (Kominkova & 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.

In a new 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 the lowest number of resident aquatic plant species when compared to sites with lower Pb concentrations (78-92 µg Pb/L) (Mishra et al., 2008). This shift toward more Pb-tolerant species is also observed in terrestrial plant communities near smelter sites (U.S. EPA, 2006). 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.

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 in natural systems. However, the evidence for Pb effects at higher levels of ecological organization is sufficient to conclude that there is a **causal relationship between Pb exposures and the alteration of species richness, species composition and biodiversity in terrestrial and aquatic ecosystems.**

2.6.11. Ecological Effects and Corresponding Pb Concentrations

There is limited evidence to relate ambient air concentrations of Pb to levels of deposition onto terrestrial and aquatic ecosystems and subsequent movement of atmospherically-deposited Pb though environmental compartments (e.g., soil, sediment, water, biota). 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. However, the proportion of observed effects of Pb attributable to Pb

from atmospheric sources is difficult to assess due to a lack of information not only on bioavailability, as affected by the specific characteristics of the receiving ecosystem, but also on deposition, and on kinetics of Pb distribution in ecosystems in long-term exposure scenarios.

Threshold levels for Pb in terrestrial and aquatic systems may serve as a tool for interpreting the effects of atmospherically deposited Pb as a component of total Pb loading. For soils, ecological soil screening levels (Eco-SSLs) have been developed by the EPA for Pb. Eco-SSLs are maximum contaminant concentrations in soils that are predicted to result in little or no quantifiable effect on terrestrial receptors. The Pb Eco-SSL values for terrestrial birds, mammals, plants and invertebrates are 11, 56, 120 and 1,700 mg Pb/kg soil (dry weight), respectively. In aquatic systems, national recommended ambient water quality criteria have been developed by the EPA Office of Water to protect aquatic life and human health in surface waters. The ambient water quality criteria for Pb are expressed as a criteria maximum concentration (CMC) for acute toxicity and criteria continuous concentration (CCC) for chronic toxicity. 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. In saltwater, these values are 210 µg Pb/L CMC and 8.1 µg Pb/L CCC, respectively. These U.S. EPA Office of Water criteria were 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.

2.7. Integration of Health and Ecological Effects Overview

The health and ecological effects considered for causal determination are summarized in the Table 2-5. The health endpoints include neurological, cardiovascular, renal, immune, hematological and reproductive effects as well as cancer. The ecological endpoints considered for causal determination are bioaccumulation, mortality, growth, physiological stress, hematological effects, developmental and reproductive effects, neurobehavioral effects, and community and ecosystem level effects. The substantial overlap between the ecological and health endpoints considered in the causal determinations allowed for the integration of the evidence across these disciplines.

Table 2-5. Summary of Causal Determinations for Health and Ecological Effects

Outcome	Human Health Causal Determination	Ecological Receptors Causal Determination
Neurological Effects[a]	Causal Relationship	Causal Relationship: Invertebrates and Vertebrates
Cardiovascular Effects	Causal Relationship	N/A
Renal Effects	Causal Relationship	N/A
Immune System Effects	Causal Relationship	N/A
Heme Synthesis and RBC Function[b]	Causal Relationship	Causal Relationship: Invertebrates and Vertebrates
Reproductive Effects and Birth Outcome[c]	Causal Relationship	Causal Relationship: Invertebrates and Vertebrates Inadequate to Infer Causal Relationship: Plants
Cancer	Likely to be a causal relationship	N/A
Bioaccumulation	The causal determination for bioaccumulation was developed respective to the impact of bioaccumulation on ecosystem services. Thus, although Pb bioaccumulates in all organisms including humans, causality was not applicable to bioaccumulation in humans.	Causal Relationship
Mortality	The strongest evidence of Pb-induced mortality in humans was observed for cardiovascular disease related mortality.	Causal Relationship: Invertebrates and Vertebrates Inadequate to Infer Causal Relationship: Plants
Growth	N/A	Causal Relationship: Plants and Invertebrates Suggestive of a Causal Relationship: Vertebrates
Physiological Stress	In Human Health, oxidative stress was considered as a 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
Community and Ecosystem Level Effects	N/A	Causal Relationship

[a] 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. The human epidemiologic evidence evaluated included a wider range of health endpoints such as cognition.

[b] The health hematological effects considered in the determination of causality were primarily heme synthesis and RBC function. The ecological evidence considered for the causal determination included heme synthesis, blood cell count, and altered serum profiles.

[c] Reproductive health effects, including effects on sperm, as well as birth outcomes such as spontaneous abortion, were considered in the causal determination. In the ecological literature, a wide range of endpoints, including embryonic development, multigenerational studies, delayed metamorphosis, and altered steroid profiles, were considered.

2.7.1. 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 principle modes of action of human health endpoints associated with Pb exposure and the concentrations at which those effects are observed. Then, effects of Pb observed in organisms in aquatic and terrestrial ecosystems (Section 2.6) are evaluated along with evidence from human and laboratory animals 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 5.2 has the potential to contribute to the development of a number of Pb-induced health effects (Table 2-6). 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. Dose captures Pb exposure concentrations in in vitro systems or in animal models when no blood Pb level was reported. The observable effect levels in humans reported in Table 2-6 are limited by the data and methods available 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.

Table 2-6. Related human health effects resulting from the MOAs of Pb and the lowest level eliciting the MOA

Mode of Action	Related Health Effects (ISA Section)	Lowest Level at which MOA Observed		References
		Blood Pb[a]	Dose[a]	
Altered Ion Status	All Heath Effects of Pb	3.5 µg/dL	50 pM, acute	Huel et al. (2008); Kern et al. (2000)
Protein Binding	Renal (5.5), Heme Synthesis and RBC Function (5.7)	6.4 µg/dL	50 µM, acute	Chen et al. (2010); Klann and Shelton (1989)
Oxidative Stress	All Heath Effects of Pb	5-10 µg/dL	10 µM, acute	Quinlan et al. (1988); Ahamed et al. (2006); Yiin and Lin (1995)
Inflammation	Neurological (5.3), Cardiovascular (5.4), Renal (5.5), Immune (5.6), Respiratory (5.6.4), Hepatic (5.9.1)	3 µg/dL	0.01 µM, acute	Kim et al. (2007); Chetty et al. (2005)
Endocrine Disruption	Reproductive Effects and Birth Outcomes (5.9), Bone and Teeth (5.9.4), Endocrine System (5.9.3)	2 µg/dL	20 ppm, acute	Krieg (2007); Wiebe and Barr (1988)
Cell Death/Genotoxicity	Cancer (5.10), Reproductive Effects and Birth Outcomes (5.8), Bone and Teeth (5.9.4)	3.1 µg/dL	50 nM, acute	Van et al. (2004); Bonacker et al. (2005)

[a]Reported as blood Pb level and dose delivered (human, laboratory animal, and in vitro data).

Ecological 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.

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 5-1). Pb will 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 calcium-dependent cell signaling, such as 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. Pb-exposure decreases evoked release of neurotransmitters, while simultaneously increasing spontaneous release of neurotransmitters through Ca^{2+} mimicry. Evidence for these effects in Pb-exposed experimental animals and cell cultures have 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 is also responsible for 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 affected by Pb are highly conserved across species accounting 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 to 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. 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 $^{\bullet}NO$, 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 will 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 animals, including cattle. However the results of these studies are mixed and require further investigation.

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 caused 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 (>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 clatogenicity 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 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.

2.8. Policy Relevant Considerations and Human Health

2.8.1. Air-to-Blood Relationships

The 1986 Pb AQCD described epidemiological studies of relationships between air Pb and blood Pb. Much of the pertinent earlier literature described in the 1986 Pb AQCD was drawn from a meta-analysis by Brunekreef (1984). In addition to the meta-analysis of Brunekreef (1984), seven more recent studies have provided data from which estimates of the blood Pb-air Pb slope can be derived for children (Table 2-7). The range of estimates from these seven studies is 1-9 µg/dL per µg/m^3, which encompasses the estimate from the Brunekreef (1984) meta-analysis of (3-6 µg/dL per µg/m^3). The Schnaas (2004) had

a particularly strong experimental design in that is the only longitudinal study in which blood Pb concentration was monitored repeatedly in individual children from age 6 months to 10 years. For children who experienced the largest declines in air Pb (i.e., from 2.8 to <0.1 μg/m^3), the predicted blood Pb-air Pb slope (adjusted for age, year of birth, SES, and use of glazed pottery) was 0.213 ln[μg/dL blood] per ln[μg/m^3 air]. The cross-sectional study done by Ranft (2008) attempted to account for potential covariates that influence blood Pb (e.g., soil Pb concentration, gender, environmental tobacco smoke, fossil heating system and parental education). It is the only study that reported a logarithmic blood Pb-linear air Pb relationship, which results in an upward curvature of the blood Pb-air Pb relationship (i.e., the blood Pb-air Pb slope increases with increasing air Pb concentration). In other studies (or based on other studies), the blood Pb-air Pb relationship was either log-log (Brunekreef, 1984; Hayes et al., 1994; Schnaas et al., 2004), which predicts an increase in the blood Pb-air Pb slope with decreasing air Pb concentration or linear (Hilts, 2003; Schwartz & Pitcher, 1989; Tripathi et al., 2001), which predicts a constant blood Pb-air Pb slope across all air Pb concentrations. These differences may simply reflect model selection by the investigators; alternative models are not reported in these studies. Because air Pb contributes to Pb in soil and indoor dusts, adjustment for the correlated covariates such as soil Pb would introduce a downward bias in the slope estimate.

Table 2-7. Summary of Estimated Slopes for Blood Pb to Air Pb Relationships in Children

Reference	Study Methods	Model Description	Blood Pb – Air Pb Slope (µg/dL per µg/m^3)
Brunekreef et al. (1984)	Location: Various countries Years: 1974-1983 Subjects: Children (varying age ranges) Analysis: Meta analysis of 18 studies	Model: Log-Log Blood Pb: 5-41 µg/dL (mean range for studies) Air Pb: 0.2-10 µg/m^3 (mean range for studies)	All children: 18[a], 6.1[b] Children <20 µg/dL: 13[a], 3.0[b]
Hayes et al. (1994)	Location: Chicago, IL Years: 1974-1988 Subjects: 0.5-6 yr (9,604 blood Pb measurements) Analysis: Regression of blood Pb screening and quarterly average air Pb	Model: Log-Log Blood Pb: 12-30 µg/dL (annual GM range) Air Pb: 0.5-1.2 µg/m^3 (annual GM range)	24[a], 5.7[b]
Hilts et al. (2003)	Location: Trail, BC Years: 1989-2001 Subjects: 0.5-6 yr (292-536 blood Pb measurements/yr) 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 median range) Air Pb: 0.03-1.1 µg/m^3 (annual median range)	6.5
Ranft et al. (2008)	Location: Germany Years: 1983-2000 Subjects: 6-11 yr (n = 843) Analysis: Pooled regression 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[c]
Schnaas et al. (2004)	Location: Mexico City Years: 1987-2002 Subjects: 0.5-10 yr (n = 321) Analysis: Regression of longitudinal blood Pb measurements and annual average air Pb data	Model: Log-Log Blood Pb: 5-12 µg/dL (annual GM range) Air Pb: 0.7-2.8 µg/m^3 (annual mean range)	4.8[a], 1.1[b]
Schwartz and Pitcher (1989), U.S. EPA (1986)	Location: U.S. Years: 1976-1980 Subjects: 0.5-7 yr (n = 7,000) Analysis: NHANES blood Pb, gasoline consumption data and Pb concentrations in gasoline	Model: Linear Blood Pb: 11-18 µg/dL (mean range) Air Pb: 0.36-1.22 µg/m^3 (annual maximum quarterly mean)	9.3
Schwartz and Pitcher (1989), U.S. EPA (1986)	Location: Chicago, IL Years: 1976-1980 Subjects: 0-5 yr (n = 7,000) Analysis: Chicago blood Pb screening, gasoline consumption data, and Pb concentrations in gasoline	Model: Linear Blood Pb: 18-27 µg/dL (mean range) Air Pb: 0.36-1.22 µg/m^3 (annual maximum quarterly mean)	7.7
Tripathi et al. (2001)	Location: Mumbai, India Years: 1984-1996 Subjects: 6-10 yr (n = 544) Analysis: Regression of blood Pb and air Pb data	Model: Linear Blood Pb: 8.6-14.4 µg/dL (regional GM range) Air Pb: 0.11-1.18 µg/m^3 (regional GM range)	3.6

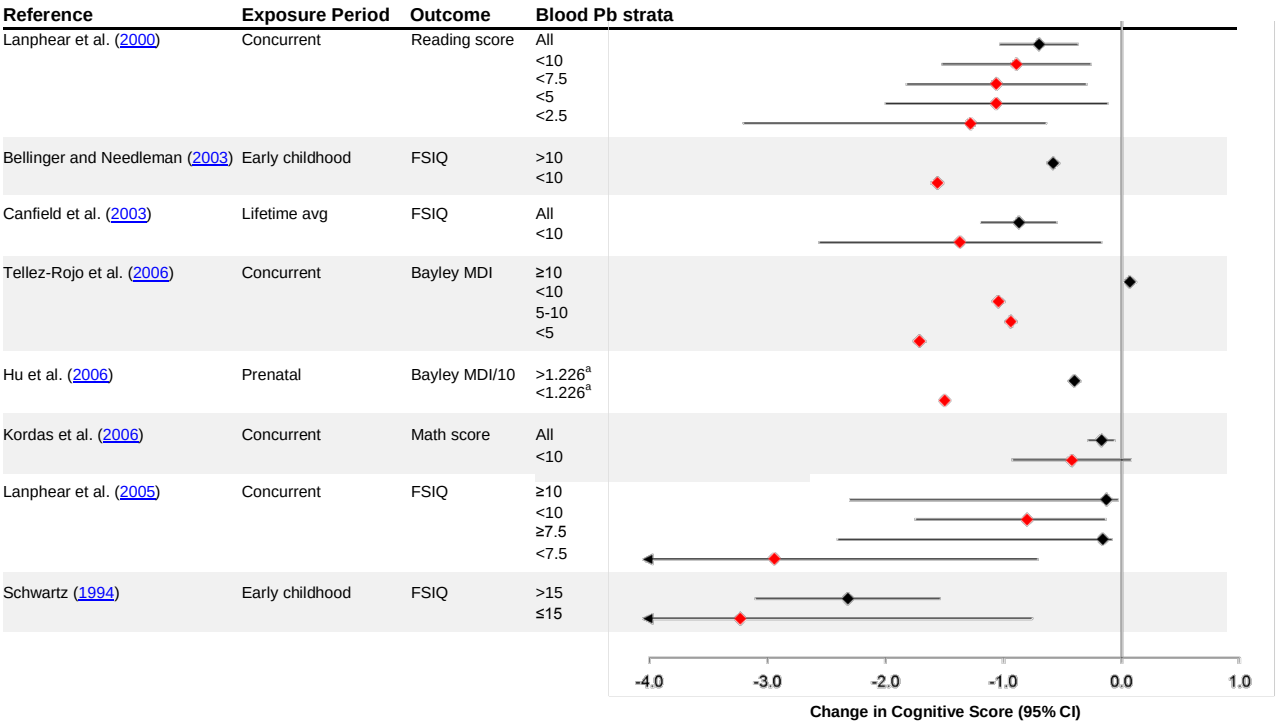
[a]At an air concentration of 0.15 µg/m^3
[b]At an air concentration of 1 µg/m^3
[c]For a change in air Pb concentration from 0.025 to 0.465 µg/m^3

GM, geometric mean

2.8.2. Concentration-Response Functions

With each successive assessment to-date, the epidemiologic and toxicological study findings show that progressively lower blood Pb levels are associated with cognitive deficits and behavioral impairments

as well as other outcomes (U.S. EPA, 2006) (Tables 2-2 and 2-3). Furthermore, in the 2006 Pb AQCD, compelling evidence for a steeper slope for the relationship between blood Pb level and children's IQ at lower blood Pb levels was presented based on 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 & Rothenberg, 2005), and several individual studies. This body of with the addition of more recent studies is presented Figure 2-2. The majority of the epidemiologic evidence from stratified analyses comparing the lower and the higher ends of the blood Pb distributions indicates larger slopes at lower blood Pb levels. Relatively few studies examined the concentration-response relationship between Pb in blood or bone and neurocognitive effects in adults. Of the studies that did examine this relationship, findings were mixed with some studies reporting a linear relationship with cognition and others reporting non-linear relationships.



Note: a = Pb levels measured in plasma of maternal blood during 1st trimester of pregnancy. FSIQ = full-scale IQ, MDI = mental development index. Effect estimates are standardized to a 1 µg/dL increase in blood Pb level. Black symbols represent effect estimates among all subjects or in highest blood Pb stratum. Red symbols represent effect estimates in lower blood Pb strata. Effect estimates without error bars are from studies that did not provide sufficient information in order to calculate 95% CIs.

Figure 2-2. Comparison of associations between blood Pb and cognitive function among various blood Pb strata.

Concentration-response relationships were examined in several epidemiologic studies of blood pressure and mortality. In a study of Korean workers, the Pb-induced increase in systolic blood pressure was better described by a log linear function of blood Pb level than by a linear function (Weaver, 2010).

Animal toxicological studies provide support for this concentration response relationship. Few studies that focused on Pb-induced hypertension in experimental animals have included more than two exposure concentrations; however these few studies appear to have a supralinear (concave downward) dose response and do not conflict with the epidemiologic findings.

Studies investigating both all-cause and cardiovascular mortality report both linear and non-linear relationships. Findings from NHANES analyses were mixed with Schober et al. (2006), reporting a linear association between the relative hazard for all cause mortality with blood Pb, and Menke et al. (2006), reporting a non-linear relationships between blood Pb level and the hazard ratio for all cause, MI, stroke, and cancer mortality. This latter analysis provided evidence for an association between blood Pb and cardiovascular mortality in the NHANES population, where the mean blood Pb level was 2.58 µg/dL, and the hazard ratio of Pb with cardiovascular mortality reached its maximum at blood Pb levels between of 6 and 7 µg/dL. Non-linear relationships between patella bone Pb and log HR for all-cause, cardiovascular, and IHD mortality were reported by Weisskopf et al. (2009).

Concentration response information was provided in a small number of studies of Pb-related nephrotoxicity in the occupational setting (Ehrlich et al., 1998; Weaver et al., 2003). Data in 267 Korean Pb workers in the oldest age tertile (mean age = 52 years) revealed no threshold for a Pb effect (beta = 0.0011, p = <0.05; regression and lowess lines shown), however the mean blood Pb level in this population was 32 µg/dL (Weaver et al., 2003).

Non-linear concentration/exposure response relationships or attenuation of these relationships at higher exposure levels is reported in the occupational literature for a variety of exposures. Explanations for this phenomenon include greater exposure measurement error, competing risks, and saturation of biological mechanisms at higher exposure levels, and exposure-dependent variation in other risk factors (Stayner et al., 2003). Non-linear concentration response functions are reported in the air pollution literature (Smith & Peel, 2010). With respect to Pb exposure, different biological mechanisms may operate at different exposure levels and/or there may be a lower incremental effect of Pb due to covarying risk factors such as low SES, poorer caregiving environment, and other higher environmental exposures. In addition, the 2006 Pb AQCD considered the explanation for the supralinear concentration response function postulated by Bowers and Beck (2006), who stated that "a supralinear slope is a required outcome of correlations between a data distribution where one is log-normally distributed and the other is normally distributed." The 2006 Pb AQCD determined that, while the conclusions drawn by Bowers and Beck may be true under certain conditions, their assumptions (e.g., that IQ are scores forced into a normal distribution) were not generally the case in the epidemiologic analyses showing a supralinear concentration response function. To support this conclusion, the 2006 Pb AQCD cited Hornung et al. (2006), which provided evidence that the IQ data used in the pooled analysis of seven studies by Lanphear et al. (2005) were not normalized and a log-linear model (a linear relationship between IQ and the log of blood Pb) provided the best fit.

The current body of evidence on the effects of Pb allows critical evaluation of several of the aforementioned explanations for the supralinear curve concentration response function in epidemiological studies. For example, in several populations, higher blood Pb levels have been measured in susceptible groups such as those with higher poverty, greater exposure to tobacco smoke, lower parental education, and lower birth weight (Lanphear et al., 2000; Lanphear et al., 2005). It has been suggested that in populations of low SES, poorer caregiving environment, and greater social stress, the incremental effect of Pb exposure may be attenuated due to the overwhelming effects of these other risk factors (Schwartz, 1994). Several studies have found significant associations of these sociodemographic risk factors with neurocognitive deficits, and Miranda et al. (2009) found that indicators of SES (i.e., parental education and enrollment in a free/reduced fee lunch program) accounted for larger decrements in EOG scores than did blood Pb level (Figure 5-10). Few studies have compared Pb effect estimates among groups in different sociodemographic strata, and the limited data are mixed. Some have found greater Pb-associated neurocognitive deficits in low-SES groups (Bellinger et al., 1990; Schwartz, 1994). In a meta-analysis of eight studies, Schwartz (1994) found a smaller decrement in IQ per 1 µg/dL increase in blood Pb level for studies in disadvantaged populations (-2.7 points [95% CI: -5.3, -0.07]) than for studies in nondisadvantaged populations (-4.5 points [95% CI: -5.6, -2.8]). It is important to note that blood Pb is associated with deficits in neurocognitive function in both higher and lower SES groups; however, it is unclear what differences there are between groups in the decrement per unit increase in blood Pb and whether these differences can explain the nonlinear dose-response relationship.

Although, the 2006 Pb AQCD did not identify a biological mechanism for a steeper slope at lower than at higher blood Pb levels such a mechanism was not ruled out. In fact, several lines of evidence support the possibility of low-dose and high dose-Pb acting through different mechanisms. For example, in mice, lower-dose Pb is associated with differential responses of the neurotransmitters dopamine and norepinephrine compared with control treatment and higher doses (Leasure et al., 2008; Virgolini et al., 2005). These differential responses of neurotransmitter systems to low-dose Pb versus a higher-dose Pb may provide mechanistic understanding of the nonlinearity of Pb-induced behavioral changes in animals and may also explain the nonlinear blood Pb-neurocognitive and neurobehavioral associations reported widely among children. Additional evidence points to differences in hormonal homeostasis by Pb exposure level. In male mice with chronic Pb exposure (PND21 to 9 months of age), basal corticosterone levels are significantly lower in the 50 ppm exposure group versus control or 150 ppm Pb.

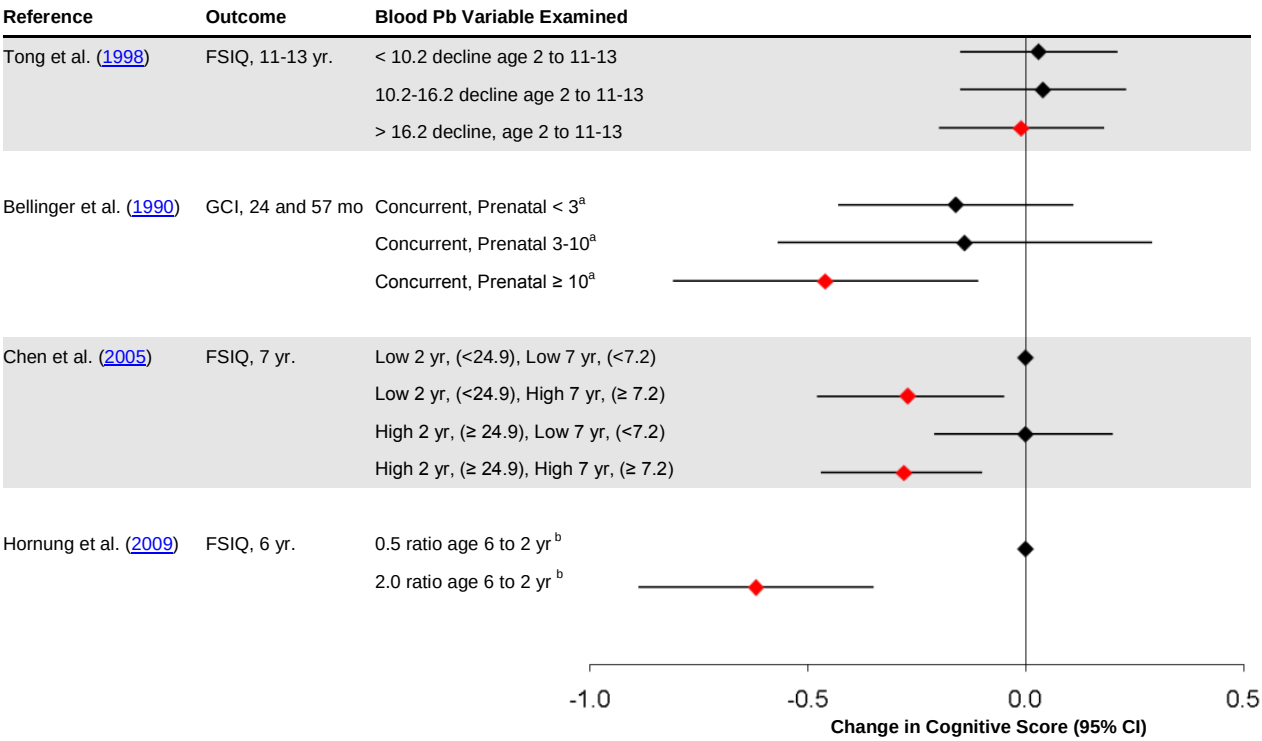
Additional mechanistic understanding comes from differences in histological changes found in Pb-exposed animals. Compared with high-dose Pb, low-dose Pb stimulates greater induction of c-fos, a marker of neuronal activation and action potential firing (Lewis & Pitts, 2004). These findings may underlie the nonlinear association between Pb exposure and learning and the U-shaped behavioral dose-responsiveness seen with amphetamine-induced motor activity in males after GLE (Leasure et al., 2008).

Sensory organ findings also show vastly different outcomes with low versus higher doses of Pb. High-dose Pb produces subnormal retinal ERGs and low-dose Pb produces supernormal ERGs in both children (Rothenberg et al., 2002) and rodents (Fox & Chu, 1988; Fox & Farber, 1988; Fox et al., 1991). Inverted U-shaped dose-response curves have been seen for rod photoreceptor numbers or neurogenesis (Giddabasappa et al., 2011), retinal thickness (Fox et al., 2010), and rod cell proliferation (Giddabasappa et al., 2011). Thus, these dichotomous histological findings are coherent with the functional retinal test or the ERG where high-dose Pb produces subnormal ERGs and low-dose Pb produces supernormal ERGs.

Hierarchical enzyme activity also may explain nonlinear Pb dose-response relationships. The phosphatase enzyme calcineurin has been shown to be inhibited by high dose Pb exposure and stimulated by low dose Pb exposure (Kern & Audesirk, 2000). At low doses of Pb, Pb displaces calcium at its binding sites on calmodulin and by acting as a calmodulin agonist at calcineurin's catalytic A subunit, stimulates calcineurin activity. At high Pb doses, Pb can bind directly to a separate calcium-binding B subunit, overriding the calmodulin-dependent effect and turning off the activity of calcineurin. Interestingly, mice with modulated calcineurin expression exhibit aberrant behavior related to schizophrenia (Miyakawa et al., 2003) or impaired synaptic plasticity and memory (Zeng et al., 2001). This example of the stimulatory effects of Pb at low doses and inhibitory effects of Pb at high doses gives another example of biological plausibility for the non-monotonic dose response of Pb reported in multiple studies.

2.8.3. Timing and Duration of Exposure

Epidemiologic studies reviewed in the 2006 Pb AQCD observed neurocognitive deficits in association with prenatal, peak childhood, cumulative childhood, and concurrent blood Pb levels. Among longitudinal studies that examined blood Pb level at multiple time points, several found that concurrent blood Pb was associated with the largest decrement in IQ. A common limitation of epidemiologic studies was the high correlation among Pb exposure metrics at different ages, making it difficult to distinguish among effects of Pb exposure at different ages and to ascertain which developmental time periods of Pb exposure were associated with the greatest risk of neurodevelopmental morbidity. Although prospective cohort studies have provided valuable information on the effects of Pb exposure at different periods of development, including the prenatal period and early childhood, the limitations noted in the previous 2006 Pb AQCD remain. Collectively, the epidemiologic evidence has not identified one unique time window of exposure that poses the greatest risk to cognitive function in children (Figure 2-3). However, toxicological studies included in the 2006 Pb AQCD demonstrated that developmental exposure to Pb was the most sensitive window for Pb-dependent neurotoxicity and recent toxicological studies continue to support this finding.



Note: [a]Effect estimates represent associations between concurrent blood Pb level and cognitive function (standardized to standard deviation) in children categorized by prenatal blood Pb level. [b]Values represent the ratio of blood Pb level at age 6 years to that at age 2 years. FSIQ = Full-scale IQ, GCI = General Cognitive Index. Cognitive function scores were standardized to their standard deviation. Effect estimates in red represent blood Pb level variables associated with the greater decrease in cognitive function.

Figure 2-3. Associations of cognitive function in children with different degrees of changes in blood Pb levels over time.

The 2006 Pb AQCD noted the importance of the duration of exposure in animal studies of a wide array of effects including neurological, cardiovascular, renal, immune and reproductive effects of Pb. Generally, epidemiologic studies of the effect of Pb exposure on human health have not been designed to assess duration of exposure needed to induce those effects. Some exceptions are cohort studies with repeated blood Pb measurements and cognitive assessments within short intervals of time during pregnancy and in the first year of life (every 3 months). Studies have reported associations between prenatal blood Pb levels and decrements in mental development (Bayley MDI) in children assessed between 3 and 6 months (Bellinger et al., 1984; Dietrich et al., 1986; Dietrich et al., 1987; Shen et al., 1998). In particular, Rothenberg et al. (1989) observed that maternal blood Pb levels from week 36 of pregnancy to delivery was associated with less ability of infants to self-quiet in the first 30 days of life. Additionally, in the Cincinnati cohort, blood Pb levels measured at neonatal day 10 also were associated

with MDI decrements at age 3 and 6 months (Dietrich et al., 1986; Dietrich et al., 1987). Consistent with the neurotoxicology literature, findings from these studies indicate that short-duration Pb exposure during critical periods of in utero or neonatal development are associated with cognitive impairments in young infants.

2.8.3.1. Persistence of Effects

The issue of persistence of the neurodevelopmental effects of low-level Pb exposure was also considered in the 2006 Pb AQCD, with some evidence suggesting that the effects of Pb on neurodevelopmental outcomes persisted into adolescence and young adulthood. However, in these studies, blood Pb levels remained relatively stable over time. Thus, the effect of concurrent exposures was not ruled out. In addition, the persistence of effect appears to depend on the duration of exposure as well as other factors that may affect an individual's ability to recover from an insult. Toxicological studies from the 2006 Pb AQCD highlighted the importance of Pb exposure in early life in promoting Alzheimer's like pathologies in the adult brain, demonstrating Pb-induced neurodegeneration and formation of neurofibrillary tangles. Recent toxicological studies continue to point to an early life window in which Pb exposure can contribute to pathological brain changes consistent with Alzheimer's disease. Blood Pb generally is not associated with Alzheimer's disease in epidemiological studies of adults. However, recent evidence indicates associations between early life ALAD activity, a biomarker of Pb exposure, and schizophrenia later in adulthood. Consistent with these findings, toxicological studies have observed Pb-induced emotional changes in males and depression changes in females.

2.8.4. Susceptible Populations and Lifestages

Potential susceptibility factors examined in Chapter 6 of this document include lifestage, sex, genes, pre-existing diseases/conditions, race and ethnicity, SES, BMI, nutrition, stress, cognitive reserve (e.g., the resilience of the mind), and co-exposure to other metals/toxicants. Studies are included in Chapter 6 if the findings for the susceptible population or lifestage were compared across strata with and without the potential susceptibility factor. By virtue of their design some cohort studies, including cohort studies of pregnant women or other populations or lifestages with no comparison group, are discussed in the endpoint-specific sections rather than in Chapter 6. This integrative summary, however, draws on evidence relating to potentially susceptible populations and lifestages appearing throughout this document. Also in Chapter 6, separate discussions of studies that evaluate factors that influence Pb exposure and uptake, and studies that examine the modification of the association of Pb with health endpoints by a possible susceptibility factor are presented. In this integrative summary both types of studies are considered together.

2.8.4.1. Children

Children may be more highly exposed to Pb compared to adults without occupational exposure to Pb, through their behaviors (e.g., hand-to-mouth contact). Blood Pb levels are highest among the youngest children and decrease with increasing age of the child (Table 6-1). Biokinetic factors that vary by age, including bone turnover and absorption, also affect blood Pb levels. Childhood, as a susceptibility factor related to Pb exposure and dose, is discussed in more detail in sectin 6.1.1.1. The kinetics of Pb, and how absorption, distribution, and elimination may vary depending on lifestage, is discussed in Section 4.2.

It is recognized that Pb can cross the placenta to affect the developing nervous system of the fetus (Sections 4.2.2.4, 5.3.2.1) and there is evidence of increased susceptibility to the neurocognitive effects of Pb exposure during several lifestages throughout childhood and into adolescence (for more detail, see Section 5.3.2.1). Further, Pb exposure is associated with effects on the renal (Section 5.5.2.3), immune (Section 5.6) and heme synthesis and RBC function (Section 5.7) of children. A limited number of studies of immune parameters, transferring saturation, and iron-deficiency anemia that stratified children by age report stronger associations among the youngest children. Childhood, as a susceptibility factor related to Pb-induced health effects, is discussed in more detail in Section 6.2.1.1.

2.8.4.2. Adults

There is evidence that both recent and/or cumulative exposure to Pb may result in health outcomes during adulthood, as indicated by consistent associations of blood Pb or bone Pb with cardiovascular diseases and mortality, renal, immune, hematological, and reproductive effects. Blood Pb and bone Pb levels tend to be higher in older adults (>65 years) compared with the general population. Mobilization (Section 4.2.2.2) of Pb from the bone stores may occur during periods of physiological stress, including older adulthood (Section 6.1.1.2). In recent studies, age was specifically examined as an effect modifier of the association of Pb with mortality, cognition and blood pressure in adults; findings for mortality were mixed while little evidence of modification by age was reported for the other specific outcomes. Toxicological studies support the plausibility of differences in susceptibility to health effects depending on lifestage.

2.8.4.3. Sex

In a recent NHANES analysis and several other studies, gender-based differences in blood Pb level were observed among the adolescent and adult age groups, with higher blood Pb levels among males. The gender-based differences were not substantial among the youngest age groups (1-5 years old and 6-11 years old). Studies of effect measure modification of the association of Pb and various health endpoints including neurological effects such as cognition, kidney function blood pressure immune effects and

cancer by sex were conducted. Overall, findings were mixed with the most consistent evidence reported for neurological endpoints. Recent epidemiologic evidence increased the consistency of collective evidence base and pointed to males having increased susceptibility for Pb-associated neurotoxicity. Toxicological studies continue to demonstrate increased susceptibility of males for endpoints such as sensory function, balance, stress hormone homeostasis, and brain membrane composition. Sex differences were also observed in toxicological studies across a wide array of endpoints including of behavior, memory, gross motor skills, obesity, and retinal decrements. See Section 6.1.2 and 6.2.2 for more details.

Hormone levels may affect susceptibility to Pb-related health effects and associations among females may vary based on hormonal status, such as menopause or ovary removal. Toxicological and epidemiologic evidence supports the potential susceptibility to Pb effects based on hormonal status with findings on delayed onset of puberty and changes to the female reproductive tract.

2.8.4.4. Race and Ethnicity

Higher blood Pb and bone Pb levels among African Americans have been documented and recent studies continue support previous findings. Further, larger proportions of both non-Hispanic blacks and Mexican American children have blood Pb levels exceeding 5 µg/dL of blood. The 2006 Pb AQCD noted that a clear downward temporal trend was apparent in NHANES data during the previous two decades but that blood Pb level was declining at different rates for groups within the population, which were defined by race, as well as income and demographic factors include age of housing. Recent data suggest that the gap in Pb exposure between African American and White subjects is decreasing, but African Americans still tend to have higher blood Pb levels. Evidence of increased association of Pb with cardiovascular outcomes among non Hispanic blacks and Mexican Americans was reported for the NHANES population. Results of other recent epidemiological studies suggest that there may be race related susceptibility for additional outcomes but the evidence is limited and confounding or modification by other factors, such as SES, may be present. See Sections 6.1.3 and 6.2.7 for additional details.

2.8.4.5. Socioeconomic Status

The 2006 Pb AQCD noted that the geometric mean blood Pb concentration varied with SES and other demographic characteristics that have been linked to Pb exposure. The gap between SES groups with respect to Pb level appears to be diminishing, with Pb level being higher but not significantly higher among lower income subjects. Lower SES individuals appear to represent a susceptible population. For example, a study of Pb and IQ reported greater inverse associations among those in the lowest SES groups. There is also 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). In contrast, there is

some evidence that Pb-associated neurocognitive effects may be larger in magnitude among higher SES populations. In a meta-analysis, Schwartz (1994) found that in studies in higher SES populations, blood Pb was associated with a greater decrement in IQ than in low SES populations. See Sections 6.1.4 and 6.2.8 for additional details.

2.8.4.6. Genes

Several genes were examined as potential modifiers the associations between Pb and health effects. Epidemiologic and toxicological studies reported ALAD and VDR variants may be health-related susceptibility factors. Although limited, evidence suggests that risk of Pb-associated neurocognitive deficits in children also may be modified by variants in genes for APOE, MTHFR, and dopamine receptors. Other genes examined that may also affect susceptibility to Pb-related health effects were DRD4, GSTM1, TNF-alpha, eNOS, and HFE. See Section 6.2.4 for additional details.

2.8.4.7. Pre-existing Conditions

Pre-existing diseases/conditions also have the potential to affect the association of Pb exposure with health endpoints have been studied in relation to autism, diabetes, and hypertension. Recent epidemiologic studies did not support modification of Pb and health endpoints by diabetes; however, past studies have found diabetics to be a more susceptible population with regard to effects on renal function. Recent epidemiological studies support finding from the 2006 Pb AQCD that hypertension is observed to be a susceptibility factor i with both renal effects and heart rate variability demonstrating stronger associations among hypertensive individuals compared to those that are normotensive. Although the evidence is limited, current research has shown that in autistic children, blood Pb level is differentially correlated with expression of immune-related genes. See Section 6.2.5 for additional details.

2.8.4.8. Nutrition and Lifestyle Factors

Body mass index (BMI), alcohol consumption, and nutritional factors were examined in recent epidemiologic and toxicological studies. Modification of associations between Pb and various health effects (mortality and heart rate variability) was not observed by BMI or 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 possibility of alcohol as a susceptibility factor. Among nutritional factors, those with iron deficiencies were observed to be a susceptible population for Pb-related health effects in both epidemiologic and toxicological studies. Other nutritional factors, such as calcium, zinc, and protein, demonstrated the potential to modify associations between Pb and health effects in toxicological studies. Recent epidemiologic studies of these factors were either not performed or observed no modification. Folate was also examined in a recent epidemiologic study of birth size but no interaction

was reported between Pb and folate. Further study of these and other nutritional factors will be useful in determining susceptibility among individuals with various nutritional levels/deficiencies. See Sections 4.2, 6.2.9, 6.2.10, and 6.2.11 for additional details.

2.8.4.9. Stress and Cognitive Reserve

Animal toxicology findings described in the 2006 Pb AQCD demonstrated interactions between Pb exposure and stress. Namely, Pb-exposed animals reared in cages with enriched environments (toys) perform better in the Morris water maze than their Pb-exposed littermates who were reared in isolation. New findings indicate a potentiating effect of stress on behavior and memory at low-dose Pb exposures. In comparison, epidemiologic evidence for such interactions has been sparse. However, consistent with historical animal studies, a recent epidemiological study indicated that positive social environment of children as characterized by maternal self-esteem, may lessen the impact of Pb exposure on neurodevelopment. Self-perceived stress was shown to modify the association of bone Pb with hypertension. In addition, a greater association of Pb with cognitive function was found in workers with lower cognitive reserve (Sections 6.2.12 and 6.2.13).

2.8.4.10. Co-exposure of Lead with Metals or Other Chemicals

The 2006 Pb AQCD reported that the majority of studies examined other chemicals as confounders and not effect measure modifiers (U.S. EPA, 2006). Although the body of evidence remains limited, recent epidemiologic studies have begun to explore the possible interaction between Pb and other metals or chemical agents. These studies report some stronger associations between Pb and various health endpoints with co-exposure to Cd, As, Mn, fluoride, tobacco smoke and urban pollutants.

Epidemiologic and toxicological studies have reported increased susceptibility to Pb-related health effects among those with high Cd levels. Modification of associations of Pb with levels of reproductive hormones and renal dysfunction were reported in epidemiologic studies. Toxicological evidence of a synergism between Pb and Cd with regard to renal toxicity supported the epidemiological evidence. In addition, exposure to Pb and As was associated with effects on immune function in children living near a smelter and a statistical interaction between the metals was observed. Studies suggest that co-exposure to As may increase the bioavailability of Pb establishing the plausibility of increased susceptibility of Pb-related health effects when co-exposed to As. An interaction was also reported between Pb and Mn (Y. Kim et al., 2009) in a study of IQ. Fl has been identified as a potential susceptibility factor in a toxicological study but has not yet been explored in epidemiologic studies. The toxicological reported that co-exposure of Pb and Fl increased Pb deposition in calcified tissues. Since Pb is acid soluble, fluoridation may increase Pb concentration in water through leaching from pipes and Pb solder. Additional details of the studies summarized, are found in Sections 6.2.14 and 6.2.15.

Recent studies suggested that Pb-associated neurotoxicity in children are exacerbated with co-exposures to environmental tobacco smoke but findings from recent epidemiologic studies examining modification by smoking for other outcomes are mixed. Exposure to urban areas with larger industrial sources and higher traffic has been suggested to increase Pb body burden and risk of Pb exposure. An ecological study reports an association of accumulated metals in the soil (e.g., Pb, Zn, Cd, Ni, Mn, Cu, Co, Cr and V) with reduced learning achievement among the students at the school (Mielke et al., 2005).

2.9. Summary

This section summarizes the main conclusions of this assessment regarding the health and ecological effects of Pb and, for health outcomes, the concentrations at which those effects are observed. The conclusions from the 2006 Pb AQCD and the causality determinations for the health and ecological effects of Pb exposure from this review are summarized in Table 2-8.

The 2006 Pb AQCD reviewed a strong body of evidence clearly substantiating the health effects of Pb at contemporary exposure levels. Neurological effects in children and cardiovascular effects in adults were the effects that were best substantiated as occurring at blood Pb concentrations as low as 5 to 10 μg/dL. Other newly demonstrated immune and renal system effects among general population groups were also emerging as low-level Pb-exposure effects of potential public health concern. New epidemiologic and toxicological studies support the findings of the previous assessement and provide additional evidence for these effects at increasingly lower levels.

The major conclusions reached in the 2006 Pb AQCD for terrestrial ecosystems focused on evidence from smelter sites or other industrial point sources with elevated levels of Pb where death of vegetation was found to cause a near-complete collapse of the detrital food web, creating a terrestrial ecosystem in which energy and nutrient flows were minimal. In aquatic ecosystems, the best documented links between Pb and effects on the environment were with effects on individual organisms. Bioaccumulation of Pb in aquatic organisms was shown to alter the aquatic environment. Further, it was noted that alteration of ecological interactions (e.g., species competitive behaviors, predator-prey interactions, and contaminant avoidance behaviors) may have negative effects on species abundance and community structure. However, the fact that both terrestrial and aquatic systems frequently contain multiple metals and other stressors made it difficult to attribute observed adverse effects to Pb.

The current document presents detailed reviews of the scientific literature on the effects of Pb in aquatic and terrestrial ecosystems, but, also integrates the evidence of effects across aquatic and terrestrial habitats. The ecological endpoints considered for causal determination are bioaccumulation, mortality, growth, physiological stress, hematological effects, developmental and reproductive effects, and neurobehavioral effects. The substantial overlap between the ecological and health endpoints considered

in the causal determinations allowed the integration of the evidence across these disciplines. This organizational scheme represents an evolution from the structure of the 2006 Pb AQCD and lends additional weight to the ecological evidence by reducing the uncertainty associated with attributing ecosystem effects to Pb rather than other metals or toxicants that co-occur with Pb since much of the evidence documenting the effects and modes of action of Pb comes from controlled animal experiments.

Table 2-8. Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb

Health Outcome	Conclusions from the 2006 Pb AQCD	Conclusions from the 2011 Ist Draft Pb ISA
Neurological Effects		Causal relationship
Neurocognitive Function and Learning	The collective body of epidemiologic studies provided clear and consistent evidence for the effects of Pb exposure on decreasing neurocognitive function in children.	Recent epidemiologic studies in children continue to demonstrate associations with IQ; most evidence emphasizes associations of blood Pb levels as low as 2 µg/dL with specific indices of neurocognitive function (e.g., verbal skills, memory, learning visuospatial processing). Among environmentally exposed adults, the most consistent findings were associations of cumulative Pb exposure metrics with cognitive deficits.
Neurobehavioral Effects	Several epidemiologic studies reported associations between Pb exposure and that ranged from inattentiveness to self-reported delinquent behaviors to criminal activities. Uncertainty remained regarding the critical time period of Pb exposure. In addition, uncertainties remained regarding whether Pb exposure was an independent predictor of neurobehavioral effects. Results from studies of ADHD were inconclusive. Suggestive relationship for both blood and bone Pb with depression and anxiety symptoms.	Recent studies in children continue to support associations of Pb exposure (blood Pb levels 3-11 µg/dL) with a range of effects from anxiety and distractibility to conduct disorder and delinquent behavior. New evidence indicates associations between blood Pb levels as low as 1 µg/dL and ADHD diagnosis and contributing diagnostic indices.
Sensory Organ Function	The selective action of Pb on retinal rod cells and bipolar cells is well documented in earlier AQCDs. There was coherence between the animal and the human literature on the effects of chronic Pb exposure on auditory function.	No new epidemiologic studies on sensory organ function. Recent toxicological studies find retinal effects in male rodents at lower blood Pb levels (~12 µg/dL)

Neurodegenerative Diseases	Several epidemiologic studies of Pb exposure and Alzheimer's disease, or dementia did not report associations association, but each study had sufficient limitations.	Recent epidemiological studies report associations with PD and essential tremor. Emerging toxicological evidence suggests that early life exposure may be associated with neurodegeneration in adult animals.
Cardiovascular Effects	Epidemiologic studies consistently demonstrated associations between Pb exposure and increased risk of CVD outcomes. Experimental toxicology confirmed Pb effects on the cardiovascular system observed in epidemiologic studies.	Causal relationship
Blood Pressure	A doubling of blood Pb level was associated with a 1 mmHg increase in systolic blood pressure and a 0.6 mmHg increase in diastolic blood pressure.	Recent studies confirm these findings. Associations of increased BP with Pb exposure observed at blood Pb levels < 2 µg/dL.
Hypertension	Suggestive evidence that cumulative Pb exposure may be associated with hypertension. Animal studies demonstrated that long-term exposure to Pb resulted in hypertension that persisted after cessation of exposure.	Recent studies, including those using bone Pb as a metric of cumulative exposure, confirm and add weight to previous findings. Associations of hypertension with Pb exposure observed at blood Pb levels < 2 µg/dL. Recent studies have emphasized the interaction of cumulative exposure to Pb with other factors including stress.
Cardiovascular Mortality	Limited evidence in support of cardiovascular mortality.	Recent studies including an NHANES analysis of the association of blood Pb with cause-specific mortality and study of older adults, which used bone Pb as an exposure metric, addressed limitations of previous studies and provide additional evidence for an association of Pb with cardiovascular mortality.
Renal Effects	Circulating and cumulative Pb exposure was associated with longitudinal decline in renal function. Experimental studies demonstrated that initial accumulation of absorbed Pb occurred primarily in the kidneys and hyperfiltration phenomenon during the first 3 months of exposure was noted.	Causal relationship Recent studies expand upon the strong body of evidence that Pb exposure is associated with kidney dysfunction including increased serum creatinine and decreased creatinine clearance at blood Pb levels < 5 µg/dL.
Immune System Effects	Epidemiologic studies suggested that Pb exposure may be associated with effects on cellular and humoral immunity including changes in serum immunoglobulin E levels in children. Toxicological evidence supported these findings and provided evidence for effects on downstream events such as inflammation and decreased host resistance.	Causal relationship Recent studies support the strong body of evidence that Pb exposure is associated with both cell-mediated and humoral immunity. The consistency and coherence of findings among related immune effects establishes the biological plausibility for epidemiologic observations of associations with infection, allergy and effects in other organ systems.

Heme Synthesis and RBC Function	Pb exposure was associated with disruption in heme synthesis in both children and adults. Toxicological studies demonstrated that Pb interferes with red blood cell survival and mobility.	Causal relationship Recent epidemiologic and toxicological studies provide strong evidence that exposure to Pb is associated with numerous deleterious effects on the hematological system including altered heme synthesis mediated through decreased ALD and ferrochelatase activities, decreased RBC survival and function, decreased hematopoiesis and increased oxidative stress and lipid peroxidation.
Reproductive Effects and Birth Outcomes	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.	Causal relationship Recent toxicological and epidemiologic studies provide strong evidence for delayed onset of puberty in males and females as well as effects on sperm. Evidence on pregnancy outcomes was inconsistent and less coherent across disciplines for preterm birth, spontaneous abortion, low birth weight, birth defects, hormonal influence and fecundity.
Cancer	Epidemiologic studies of highly exposed workers suggested a relationship between Pb and cancers of the lung and the stomach; the evidence was limited by confounding by 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.	Likely causal relationship Some epidemiologic evidence supporting associations between Pb and cancer with the strongest evidence from animal toxicology between Pb and cancer, genotoxicity/clastogenicity, or epigenetic modification.

Ecological/ Welfare Effect	Findings from 2006 Pb AQCD	Conclusions in the 2011 1st Draft ISA
Bioaccumulation as it affects Ecosystem Services	Atmospheric Pb pollution has resulted in accumulation of Pb in terrestrial and aquatic systems throughout the world. In field studies in aquatic systems, Pb has been shown to significantly alter the aquatic environment through bioaccumulation and alterations of community structure and function. Due to low solubility of Pb in water, dietary Pb (i.e., Pb absorbed to sediment, particulate matter and food) may contribute substantially to exposure and toxicity.	Causal relationship There is compelling evidence from several decades of research and in recent studies that Pb bioaccumulates in plants, invertebrates and vertebrates in terrestrial and aquatic ecosystems. Pb deposited on the surface of, or taken up by organisms has the potential to contribute to exposure and toxicity in primary and secondary order consumers and alter the services provided by ecosystems (i.e., provisioning services).
Mortality	No information on mortality in plants. Effects of Pb on invertebrates and vertebrates include decreased survival.	Inadequate to infer a causal relationship for plants Insufficient evidence for mortality in plants. Causal relationship in vertebrates and invertebrates Recent studies provide additional evidence for Pb effects on mortality in invertebrates and vertebrates.
Growth	Evidence of growth effects in algae, aquatic plants, soil invertebrates and aquatic invertebrates. Limited evidence in avian and mammalian consumers.	Causal relationship in plants and invertebrates Recent studies on growth in algae and invertebrates find effects of Pb at lower concentrations than previously, and additional evidence for growth effects in plants. Suggestive causal relationship in vertebrates Limited studies considered effects on growth in vertebrates.
Physiological Stress	Pb exposure may cause lipid peroxidation and changes in glutathione concentrations. There are species differences in resistance to oxidative stress.	Causal relationship Recent studies support the strong body of evidence for upregulation of antioxidant enzymes and increased lipid peroxidation associated with Pb exposure in many species of plants, invertebrates and vertebrates.
Hematological Effects	Pb effects on heme synthesis were documented in the 1986 AQCD and continue to be studied in aquatic and terrestrial biota. Changes in ALAD are not always related to adverse effects but may simply indicate exposure. Numerous studies have reported the effects of Pb exposure on blood chemistry in aquatic and terrestrial biota.	Causal relationship Recent studies expand the evidence for Pb effects on ALAD enzyme activity in bacteria, invertebrates, and vertebrates and altered serum profiles and blood cell counts in vertebrates.

Development and Reproduction	No information on reproduction in plants. Limited new evidence in invertebrates and vertebrates.	Inadequate to infer a causal relationship for plants There are an insufficient number of studies that consider Pb effects on plant reproduction. Causal relationship in invertebrates and vertebrates Recent studies expand the evidence for Pb effects on embryonic development as well as for multi-generational effects in invertebrates. In vertebrates, there is new evidence for delayed metamorphosis and altered steroid profiles in the few species studied.
Neurobehavior	Exposure to Pb has been shown to affect brain receptors in fish. Exposure to Pb in laboratory studies and simulated ecosystems may alter species competitive behaviors, predator-prey interactions and contaminant avoidance behaviors.	Causal relationship Recent studies identify possible molecular targets for Pb neurotoxicity in invertebrates and fish. There is new evidence in a few invertebrate and vertebrate species for behavioral effects associated with Pb exposure.
Community and Ecosystem Level	Effects of Pb difficult to interpret because of the presence of other stressors including metals.	Causal Relationship Uptake of Pb into aquatic and terrestrial organisms and subsequent effects on survival, reproduction, growth, behavior and other physiological variables at the species scale is likely to lead to effects at the population, community and ecosystem scale. There is additional evidence for effects of Pb in soil microbial communities, and in sediment-associated and aquatic plant communities.

Chapter 2 References

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Chapter 3. Ambient Lead: Source to Concentration

3.1. 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 new data and studies. This includes new knowledge of Pb fate and transport, the latest developments in monitoring methodologies, and recent data describing Pb concentrations as a function of size range. 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, 2006).

Section 3.2 provides an overview of the primary and secondary sources of air Pb. Section 3.3 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 3.4. Ambient Pb concentrations, their spatial and temporal variability, size distributions of Pb-bearing particulate matter (PM), and associations with copollutants are characterized in Section 3.5. Concentrations of Pb in non-air media and biota are described in Section 3.6.

3.2. Sources of Atmospheric Lead

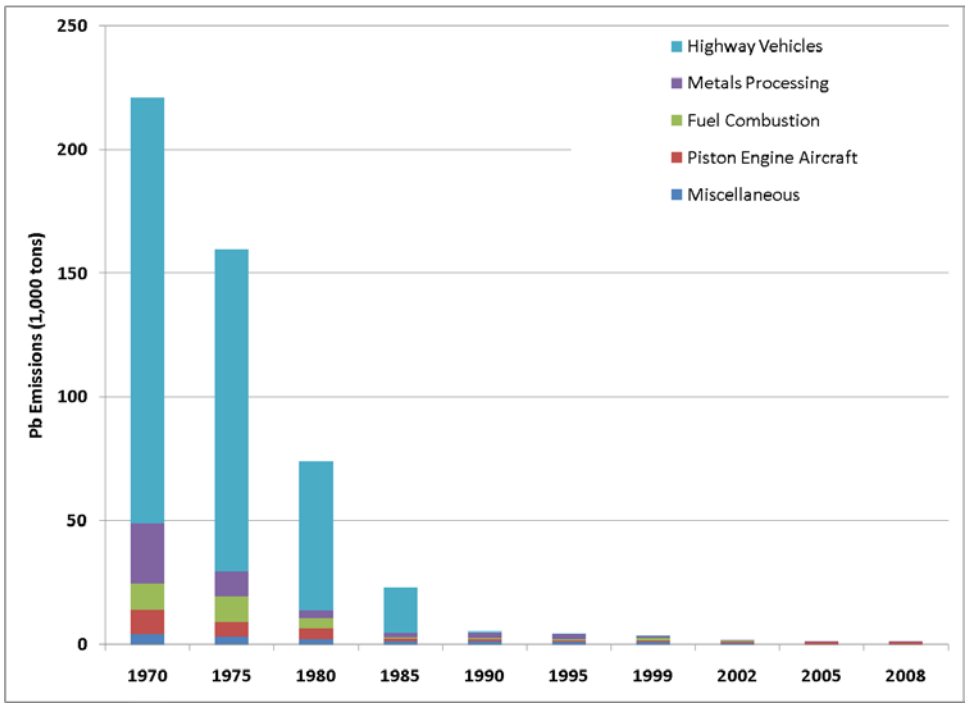
The following section reviews updated National Emissions Inventory (NEI) data from 2008 (U.S. EPA, 2011), which is the most recently available quality-assured Pb emissions data. This section also reviews updated information from the peer-reviewed literature regarding sources of ambient Pb. Detailed information about processes for primary and secondary anthropogenic emissions and naturally-occurring emissions can be found in the 2006 Pb AQCD (U.S. EPA, 2006). The papers cited herein generally utilized PM sampling data, because ambient airborne Pb readily condenses to PM. The 2006 Pb AQCD (U.S. EPA, 2006) employed the 2002 NEI (U.S. EPA, 2008a) or source analysis and 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

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

from industrial processes, incineration, and utilities, each contributing less than 5% (53%). Since that time, states have additional information to the inventory. The mobile source category included combustion products from organic Pb antiknock additives used in piston-engine aircraft (hereafter referred to piston aircraft emissions).

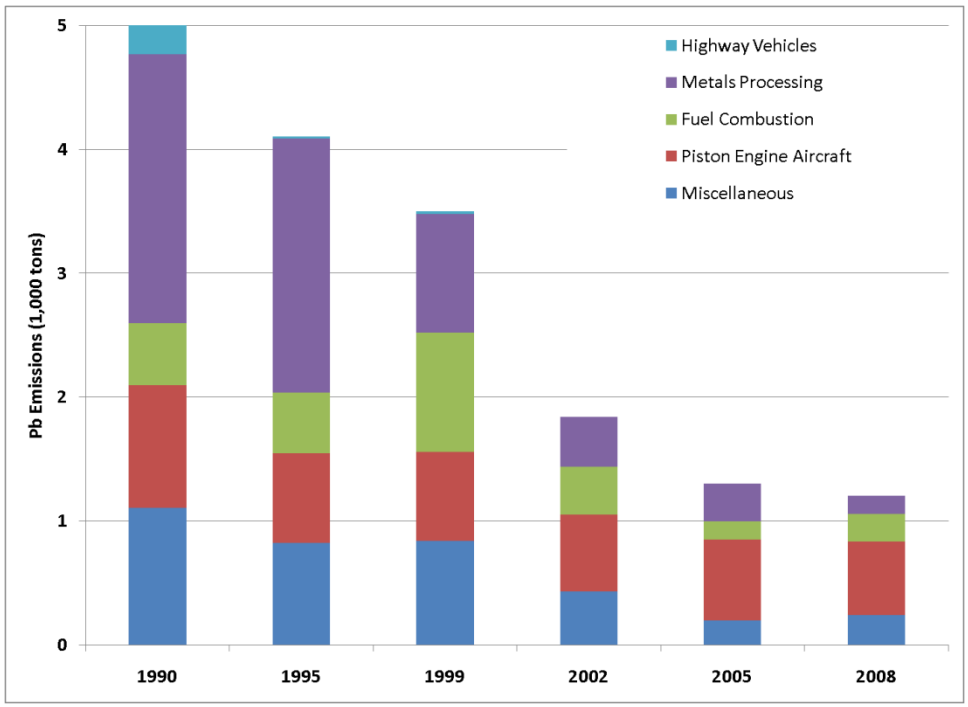
3.2.1. National Emissions Inventory

Emissions of Pb have dropped substantially over the past forty years, as shown in Figure 3-1 and Figure 3-2. 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, 2006). This action resulted in a 98% reduction in Pb emissions from 1970-1990. Total Pb emissions over the period 1990-2008 decreased an additional 77%, from 5,200 tons in 1990 to 1,200 tons in 2008. Subsequent emissions reductions are related to enhanced control of the metals processing industry. In 1990, metals processing accounted for 42% (2,200 tons) of total Pb emissions. By 2008, metals processing accounted for 12% (150 tons) of total emissions. This represented more than an order of magnitude decrease in Pb emissions from metals processing. At the same time, emissions from piston engine aircraft varied only slightly over this time period. In 1990, off-highway Pb emissions were 990 tons and represented 19% of total Pb emissions. In 2008, off-highway Pb emissions from piston engine aircraft were slightly lower at 590 tons, which comprised 49% of all Pb emissions. “Miscellaneous” emissions from other industrial processes, solvent utilization, agriculture, and construction comprised 20% of emissions (240 tons) in 2008.



Source: U. S. EPA (2011)

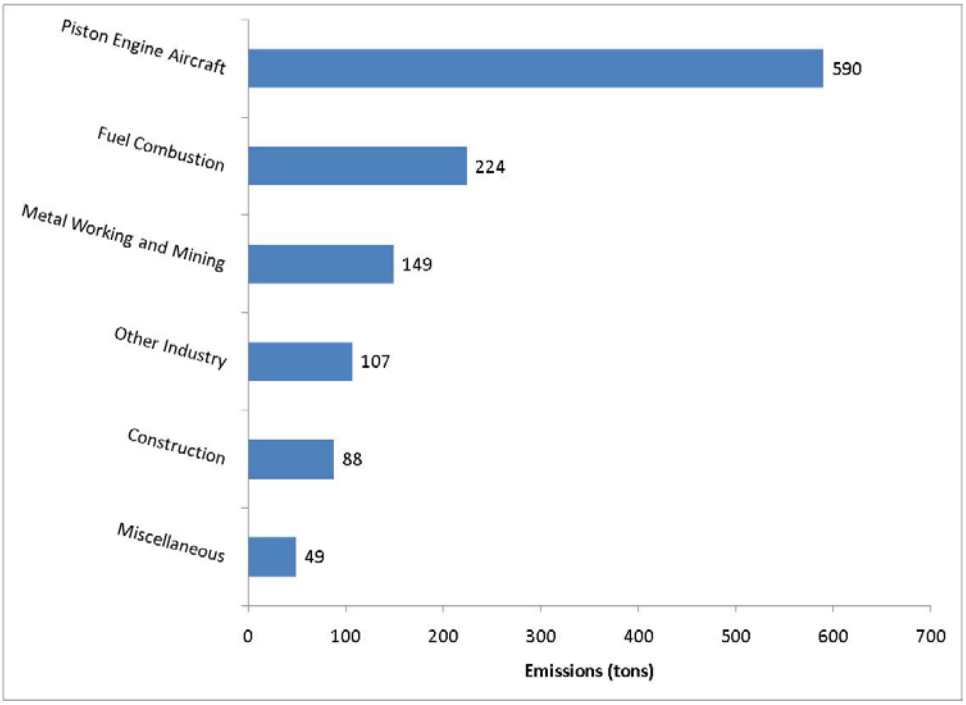
Figure 3-1. Trends in Pb emissions (thousand tons) from stationary and mobile sources in the U.S., 1970-2008.



Source: U. S. EPA (2011)

Figure 3-2. 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 point source emissions estimated by the 2008 NEI are shown in Figure 3-3. Emissions from piston engine aircraft emissions comprised nearly half of all emissions (590 tons). Industrial fuel combustion contributed 220 tons (18%) of Pb emissions in 2008, followed by metal working and mining (12%), other industry (9%), dusts from construction (7%) and miscellaneous contributions from agriculture, solvent utilization, and operation of commercial marine vessels and locomotives (4%) (U.S. EPA, 2011). 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.

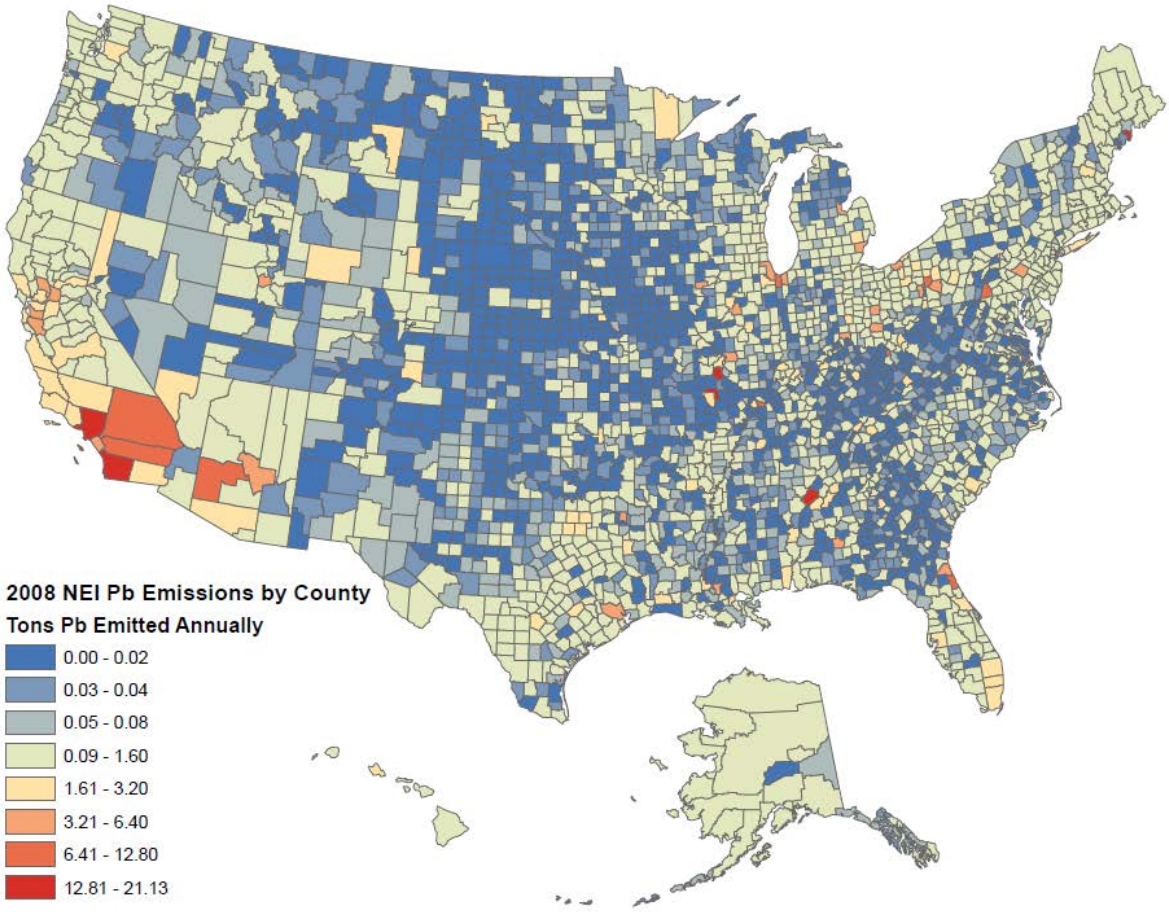


Source: U. S. EPA (2011)

Figure 3-3. Nationwide stationary source Pb emissions (tons/yr) in the U.S. by source sector in 2008.

There is substantial variability in Pb emissions from stationary sources across U.S. counties, as shown in Figure 3-4 for the continental U.S. The emissions levels, shown in units of tons, vary over several orders of magnitude. Ninety-four percent of U.S. counties had 2008 emissions below 1 ton, and 50% of counties had 2008 emissions below 0.044 tons. The upper 0.1% of stationary emissions came from thirty-three counties. This category included all counties emitting more than 3.8 tons of Pb in 2008. Jefferson County, MO was the highest emitting county, with over 21 tons of airborne Pb emissions in

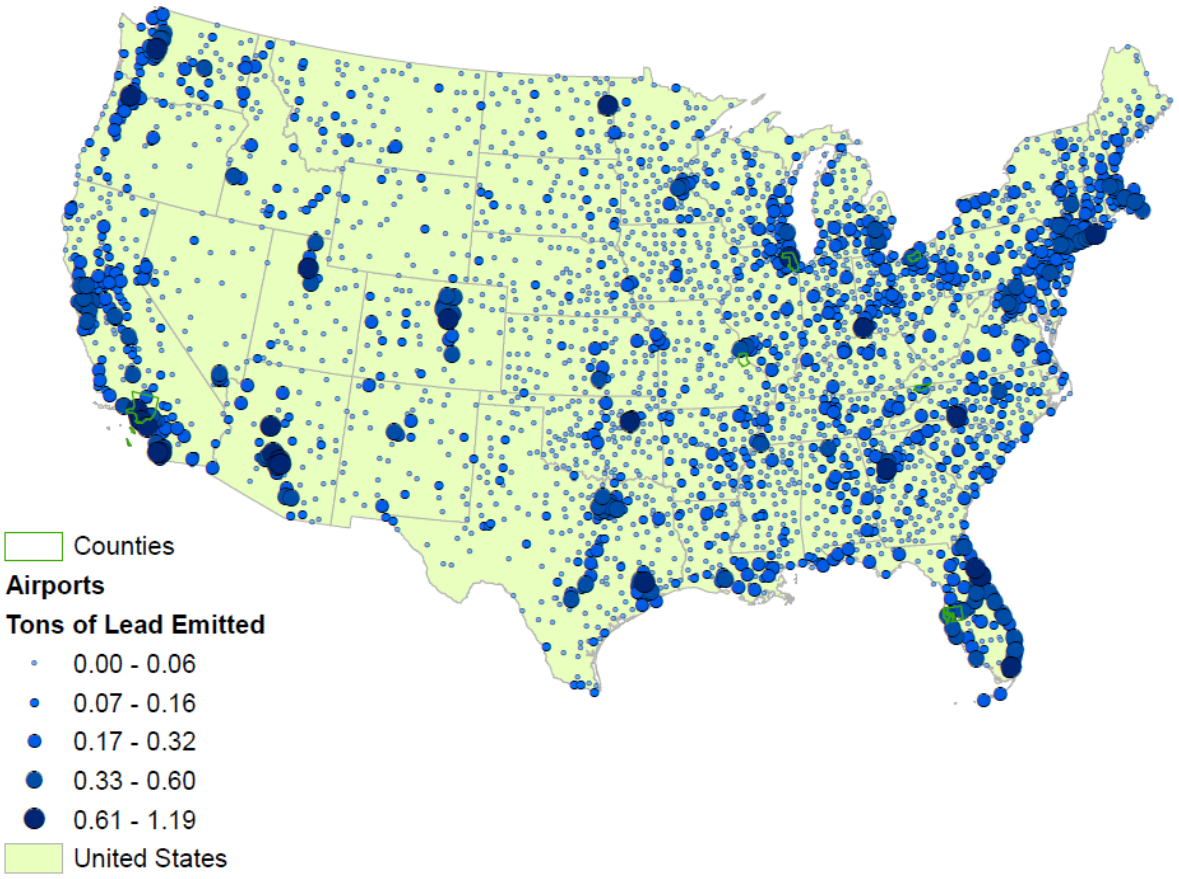
2008. Jefferson County is home to the Doe Run primary Pb smelting facility, which is the only remaining operational primary Pb smelter in the U.S.



Source: U.S. EPA (2011)

Figure 3-4. County-level Pb emissions (tons) from stationary sources in the U.S. in 2008.

Pb emissions from piston engine aircraft operating on leaded fuel occur at approximately 20,000 airports across the U.S. Figure 3-5 displays Pb emissions (tons) at airports around the continental U.S. The map illustrates that airport emissions tend to be elevated around highly populated metropolitan regions, which typically have multiple airports with varying activity levels. Clusters of airports around metropolitan areas and megapolitan regions (multiple contiguous metropolitan areas) are notable from Figure 3-5. Among these sites, piston aircraft emissions at airports within twenty-eight counties cumulatively emitted greater than one ton of Pb in 2008 U.S. EPA (2011). Additionally, within the 2008 NEI, there were estimates of Pb emissions during flight from piston aircraft. These estimates are provided by state and cumulatively account for 296 tons in 2008.



Source: U.S. EPA (2011)

Figure 3-5. Pb emissions (tons) at airports in the U.S. in 2008. The size of the symbol indicates the magnitude of Pb emissions at each airport.

3.2.2. Anthropogenic Sources

Anthropogenic Pb source categories are organized below in order of magnitude reported on the 2008 NEI (U.S. EPA, 2011), with emissions from piston engine aircraft being the highest and resuspended dust from previously deposited Pb being substantially lower.

3.2.2.1. Lead Emissions from Piston Engine Aircraft Operating on Leaded Aviation Gasoline and Other Non-Road Sources

The largest source of Pb in the NEI is from piston engine aircraft operating on leaded aviation gasoline emissions (U.S. EPA, 2011). Murphy et al. (2007) cited fuel consumption estimates provided by the U.S. Department of Energy indicating that 575,000 kg/yr of Pb is used in piston engine aircraft fuel, and based on the 1999 NEI, that 490,000 kg/yr (85%) become airborne upon combustion. Levin et al. (2008) point out that emissions from piston engine aircraft are exempt from reporting to the EPA Toxic

Release Inventory. Levin et al. (2008) summarized findings from environmental protection departments of the State of Illinois, the U.S., and Canada regarding ambient Pb concentrations at and near airports. The Canadian report noted average and maximum air Pb levels were 0.030 and 0.30 μg/m^3, respectively, compared with background levels of 0.007 and 0.018 μg/m^3 (Conor Pacific Environmental Technologies Inc, 2000). The Illinois report noted that air Pb concentrations were elevated downwind of O'Hare airport compared with upwind levels (Illinois Environmental Protection Agency, 2002). 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. Fuel consumption rates can be obtained for some engine/aircraft combinations by running FAA's Emissions and Dispersion Modeling System (FAA, 2011). The ASTM specification for the maximum Pb content is "100 Low Lead", the most commonly used leaded avgas, is 2.12 g of elemental Pb/gallon (ASTM, 2007).

Dynamometer testing has indicated that Pb emissions from piston engine aircraft fuel combustion can occur in the particulate and gaseous form. 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 avgas. The additives had trace levels of Pb that exist in gas phase when temperatures are higher than 650 C, below which they condense to particulate phase. 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 to act as a Pb "scavenging agent." When ethylene dibromide reacts with Pb, it forms Pb bromide and Pb oxybromides, which are more volatile.

3.2.2.2. Fugitive Emissions from Metals Processing and Mining

Fugitive emissions from secondary Pb processing can be substantial over the course of a year, but they are difficult to estimate. Goyal et al. (2005) estimated fugitive emissions using concentration data obtained from samplers sited in close vicinity of secondary Pb recovery 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 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·sec at the Florida site, 9.4×10^{-7} to 2.0×10^{-6} g/m^2·sec for the Texas site, and 8.8 x 10^{-7} to 1.1×10^{-6} g/m^2·sec 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/sec, the Texas site median ranging from 8.4×10^{-2} to 8.6×10^{-2} g/sec, and the New York site ranging from 8.4×10^{-3} to 1.0×10^{-2} g/sec. Additionally, the Bayesian hierarchical model was used to estimate fugitive Pb emissions nationwide using concentration data as prior information. Nationwide median fugitive emissions were estimated to be 9.4×10^{-7} to 3.3×10^{-6} g/m^2·sec.

Waste from current or defunct mines has been shown to present an additional fugitive source of Pb. For example, Zheng et al. (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 $PM_{10-2.5}$ and $PM_{2.5}$. They estimated that mine waste was responsible for 88% of Pb in $PM_{10-2.5}$ samples and 40% of Pb in $PM_{2.5}$ samples.

3.2.2.3. Fossil Fuel Combustion

Fossil fuel combustion accounts for roughly 12% of Pb emissions in the U.S. 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 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 tons Pb/yr to the atmosphere. There were no emission factors for crude oil or residual oil but these represent potentially large sources (up to 100-500 tons/yr and up to 25-700 tons/yr, respectively). 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. 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 (J. M. Pacyna & Pacyna, 2001; Witt et al., 2006). The Pb isotope ratio values (mainly $^{206}Pb/^{207}Pb$) for coals 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.

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/yr worldwide. Using a separate global model, Niisoe et al. (2010) calculated emissions of Pb from coal combustion in Japan during 2000 to be 900 tons/yr, based on 9×10^7 tons/yr coal combustion and an emission factor of 10 g Pb/ton. The equivalent value for Pb emissions from China was 56,000 tons Pb/yr. Calculated Pb concentrations in surface air for China agreed with this value within a factor of two, although there was a systematic underestimation suggesting an incomplete knowledge of the Pb emissions (Niisoe et al., 2010). It was notable, however, that the

calculated emissions from Chinese coal combustion make up a substantial proportion (~66%) of the total global Pb emissions from fossil fuels detailed in Rauch and Pacyna (2009).

Tan et al. (2006) compared several emissions sources in Shanghai, China. They estimated emission values for on-road exhaust from use of Pb-free gasoline (238 ± 5 mg/kg), vehicle exhaust from leaded on-road gasoline (7,804 ± 160 mg/kg), coal combustion (1,788 ± 37 mg/kg), metallurgic dust (6,140 ± 130 mg/kg), soil (11.7 ± 0.3 mg/kg), and cement (103 ± 2 mg/kg). Pb-free automobile gasoline has been in use in Shanghai since 1997. The isotope ratios for each of these emission sources were determined. Based on the 4.4×10^7 tons of coal combusted annually in Shanghai, an average coal Pb concentration of 13.6 ± 6.6 mg/kg, and an emission factor of 0.5, approximately 300 tons Pb was being emitted annually in association with fine PM. They concluded that a major priority should be to reduce Pb emissions from coal combustion now that the contribution from vehicle exhaust emissions has decreased.

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 (W. Wang et al., 2006). This seasonal relationship contrasts with observations for the U.S. described in the 2006 Pb AQCD (U.S. EPA, 2006) which indicated that for West Virginia, higher emissions from power stations occurred in summer months. The increased energy use in summer periods in the U.S. may be attributable to increased requirements for air-conditioning.

3.2.2.4. Other Industrial Sources

Several Pb isotope studies have been used to distinguish contributions from industrial activities. For example, in northern China, Wang et al. (2006) noted that, in the response to decreasing atmospheric Pb concentrations in total suspended particles (TSP) samples collected from 1994 to 1998, the $^{206}Pb/^{207}Pb$ isotope ratio showed a related trend with values of ~1.149 in 1994 increasing to ~1.161 in 1998. The Pb concentration and isotope ratio values then remained approximately constant from 1998 through to 2001. Although this was consistent with a decreasing contribution of Pb from on-road gasoline, the ratio values were still lower than those for Chinese coal [$^{206}Pb/^{207}Pb$ ~1.18 in Mukai et al. (2001)], suggesting that local Pb ore sources typically have lower $^{206}Pb/^{207}Pb$ ratios. The range for Chinese ores was 1.081 to 1.176, with lower values corresponding to ores from northern China (Mukai et al., 2001).

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. Deconvolution of sources was attempted using Pb isotopes, but one major area of uncertainty was the amount and the isotope composition of Pb emanating from incineration plants, particularly in the U.K. 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. There have, however, been other environmental studies concerning incineration, and these give a highly consistent value 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 ~1.155. 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 1.1427-1.1576 and 2.4260-2.4346, respectively, i.e., quite a narrow range (de la Cruz et al., 2009 and references therein).

3.2.2.5. Roadway-Related Sources

Contemporaneous Emissions from Vehicle Parts

Contemporaneous 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 every automobile in the U.S. in order to balance the tires, and may become loose and fall off. Ambient air Pb concentrations near heavily trafficked areas may be related to use of Pb-based wheel weights that are prone to dislodgement. On pavement they may be ground into fine PM by the pounding forces of traffic (Root, 2000). For example, Schauer et al. (2006) measured Pb emissions in two traffic tunnels and found that the fraction of Pb in $PM_{2.5}$ was no more than 17% of Pb measured in PM_{10}. 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 low but substantial quantities of Pb in PM_{10} (0.02 ± 0.01 mg/g) and $PM_{2.5}$ (0.01 ± 0.00 mg/g) for semi-metallic brake pads and in PM_{10} (0.01 ± 0.00 mg/g) for low-metallic brake pads. 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 Pb were emitted from brake wear each year, compared with 2.6 kg of Pb from tire tread wear; an estimated 549 kg 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 are 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.

Unleaded Fuel

Unleaded fuel contains Pb as an impurity within crude oil (E. G. Pacyna et al., 2007). Schauer et al. (2006) measured Pb in $PM_{2.5}$ from tailpipe emissions and observed significant quantities in on-road gasoline emissions (83.5 ± 12.80 mg/kg) and higher but non-significant quantities in diesel emissions (137 ± 133 mg/kg). Hu et al. (2009) investigated the heavy metal content of diesel fuel and lubricating oil. They found <1-3 ppm Pb in samples of lubricating oil. Hu et al. (2009) 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 dynamic 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 MMAD. The Tan et al. (2006) study cited in Section 3.2.2.3 for Shanghai, China, where unleaded fuel has been in use since 1997, illustrated that Pb emissions from unleaded on-road gasoline were substantially lower than Pb emissions from coal combustion. Assuming that the natural Pb content of unleaded fuels in the U.S. is similar, it is unlikely that road vehicle combustion of unleaded on-road gasoline is currently a major contributor to total Pb emissions in the U.S.

3.2.2.6. Deposited Lead

Soil Pb can serve as a reservoir for deposited Pb. The following subsections describe studies of previously deposited Pb from industrial, historical leaded on-road emissions, and urban sources such as paint and building materials. The 2006 Pb AQCD (U.S. EPA, 2006) 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. Since publication of the 2006 Pb AQCD (U.S. EPA, 2006), further analysis of the Harris and Davidson (2005) paper has revealed that the contributions of Pb from piston engine aircraft were underestimated compared with the 2002 NEI. Assumptions of spatial uniformity incurred by the "continuously stirred reactor" mass balance model and for mixing layer height used by Harris and Davidson (2005) were also not valid because Pb concentrations are spatially heterogeneous at the urban scale; see Section 3.5. Therefore, the estimate of 90% of airborne Pb from resuspension is not employed in the current assessment. Currently, 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.

Lead from Industrial Sites

Several studies have indicated elevated levels of Pb in soil exposed to industrial emissions, including brownfield sites (Deng & Jennings, 2006; Dermont et al., 2010; Hofer et al., 2010; Jennings &

Ma, 2007; Sriskandan et al., 2007; van Herwijnen et al., 2007; Verstraete & Van Meirvenne, 2008). It is possible that Pb in soil serves as a source of airborne Pb. Laidlaw and Filipelli (2008) reviewed the literature on Pb resuspension from soil and then analyzed IMPROVE data to explore conditions under which Pb may become resuspended. They observed a seasonal pattern in concentration of soil resuspended in the atmosphere, and they also found that 83% of the variability in concentrations of soil in the atmosphere was predicted by the variability in meteorology and soil moisture content. The authors concluded that seasonality and climate parameters could not be eliminated in relation to ambient Pb concentrations. Such mechanisms are described in more detail in Section 3.3.

Lead from Paint and Building Materials

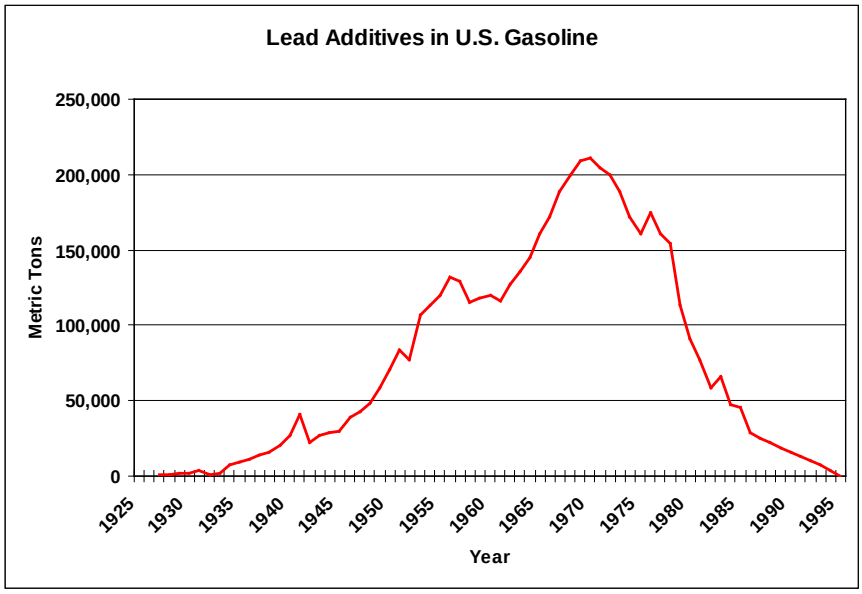
Exterior painted structures have long been known to be a source of ambient Pb (U.S. EPA, 2006). Recent studies support older findings. Mielke and Gonzales (2008) sampled exterior paint chips from 25 homes in New Orleans, LA, and they found elevated Pb levels in 24 of the 25 tested exterior paints (median: 36,000 mg/kg). 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 comparison was significant ($p < 0.006$ at one site and $p < 0.0001$ at 4 other sites), with median Pb concentrations under the steel structures being between 2.5 to 11 times higher than median Pb concentrations not near a structure.

The studies described above considered paint as a source of Pb dust through gradual abrasion of the painted surfaces. However, ambient conditions may also affect the availability of Pb in paints. Edwards et al. (2009) performed experiments to simulate one week of exposure of Pb-based paints to elevated levels of O_3 (11.3 ± 0.8 ppm or 150 times the level of the 8-h NAAQS) and NO_2 (11.6 ± 0.9 ppm, or 220 times the level of the annual NAAQS). Following NO_2 exposure, the Pb availability in wipe samples increased by a median of 260% ($p < 0.001$), and following O_3 exposure, the Pb availability increased by a median of 32% ($p = 0.004$). Edwards et al. (2009) state that the high O_3 and NO_2 concentrations simulated in the chamber were equivalent of 4.3 and 3.7 years of exposure at 50 and 60 ppb, respectively.

Building demolition was listed as a source of urban Pb dust in the 2006 Pb AQCD (U.S. EPA, 2006). In a follow-up study to previous work cited therein, Farfel et al. (2005) observed that Pb dust surface loadings increased by 200% in streets, by 138% in alleys, and by 26% in sidewalks immediately following demolition of an old building. One month later, Pb dust loadings were still elevated in alleys (18%) and sidewalks (18%), although they had decreased in streets by 29%. 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.

Lead 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 still may be persistent in the environment as a potential source of airborne Pb. The historical combustion of leaded on-road gasoline has been estimated from documents submitted by Ethyl Corporation to the U.S. Senate ("Airborne Lead Reduction Act of 1984," 1984) and a report by the U.S. Geological Survey (USGS, 2005); see Mielke et al. (2010b). These estimates are presented in Figure 3-6. 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 non-highway applications, including piston engine aircraft fuel, racing fuels, farm tractors, snowmobiles, and boats.

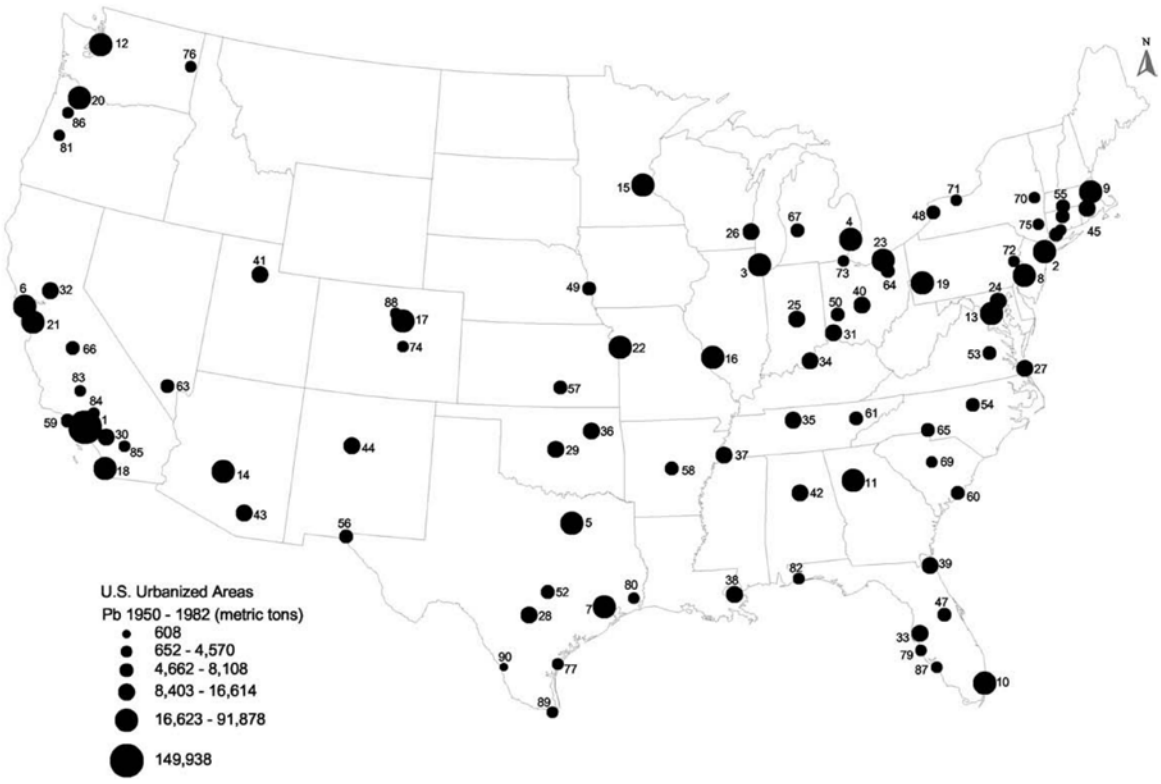


Source: Used with permission from Pergamon Press, Mielke et al. (2010b).

Figure 3-6. Total U.S. Pb additives in on-road gasoline used in on-road vehicles, 1927-1995. Estimates were derived from the proceedings of the U.S. Senate hearings on the Airborne Pb Reduction Act of 1984, S. 2609 ("Airborne Lead Reduction Act of 1984," 1984) and the U.S. Geological Survey Pb end use statistics (USGS, 2005).

The particle sizes of Pb emissions from on-road sources were estimated by the U.S. EPA (1986), which indicated that 75% of Pb additives were emitted as exhaust. The tonnages of relatively large >10 μm mass median aerodynamic diameter (MMAD) Pb-PM probably settled locally, especially in high traffic urbanized areas where soil Pb, from historic emissions as well as contemporaneous sources, are elevated adjacent to roadways and decrease with distance away from the roadway (Laidlaw & Filippelli, 2008). EPA (1986) indicated that 35% of the PM were < 0.25 μm in MMAD. The majority of PM, by number, emitted from automobiles was in the ultrafine size range (Londahl et al., 2009). However, Londahl et al. (2009) did not include ethylene dibromide to the fuel in these experiments. As described in the Gidney et al. (2010) study referenced in Section 3.2.2.1, it was found that ethylene dibromide acted to scavenge Pb in PM to form more volatile Pb bromide and Pb oxybromide to produce gaseous Pb emissions.

The use of Pb additives also resulted in a national scale of influence. For example, various sized urbanized areas of the U.S. have different amounts of vehicle traffic associated with Pb (Mielke et al., 2010a). Figure 3-7 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. The implication of this figure is that the soil Pb concentration will be proportional to the magnitude of historic on-road emissions in each city. It is recognized that the amount of soil turnover since 1982 may have varied substantially among the cities illustrated in Figure 3-7, depending on the amount of highway construction in those cities. Hence, the map may overestimate potential amounts of Pb in soil, and consequently of airborne Pb from resuspended soil, in some of the cities illustrated.



Source: Used with permission from Pergamon Press, Mielke et al. (2010b)

Figure 3-7. Estimated Pb aerosol inputs from on-road gasoline into 90 U.S. urbanized areas (UAs), from 1950 through 1982.The numbers on the map are rankings of each UA. The size of each dot refers to the magnitude of motor vehicle gasoline-related emissions for each group of UAs. The extremes are, Los Angeles UA (ranked #1) and Laredo, Texas (ranked #90). Some of the UAs have been used as sites in soil Pb studies, as indicated in Table 3-9.

3.2.3. Source Attribution

3.2.3.1. Lead Speciation and Source Apportionment

The following section describes new findings with respect to speciation of Pb content in aerosols. Analytic techniques for speciation are explicated in Section 3.4. Forms of Pb commonly observed in the environment are presented in Table 3-1 to serve as a reference for the categories of Pb sources described in Sections 3.2.1 and 3.2.2. Detailed descriptions of the related chemistry were presented in the 2006 Pb AQCD (U.S. EPA, 2006).

Table 3-1. Pb compounds observed in the environment

Emission Source	Observed Pb Compounds
Minerals	PbS (Galena)
	PbO (Litharge, Massicot)
	Pb_3O_4 (Minimum or "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 1150 K)
	$PbCl_2$ (Low rank coals, above 1150 K)
	$PbSO_4$ (Below 1150 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
Motor Vehicle Exhaust (Combustion of Leaded Fuel)[1]	PbBrCl
	$PbBrCl\text{-}2NH_4Cl$
	$PbBrCl\text{-}NH_4Cl$
Roadside Dust	$PbSO_4$, Pb^0, $PbSO_4(NH_4)SO_4$, Pb_3O_4, $PbO\text{-}PbSO_4$ and $2PbCO_3\text{-}Pb(OH)_2$
Other mobile sources:	
Brake wear, wheel weights	Pb^0
Racing vehicle emissions	Pb halides
Aircraft Engine Wear	Pb^0
Lawn Mowers	Pb halides (battery leakage)

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

Chemical speciation of Pb by source was reviewed in the 2006 Pb AQCD (U.S. EPA, 2006), and is fairly well understood and varies considerably between sources. Pb components from Pb smelters are mainly elemental Pb (Pb^0), Pb sulfide (PbS), Pb sulfates ($PbSO_4$, $PbO\text{-}PbSO_4$), and Pb oxide (PbO), with Pb carbonate ($PbCO_3$) and Pb silicates. Other smelters also produce Pb, mainly as Pb oxide (PbO). Pb in coal combustion emissions is mainly in the form of Pb sulfide (PbS) and Pb sulfate ($PbSO_4$) with some Pb selenide (PbSe) as well as sulfates, oxides and chlorides. In wood combustion emissions $PbCO_3$ and Pb oxides are important. Waste incineration produces mainly Pb chloride ($PbCl_2$) and PbO. Resuspended mining soils were reported to be abundant in $PbCO_3$ and $PbSO_4$, as well as a variety of complex salts containing phosphates, hydroxides, chlorides, oxides, and silicates. On-road engine exhaust from leaded gasoline use contained mostly Pb bromide and Pb chloride salts, including PbBrCl, $PbBrCl\text{-}NH_4Cl$, and $PbBrCl\text{-}2NH_4Cl$. Road dust is rich in $PbSO_4$, Pb^0, oxides, carbonates, and hydroxides. Motor vehicles also contribute Pb^0 from brake wear and wheel weights.

The 2006 Pb AQCD (U.S. EPA, 2006) described the atmosphere as the major environmental transport pathway for Pb, with Pb primarily present in submicron aerosols. Although not directly addressed in the 2006 Pb AQCD (U.S. EPA, 2006), organolead vapor emissions were extensively discussed in the 1986 Pb AQCD (U.S. EPA, 1986) which concluded that they were primarily emitted

during manufacture, transport, and handling of leaded on-road gasoline. Organolead vapors contributed less than 10% of vehicular Pb tailpipe emissions when leaded on-road gasoline was still in use. Studies of Pb emissions within enclosed microenvironments where automobiles were the dominant Pb source cited within the 1986 Pb AQCD (U.S. EPA, 1986), reported that organic Pb vapors contributed less than 20% of total vehicular Pb emissions. More recent studies support this (Shotyk et al., 2002). The 20% estimate of organic Pb vapors from the previous studies of on-road Pb emissions may potentially provide an upper bound for organic Pb vapors from current piston engine aircraft emissions.

Several recent studies have used speciation techniques either simply to determine the chemical composition of emissions or for source attribution. In urban environments, airborne Pb concentrations are likely a mix of various sources. Ogulei et al. (2006) performed source apportionment of $PM_{2.5}$ samples in Baltimore, MD and found multiple industrial, urban, and background influences. Sixty-three percent of the Pb was associated with incineration, while 20% was associated with a wildfire episode from which PM was transported from Quebec, 8% was associated with secondary nitrate, 6% was associated with operations at a steel plant, and 3% was associated with local gasoline traffic. Dillner et al. (2006) analyzed the composition of $PM_{2.5}$ and TSP samples in Beijing, China and found that Pb comprised roughly 0.2% of TSP and 1.4% of $PM_{2.5}$ during industrial pollution events, 0.1% of TSP and 0.5% of $PM_{2.5}$ during urban pollution events, and 0.05% of TSP and 0.4% of $PM_{2.5}$ during dust storms. During industrial pollution events, the authors note that the amount of Pb in $PM_{2.5}$ can be substantial.

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). Pb speciation emissions from a sintering plant, a major component of the steel making process, were reported for the first time, with cerussite, a Pb carbonate ($PbCO_3$-$2H_2O$), emerging as the most abundant species (Sammut et al., 2010). The predominance of carbonates as major Pb species in industrial emissions is unusual. Choel et al. (2006) confirmed that Pb was strongly associated with sulfur in smelter emission PM, and that Pb sulfates and Pb oxy-sulfates were the most abundant species, with important contributions from Pb oxides. Zhang et al. (2009) used single particle aerosol mass spectrometry (ATOFMS) to speciate Pb-bearing PM in Shanghai, China in 2007. PM containing Pb along with OC and/or EC was attributed to coal combustion processes; this accounted for roughly 45% of Pb-bearing PM. PM producing high correlations between Cl and Pb were ascribed to waste incineration, while Pb-bearing PM with a strong phosphate signal was attributed to the phosphate industry.

A few recent studies have used speciation techniques to characterize Pb and other components of PM_{10}, $PM_{2.5}$, and PM_1. 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. Approximately two-thirds of PM_1 consisted of secondary aerosols, e.g. mainly sulfate, nitrate and primary/secondary organics. The remaining third included PM from biomass burning, fossil fuel combustion and various industrial sources. For the latter group, strong Pb-Zn-K-Na associations were

observed. 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. Wojas and Almquist (2007) used ICPMS to characterize trace metals in $PM_{2.5}$, PM_{10}, and TSP samples obtained in Oxford, OH. They observed that Pb was highly correlated with several other elements (Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Ni, Pb, Sb, Si, and Zn), suggesting that Pb and copollutants emanated from a variety of sources including road dust and fuel combustion. Similarly, a study by Moffet et al. (2008) found that Pb-Zn-Cl 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. The unique single particle chemical associations closely matched signatures indicative of waste incineration. A study of PM_{10} and $PM_{2.5}$ collected in Shanghai, China and analyzed using extended X-ray absorption fine structure spectroscopy (EXAFS), found that the main chemical forms of Pb were $PbCl_2$ (41 ± 4%), $PbSO_4$ (37 ± 2%) and PbO (22 ± 3%) (Tan et al., 2006). There was no significant difference in the chemical forms of Pb between the PM_{10} and $PM_{2.5}$ samples. The main sources of these forms of Pb, based on Pb isotopic composition, were coal combustion, metallurgic dust and vehicle exhaust emissions (none though from leaded on-road gasoline). Approximately 83% Pb was in the <2.5 µm size range. Murphy et al. (2007) found that the volatility of Pb and its compounds such as PbO results 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 solubility (Barrett et al., 2010). Variations in the relative proportions of Pb-containing compounds may account for the difference in Pb solubility in aerosols (Fernández Espinosa & Ternero-Rodríguez, 2004; Tan et al., 2006; von Schneidemesser et al., 2010).

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 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, Al, K, Fe, Zn, Mo, Ag, Ba). 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. For urban aerosols collected in the U.S., two types of Pb-PM were found; in the main class, Pb was found together with K and usually also Zn. There were also minor amounts of Na, EC and organic carbon (OC) including amines. The second, minor class contained Pb together with Na, K, Zn, smaller amounts of Fe, EC and OC. The size distribution of the first group of Pb-PM usually peaked around 200 nm.

Sample solubility can inform speciation efforts. For example, a study involving weak acid leaching was carried out by Erel et al. (2006). The transport of anthropogenic pollution by desert dust in the eastern Mediterranean region was studied by determining major and trace element concentrations, organic pollutants, and Pb isotope ratios. PM_{10} samples were collected during 10 dust storms in 2001-2003. Most samples were polluted to some extent with pollutants released by weak acid (0.5 M HNO_3) extraction (including carbonates, oxides and surface-bound fractions). From the Pb isotope data, most of the Pb came from recently north African emissions and from past Israeli emissions (Pb now residing in Israeli soils).

3.2.3.2. Lead 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 & 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 & 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 & 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 (Hsu et al., 2006; Knowlton & Moran, 2010; Noble et al., 2008; 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 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.

3.3. Fate and Transport of Lead

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 the contribution of atmospheric Pb to total Pb exposure. Figure 3-8 describes relevant Pb transport pathways through environmental media discussed in this chapter and their relationship to key environmental exposure pathways for which some or all of the Pb is processed through

the atmosphere. This discussion includes new research on atmospheric transport of Pb, atmospheric deposition and resuspension of Pb, Pb transport in surface waters and sediments, and Pb transport in soil.

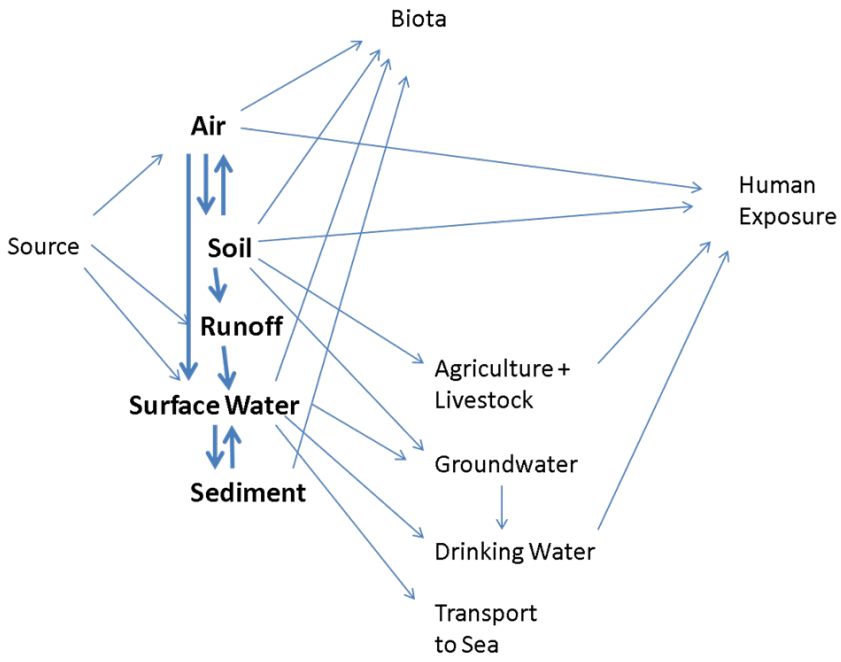


Figure 3-8. Fate of atmospheric lead. Media through which Pb is transported and deposited are shown in bold.

3.3.1. Air

The 2006 Pb AQCD (U.S. EPA, 2006) 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 has not only led to lower atmospheric concentrations in areas impacted by vehicles (Section 3.5), but a pervasive pattern of decreasing atmospheric Pb deposition and decreasing concentrations in other environmental media even at great distances from sources.

The 2006 Pb AQCD (U.S. EPA, 2006) 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 wet deposition as 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/sec and dry deposition fluxes ranging from 0.04 to 4 $mg/m^2 \cdot yr$. 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 United States ranged from 0.3 to 1.0 $mg/m^2 \cdot yr$. 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 $mg/m^2 \cdot yr$ 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, 2006) concluded that resuspension by wind and traffic contribute to airborne Pb near sources. Pb in resuspended road dust exhibited a bimodal size distribution, but mass was predominantly associated with coarse PM. The Pb fraction in resuspended dust ranged from 0.002 to 0.3%, with the highest fractions observed for paved road dust and lowest for agricultural soil.

3.3.1.1. Transport

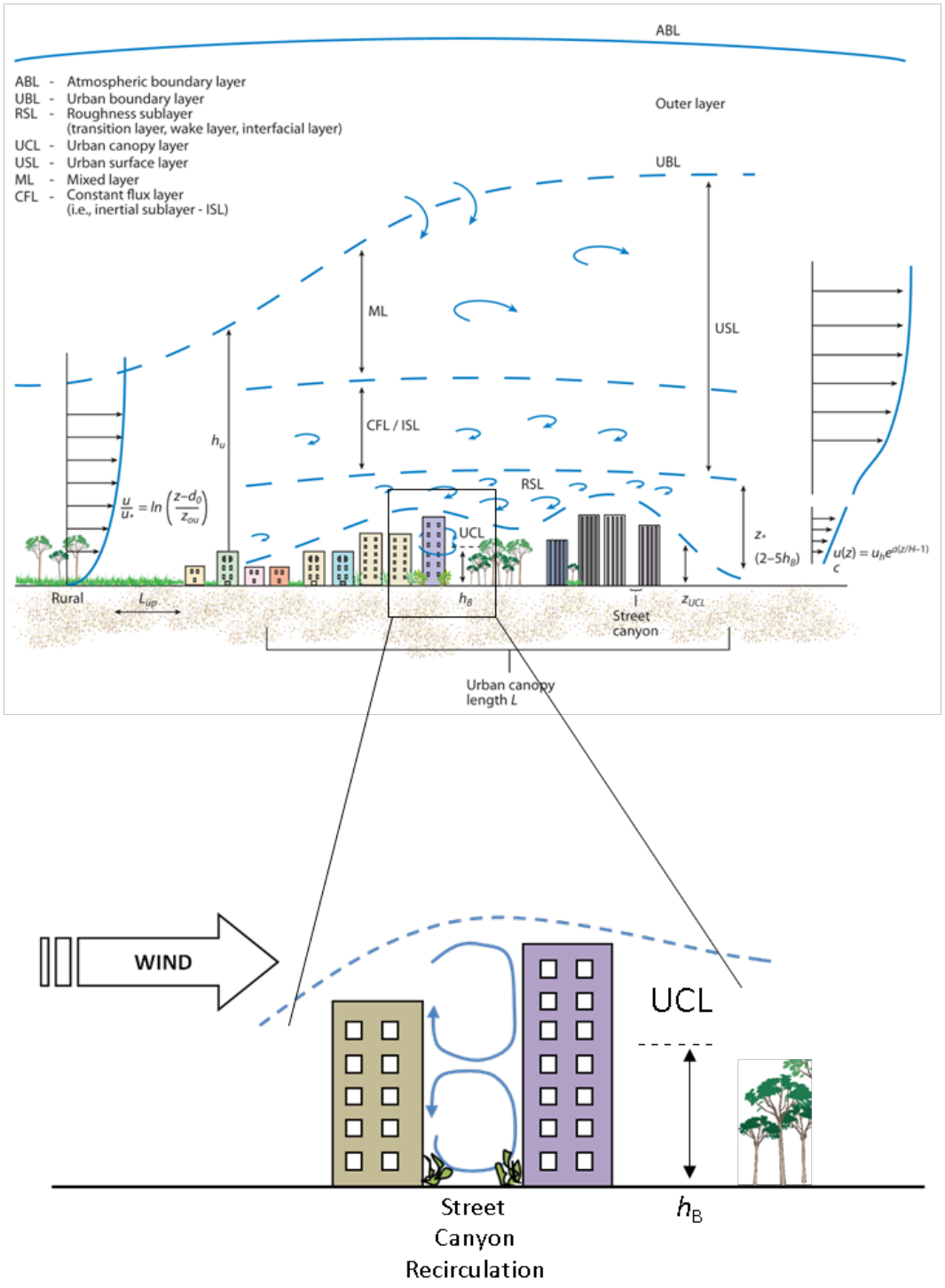
New research on long range transport as well as transport of Pb in urban areas has advanced the understanding of Pb transport in the atmosphere. While the 2006 Pb AQCD described long range Pb transport as essentially a process of submicron PM transport (U.S. EPA, 2006), much of the recent research on 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 and concluded that toxic metals were often associated with coarse PM. 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 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. PbSO4 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.

Transport and Dispersion Mechanisms in Urban Environments

Several major U.S. sources of Pb emissions are located in urban areas. The urban environment can be considered unique 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. This section focuses on special features of urban environments and upon processes that influence the distribution and redistribution of Pb-bearing PM.



Source: Used with permission from Annual Reviews, Fernando (2010)

Figure 3-9. Scales of turbulence within an urban environment.Top: multiple scales within the atmospheric boundary layer. Bottom: illustration of airflow recirculation within a single street canyon located in the urban canopy layer.

As shown in Figure 3-9, 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 (see Figure 3-9), which result in lower mean components of air movement, higher turbulence components, and higher sheer stress within the canyon compared with open field conditions (Britter & Hanna, 2003; Kastner-Klein & Rotach, 2004). 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 (Kastner-Klein et al., 2003; Kastner-Klein et al., 2001; Soulhac et al., 2009). All of these factors have the potential to influence human exposure to atmospheric Pb in urban areas with substantial Pb emissions.

3.3.1.2. Deposition

Wet Deposition

The 2006 Pb AQCD (U.S. EPA, 2006) 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. Recent research confirms the general trend described in the 2006 Pb AQCD (U.S. EPA, 2006) 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. This could also explain the greater importance of dry deposition near sources because coarse mode PM makes a greater contribution to PM mass. Although recent observations are consistent with these trends 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

New measurements of Pb dry deposition fluxes are similar to those reported in the 2006 Pb AQCD (U.S. EPA, 2006), except in industrialized urban areas, where it is considerably greater. 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 an urban industrial site than for New Brunswick. 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. Elements from anthropogenic sources, including Pb, were generally associated with fine PM. In a study of Tokyo Bay (Sakata & Asakura, 2008), reported an average dry deposition velocity of 1.06 cm/sec, which is at the upper end of dry deposition velocities reported in the 2006 AQCD (U.S. EPA, 2006). They also reported that dry deposition fluxes were greater in industrially impacted urban areas, ranging from 12-17 $\mu g/m^2 \cdot yr$, more than 10 times the upper bound of the range reported in the 2006 Pb AQCD (U.S. EPA, 2006).

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, 2006). Watmough and Dillon (2007) found that the bulk annual deposition of Pb in a central Ontario forested watershed during 2002-2003 was 0.49 $mg/m^2 \cdot yr$; this was lower than the value of 1.30-1.90 $mg/m^2 \cdot yr$ 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 took place before the start of the Watmough and Dillon (2007) study.

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, new 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.

3.3.1.3. Resuspension of Lead from Soil to Air after Lead Deposition

As described in Section 3.2, the greatest Pb emissions in the United States occur in locations near major specific point sources, including airports, 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, 2006) concluded that resuspension by wind and traffic can be a substantial source of airborne Pb above background levels near sources, with resuspended dust accounting for between 0.002 to 0.3% of PM mass. Since then, results from several studies have provided support for a substantial contribution from resuspension by indicating a smoothed soil Pb concentration profile that decreases with distance from various sources, including city centers (Laidlaw & Filippelli, 2008), major freeways (Sabin, Lim, Venezia, et al., 2006), and steel structures with abrading paint (Weiss et al., 2006). The smoothed profile is consistent with continual Pb resuspension and deposition due to atmospheric turbulence. Recent Pb speciation results also indicate a substantial 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 consistent with Pb-humate as the major form of Pb in airborne PM, suggestive of soil resuspension since the local near-surface soils had high humic content (Pingitore et al., 2009).

Recent research on urban PM transport is also 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 relationship 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 was applied to a road and then the grit’s dispersion by traffic was measured over time to simulate resuspension and transport of a trace metal such as Pb. 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.

New 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 (Lara-Cazenave et al., 1994), 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. Together these results demonstrate that under in the vicinity of previous major emission sources and the absence of current major sources, resuspension can make a substantial contribution to atmospheric Pb concentrations.

3.3.2. Water

As described in the 2006 Pb AQCD (U.S. EPA, 2006), atmospheric deposition has been identified as the largest source of Pb in surface waters, but urban runoff and industrial discharge are also important. 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 by flushing, evaporation and sedimentation. 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. As described in the 2006 Pb AQCD (U.S. EPA, 2006), metals in waterways are transported primarily as soluble chelates and ions, or adsorbed on colloidal surfaces, including secondary clay minerals, iron and manganese oxides or hydroxides, and organic matter, and adsorption on organic or inorganic colloids is particularly important for Pb. The extent of sorption is 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 environmental removal by sulfides is particularly important.

Although atmospheric deposition was identified as the largest source of Pb in surface waters in the 2006 Pb AQCD (U.S. EPA, 2006), runoff from storms was also identified as an important source. A substantial portion of Pb susceptible to runoff is originates from atmospheric deposition. The 2006 Pb AQCD (U.S. EPA, 2006) concluded that important contributors to Pb in dust on roadways included vehicle wear, vehicle emissions, road wear, fluid leakage, and atmospheric deposition. Runoff from buildings due to paint, gutters, roofing materials and other housing materials were also 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.

3.3.2.1. Lead Transport in Water and Sediment

Recent publications provide additional detail regarding Pb adsorption on iron rich and organic rich colloids. Correlation between Pb concentration in unfiltered water with total Fe was observed (Hassellov & von der Kammer, 2008), which is consistent with previous research using cross flow filtration (Pokrovsky & Schott, 2002; Ross & 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 & 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 & 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 & 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 iron 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). This was likely due to the presence of organic-bound

colloids smaller than 0.45 µm rather than true Pb dissolution (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 iron and organic rich colloids. In addition, new results indicated that although the 2006 Pb AQCD (U.S. EPA, 2006) 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.

3.3.2.2. Deposition of Lead within Bodies of Water and in Sediment

As described in the 2006 Pb AQCD (U.S. EPA, 2006), 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 & Stephens, 2008). Urbanized areas tend to have greater aquatic Pb loads, as several studies have shown the strong positive correlation between population density and river or lake sediment Pb concentrations (Chalmers et al., 2007; Horowitz et al., 2008). 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 TSP, 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. (2010) reported that PM Pb accounted for 85 ± 15% and 50 ± 22% in Boston Harbor and Massachusetts Bay, respectively, while Lai et al. (2008) 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 & Berry, 2010). Several new advances in the study of Pb cycling in these complex environments have been described in recent publications. Li et al. (2010) 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 PM-Pb 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 m/day has been used in some models of Pb transport and distribution in aquatic-sediment systems (L. Li et al., 2010). Surface sediment Pb concentrations for various continental shelves were collated and compared by Fang et al. (2009); see Table 3-2.

Table 3-2. Surface sediment Pb concentrations for various continental shelves; see Fang et al. (2009) and references therein.

Location	Digestion solution	Pb (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

3.3.2.3. Flux of Lead 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. (J. L. 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 (J. L. 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). 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 (G. Lee et al., 2008). Processes governing Pb release from lake sediments, including microbial reductive dissolution of Fe, biogenic sulfide production and metal sorption-desorption, have been investigated and results indicated that release of Pb from sub-oxic and anoxic zones of the sediment act as a Pb source to the overlying water of the lake (Sengor et al., 2007).

Sediment resuspension from marine environments is similarly important, with disturbance of bed sediments by tidal action in estuarine areas resulting in a general greater capacity for re-suspension of PM. 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 (L. Li et al., 2010). 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).

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.

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, 2006), and 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.

3.3.2.4. Lead in Runoff

Runoff is a major source of Pb in surface waters. This complicates any evaluation of the contribution of atmospheric Pb to surfaces waters, 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 deposited from the atmosphere. The 2006 Pb AQCD (U.S. EPA, 2006) identified important contributors to Pb pollution in dust associated with roadways, such as vehicle wear, vehicle emissions, road wear, fluid leakage, and atmospheric deposition That review identified contributors to runoff from buildings, such as paint, gutters, roofing materials and other housing materials. The 2006 Pb AQCD (U.S. EPA, 2006) also 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 runoff 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 roadway and urban runoff and snow melt.

Severe contamination due to export of anthropogenic Pb to adjacent ecosystems via sewage systems (urban runoff and domestic wastewater) and to a lesser extent by direct atmospheric deposition has been documented (Soto-Jiménez & 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 (Al-Malack, 2001; Lasheen et al., 2008; Weiss et al., 2006).

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 & McCready, 2009).

Several new studies reported that the size distribution of 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 & 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 whilst 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 from road surfaces using , 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 Cr and 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, 2006), 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. (J. Sansalone et al., 2010) compared Pb-containing PM size distributions from 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. (2006) 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. (2006) investigated a catchment that drained towards a wide grassy area and found that Pb was mainly associated with dissolved organic matter (DOM). The study suggested that interaction of the rainfall-runoff with the grassy area may have resulted in removal of PM-bound Pb and hence in the association of Pb with DOM. PM amount and size can also be influenced by the runoff surface. Guo et al. (2006) found that Pb entering the engineered PER system was mainly in the dissolved fraction 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, Pb was strongly correlated with TSP, no relationship between total suspended solids and rainfall, rain intensity, antecedent dry days or runoff event duration were observed, and traffic volume also did not appear to have an effect They concluded that runoff composition from site to site could be highly variable. Most other studies, however, 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 PM 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 (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 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 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 (U.S. EPA, 2006). The relocation of snow means that the area receiving the snowmelt is not necessarily the same area that which 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 (J. J. Sansalone & 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 & 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 coarser PM (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 & 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, 2006), including several recent publications on a forested watershed (Lake Plastic) in central Ontario (Landre et al., 2009, 2010; Watmough & Dillon, 2007) and nearby Kawagama Lake, Canada (Shotyk & 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 it 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 rapid 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 that had been transported from throughout the soil profile and 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, particularly since higher concentrations of previously deposited anthropogenic Pb are usually found in the near-surface sections of upland U.K. soils (e.g., (Farmer et al., 2005)).

In another study Rothwell et al. (2007) observed stormflow Pb concentrations almost three times higher than those reported by Graham et al. (2006) for northeastern Scotland. The generally high dissolved Pb stores and high stream water DOC concentrations (Rothwell, Evans, Daniels, et al., 2007). In a separate study, Rothwell et al. (2007) 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.

Several investigators considered a Pb isotope study by Dunlap et al. (2008) of a large (>160,000 km^2) riparian system (the Sacramento River, CA), which showed that the present day flux of Pb 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% Pb was derived from past on-road gasoline emissions and 33-43% was from hydraulic Au mining sediment. The flow into the Sacramento River from these sources is an ongoing process. Periods of high surface runoff, however, mobilize additional fluxes of Pb from these two sources and carry them into the river. These pulses of Pb, driven by rainfall events, suggest a direct link between local climate change and transport of toxic metals in surface waters (Dunlap et al., 2008).

Rothwell et al. (2007) 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 likely to continue due to nitrogen leaching from the surrounding catchment and this may increase with nitrogen saturation (Curtis et al., 2005). Rothwell et al. (2007) 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 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 PM size and composition, indicating that size distributions for Pb-containing PM in runoff water varies from study to study and from location to location, and that Pb is frequently associated with chromate near roads, probably from paint used to mark road lines. 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 or when grass is present, and 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, and the understanding of Pb runoff has become more complete since publication of the 2006 Pb AQCD (U.S. EPA, 2006).

3.3.3. Soil

The 2006 Pb AQCD (U.S. EPA, 2006) 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 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 was associated with Mn and Fe oxides or carbonate.

3.3.3.1. Deposition of Lead onto Soil from Air

As described in the 2006 Pb AQCD (U.S. EPA, 2006), a considerable amount of Pb has been deposited from air onto soils in urban areas and near stationary sources and mines, and soil Pb concentrations can reach several thousand mg/kg. Major sources in urban soil were identified as automotive traffic (prior to when leaded on-road gasoline was phased out), and deteriorating Pb-based paint, with the highest Pb concentrations observed where traffic and population density were the greatest. Highest concentrations were found in city centers and near roadways, and several studies reported concentrations falling off rapidly with distance and depth of soil layers near roads. High Pb soil concentrations were also observed near stationary sources such as smelters and battery disposal operations, also decreasing 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 urban soils due to ongoing emissions from nearby mining and smelting activities are continuing to impact on the urban environment (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 $mg/m^2 \cdot yr$ to about 3 $mg/m^2 \cdot yr$ with fair agreement between locations in Canada, Scandinavia, and Scotland and showed a substantial decrease compared to when leaded on-road gasoline

was in widespread use (Fowler et al., 2006; Graham et al., 2006; Shotbolt et al., 2008; Watmough & Dillon, 2007).

Differences between throughfall and litterfall in forested areas have also 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).

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. 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 3.3.3.2. Higher Pb concentrations at greater altitudes were also found in Japan, especially above 600 m (Takamatsu et al., 2010).

There is wide agreement that atmospheric deposition due to long-range transport from industrial areas has been the major source of Pb to remote surface soils over the past decades, e.g. (Steinnes et al., 2005). However, another hypothesis proposes that the gradual increase in Pb content in O-horizon soil with changing latitude is attributable to "plant pumping and organic binding" rather than to atmospheric deposition (Rasmussen, 1997; Reimann et al., 2008; Reimann et al., 2001).

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, and that productive plants were able to accumulate more particles from the air and that rates of net Pb accumulation by the mosses were in excellent agreement with the 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 was consistent with deposition fluxes reported in the 2006 Pb AQCD (U.S. EPA, 2006), and demonstrated consistently that Pb deposition to soils has decreased since the phase-out of leaded on-road gasoline. Additional research highlighted the importance of taking forest cycling and litter throughput account in estimating input by deposition. Follow-up studies in several locations indicated little change in soil Pb concentrations since the phase-out of leaded on-road gasoline, consistent with the high retention reported for Pb in soils. Finally, although there has been considerable discussion of plant pumping as an alternative hypothesis for explaining the increases in soil

concentrations, much evidence is more consistent with atmospheric deposition as the explanation for observed increases in soil Pb concentration.

3.3.3.2. Sequestration of Lead 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, 2006). 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).

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 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 high quality 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 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 O soil horizons in 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 roadsides 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 3-10 in Section 3.3.3.3).

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 and concluded that Pb adsorption to the bacterial cell walls may be important with respect to Pb transport in soils. This new area of research provides important evidence 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 & Giammar, 2007). Overall, in agreement with the 2006 Pb AQCD (U.S. EPA, 2006), 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.

3.3.3.3. Movement of Lead 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, 2006). 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 & 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 S or 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. (2006) 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. (2006) 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. (2008) considered the biogeochemical behavior of atmospherically derived Pb in boreal forest soils in Sweden (Figure 3-10). The estimated annual losses via percolating soil water were ~2 mg/m^2·yr (Klaminder, Bindler, & Renberg, 2008) and so the annual loss, assumed to be from the mor layer, was greater than the atmospheric input of ~0.5 mg/m^2·yr. 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, Bindler, Emteryd, et al., 2006). These values were greater than the values of 2-150 years determined for U.S. forest soils, e.g. (J. Kaste et al., 2003; Watmough et al., 2004) 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.

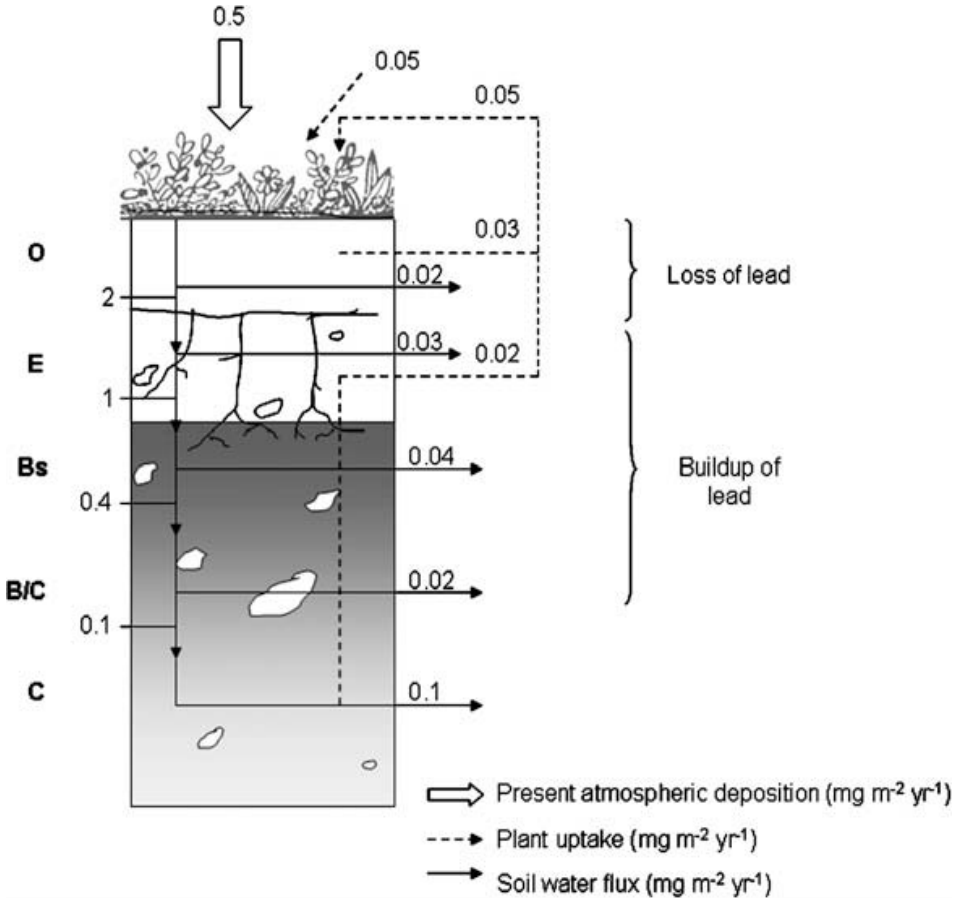


Figure 3-10. Schematic model summarizing the estimated flux of Pb within a typical podzol profile from northern Sweden using data from Klaminder et al. (2006).The atmospheric deposition rate is from (Klaminder, Bindler, Emteryd, et al., 2006), the plant uptake rates from (Klaminder et al., 2005) and estimated soil-water fluxes from (Klaminder, Bindler, Laudon, et al., 2006).

Klaminder et al. (2008) investigated in more detail the distribution and isotopic signature of Pb persisted 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 concentrations changes within the mor layer.

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/yr and 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 on non-forest soils and enhanced mobility on Fe and OM colloids. Pb was strongly retained on acidic Mediterranean soil columns, with association of Pb with the exchangeable, OM and crystalline Fe oxide fractions appearing to favor mobility while association with Mn oxides and amorphous Fe oxides was linked with semi-irreversible retention of Pb in the solid phase (Garrido et al., 2008). 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.

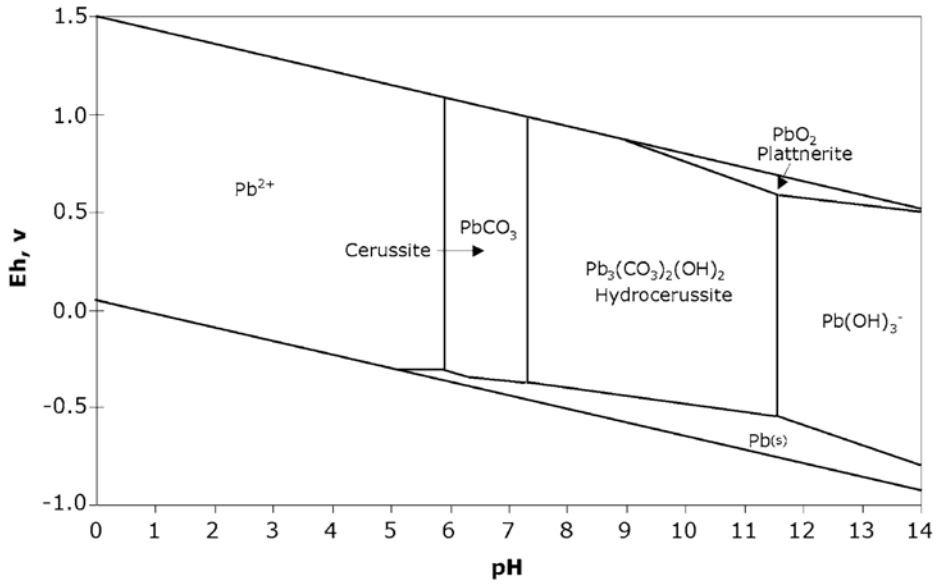
Soil pH value is probably the single most important factor affecting solubility, mobility and phytoavailability but reducing conditions also results in increased Pb mobility, with the release of Pb into an anoxic soil solution due to the combined effect of Fe(III) reductive dissolution and dissolved 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 & 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 (S. Roulier et al., 2008).

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-horizonof 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 were more Pb was mobile in weathered topsoil than in the unweathered subsoil. 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 (Schuwirth et al., 2007). 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 3-11, 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 & Rimstidt, 2009).



Source: Used with permission from Elsevier Publishing, Scheetz and Rimstidt (2009)

Figure 3-11. 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; J. M. Kaste et al., 2006). As was concluded in the 2006 Pb AQCD (U.S. EPA, 2006), 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.

3.4. Monitoring of Ambient Lead

3.4.1. Ambient Measurement Techniques

3.4.1.1. Size-Selective PM Monitoring for Lead Concentrations

Ambient Pb is present in the atmosphere as PM and distributed over a wide range of PM sizes. In recognition of the role of all PM sizes in ambient air Pb exposures, including the ingestion of PM deposited onto surfaces, the indicator for the Pb NAAQS is Pb in TSP. As described in Chapter 4, ingestion of deposited Pb can be a substantial contributor to total Pb exposure. Additionally, a substantial fraction of atmospheric Pb may be associated with PM larger than 10 µm (ultracoarse PM). However, the variability of capture efficiency for TSP using current TSP samplers is considerably greater than the capture efficiency for PM_{10} using current PM_{10} samplers. For example, the symmetrical design of Federal Reference Method (FRM) samplers for PM_{10} makes their collection efficiency independent of wind direction, and collection efficiency is independent of wind speed under typical sampling conditions. While the collection efficiency of TSP samplers is nearly 100% for fine PM up to 5µm diameter, there is much greater variability associated with collection of larger PM (Wedding et al., 1977). For example, using the FRM for TSP, 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 (Rodes & Evans, 1985). Effective D_{50} (size at 50% efficiency) was observed to decrease from 50 µm at a 2 km/h wind speed to 22 µm at 24 km/h (Wedding et al., 1977).

Recognizing the variability in capture efficiency associated with TSP samplers and the potential benefit of an indicator with lower measurement variability, the last NAAQS review considered whether the indicator for the Pb NAAQS should be revised from one based on Pb-TSP to one based on Pb-PM_{10}. The final decision in the review was to retain the Pb-TSP indicator. The rationale for this decision included recognition of exposure due to Pb-TSP that would not be captured by PM_{10} sampling, the paucity of information documenting the relationship between Pb-PM_{10} and Pb-TSP at the broad range of Pb sources in the U.S., and uncertainty regarding the effectiveness of a Pb-PM_{10}-based NAAQS in controlling ultracoarse Pb-PM near sources where Pb concentrations are highest (73 FR 66991). Changes

were made to the monitoring and data handling provisions, however, to allow for the siting of Pb-PM_{10} monitors for compliance purposes in locations remote from sources, where the evidence indicates that airborne Pb is predominantly in the PM_{10} size fraction (73 FR 66964). For Pb-PM_{10} monitoring to be allowed under these regulations, the maximum three-month average Pb concentration at a site must not exceed 0.10 $\mu g/m^3$ over a three-year time period. Additionally, if a Pb-PM_{10} monitor is sited near a source, the majority of the particle-bound Pb must be smaller than 10 μm (40 CFR Part 58).

3.4.1.2. Federal Reference Method and Federal Equivalence Method Evaluation

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 federal equivalence methods (FEM). Measurements for determinations of NAAQS compliance must be made with FRMs or FEMs. As of August 2010, 1 manual reference method and 24 manual equivalent methods had been approved for Pb (http://www.epa.gov/ttn/amtic/criteria.html). The FRM for Pb was promulgated in 1979 and is based on flame atomic absorption spectroscopy (AAS) (40 CFR Part 50). The FRM provides for collection of PM by high volume sampling and analysis of the PM for Pb by atomic absorption spectrometry. Ambient air suspended in PM is collected on a glass fiber filter for 24 hours using a high volume air sampler. The analysis of the 24-hour samples may be performed for either individual samples or composites of the samples collected over a calendar month or quarter. 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. Inductively-coupled plasma mass spectrometry (ICPMS) is under consideration as the new FRM for Pb-TSP.

PM_{10} monitoring can be used in limited circumstances to measure Pb concentration. (40 CFR 58). The proposed method is based on sampling requirements for an existing Federal Reference Method for the Determination of Coarse PM as $PM_{10} - PM_{2.5}$ ("Reference Method for the Determination of Coarse Particulate Matter as PM10-2.5 in the Atmosphere," 2010), which requires a specially approved PM_{10C} sampler that meets more demanding performance requirements than conventional PM_{10} samplers. Ambient air is drawn through an inertial particle size separator for collection on a polytetrafluoroethylene (PTFE) filter. The analysis method for the FRM is based on x-ray fluorescence spectrometry. In addition, several FEM have been approved based on a variety of principles of operation have been approved, including: inductively coupled plasma optical emission spectrometry, or ICPMS. Specifications for Pb monitoring are designed to help states demonstrate whether they have met compliance criteria. Operational parameters required under Appendix G of 40 CFR Part 50 are listed in Table 3-3.

Table 3-3. Specifications for Pb monitoring

Parameter	Specification[a]
Range	0.7-7.5 µg Pb/m^3
Sensitivity	0.2-0.5 µg Pb/mL for 1% change in absorbance
Lower Detectable Limit	0.07 µg Pb/m^3

[a]Assumes sample volume of 2,400 m^3.

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.

3.4.1.3. Chemical Speciation Network, IMPROVE, and National Air Toxics Trends Network Monitors

In addition to being monitored for regulatory purposes in the SLAMS network, Pb is also monitored in three other sampling networks. Pb is monitored at 53 monitoring sites as a part of the Chemical Speciation Network. Participating monitoring agencies responsible for site operation are given flexibility in sampler design, with filter collection media best-suited for the analysis of specific components (U.S. EPA, 1999a). Several samplers are approved for CSN monitoring, all of which collect bulk PM species with multiple channels containing different types of filters appropriate for speciation sampling. Pb is one of 33 elements in $PM_{2.5}$ collected on Teflon filters every third day and analyzed by energy dispersive X-ray fluorescence spectrometry.

Pb is also monitored at 110 aerosol visibility-monitoring sites as a part of the Interagency Monitoring of Protected Visual Environments (IMPROVE) program. An additional 59 aerosol samplers that are not directly operated through the program are operated following IMPROVE protocols.

IMPROVE is a cooperative effort by Federal and state organizations to protect visibility in 156 national parks and wilderness areas as described in the 1977 amendments to the Clean Air Act. Objectives are: (1) to establish current visibility and aerosol conditions in high priority (class I) areas for visibility protection; (2) to identify chemical species and emission sources responsible for existing man made visibility impairment (3) to document long-term trends for assessing progress towards visibility goals; and (4) to provide regional haze monitoring representing protected federal areas in accordance with the regional haze rule. The IMPROVE sampler operates with four sampling modules, three for $PM_{2.5}$ and one for PM_{10}. Pb is not measured in PM_{10}, but one of the three $PM_{2.5}$ modules contains a Teflon filter used for determination of gravimetric mass, absorbance, and elemental analysis by Particle Induced X-Ray Emission (PIXE) and XRF. A total of 9 elements are determined by XRF, including Pb (University of California Davis, 1995).

Pb in PM_{10} is also monitored in the National Air Toxics Trends Station (NATTS) network (ERG, 2009). PM 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, 1999b). Pb is one of seven core metals collected on Teflon filters and analyzed by ICPMS. The NATTS network was developed to fulfill the need for consistent data quality of long-term monitoring data on hazardous air pollutants of consistent data quality, for use in assessing trends and emission reduction program effectiveness, assessing and verifying air quality models, exposure assessments, emission control strategies, and as direct input for receptor modeling. As of December 2009, the network consisted of 27 monitoring stations, including 20 urban and 7 rural stations operating on a one in six day sampling frequency. Typically more than 100 pollutants are measured at each site, and monitoring is required for nineteen species, including Pb. Pb monitoring is also required for $PM_{2.5}$ samples at NCore monitoring sites beginning no later than January 1, 2011, and monitoring of Pb in coarse PM (PM_{10}-$PM_{2.5}$) is also likely to be included (U.S. EPA, 2006).

3.4.1.4. Other Measurement Methods for Total Lead

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. It is the method used for Pb analysis in the IMPROVE network. 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 (Ariola et al., 2006; Chan et al., 2008; Cong et al., 2007; Johnson et al., 2006; Johnson et al., 2008; Sanchez-Ccoyllo et al., 2009; Wåhlin et al., 2006) (Cohen et al., 2010; Waheed et al., 2010).

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 & 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 in 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 Lead Methods

Anodic stripping voltammetry, atomic emission spectroscopy, and colorimetry have also been used for measurement of atmospheric Pb (Finlayson-Pitts & 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 amalgam on a mercury 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 atomic absorption spectroscopy 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.

3.4.1.5. 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 and manganese oxides, bound to OM, and residual. Extraction was carried out with successively stronger solutions, starting with magnesium chloride 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 & 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 (Canepari et al., 2006; Falta et al., 2008; Voutsa & 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, 2009), 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 & 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 (Birmili et al., 2006; Dos Santos et al., 2009), 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; Poykio et al., 2007; Sillanpaa et al., 2005; Smichowski et al., 2008) and locations (Al-Masri et al., 2006; Annibaldi et al., 2007; Canepari et al., 2006; Cizmecioglu & Muezzinoglu, 2008; Dahl et al., 2008; Dos Santos et al., 2009; Fujiwara et al., 2006; Gutierrez-Castillo et al., 2005; Heal et al., 2005; Perrino et al., 2010; Richter et al., 2007; Sato et al., 2008; W. Wang et al., 2006; Yadav & Rajamani, 2006), leading to a better understanding of mobility characteristics of Pb in airborne PM.

3.4.1.6. Speciation Techniques

XAFS

There have been few attempts to speciate Pb in atmospheric PM into individual species. 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 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 quantify Pb complexed with humic substances from soil in road dust (Pingitore et al., 2009) and to investigate the speciation of atmospheric Pb in soil after deposition (X. Y. Guo et al., 2006). 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- 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 carried out by coupling a chromatographic separation step with a mass spectrometry-based multi-element detection systems 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 and high performance liquid chromatography. Detection systems most commonly used are 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 & 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 (Jitaru et al., 2004; Leal-Granadillo et al., 2000; Poperechna & Heumann, 2005).

3.4.1.7. Continuous Lead 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 (T. A. Cahill et al., 1987; Raabe et al., 1988) 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 (Bench et al., 2002; C. F. Cahill, 2003). The University of Maryland Semi-continuous Elements in Aerosol Sampler (Kidwell & Ondov, 2001, 2004) uses direct steam injection into promote condensational growth of sampled 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). Other high time resolution methods suitable for Pb analysis in PM are under development, including near real-time XRF analysis.

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). This technique can also be considered as an effective method for real time Pb measurement in PM (Silva & 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 (Bein et al., 2007; Johnson et al., 2008; Pekney et al., 2006; Reinard et al., 2007; Snyder et al., 2009) or specifically targeted (Moffet, de Foy, et al., 2008; Murphy et al., 2007; Salcedo et al., 2010) Pb measurements.

3.4.2. Ambient Network Design

Ambient air Pb concentration is detected by FRM monitors at state and local monitoring stations (SLAMS) reporting data used for NAAQS compliance to the Air Quality System (AQS). Monitoring requirements for Pb have evolved over the past ten years. Monitoring for ambient Pb levels has been required for all major urban areas where ambient air Pb measurements have been elevated near or beyond the level of the NAAQS. Alternately, state and local agencies have located Pb monitoring stations in proximity to Pb point source emissions. Prior to 2006, monitoring sites were established where sources

emitted 5 or more tons/yr or where smaller stationary sources were located in proximity to populated neighborhoods.

Pb monitoring requirements have experienced several changes since publication of the last Pb AQCD (U.S. EPA, 2006). In 2008 revisions for the Pb NAAQS were announced, and new monitoring requirements to support NAAQS revision called for expanded monitoring at sources that emit Pb at a rate of 1.0 or more tons/yr and non-source oriented monitoring at each Core Based Statistical Area (CBSA) with a population of 500,000 or more ("National Ambient Air Quality Standards for Lead (final rule)," 2008). This corresponded to approximately 100 non-source oriented monitors. Some of the new monitors were required to become operational by January 1, 2010, with the remainder operational by January 1, 2011. Subsequent revisions to these requirements have been promulgated, including reduction of the source oriented monitoring threshold from 1.0 tons/yr to 0.5 tons/yr and replacing the requirement for population-based CBSA monitoring with a requirement for non-source oriented Pb monitoring at National Core multipollutant monitoring network (NCore) sites in CBSA's with a population of 500,000 or more (75 FR 81126). 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, DC, will have at least one urban station.

Data used in this chapter are from the period 2007-2009. The number of source oriented and non-source oriented monitors changed each analysis year because the monitoring requirements changed over this time. Monitors were designated to be source-oriented if they were designated in AQS as source oriented, or they were located within one mile of a 0.5 ton/yr or greater source, as noted in the 2005 NEI (U.S. EPA, 2008a). Non-source oriented monitors were those monitors not considered to be source oriented. This loosening of the restrictions was intended to accommodate the 2007-2008 data that were obtained before the latest monitor designation requirements were implemented.

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. While monitoring in multiple networks improves geographic coverage, measurements between networks are not directly comparable in all cases because different PM size ranges are sampled in different networks. Depending on the monitoring network, Pb is monitored either in TSP, PM_{10}, or $PM_{2.5}$.

3.4.2.1. Monitor Siting Requirements

Spatial scales defined for Pb monitoring range from microscale to regional scale (40 CFR Part 58):

- Microscale: Defines the concentrations in air volumes associated with area dimensions ranging from several meters up to about 100 m. This scale applies to areas in close proximity to Pb point sources.
- Middle Scale: Defines the concentration typical of areas up to several city blocks in size with dimensions ranging from about 100 m to 0.5 km. This scale generally represents Pb air quality levels in areas up to several city blocks.
- Neighborhood Scale: Defines concentrations within some extended area of the city that has relatively uniform land use 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.
- Urban Scale: Defines concentrations within area of city-like dimensions on the order of 4 to 50 km. Such stations would be used to present ambient Pb concentrations over an entire metropolitan area with dimensions in the 4 to 50 km range. An urban scale station would be useful for assessing trends in citywide air quality and the effectiveness of larger scale air pollution control strategies.
- Regional Scale: Defines usually an area of reasonably homogeneous geography without larges sources, and extends from tens to hundreds of kilometers. This large scale of representativeness has not been used widely for Pb monitoring, but provides important information on background air quality and inter-regional pollutant transport.

Since the majority of Pb emissions mass comes from point sources, such as metals processing facilities, waste disposal and recycling, and fuel combustion, the SLAMS network is primarily used to assess the air quality impacts of Pb point sources. A second purpose of the SLAMS network is to determine the broad population exposure from any Pb source. The most important spatial scales to characterize the emissions from point sources are the micro, middle, and neighborhood scales.

Background information such as point source emissions inventories, climatological summaries, and local geographical characteristics are used to identify areas where monitoring is necessary. After siting each Pb station, specific siting criteria must be fulfilled for Pb monitoring in the SLAMS network. Micro and middle scale monitors must be 2-7 m above ground. All other scale monitors must be 2-15 m above ground. Monitors must be more than 2 m from supporting structures. Monitors must be more than 10 m from trees. Microscale monitors designed for monitoring traffic related Pb must be 2-10 m from

roadways. Distance from roadways for other scales depends on the purpose and scale of the monitor and the level of traffic on the roadway.

3.4.2.2. Spatial and Temporal Coverage

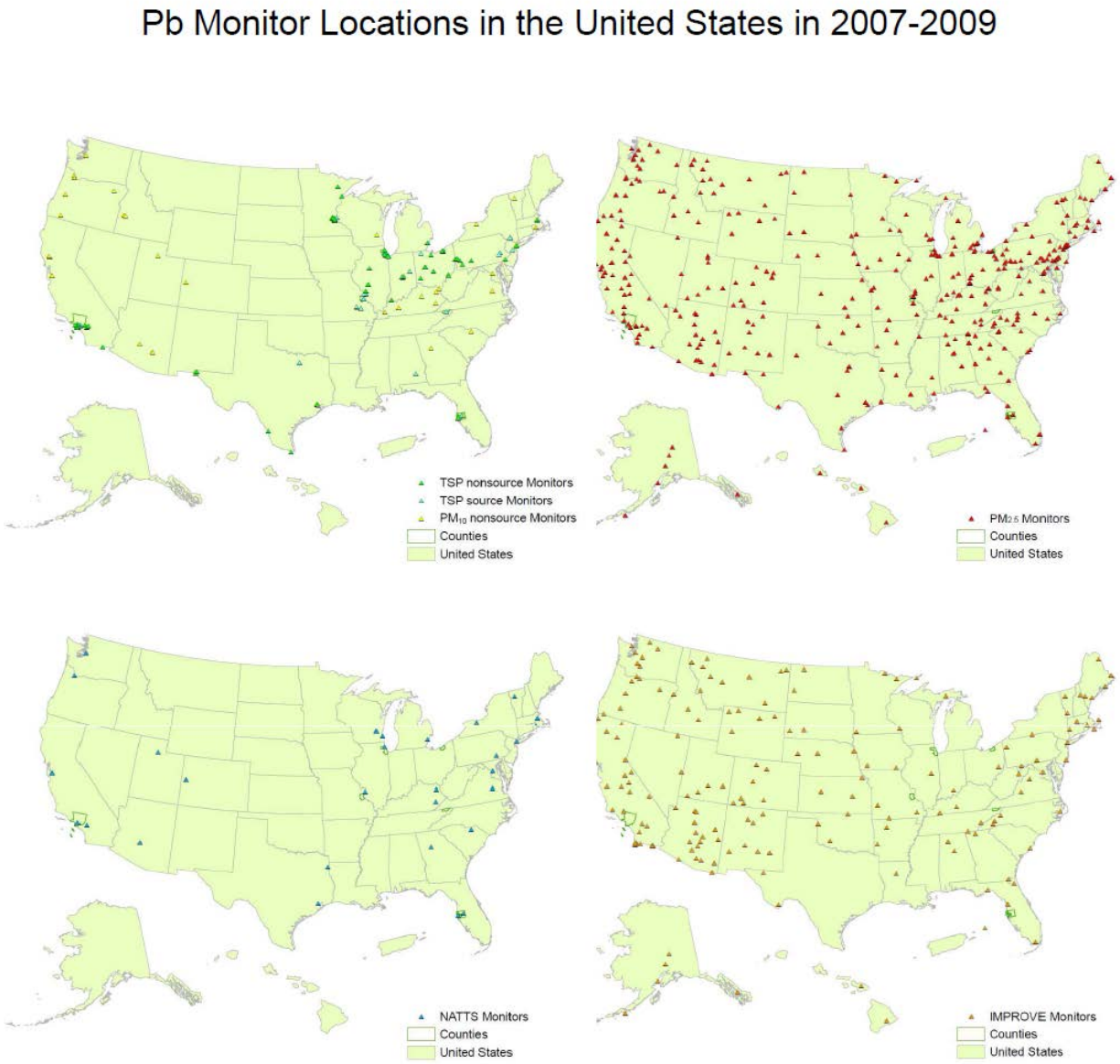


Figure 3-12. Pb monitoring sites for SLAMS, CSN, NATTS, and IMPROVE networks, 2007-2009.

Figure 3-12 shows Pb monitoring sites for all networks. The top left map shows the SLAMS monitors reporting to the AQS system from 2007 to 2009. Monitors are indicated as Pb-TSP source

oriented, Pb-TSP non-source oriented, and PM_{10} non-source oriented. Source and non-source designations used in the data analysis in Section 3.5 are indicated on this map. The top right map shows $PM_{2.5}$ monitors from the CSN. The bottom left map shows National Air Toxics Trend Networks sites, and the bottom right map shows IMPROVE monitoring sites. There is a high density of FRM monitors in some cities containing Pb sources, including Los Angeles, St. Louis, Pittsburgh, and Minneapolis. As a result, population coverage varies across cities, with those cities with major Pb sources having greater coverage. Coverage for $PM_{2.5}$ in the CSN and IMPROVE network is geographically comprehensive. In comparison, the FRM sites are more representative of source effects. NATTS sites have less extensive national coverage.

3.5. Ambient Air Lead Concentrations

The following section summarizes data on ambient air Pb concentrations during the years 2007-2009. The section begins with a description of Pb concentrations observed in TSP, PM_{10}, and $PM_{2.5}$ at source oriented and non-source oriented monitors across the U.S. Next, seasonal patterns and multi-year trends of Pb concentration are presented for the U.S. It is notable that Pb concentrations have declined substantially over the past 40 years; this is described further in Section 3.5.2. An examination of AQS data and the peer-reviewed literature is provided to evaluate the size distribution of Pb-bearing airborne PM under varied ambient conditions. Finally, the relationship between Pb concentration and concentrations of copollutants are presented. Summary information is presented within this section, and detailed data are included in an Appendix to this chapter.

3.5.1. Spatial Distribution of Air Lead

3.5.1.1. Variability across the U.S.

This section presents nationwide Pb concentration data measured using source oriented and non-source oriented TSP FRM monitors, PM_{10} monitors, and $PM_{2.5}$ monitors from the CSN for 2007-2009. This information is useful to develop a sense of variability in Pb concentrations at a national scale. For this analysis, source oriented monitors encompassed all those listed as “source oriented” in the AQS, based on state agency reporting, plus those within one mile of a facility emitting 0.5 ton/yr or more. Non-source oriented monitors were those monitors in the system not designated to be source oriented (Figures 3-13 though 3-16), the majority of U.S. counties do not have a Pb monitor.

Concentrations of Pb Measured using TSP Monitors

Source oriented maximum 3-month average Pb data were obtained for 22 counties across the U.S. during the period 2007-2009. Figure 3-13 illustrates that the level of the NAAQS was exceeded in fourteen counties where source oriented monitoring was performed. Summary data are presented below in Table 3-4, and detailed data for the one-month and three-month average and maxima source oriented Pb-TSP concentrations are provided in Tables 3A-1 through 3A-4 in the Appendix. The mean was skewed towards the 75th percentile of the distribution for both the monthly and three-month data sets. The primary difference between the one-month average and three-month rolling average data sets occurs at the upper tails of the distribution. Data for sites at which national maxima were reached for 2007-2009 are presented in Table 3-5. The highest monthly and three-month average concentrations occurred at the same sites: Herculaneum, MO followed by Los Angeles, CA. The highest annual site max 1-month value occurred in Cook County, IL in 2008, followed by Iron County, MO in 2008 and Hillsborough County, FL in 2007. The Herculaneum and Los Angeles sites were also above the 90th percentile annual 3-month site max Pb concentrations. The highest annual site max 3-month concentrations occurred in Herculaneum in 2008, Los Angeles in 2008, and Iron County, MO in 2008. The majority of data were below the level of the NAAQS over the 3-year period, but high values at a subset of source oriented monitoring locations tended to skew the nationwide distribution of Pb concentration data upwards.

Table 3-4. Summary data for source oriented Pb monitors across the U.S.

	Mean, $\mu g/m^3$	Median, $\mu g/m^3$	95th%, $\mu g/m^3$	99th%, $\mu g/m^3$	Max, $\mu g/m^3$
Monthly	0.24	0.070	0.98	2.1	4.4
3-mo rolling avg	0.24	0.080	0.98	1.9	2.9

Table 3-5. Summary data for sites at which source oriented statistics are at a maxima

County	AQS	Highest Monthly Mean, $\mu g/m^3$	Highest 3-mo Mean, $\mu g/m^3$	Highest Monthly Annual Site Max, $\mu g/m^3$ (Year)	Highest 3-mo Annual Site Max, $\mu g/m^3$ (Year)
Jefferson, MO	290990015	1.3	1.3		2.9 (2008)
Jefferson, MO	290990004	1.1	1.1		
Los Angeles, CA	060371405	0.86	0.93		2.5 (2008)
Chicago, IL	180350009			4.4 (2008)	
Iron, MO	290930016			4.2 (2008)	2.5 (2008)
Hillsborough, FL	120571066			3.6 (2007)	

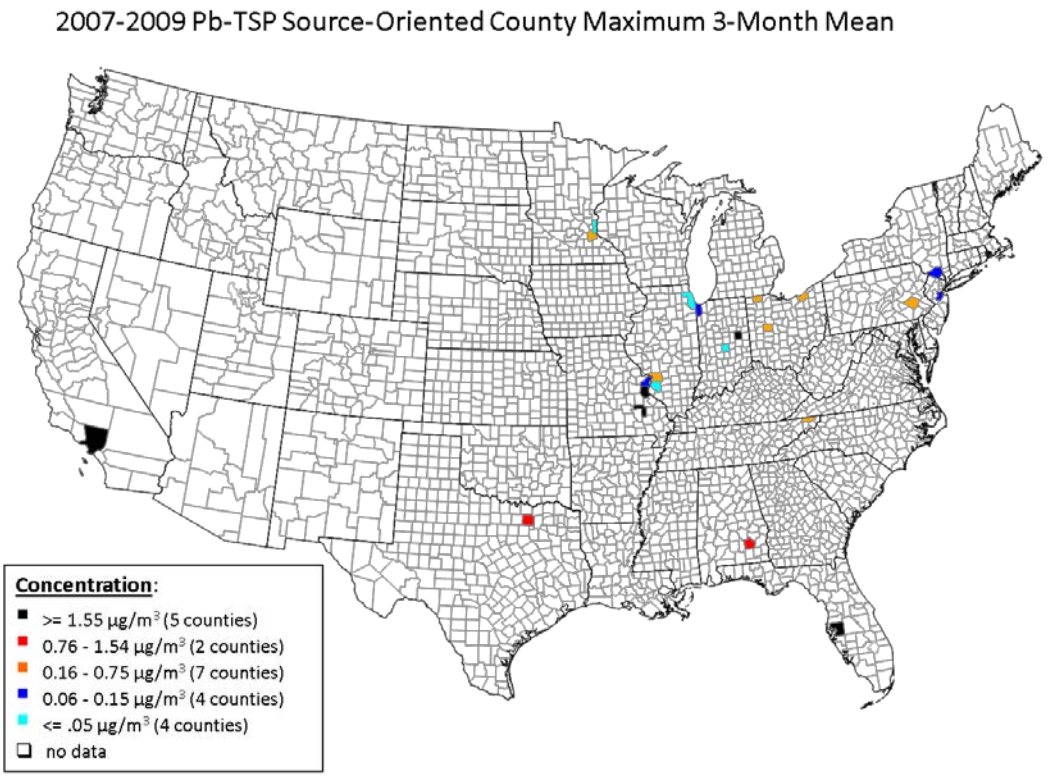


Figure 3-13. Highest county-level source oriented Pb-TSP concentrations (µg/m^3), maximum 3-month average, 2007-2009.

Non-source oriented maximum 3-month average Pb concentration data were obtained with TSP monitors for 36 counties across the U.S. during the period 2007-2009; non-source PM_{10} monitoring data are not included here because they were obtained at select sites in 2009 only. The median for monthly and 3-month rolling average data was 0.010 µg/m^3. Detailed data for site-specific Pb-TSP concentrations are provided in Tables 3A-5 and 3A-6 in the Appendix to this chapter. Nationwide, the mean monthly average non-source oriented Pb-TSP concentration for 2007-2009 was more than an order of magnitude lower than the source-oriented data. Collectively, these data indicate that non-source oriented monitors tend to measure concentrations an order of magnitude lower than the level of the NAAQS.

Concentrations of Pb Measured using PM_{10} Monitors

Figure 3-14 displays maximum 3-month average county-level data for Pb in PM_{10} concentrations for 36 counties in which measurements were obtained. The data presented here are not compared to the level of the NAAQS because PM_{10} monitors were not incorporated into the non-source oriented monitoring network until 2009. 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.

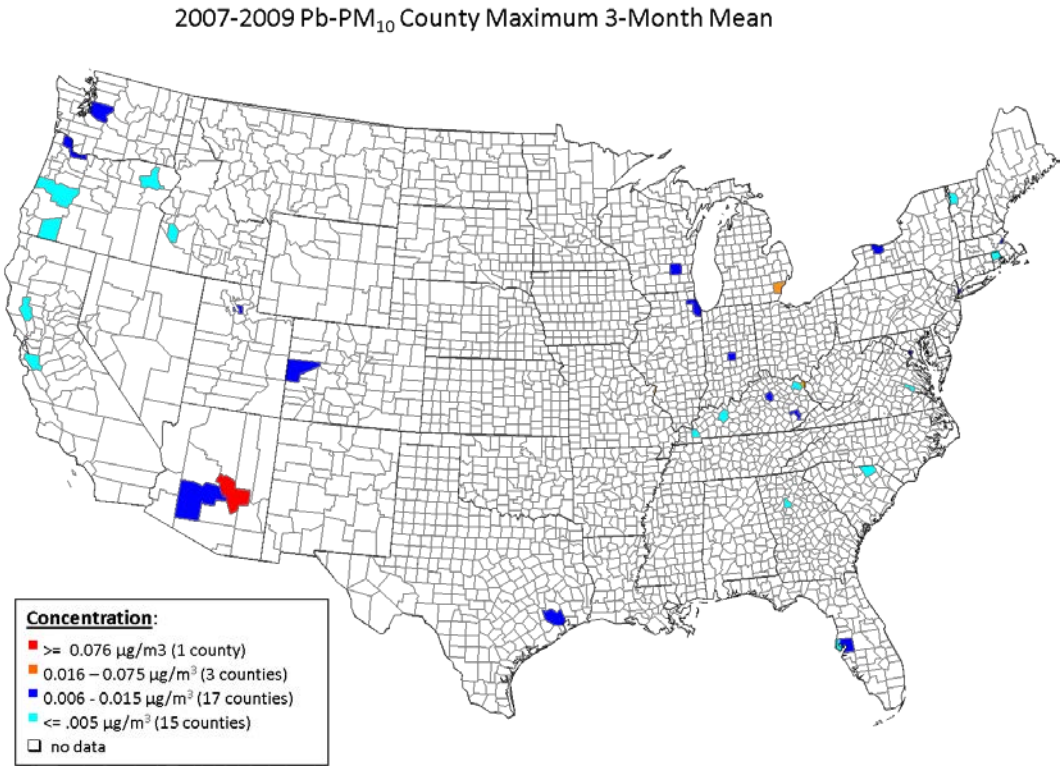


Figure 3-14. Highest county-level Pb-PM$_{10}$ concentrations (µg/m^3), maximum 3-month average, 2007-2009.

Figure 3-15 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 level of the NAAQS because $PM_{2.5}$ monitors are not deployed for that purpose. 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.

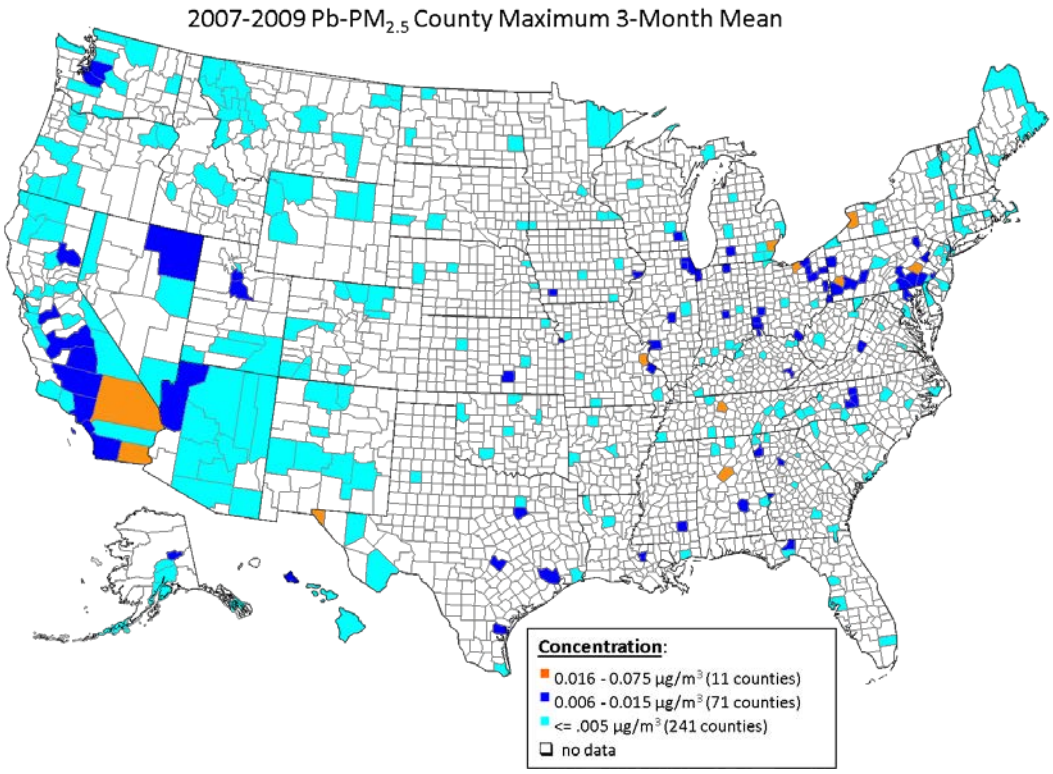


Figure 3-15. Highest county-level Pb-$PM_{2.5}$ concentrations (µg/m^3), maximum 3-month average, 2007-2009.

3.5.1.2. 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, spatial variability of Pb concentrations in urban areas may also be high. Such variability may not be detected if one or a small number of central site monitors is in use, so cities with multiple monitors can be used to characterize intra-urban variability. Intra-urban variability in Pb concentrations reported to AQS at the individual county level was described in detail in the Appendix in Section 3A.2. County-level data were used because PM-bound airborne Pb tends to settle over short distances to produce large spatiotemporal variability, as described in Section 3.3.1 and revisited in Section 3.5.3. 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 combinations of source and urban contributions of Pb. Maps and wind roses are presented in the 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.

When collectively reviewing the data from the six counties, it became apparent that spatial and temporal variability of Pb concentrations were commonly high. 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, the 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. As described in Section 3.3, 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 for the monitoring data analyzed in the Appendix. Additionally, resuspension of wheel weights and soils, emission of trace Pb during on-road gasoline combustion, and urban background levels of Pb are uncertain influential factors in ambient Pb concentrations. This is consistent with field studies to characterize Pb concentrations that are described in the literature.

A number of studies have characterized how Pb-bearing PM is distributed over the neighborhood scale in the air. Martuzevicius et al. (2004) examined the spatial variability of $PM_{2.5}$ samples obtained in the greater Cincinnati, OH area at 6 urban, 4 suburban, and 1 rural site and found that Pb concentration in $PM_{2.5}$ had a coefficient of variation (CV, defined as the standard deviation of site measurements divided by the average) 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. (2006) measured Pb-PM with 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. Sabin et al. (2006) also reported deposition flux at the same sites, which ranged from 8.3 to 29 μg/m^2·day at the rural sites, with a CV of 48%, and was 1.4 ng/m^3 at the rural site. Han et al. (2007) found that Pb concentrations within resuspended road dust were higher at an inner-city ring road and an industrial site compared with residential or construction sites. Li et al. (2009) observed that Pb concentration in $PM_{2.5}$ samples was 2.3-3.0 times higher near a bus depot than next to a rural-suburban road. Ondov et al. (2006) measured Pb-$PM_{2.5}$ concentration at three Baltimore sites, one of which was industrial and the other two of which were considered "receptor" sites. Average Pb-$PM_{2.5}$ concentrations at the different sites were 8.3 ng/m^3 at the industrial site and 1.9 ng/m^3 and 7.2 ng/m^3 at the receptor sites. As a group, these studies support the analysis of intra-urban AQS data by illustrating that intra-urban variability is most strongly related to type, strength, and location of sources.

3.5.2. Temporal Variability

The following section presents 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.

3.5.2.1. Multi-year Trends

Figure 3-16 illustrates the trend in ambient air TSP-Pb concentrations during the years 1980-2009. Over this time period, average air Pb concentrations have declined by 91% from 1.3 $\mu g/m^3$ (in 1980) to 0.12 $\mu g/m^3$ (in 2009). The median concentrations have declined by 97% from 0.87 $\mu g/m^3$ (in 1980) to 0.025 $\mu g/m^3$ (in 2009). While the sharpest drop in Pb concentration occurred during 1980-1990 as a result of the phase-out of Pb antiknock agents in on-road fuel, a declining trend can also be observed between 1990 and 2009 following reductions in industrial use and processing of Pb, as described in Section 3.2.1. In 1990, the median Pb concentration was 0.19 $\mu g/m^3$ and the average Pb concentration was 0.55 $\mu g/m^3$ to yield 87% and 78% reductions, respectively, from 1990 to 2009. Average concentrations in these calculations and in Figure 3-16, are heavily influenced by the source oriented monitors in the network. New Pb concentration data from expansion of the source oriented portion of the network in 2010 will allow for greater assessment of changes of Pb concentrations on nationwide statistics and trends.

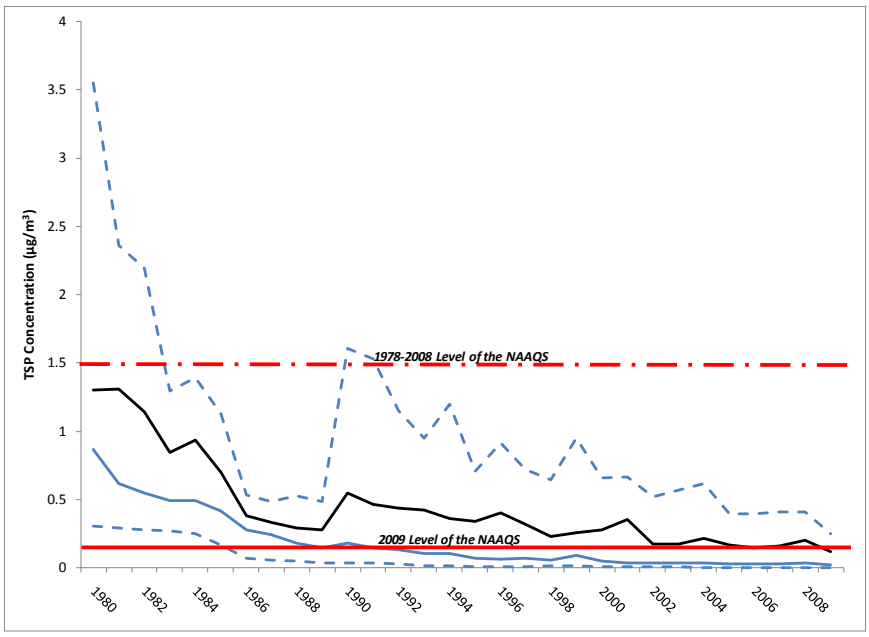


Figure 3-16. National trends in Pb concentration (µg/m^3), all FRM monitors, 1980-2009.Average concentration is shown by the solid black line, median concentration is shown by the solid blue line, and 10th and 90th percentiles are plotted with dashed lines. The red lines on the plot illustrate former and current levels of the Pb NAAQS.

Figure 3-17 and Figure 3-18 show ambient Pb concentrations from 1990 to 2009 for source oriented monitors and non-source oriented monitors, respectively. In both cases concentration data are consistent with a downward trend, and concentrations were considerably lower at the end of the period than at the beginning of the period.

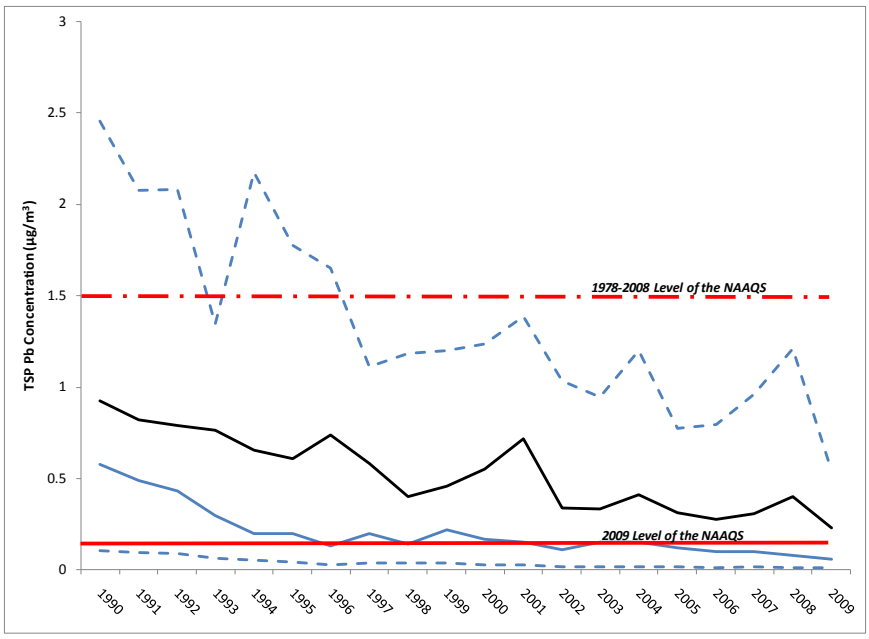


Figure 3-17. National trends in Pb concentration (µg/m^3), source oriented FRM monitors, 1990-2009.Average concentration is shown by the solid line, and the 10th and 90th percentiles are shown by the dashed lines.

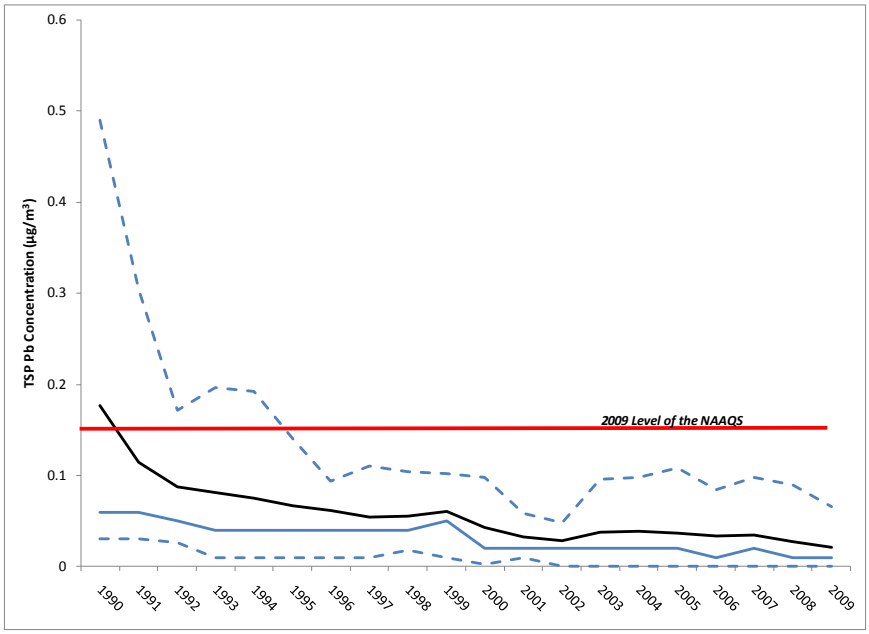


Figure 3-18. National trends in Pb concentration (µg/m^3), non-source oriented FRM monitors, 1990-2009. Average concentration is shown by the solid line, and the 10th and 90th percentiles are shown by the dashed lines.

For source oriented monitors, average concentrations decreased from 0.93 µg/m^3 to 0.23 µg/m^3 (75% decline) and upper 90th percentile concentrations decreased from 2.5 µg/m^3 to 0.54 µg/m^3 (78% decline) over the 20-year period. A portion of the decrease can be attributed to reductions in emissions from the Herculaneum, MO smelter between 2001 and 2002 (U.S. EPA, 2010). An abrupt decrease in average concentrations between these years is evident in Figure 3-17. Note that the number of monitors contributing to these statistics increased from 29 during 1990-1999 to 47 for 2000-2009.

For non-source oriented monitors, average concentrations decreased from 0.18 µg/m^3 to 0.020 µg/m^3 (88% decline) and upper 90th percentile concentrations decreased from approximately 0.49 µg/m^3 to approximately 0.05 µg/m^3 (87% decline) over the 20-year period. Average concentrations near stationary sources were 5 to 12 times typical concentrations from non-source oriented monitoring locations between 1990 and 1999; during the subsequent decade, average source oriented Pb concentrations were 8 to 22 times higher than non-source concentrations (U.S. EPA, 2010). This differential likely reflects the absence of Pb emissions from automobiles during 2000-2009. The number of monitors contributing to these statistics increased from 29 during 1990-1999 to 53 for 2000-2009.

When both source oriented and non-source oriented monitoring sites are considered, average Pb concentrations decreased by 73% between 2001 and 2008 for maximum 3-month average concentrations at 24 sites that are near large stationary sources and 101 sites that are not near stationary sources (U.S. EPA, 2010).

3.5.2.2. Seasonal Variations

This section outlines seasonal variability among Pb monitors on a nationwide basis. Seasonal variation may provide insight related to differential influences of sources and climate throughout a year. Additionally, the magnitude of concentrations within the monthly data distributions and of variations between months sheds light on the influence of season as well as on differences between source oriented, non-source oriented, PM_{10}, and $PM_{2.5}$ data.

The average of Pb concentrations over all monitoring sites is higher in fall and lower in winter than other seasons. Monthly average Pb concentrations averaged over multiple sites and over 3 years from 2007-2009 are shown in for Pb-TSP from source oriented monitors (Figure 3-19), Pb-TSP from non-source oriented monitors (Figure 3-20), Pb-PM_{10} (Figure 3-21), and Pb-$PM_{2.5}$ (Figure 3-22). For source oriented Pb-TSP (Figure 3-19), monthly average concentrations were determined from between 146 and 154 samples in each month. The highest monthly average concentrations were observed in March, April, and November, and exceeded 0.26 µg/m^3. For non-source oriented TSP (Figure 3-20), 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 average

concentrations, each of which were below 0.015 µg/m^3, but concentrations were similar between spring, summer and fall months.

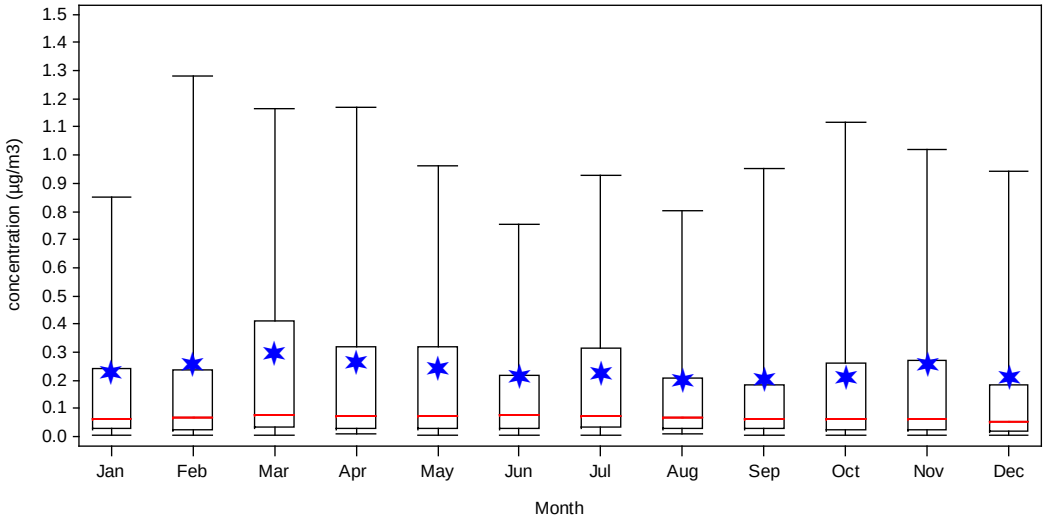


Figure 3-19. Monthly source oriented Pb-TSP average (µg/m^3) over 12 months of the year, 2007-2009.Box and whisker plots are used for each month, with the box comprising the interquartile range of the data and the whiskers comprising the range within the 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

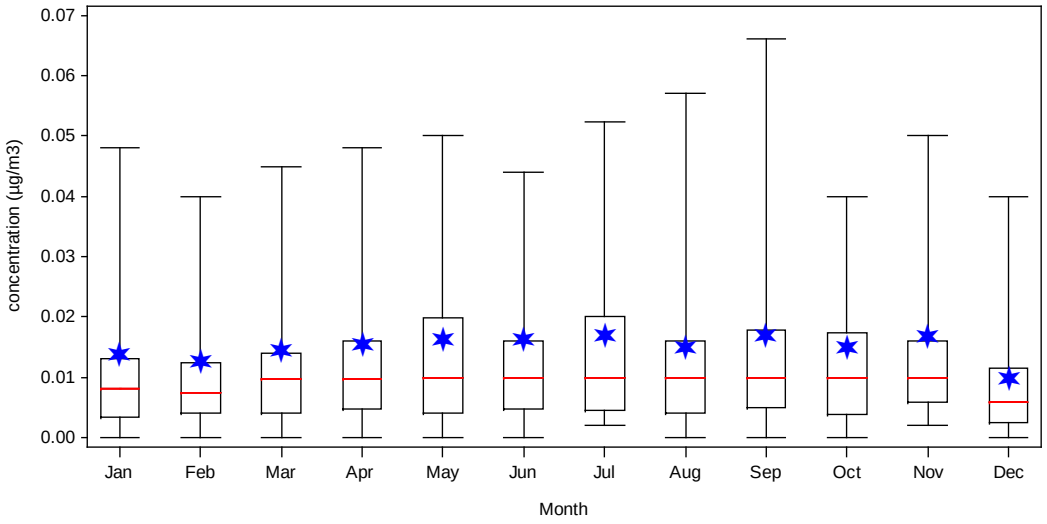


Figure 3-20. Monthly non-source oriented lead-TSP average (µg/m^3) over 12 months of the year, 2007-2009.Box and whisker plots are used for each month, with the box comprising the interquartile range of the data and the whiskers comprising the range within the 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

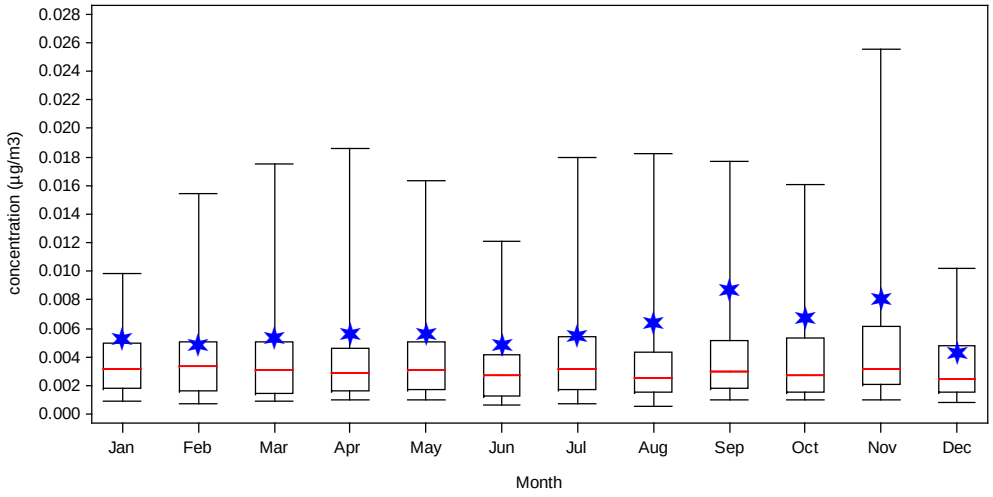


Figure 3-21. Monthly lead-PM$_{10}$ average (µg/m^3) over 12 months of the year, 2007-2009.Box and whisker plots are used for each month, with the box comprising the interquartile range of data and the whiskers comprising the range from 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

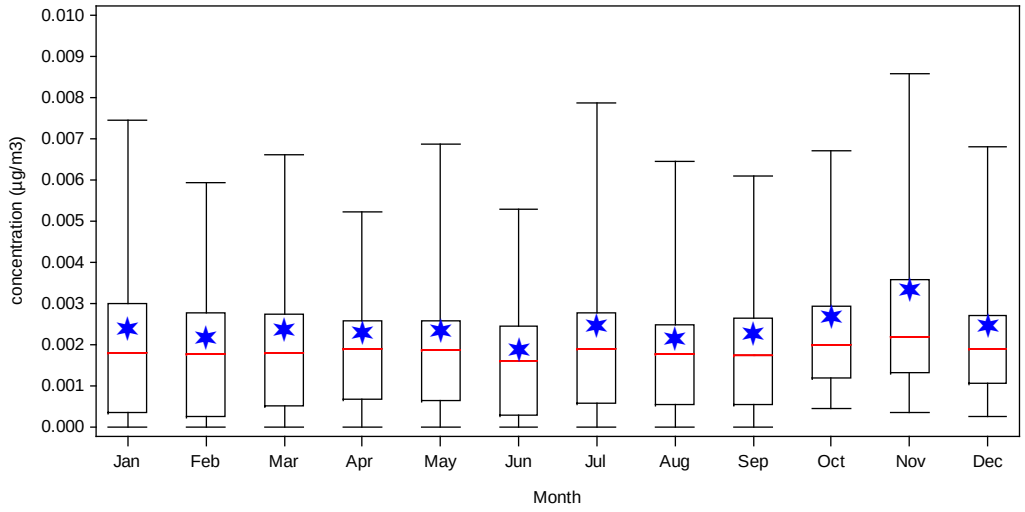


Figure 3-22. Monthly lead-$PM_{2.5}$ average (µg/m^3) over 12 months of the year, 2007-2009.Box and whisker plots are used for each month, with the box comprising the interquartile range of the data and whiskers comprising the range from 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

For both Pb-PM_{10} (Figure 3-21) and Pb-$PM_{2.5}$, (Figure 3-22) monthly average concentrations are considerably higher in the fall than in other seasons, with lowest the three highest monthly average concentrations observed in September, October, and November, and the average September concentration more than double the average December concentration. 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. Some of the Pb-$PM_{2.5}$ concentrations were below Method Detection Limits and the absolute difference in monthly average concentrations between fall and other seasons for Pb-$PM_{2.5}$ of 0.001 µg/m^3 is extremely small. In spite of this the observed trends for $PM_{2.5}$ are consistent with the PM_{10} observations. Whether the seasonal trend for Pb-PM_{10} and Pb-$PM_{2.5}$ differs from trends for both source oriented and non-source oriented Pb-TSP because of the difference in source proximity, sampling locations or difference in size range sampled is not clear.

Although details of the seasonal trends varied with PM size range and source proximity, the data as a whole indicate that average monthly concentrations in the fall were consistently higher than the lowest average monthly concentrations, and that average monthly concentrations in the winter were consistently lower than the highest average monthly concentration regardless of PM size range or source proximity. Overall, there this indicates a clear tendency toward higher fall concentrations and lower winter concentrations. These results are consistent with observations at a single location by Melaku et al. (2008)

that atmospheric Pb concentrations in fall were higher than in winter in an intensive study of urban Washington DC, but not consistent with their observations of higher summer concentrations than fall.

3.5.3. Size Distribution of Lead-Bearing PM

Size-selective monitoring data from AQS and the literature is examined in this section to improve understanding of the size distribution of Pb-bearing PM. This information informs monitoring strategies because high content or correlation of PM_{10} with TSP may allow for expanded usage of PM_{10} within the FRM monitoring network. Additionally, size distribution data enhances understanding of the relationship between sources and characteristics of airborne Pb-bearing PM.

3.5.3.1. AQS Data Analysis

This section employs AQS data for Pb concentrations from co-located TSP, PM_{10}, and/or $PM_{2.5}$ FRM 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 the 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 monitors and average concentration ratios are available per collocation site in Table 3A-13 in the Appendix. For the comparison between Pb-TSP and Pb-PM_{10}, 27 sites were available for analysis. A summary of these data are provided in Table 3-6. Overall, the average correlation, ρ, was moderate, although the wide range across monitors indicates suggests site-to-site variability. The average ratio of Pb-PM_{10} to Pb-TSP concentrations was relatively high at 0.88. When broken down by land type, the average ρ went up slightly for urban and city center land use, with a lower average ratio of concentrations. For suburban sites, the average ρ was reduced, but the average ratio of concentrations was near unity. Average ratio of concentrations greater than one suggest that some portion of the TSP was not collected due to instrument biases, as discussed in Section 3.4.

Table 3-6. Summary of comparison data for co-located lead-TSP and lead-PM_{10} monitors.

Monitors	Correlation			Average Ratio		PM_{10}:TSP
	Average	Standard Deviation	Range	Average	Standard Deviation	Range
Overall	0.65	0.30	0.00-0.99	0.88	0.51	0.33-2.60
Urban and City Center	0.73	0.25	0.29-0.99	0.73	0.19	0.54-1.10
Suburban	0.55	0.33	0.00-0.98	0.99	0.74	0.33-2.60
Rural	-	-	-	-	-	-

Forty-five sites were available for analysis to compare Pb-TSP with Pb-$PM_{2.5}$. A summary of these data are provided in Table 3-7. Overall, the average ρ was moderate. As for the TSP to PM_{10} comparison, a wide range of correlations suggests that the tracking of the TSP and $PM_{2.5}$ time series was quite variable between sites and source influences. The average ratio of Pb-$PM_{2.5}$ to Pb-TSP concentrations was somewhat high, but with several monitors having ratios above one, sampler bias may have influenced these statistics. When broken down by land type, the average ρ was somewhat reduced for urban and city center land use. For suburban sites the average ratio of concentrations was lower, with some of the same sampling bias issues. For rural sites, the average ρ was much higher. Correlation between $PM_{2.5}$ and TSP may suggest commonality of sources or processes influencing both size fractions at the same time.

Table 3-7. Summary of comparison data for co-located Pb-TSP and Pb-$PM_{2.5}$ monitors

Monitors	Correlation			Average Ratio		$PM_{2.5}$:TSP
	Average	Standard Deviation	Range	Average	Standard Deviation	Range
Overall	0.50	0.25	0.03-0.95	0.79	0.49	0.11-2.34
Urban and City Center	0.43	0.26	0.03-0.90	0.75	0.54	0.11-2.34
Suburban	0.55	0.23	0.16-0.95	0.66	0.29	0.36-1.57
Rural	0.71	0.24	0.37-0.90	1.36	0.39	0.83-1.76

Pb-PM_{10} and Pb-$PM_{2.5}$ data were compared for 50 sites. A summary of these data are provided in Table 3-8. Overall, the average ρ was moderately high, with perfect correlation at a Providence, RI site (AQS ID: 440070022) and very high correlation at several other sites. The average ratio of Pb-$PM_{2.5}$ to Pb-PM_{10} concentrations was 1.01, suggesting that some bias existed in the data so that $PM_{2.5}$ was often higher than PM_{10} concentration. Data for urban and city center and suburban sites were similar for correlations and average ratios of concentration. For rural sites, the average ρ was slightly lower, and the range of ratios of concentrations showed that $PM_{2.5}$ concentrations were almost always higher than PM_{10} concentrations, suggesting bias among the rural monitors.

Table 3-8. Summary of comparison data for co-located Pb-PM_{10} and Pb-$PM_{2.5}$ monitors.

Monitors	Correlation			Average Ratio		$PM_{2.5}$: PM_{10}
	Average	Standard Deviation	Range	Average	Standard Deviation	Range
Overall	0.76	0.22	0.15-1.00	1.01	0.37	0.45-2.15
Urban and City Center	0.72	0.24	0.15-1.00	0.85	0.38	0.45-2.15
Suburban	0.81	0.17	0.34-0.98	1.08	0.35	0.61-1.89
Rural	0.66	0.28	0.22-0.99	1.23	0.29	0.97-1.63

In a few cases, Pb-TSP, Pb-PM_{10}, and Pb-$PM_{2.5}$ monitors were co-located simultaneously, so that more information regarding size distribution can be discerned. For example, in urban Jefferson County, AL (AQS: 010730023), the average ratio of $PM_{2.5}$ to TSP was 0.84, while the average ratio of PM_{10} to TSP was 0.80 for the years 2005-2006. These reported values suggested that most of the PM was in the

fine mode. But, the data also indicated instrumentation bias with either the $PM_{2.5}$ or PM_{10} monitors or both, because the average concentration ratio was greater than one. In a suburban portion of Cook County, IL (AQS: 170314201), the $PM_{2.5}$ to TSP average ratio was reported to be 0.36, and the PM_{10} to TSP average ratio was reported to be 0.39. This suggested that the majority of the sample mass was from particles larger than 10 μm, but that the distribution might have been bimodal since there was little difference between $PM_{2.5}$ and PM_{10}. Note that only some of the years reported for all three monitors overlapped. In suburban Wayne, MI (AQS: 261630033), the $PM_{2.5}$ to TSP average ratio was reported to be 0.49, and the reported PM_{10} to TSP ratio was reported to be 0.84. This suggests a smoother distribution with one mode likely in the large fine or small coarse region of the distribution. Note that the $PM_{2.5}$ to TSP collocation includes one year of data more than the PM_{10} to TSP collocation. Taken together, these findings imply that the Pb-bearing PM size distribution varies substantially by location.

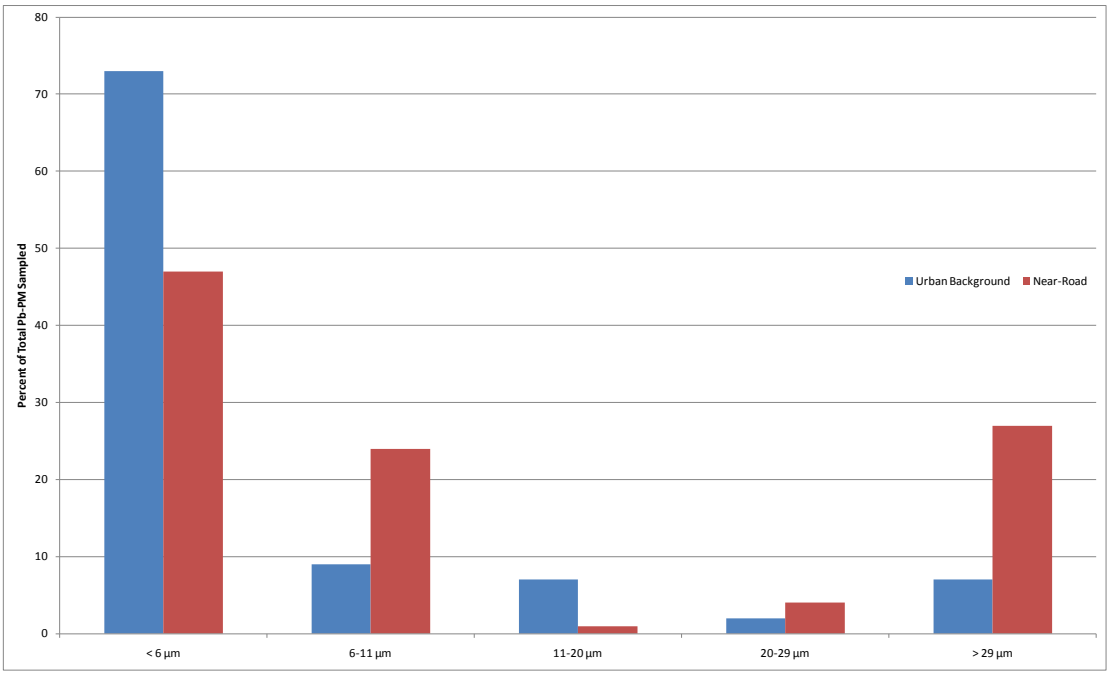
3.5.3.2. Size Distribution Studies in the Literature

Several studies in the literature have been designed to characterize the size distribution of Pb concentrations in the vicinity of sources. The following section describes studies that have measured more than one size fraction of Pb-bearing PM in the vicinity of industrial, urban, and/or traffic-related sources. Some discussion of the variability of size distribution over space and time is also provided.

Size distributions of Pb-bearing PM have been measured near several industrial sites. For example, Bein et al. (2006) measured the size distribution of Pb in PM from the Pittsburgh superfund site using rapid single particle mass spectrometry and a Multi-Orifice Uniform Deposit Impactor (MOUDI). The Pittsburgh, PA, superfund site had seventeen major PM sources within a 24-km radius. 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. Singh et al. (2002) measured the mass distribution of Pb-PM in the coarse and fine PM size ranges for the Downey industrial site along the Alameda industrial corridor in Los Angeles and a site approximately 90 km downwind in Riverside, CA. At the industrial site, the Pb-PM size distribution was unimodal with a concentration peak in the 100-350 nm size range with 34% of the particles in this size bin. 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, comprising 42% and 26% of the mass measured as PM_{10}, respectively. Pb in the fine range only comprised 13% of the particles in the 100-350 nm bin. 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.

Industrial operations associated with Pb emissions include metal works and incineration. Dall'Osto et al. (2008) measured the size distribution of Pb emissions from a steel works facility in a coastal town within the United Kingdom (U.K.). A MOUDI was employed to measure concentrations in the coarse to fine PM size range. 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. Weitkamp et al. (2005) measured Pb-bearing $PM_{2.5}$ concentrations across the river from a coke plant in the Pittsburgh, PA area. Pb was measured to comprise 0.088% of the $PM_{2.5}$ mass, and background-corrected Pb mass concentration was reasonably correlated with background-corrected $PM_{2.5}$ mass concentration ($R^2 = 0.55$). Pekey et al. (2010) measured $PM_{2.5}$ and PM_{10} concentrations in a heavily industrialized area of Kocaeli City, Turkey and obtained an average $PM_{2.5}$ concentration of 47 ng/m^3 during summer and 72 ng/m^3 during winter. Average PM_{10} concentration was 78 ng/m^3 during summer and 159 ng/m^3 during winter, to produce $PM_{2.5}/PM_{10}$ ratios of 0.60 during summer and 0.45 during winter.

Traffic can be a source of resuspension of Pb from deposited contemporaneous wheel weights or industrial emissions or historic sources via traffic-induced turbulence. Sabin et al. (2006) compared the size distribution of coarse Pb-PM captured at an urban background site and at a location 10 m from the I-405 Freeway in the southern California air basin; data from Sabin et al. (2006) are displayed in Figure 3-23. 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. Over all size fractions, the near-road site had a Pb concentration of 17 ng/m^3, compared with an urban background concentration of 9.7 ng/m^3. Sabin et al. (2006) 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. In a near-road study conducted in Raleigh, NC, Hays et al. (2011) note that the concentration of Pb in ultrafine, fine, and coarse size ranges was roughly constant at 50 mg/kg. The Pb 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. 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 wheel weights rather than historic Pb on-road gasoline emissions. Pb dust was shown by Bukowiecki et al. (2010) to be significantly higher at roadside samples compared with urban background when the PM was in the coarse mode, measured as $PM_{10-2.5}$, but not for fine modes measured as $PM_{2.5-1}$ and $PM_{1-0.1}$. Chen et al. (2010) measured Pb in PM_{10}, $PM_{2.5}$, and $PM_{0.1}$ 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, Pb in $PM_{0.1}$ was approximately 15 times higher than by the roadside. The authors suggest that particle-bound Pb was emitted from on-road gasoline and diesel engines. This could possibly be attributed to residual Pb in unleaded gasoline.



Source: Adapted, with permission from Elsevier Publishing, from Sabin et al. (2006).

Figure 3-23. Comparison of urban background and near-road size fractions of lead-bearing PM.

Several studies have suggested that near-road ambient air Pb samples are derived from non-road sources. Harrison et al. (2003) measured the distribution of Pb in PM_{10} at a roadside sampler in Birmingham, U.K.. The size distribution was unimodal with approximately 2% of the Pb mass (0.5 ng/m^3) above the 10 µm cut point, 12% (3 ng/m^3) in the 2-10 µm bin, 9% (2 ng/m^3) in the 1-2 µm bin, 53% of the Pb mass (14 ng/m^3) in the 0.2-1.0 µm bin, and 24% (7 ng/m^3) 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 traffic ($\beta = 0.067$, $R^2 = 0.38$) 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. During winter, Pb concentrations were twice as high as during the summer, and they were also higher when winds blew from the east. Brüggemann et al. (2009) suggested that this finding reflected coal burning sources rather than road dust resuspension. Wang et al. (2006) observed a bimodal Pb distribution in a heavily trafficked area of Kanazawa, Japan with incineration and generation facilities also nearby. They observed a bimodal distribution with modes at the 0.65-1.1 µm and the 3.3-4.7 µm size bins. Wang et al.'s (2006) source apportionment work in this study suggested that the fine mode derives from incineration and combustion of oil and coal.

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. 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, average Pb-$PM_{2.5}$ concentration was 30 ng/m^3 during the day and 92 ng/m^3 at night, and average Pb-PM_{10} concentration was 59 ng/m^3 during the day and 162 ng/m^3 at night, to yield $PM_{2.5}/PM_{10}$ ratios of 0.51 during the day and 0.57 at night. At the suburban site, average Pb-$PM_{2.5}$ concentration was 15 ng/m^3 during the day and 34 ng/m^3 at night, and average Pb-PM_{10} concentration was 24 ng/m^3 during the day and 42 ng/m^3 at night, to yield $PM_{2.5}/PM_{10}$ ratios of 0.63 during the day and 0.81 at night. Rural measurements were only made for Pb-PM_{10} and averaged 6 ng/m^3 during the day and 5 ng/m^3 at night. Goforth et al. (2006) measured TSP and $PM_{2.5}$ in rural Georgia and observed a $PM_{2.5}$ concentration of 6 ng/m^3 and a TSP concentration of 15 ng/m^3. Makkonen et al. (2010) measured concentrations of Pb in PM_1, $PM_{2.5}$, and PM_{10} during a spate of wildfires in rural southeastern Finland. They found that the ratio of PM_1/PM_{10} varied substantially from day to day (examples provided of 64% on 8/14/07 and 35% on 8/25/07, with $PM_{2.5}/PM_{10}$ 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. Birmili et al. (2006) compared concentrations of Pb in PM at various traffic and background sites in Birmingham, U.K.. captured 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-$PM_{1.5\text{-}3.0}$. Roadside concentrations were low. During the day, Birmili et al. (2006) measured 0.4 ng/m^3 for Pb-$PM_{0.5}$ and 1.2 ng/m^3 for Pb-$PM_{1.5\text{-}3.0}$. At night, roadside concentrations reduced to 0.17 ng/m^3 for Pb-$PM_{0.5}$ and 0.6 ng/m^3 for Pb-$PM_{1.5\text{-}3.0}$. 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-$PM_{1.5\text{-}3.0}$. Remote background concentrations were on 0.16 ng/m^3 for Pb-$PM_{0.5}$ and 0.03 ng/m^3 for Pb-$PM_{1.5\text{-}3.0}$. Brüggemann et al. (2009) measured roadside distribution of Pb in PM in Dresden, Germany 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. The distribution of data along the curves did not change substantially under the different conditions examined. When winds came from the east, the total concentration was approximately 22 ng/m^3, compared with a concentration of approximately 13 ng/m^3 when winds came from the west. Total winter concentrations of Pb were approximately 26 ng/m^3, while summertime concentrations were roughly 11 ng/m^3.

3.5.4. Lead 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 Pb 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. Spearman correlations of monitored TSP-Pb concentrations with concentrations of other criteria pollutants are summarized in Figure 3-24 for 2007-2008 data from 129 monitoring sites, and in Figure 3-25 for 2009 data from 16 monitoring sites. 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, ranging from 44 non-source oriented sites for the association of Pb with SO_2 to 81 sites for the association of Pb with PM_{10}. In Figure 3-24, and fewer for each copollutant in Figure 3-25. Each of these figures illustrates co-pollutant correlations across the U.S. Additionally, seasonal correlations between Pb and co-pollutants are provided in Figures 3A-19 through 3A-21 in the Chapter 3 Appendix, with seasonal co-pollutant measurement data from the literature (Table 3A-14). As evident in each figure, there were considerably fewer source-oriented sites available for co-located comparisons.

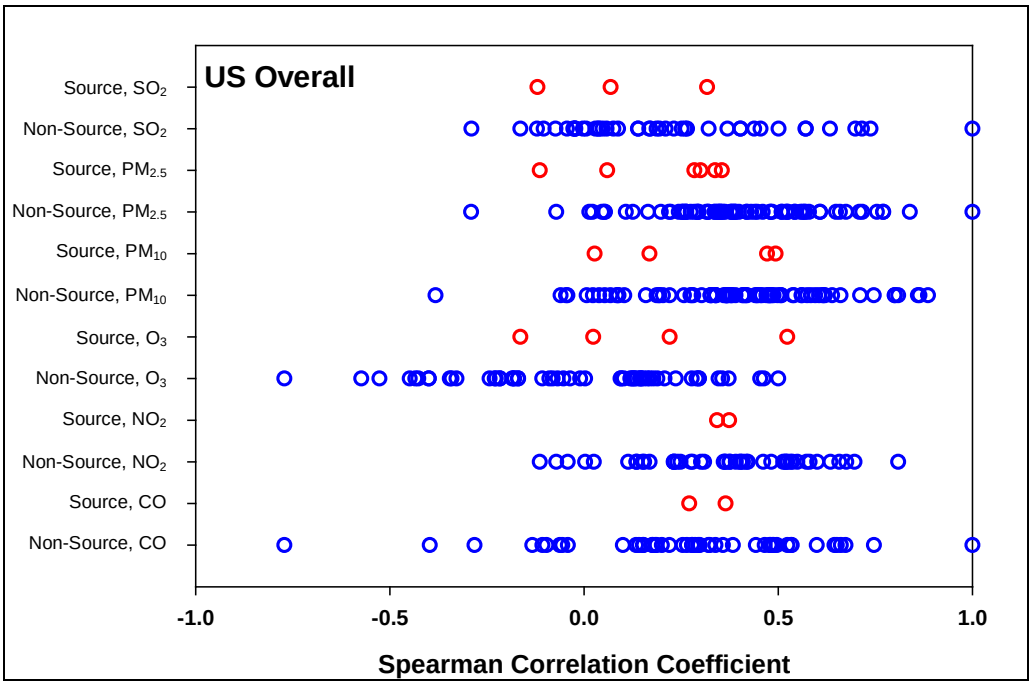


Figure 3-24. Correlations of monitored Pb-TSP concentration with copollutant concentrations, 2007-2008.

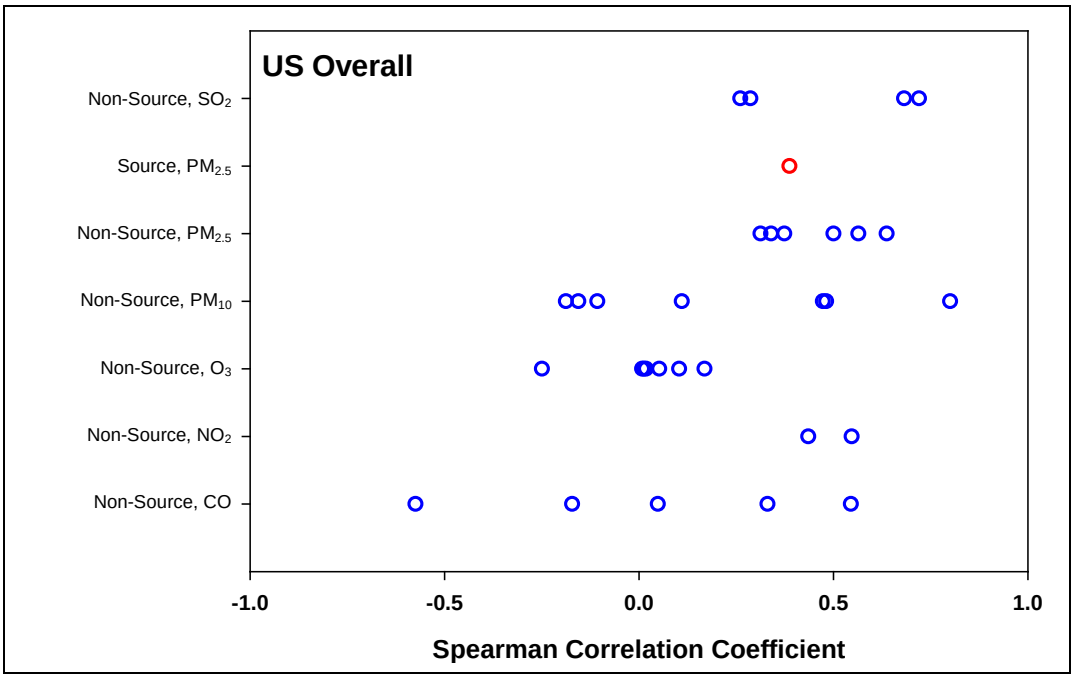


Figure 3-25. Correlations of monitored Pb-TSP concentration with co-pollutant concentrations, 2009.

Overall, Pb was most strongly associated with $PM_{2.5}$, PM_{10} and NO_2 (median R = 0.38 to 0.41), with positive Spearman correlation coefficients observed at nearly all sites. However, Pb was just as strongly associated with CO in fall and winter (median R = 0.48 to 0.58). Such correlations may suggest common sources affecting the pollutants. Overall correlation coefficients between Pb and SO_2 and between Pb and CO were also positive at most sites, but associations were generally weaker (median R = 0.29 for CO, 0.17 for SO_2). The poorest associations were observed between Pb and O_3 (median R = 0.00). Although the overall associations of Pb concentration with PM_{10} and $PM_{2.5}$ concentrations were similar, the association with PM_{10} was stronger in the spring and the association with $PM_{2.5}$ stronger in summer and fall. The strongest associations between Pb and other criteria pollutants were observed in fall and winter, and the weakest in summer.

The relationship between Pb and other species in $PM_{2.5}$ is explored in Figure 3-26, which describes data from 3 years of CSN results. These data provide a national perspective on relationships between the various bulk and elemental species monitored in the CSN network. The strongest association was with Zn (median R = 0.51). Br, Cu, and K concentrations also exhibited moderately strong associations with Pb concentrations (median R = 0.40 to 0.41). Such correlations may suggest some common sources affecting the pollutants. Other species more useful for as diagnostic indicators of crustal, general combustion, industrial emission, and coal combustion processes exhibited weaker, but still remarkable associations with Pb, including crustal elements (median R = 0.32), EC (median R = 0.32), Mn and Fe (median R = 0.32 and 0.34, respectively), S (median R = 0.27), and Se (median R = 0.27). Except for S, in summer

associations with each of these species were much weaker than in other seasons, with a Spearman Correlation Coefficient greater than R = 0.3 observed only for Zn (median R = 0.37). The weakest associations were with As, Cl, Hg, Ni, NO_3, and Na (median R = -0.03 to 0.10).

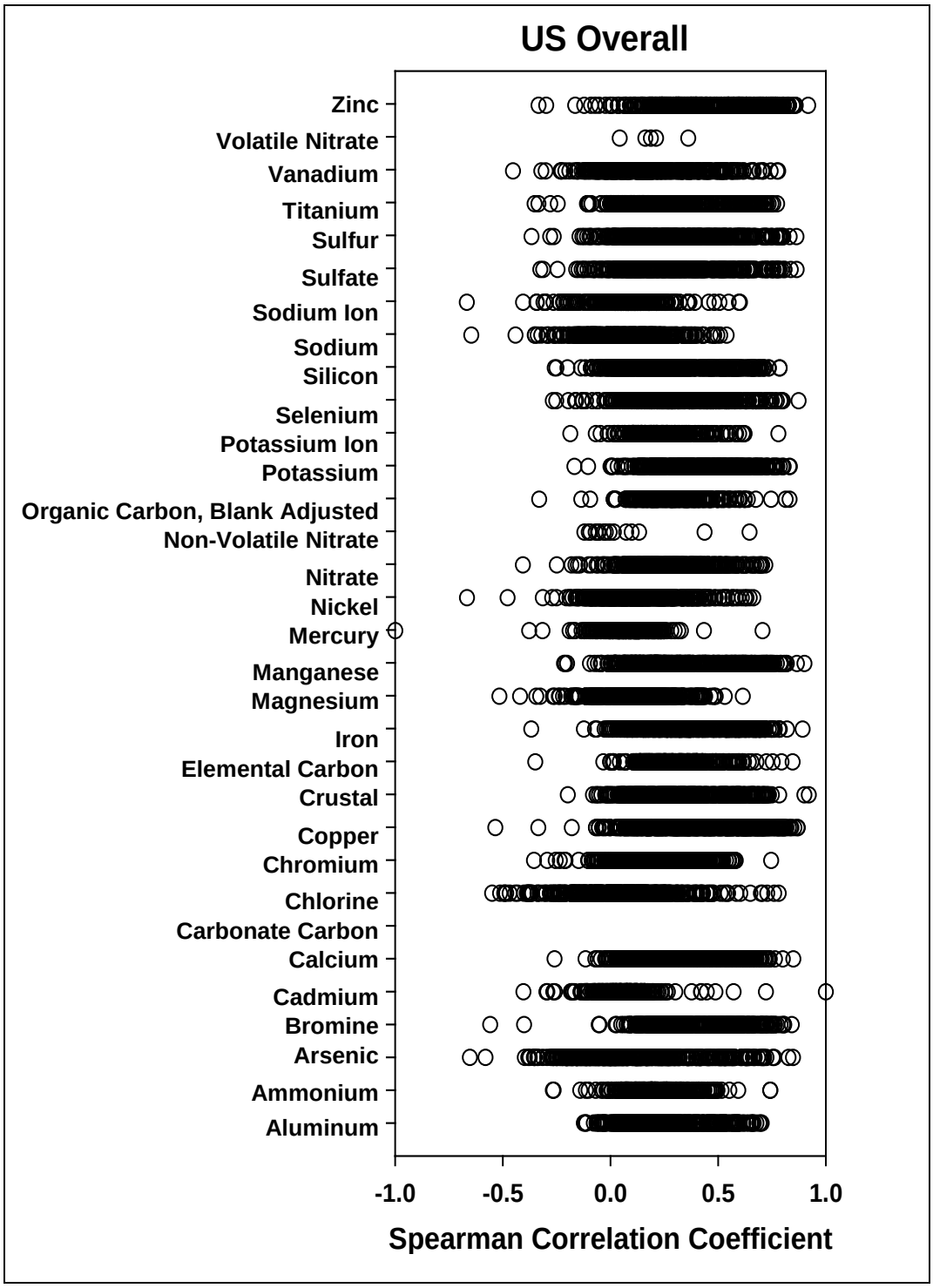


Figure 3-26. Correlations of monitored lead-$PM_{2.5}$ concentration with copollutant concentrations, 2007-2009.

3.6. Ambient Lead Concentrations in Non-Air Media and Biota

There have been some major recent research efforts to characterize geographic and temporal trends in Pb concentrations in 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, 2006) 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. Pb accumulation occurs more readily for Pb salts applied to soils than for 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, 2006) 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, 2006) 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.

3.6.1. Soils

Several studies suggest that soil can act as a reservoir for contemporaneous and historical Pb emissions. In a recent review of soil data collected from 90 U.S. cities, Mielke et al. (2010b) cited studies, some of which were 35 years old but many from the last 15 years, reporting that median soil Pb concentrations ranged from 16 to 262 mg/kg, with maximum levels ranging from 461 to 348,000 mg/kg (see Table 3-9). Soil Pb was thought to originate from present-day sources, such as industry, debrided paint, and piston engine aircraft fuel, as well as historic sources, such as on-road gasoline emissions, as described in Section 3.2.

Emissions trends have shown that industrial activities are now one of the largest sources of Pb following phase out of Pb in on-road gasoline. Pruvot et al. (2006) compared urban and agricultural soils 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. In soil samples obtained near a defunct smelter in El Paso, TX, in 1999 and 2005, Pingatore et al. (2009) found that TSP concentration was predicted strongly by concentrations of Pb-humate, which is created by sorption of Pb onto humic substances in soil. Spalinger et al. (2007) compared soil Pb samples from surrounding towns with those from the Bunker Hill Superfund remediation site in Idaho. Median background soil Pb concentrations was 48 mg/kg, while the median soil Pb concentration at Bunker Hill was 245 mg/kg.

Table 3-9. Outdoor soil Pb levels in various cities within the U.S.

	Study Year	n	Min[a]	Med[a]	Max[a]
Background soil Pb, U.S.	2001	1,319		16.5	
City-State					
Los Angeles, California	2010	550	9		216,174
Los Angeles, California	1995	343			
Chicago Illinois	2008	57			
Chicago Illinois	1987	667			
Chicago-Urban Parks	1986	255	12	262	1,312
Chicago-Suburban Parks	1986	245	12	87	1,637
Illinois, Rural Parks	1986	177	12	37	937
Detroit, Michigan	2003	59	13	189	1,345
Detroit-Suburbs, Michigan	2003	76	4	16	810
Pontiac, Michigan	2003	38	15	86	495
Oakland, California	1995	358	7		347,900
Alameda, California	1993	138	22		3,187
Boston, Massachusetts	1988	195	7		13,240
Miami, Florida	2004	240	2		1,060
Seattle, Washington	1991	51	150		74,000
Washington, D.C.	1995	240	12		6,015
Minneapolis/St. Paul, Minnesota	1984	90	5		7,650
Minneapolis, Minnesota	1988	898	1	230	20,136
St. Paul, Minnesota	1988	832	1	170	7,994
Duluth, Minnesota	1988	229	1	144	11,110
St. Cloud, Minnesota	1988	124	1	41	1,952
Rochester, Minnesota	1988	165	1	25	1,930
Outstate farms Minnesota	1988	781	1	31	7,111
Cleveland, Ohio	2006	50	19		811
Baltimore, Maryland	2008	122	0.01		5,620

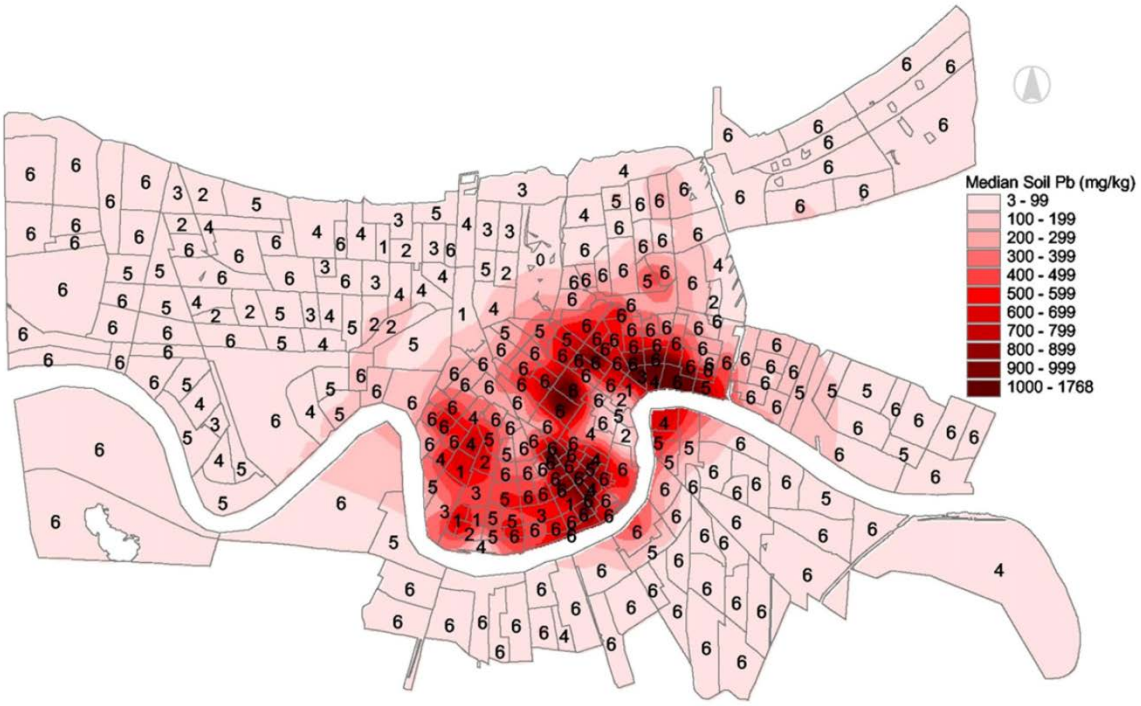
	Study Year	n	Min[a]	Med[a]	Max[a]
Baltimore, Maryland	1983	422	1	100	10,900
Indianapolis, Indiana	2005	116	46		565
Milwaukee, Wisconsin summary data	1994	372	1	160	880
Milwaukee, Wisconsin cent city + North and South Side	1994	256	1	240	7,220
Milwaukee, Wisconsin suburbs	1994	122	1	50	1,780
Cincinnati, Ohio	1990	60	2		3,166
Cincinnati, Ohio-Childcare centers	2008	69	17		4,636
Cincinnati, Ohio	1990	60			
Sacramento, California	1995	232	57	229	320,834
Tampa, Florida	1994	146	<1	100	9,160
New Orleans, Louisiana Survey 1	2005	4,026	18	134	183,588
New Orleans, Louisiana Survey 2	2005	5,467	3	100	52,798
Louisiana Orleans Parish	1997	~1,540	<25	>200	<1,000
Louisiana Lafourche Parish	1997	~190		<25	
Albuquerque, New Mexico	1981	43	3		5,280
Omaha, Nebraska	1979	176			
Dayton, Ohio	1983	22	22		461
El Paso, Texas	2009	94	<20		8700
Honolulu, Hawaii	1988	18			
Charleston, South Carolina	1975	164	9		7,890
New Haven, Connecticut	1982	487	30		7,000
Corpus Christi, Texas	1987	485	21		2,969
Pueblo, Colorado	2006	33			
Connecticut	2008	174	<10		2,200
Gainesville, Florida	2004	202	2.13		1,091
Champaign Illinois	1976	116	20		1,061
Louisiana and Minnesota	1993	6,342			Urban-rural comparisons
Southeastern. Michigan	2004	171	3		7,400
Mt. Pleasant, Michigan	1992	189	100		16,839
Syracuse, New York	2009	2,998	45		
Syracuse, New York	2002	162			
Syracuse, New York	2002	194		80 (GM)	
Lubbock, Texas	2008	52		35	
Maine urban soils	1989	100			

[a]Minimum, median, and maximum values are reported in units of mg/kg for individual cities cited in the review paper.

Source: Used with permission from Elsevier Publishing, Mielke et al. (Mielke et al., 2010b).

Several studies explore the relationship between soil Pb concentration and land use. For example, the Mielke et al. (2010b) review also found that soil Pb concentrations tended to be higher within inner-city communities compared with neighborhoods surrounding city outskirts. Laidlaw and Filippelli (2008) displayed data for Indianapolis, IN showing the Pb concentration at the soil surface had a smoothed "bull's eye" pattern, which suggested that the Pb in soil is continually resuspended and deposited within the urban area so that smooth air and soil concentration gradients emanating from the city center could be created over time. 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 & Laidlaw, 2010). Similarly, Filippelli et al. (2005) reported 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 be smoothed over time from resuspension and deposition with contributions from historic sources of on-road gasoline and Pb paint. 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 in Figure 3-27. Brown et al. (2008) also measured soil Pb concentration along three transects of Lubbock, TX and observed that soil Pb decreased with increasing distance from the city center, which was the oldest part of the city.



Source: Used with permission from Elsevier Publishing, Mielke et al. (2007)

Figure 3-27. Map of median Pb content in soil in New Orleans.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.

The amount of Pb within the inner-city is likely not from a single source but instead composed of all modern and historic sources of Pb dust that have been released in the city including Pb from several local industries, Pb dust from pulverized wheel weights, deteriorated Pb-based paint, Pb additives to on-road gasoline, and defunct incinerators that once dotted New Orleans prior to being shut down by EPA in the early 1970's. Similarly, Mielke et al. (2008) compared soil Pb concentrations 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 in New Orleans, Mielke et al. (2007) observed median and maxima deposition values 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 in Boston, MA and estimated that 60% of the soil Pb could be attributed to historic Pb on-road gasoline emissions in an urban area of the city while 14% could be attributed to historic Pb on-road gasoline emissions in a suburban area. The remainder of the Pb was attributed to paint degradation.

Several studies have examined the effects of roadways on Pb content in roadside dust. In an analysis of the relationship between land use parameters and Pb concentration in soil in Los Angeles, Wu et al. (2010) observed that soil Pb concentration was higher near freeways and major traffic arteries compared with other locations. The age of the land parcel (square-root transformed), length of highway within a 1,000 m buffer, and length of local road within a 20 m buffer in which the sample was obtained were significant predictors of Pb. Home age within 30 m of a soil sample and road length within 3,000 m 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. Amato et al. (2009) observed that deposited PM_{10} onto roadways, measured as dust samples, in Barcelona, Spain was differentially enriched with Pb. Pb concentration in PM_{10} was highest at ring roads (229 ppm) and in the city center (225 ppm), followed by demolition and construction sites (177 ppm) and near a harbor (100 ppm). Joshi et al. (2008) also observed Pb dust concentrations to be highest at industrial sites followed by commercial and residential sites in Singapore.

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., 2006; Weiss et al., 2006). Caravanos et al. (2006) used GIS to examined 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. (2003, 2005) measured Pb dust within 100 m 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. 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) (Farfel et al., 2005).

Pb can be present 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 m of the sand, and 6.4% was recovered at a depth of 0.3-0.45 m. Pb oxides were observed to be the dominant species in the contaminated sand. Berthelot et al. (2008) studied soil Pb concentrations in grounds used for testing military tanks and munitions and measured soil Pb levels to range from 250 to 2,000 mg/kg.

Soil Pb variability depends on the strength and prevalence of nearby sources. Griffith et al. (2002) investigated spatial autocorrelation of soil Pb concentration at three sites: urban Syracuse, NY, rural Geul River, The Netherlands, and an abandoned Pb Superfund site in Murray, UT. 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. These results suggest that soil Pb concentration tends to be spatially heterogeneous in the absence of a source. Later work on the spatial distribution of metals in Syracuse 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.

3.6.2. 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. Although, Pb was measured in snow, water, sediment, lichen and fish during the multiyear project, this metal was not quantified in air samples.

In the WACAP study, bioaccumulation of airborne contaminants was demonstrated on a regional scale in remote ecosystems in the Western U.S. Contaminants were shown to accumulate geographically based on proximity to individual sources or source areas, primarily agriculture and industry. 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.

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 preindustrial 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. A clear decline in Pb concentrations in sediments after the discontinued use of leaded on-road gasoline was observed at almost all WACAP locations in the for nearly all WACAP sites in the conterminous 48 states. 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. Pb was also consistently observed in WACAP fish and wildlife samples.

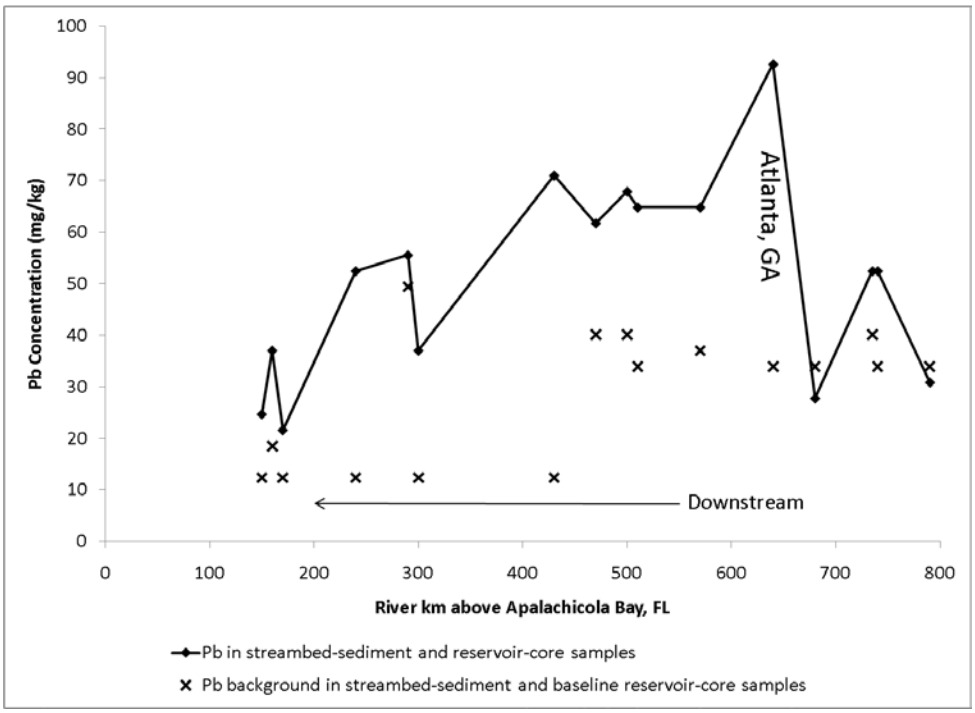
Data from select regions of the U.S. illustrate that Pb concentrations in surface waters and sediment are likely to be higher in urbanized areas compared with rural locations. Table 3-10 presents data from seven metropolitan areas (Cobb et al., 2006). Differences among the intraurban concentration ranges illustrate a high level of spatial variability within individual cities as well as high interurban variability. The rural New Orleans site reported relatively low Pb sediment concentrations, and the highest Pb sediment concentrations were reported for the city of New Orleans. Figure 3-28 and Figure 3-29 illustrate 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. The data also illustrated that Pb concentrations in sediment have declined in the U.S. since 1975 (Figure 3-29), prior to the phase-out of on-road leaded gasoline.

Table 3-10. Sediment concentrations in various cities, prior to 2005

City	Avg Pb Concentration (mg/kg)[a]	Pb Concentration Range (mg/kg)[a]
Baltimore, MD		1-10,900
Miami, FL	275	25-1612
Mt. Pleasant, MI	320	100-840
New Orleans, LA	784	31.7-5195
New Orleans, LA (rural outskirts)	11	4.8-17.3
St. Louis, MO	427	35-1860
Syracuse, NY	80	20-800

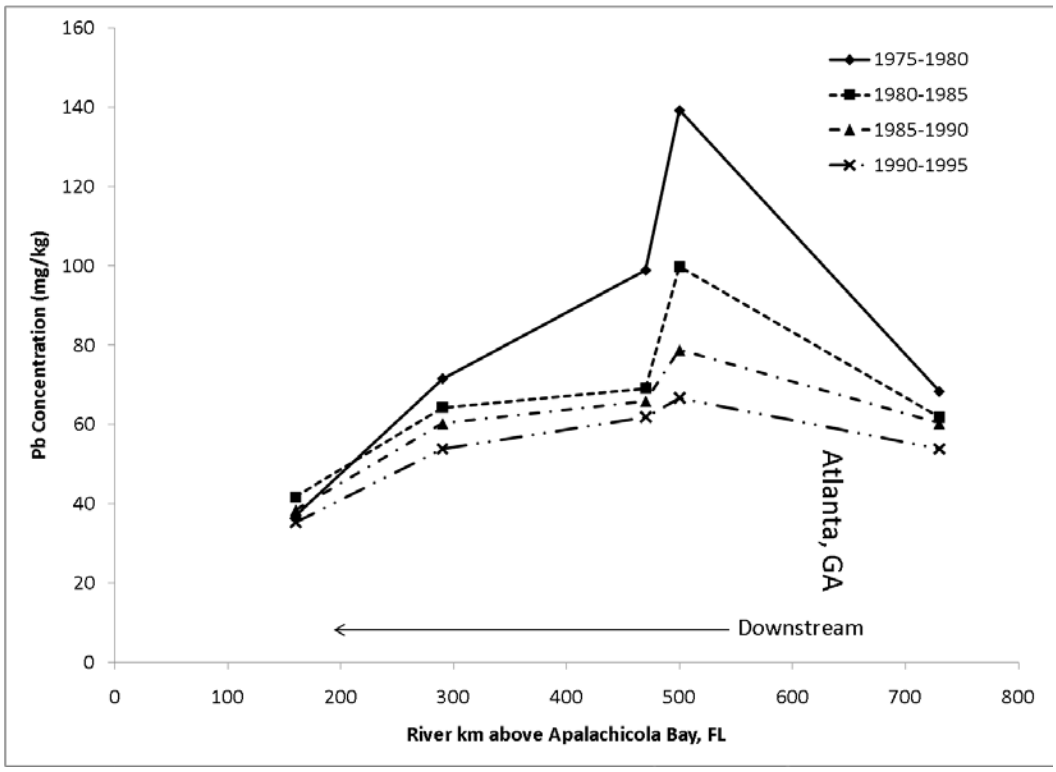
[a]Dry weight basis.

Source: Used with permission from the American Chemical Society, Cobb et al. (2006).



Source: Used with permission from the American Chemical Society, Callender and Rice (2000).

Figure 3-28. Sediment core data (1992-1994) for the lakes and reservoirs along the Apalachicola, Chattahoochee, and Flint River Basin (ACF), which feeds from north of the Atlanta, GA metropolitan area into the Gulf of Mexico at Apalachicola Bay in the Florida panhandle.Note that 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.



Source: Used with permission from the American Chemical Society, Callender and Rice (2000).

Figure 3-29. Sediment core data (1975-1995) for the lakes and reservoirs along the Apalachicola, Chattahoochee, and Flint River Basin (ACF), which feeds from north of the Atlanta, GA metropolitan area into the Gulf of Mexico at Apalachicola Bay in the Florida panhandle.Note that 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.

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. 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. However,

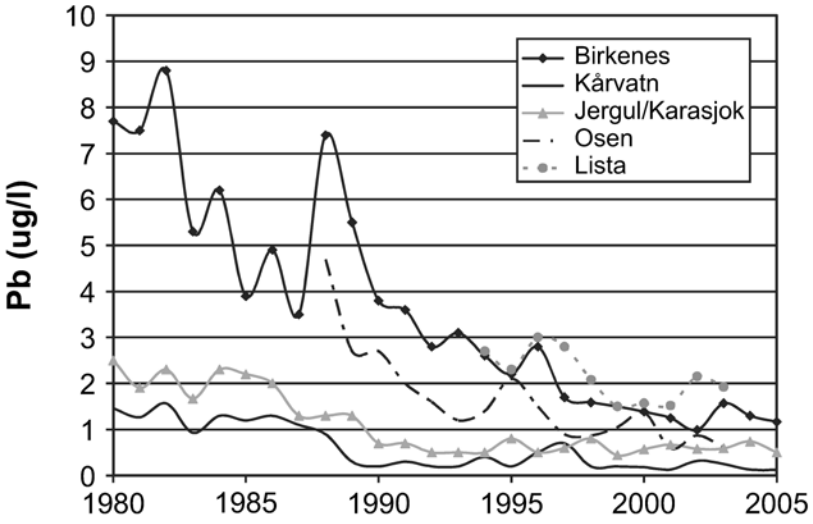
several studies noted no appreciable increase in Pb concentration within Lake Pontchartrain soils and sediments (Abel et al., 2010; Abel et al., 2007; Cobb et al., 2006; Presley et al., 2006; K. J. Schwab et al., 2007). Shi et al. (2010) analyzed Lake Pontchartrain sediment samples using a factored approach and found that most Pb was sequestered in carbonate-rich, iron oxide-rich, and magnesium 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.

3.6.3. Rain

Recent results from locations outside the United States 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, 2006), 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 1989-90, 1990-91 and 2002-03, 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 & 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 3-30). 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%). However, similarly to the situation in the U.S., the greatest reductions occurred by the late 1990s and only relatively minor reductions have occurred thereafter; see Figure 3-30.



Source: Used with permission from Pergamon Press, Berg et al. (2008)

Figure 3-30. 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. Since the outright ban on the use of leaded on-road gasoline in 2000, however, the ratio of Pb concentrations in rainwater to U.K. 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, 2006), 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, 2006) that wet deposition fluxes have greatly decreased since the removal of Pb from on-road gasoline.

3.6.4. 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. 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.

Lee et al. (2008) collected 42 snow samples during the period autumn 2004-summer 2005 from a 2.1 m 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) (collated in 2008). 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/Alsnow:Pb/Alcrust), 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. K. Lee et al. (2008) 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. (2008) suggested that these Pb concentrations were much higher than expected and that their snow samples may have suffered from contamination during sampling and analysis.

3.6.5. Natural Waters

Shotyk and Krachler (2007) measured 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 & 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) and contemporary snow (Johnson and Parnell). 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 and that for contemporary snow was 672 (Johnson, n = 6; Parnell, n = 3) ng/L. Shotyk et al. (2010) gave additional values for Pb in contemporary snow samples and these were again 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. 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, 2006). 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 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 far from 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 whilst 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.

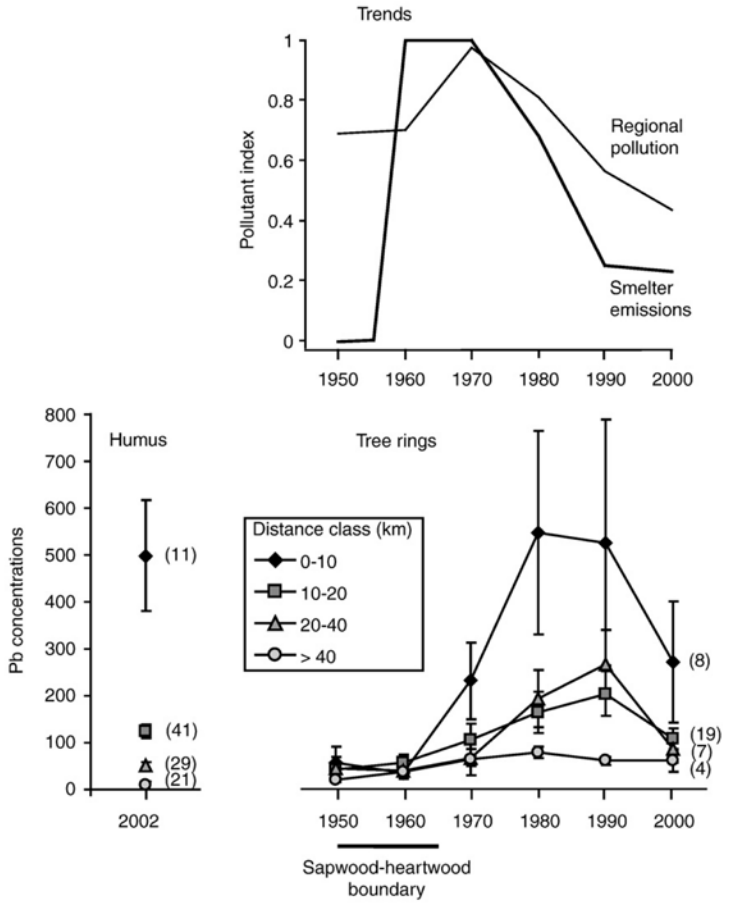
3.6.6. Moss

Mosses can be used effectively for monitoring trends in Pb deposition as demonstrated in many studies (Harmens et al., 2008; Harmens et al., 2010). 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 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).

3.6.7. Grass, Foliage, and Tree Rings

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. (2008) 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; see

Figure 3-31. Both Pb concentrations and Pb isotope ratios declined with distance from the smelter (Aznar, Richer-Lafleche, et al., 2008; Aznar, Richer-Laflèche, et al., 2008).



Source: Used with permission from Elsevier Publishing, Aznar et al. (2008)

Figure 3-31. Trends in regional pollution near a copper smelter in Canada and Pb concentrations at the boundary of heartwood trees within roughly 75 km of the smelter.

3.6.8. 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).

3.7. Summary

3.7.1. Sources of Atmospheric Lead

The 2006 Pb AQCD (U.S. EPA, 2006) 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 1990 and then by an additional 77% from 1990 to 2008, at which time emissions were 1,200 tons/yr. Industrial processes, including metals processing and industrial fuel combustion, had replaced mobile sources as the primary source of Pb to the atmosphere by the 2006 Pb AQCD (U.S. EPA, 2006). More recent data from the 2008 NEI (U.S. EPA, 2011) illustrate that piston engine aircraft emissions now comprise the largest share (~49%) of total atmospheric Pb emissions; the 2008 NEI (U.S. EPA, 2011) estimated that 590 tons of Pb were emitted from aircraft point sources. Other sources of ambient air Pb, in approximate order of importance, include metals processing, fossil fuel combustion, other industrial sources, roadway related sources, and historic Pb.

Chemical speciation of Pb had been fairly well characterized in the 2006 Pb AQCD (U.S. EPA, 2006). Estimates from the 1986 Pb AQCD (U.S. EPA, 1986) for organic on-road Pb emissions provides an upper bound for organic vapor emissions of 20% of total Pb dibromide and Pb bromide emissions from piston engine aircraft. Recent speciation studies of smelting and battery recycling operations have shown that PbS and Pb sulfates are abundant within the emissions mixture for such industrial operations.

3.7.2. Fate and Transport of Lead

The atmosphere is the main environmental transport pathway for Pb, and on a global scale atmospheric Pb is primarily associated with fine PM. On a global scale, Pb associated with fine PM is transported long distances and found in remote areas. Global atmospheric Pb deposition peaked in the 1970s, followed by a more recent decline. On a local scale, Pb concentrations in soils (including urban areas where historic use was widespread) can be substantial, and coarse Pb-bearing PM experiences cycles of deposition and resuspension that serve to distribute it. Both wet and dry deposition are important removal mechanisms for atmospheric Pb. Because Pb in fine PM is typically fairly soluble, wet

deposition is more important for fine Pb. In contrast, Pb associated with coarse PM is usually insoluble, and removed by dry deposition. However, local deposition fluxes are much higher near local industrial sources, and a substantial amount of emitted Pb is deposited near sources, leading to high soil Pb concentrations. Resuspension by wind and traffic can be an important source of airborne Pb near sources where Pb occurs in substantial amounts in surface dust.

In water, Pb is transported as free ions, soluble chelates, or on surfaces of iron and organic rich colloids, and water columns behave as important reservoirs of Pb. In 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 a disproportionate amount of Pb is removed by runoff 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. In anoxic sediments removal by sulfides is particularly important. 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, and sediment concentrations tend to follow those in overlying waters. Resuspended Pb is largely associated with OM or iron and manganese particles. This resuspension of contaminated sediments strongly influences the lifetime of Pb in water bodies and can be a more important Pb source than atmospheric deposition. Resuspension and release from sediments largely occurs during discrete events related to storms.

A complex variety of factors that 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, leading to high soil concentrations in those locations, while fine Pb-PM can be transported long distances, leading to contamination of remote areas. Surface waters act as an important reservoir, with Pb lifetimes largely controlled by deposition and resuspension of Pb in sediments. Pb retention in soil depends on Pb speciation and a variety of factors intrinsic to the soil.

3.7.3. Ambient Lead Monitoring

In recognition of the role of all PM sizes in ambient air Pb exposures, including the ingestion of particles deposited onto surfaces, the indicator for the Pb NAAQS is Pb in Pb-TSP. Although there is a lower rate of error in estimating ambient Pb from Pb-PM_{10} monitoring than from Pb-TSP monitoring, the Pb-TSP indicator was retained in 2008 because ingestion after deposition in the upper respiratory tract was considered an important component of Pb exposure. A new FRM for Pb-PM_{10} has been implemented in which ambient air is drawn through an inertial PM size separator for collection on a PTFE filter. Several FEMs have also been approved. The FRM is based on flame AAS. ICPMS is under consideration as a new FRM for Pb-TSP.

Monitoring for ambient Pb levels is required for all areas where Pb levels have been shown or are expected to contribute to maximum concentrations of 0.10 $\mu g/m^3$ or greater over a three-year time period. Pb is monitored routinely at SLAMS that report data used for NAAQS compliance to the AQS database. Pb monitoring requirements have experienced several changes since publication of the 2006 Pb AQCD (U.S. EPA, 2006). In addition to FRM monitoring, Pb is also routinely measured in smaller PM fractions in the CSN, IMPROVE, and the NATTS networks, and is planned for the NCore network. 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 $PM_{2.5}$. Monitors reporting to the AQS were considered for the purpose of this ISA to be source oriented if they were designated in AQS as source oriented, or they were located within 1 mile of a 0.5 ton/yr or greater source, as noted in the 2005 NEI (U.S. EPA, 2008a). Non-source oriented monitors were those monitors not considered to be source oriented based on these two criteria.

3.7.4. Ambient Air Lead Concentrations

Ambient air Pb concentrations have declined drastically over the period 1980-2009. The median annual concentrations for all monitors have dropped by 97% from 0.87 $\mu g/m^3$ in 1980 to 0.025 $\mu g/m^3$ in 2009. While the sharpest drop in Pb concentration occurred during 1980-1990, a declining trend was observed between 1990 and 2009. A smaller reduction was observable among source oriented Pb concentration (56%) and non-source oriented Pb data (51%) for 2000-2009.

AQS data for source oriented and non-source oriented monitoring were analyzed for 2007-2009. For source oriented monitoring, the 3-month rolling average was measured to be above the level of the NAAQS in 14 counties across the U.S. Fourteen monitoring sites had maximum 3-month rolling average values that exceeded the level of the NAAQS. The maximum 3-month rolling average concentrations ranged from 0.17-2.9 $\mu g/m^3$.

Pb concentrations, seasonal variations, inter-monitor correlations, and wind data were analyzed for six counties: Los Angeles County, CA, Hillsborough County, FL, Cook County, IL, Jefferson County, MO, Cuyahoga County, OH, and Sullivan County, TN. These sites were selected for analysis because they contained a mix of source oriented and non-source oriented monitors in urban areas. Spatial and temporal variability of Pb concentrations in each county were commonly high. Meteorology, distance from sources with respect to the monitors, and source strength all appeared to influence the level of concentration variability across time and space. PM size distribution also influenced how far the particle will travel upon initial emission or resuspension before being deposited.

Size distribution of Pb-bearing PM was demonstrated to vary substantially for several studies presented, depending on the nature of Pb sources and proximity of the monitors to the Pb sources. AQS data were also used to estimate the size distribution of Pb-bearing PM at several sites with co-located Pb monitors. On average, Pb-TSP and Pb-PM_{10} were moderately correlated, but the correlation improved for urban and city center land use types compared with all data. When comparing Pb-TSP with Pb-$PM_{2.5}$, correlations were lower in urban and city center areas compared with suburban and rural sites. A relationship between land use type and correlation was less obvious when comparing Pb-PM_{10} with Pb-$PM_{2.5}$. For urban and city center types, average ρ was fairly high. Average ρ increased for suburban sites but decreased for rural sites. Variation in correlation of size-fractionated Pb samples among different land use types may reflect differences among sources among land use types.

Pb concentrations exhibit varying degrees of association with other criteria pollutant concentrations. Overall, Pb was moderately associated with $PM_{2.5}$, PM_{10} and NO_2, with positive Spearman correlation coefficients observed at nearly all sites. However, Pb was just as strongly associated with CO in fall and winter The poorest associations were observed between Pb and O_3. Among trace metals, the strongest association was with Zn. Br, Cu, and K concentrations also exhibited moderate associations with Pb concentrations. Such correlations may suggest some common sources affecting the pollutants.

3.7.5. Ambient Lead 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. After the phase-out of Pb from on-road gasoline, concentrations in these media decreased to varying degrees. In rain, snowpack, and surface waters, Pb concentrations have decreased considerably following elimination of leaded on-road gasoline. 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 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. Although 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 emissions reductions from other sources.

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Chapter 3 Appendix

3.8. Variability across the U.S.

Table 3A-1. Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2007-9.Sites listed in the bottom six rows of the table fall in the upper 90th percentile of the data pooled by site.

Year	Season	State/ County	State	County name	Site ID	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics																			
2007-2009						1,802		0.2353	0	0	0.007	0.012	0.028	0.070	0.254	0.670	0.976	2.061	4.440
2007						554		0.2500	0	0	0.008	0.012	0.028	0.070	0.290	0.706	1.085	1.982	3.620
2008						622		0.2803	0	0	0.008	0.013	0.034	0.080	0.300	0.741	1.164	2.501	4.440
2009						626		0.1774	0	0	0.006	0.010	0.023	0.063	0.194	0.535	0.787	1.280	2.438
	Winter					443		0.2324	0	0	0.006	0.010	0.025	0.064	0.224	0.575	1.111	2.440	3.103
	Spring					447		0.2695	0	0	0.008	0.013	0.031	0.077	0.333	0.794	1.085	2.035	3.123
	Summer					458		0.2142	0	0	0.008	0.013	0.032	0.074	0.265	0.652	0.852	1.384	4.440
	Fall					454		0.2260	0	0	0.007	0.012	0.026	0.064	0.226	0.670	1.006	1.565	4.225
Nationwide statistics, pooled by site																			
2007-2009						56		0.2384	0.001	0.001	0.010	0.014	0.031	0.098	0.350	0.730	0.862	1.306	1.306
2007						48		0.2547	0.000	0.000	0.010	0.014	0.031	0.090	0.323	0.783	1.048	1.520	1.520
2008						53		0.2848	0.001	0.001	0.011	0.021	0.032	0.121	0.344	0.818	1.226	1.542	1.542
2009						53		0.1779	0.000	0.000	0.007	0.011	0.029	0.075	0.219	0.453	0.718	0.917	0.917
	Winter					56		0.2353	0.001	0.001	0.008	0.010	0.033	0.088	0.290	0.654	1.205	1.389	1.389
	Spring					56		0.2759	0.000	0.000	0.008	0.017	0.035	0.093	0.425	0.863	1.119	1.605	1.605
	Summer					56		0.2136	0.000	0.000	0.008	0.016	0.034	0.095	0.289	0.654	0.802	1.170	1.170
	Fall					55		0.2286	0.001	0.001	0.012	0.017	0.029	0.071	0.389	0.651	1.031	1.243	1.243
Statistics for individual counties (2007-2009)																			
		01109	AL	Pike		44	2	0.5110	0.070	0.070	0.083	0.094	0.190	0.355	0.710	1.277	1.356	1.434	1.434
		06037	CA	Los Angeles		108	5	0.2425	0.002	0.002	0.008	0.012	0.037	0.073	0.188	0.698	0.942	2.501	2.880
		12057	FL	Hillsborough		62	2	0.2939	0.014	0.014	0.020	0.040	0.080	0.139	0.333	0.600	0.840	3.620	3.620
		17031	IL	Cook		144	4	0.0241	0.010	0.010	0.010	0.010	0.014	0.022	0.030	0.040	0.046	0.070	0.084
		17119	IL	Madison		36	1	0.1088	0.022	0.022	0.024	0.028	0.037	0.058	0.138	0.300	0.325	0.454	0.454
		17163	IL	Saint Clair		36	1	0.0246	0.010	0.010	0.010	0.013	0.015	0.023	0.030	0.045	0.054	0.058	0.058
		18035	IN	Delaware		72	2	0.3318	0.034	0.034	0.045	0.056	0.094	0.202	0.341	0.639	0.980	4.440	4.440
		18089	IN	Lake		36	1	0.0297	0.004	0.004	0.006	0.007	0.017	0.025	0.045	0.060	0.063	0.065	0.065
		18097	IN	Marion		71	2	0.0198	0.003	0.003	0.005	0.007	0.010	0.016	0.028	0.040	0.046	0.057	0.057
		27037	MN	Dakota		35	1	0.2872	0.062	0.062	0.072	0.098	0.134	0.232	0.412	0.620	0.670	0.730	0.730
		27163	MN	Washington		31	1	0.0006	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.004	0.004
		29093	MO	Iron		144	4	0.4060	0.007	0.010	0.021	0.029	0.045	0.094	0.658	0.971	1.437	2.557	4.225
		29099	MO	Jefferson		296	10	0.6096	0.011	0.019	0.071	0.124	0.212	0.454	0.798	1.353	1.764	2.440	3.123
		29189	MO	Saint Louis		36	1	0.0361	0.005	0.005	0.005	0.005	0.008	0.050	0.050	0.050	0.050	0.066	0.066
		34023	NJ	Middlesex		12	1	0.0101	0.007	0.007	0.007	0.007	0.008	0.008	0.008	0.010	0.034	0.034	0.034
		36071	NY	Orange		106	3	0.0271	0.003	0.003	0.004	0.005	0.007	0.020	0.037	0.058	0.079	0.103	0.134
		39035	OH	Cuyahoga		108	3	0.0473	0.004	0.004	0.007	0.008	0.013	0.024	0.060	0.130	0.190	0.210	0.220
		39051	OH	Fulton		33	1	0.2434	0.015	0.015	0.055	0.066	0.093	0.190	0.360	0.510	0.620	0.690	0.690
		39091	OH	Logan		68	2	0.0763	0.020	0.020	0.020	0.030	0.040	0.070	0.090	0.120	0.190	0.290	0.290
		42007	PA	Beaver		32	1	0.1090	0.045	0.045	0.054	0.063	0.070	0.100	0.132	0.173	0.195	0.284	0.284
		42011	PA	Berks		108	3	0.1053	0.030	0.033	0.037	0.040	0.050	0.073	0.131	0.242	0.302	0.360	0.542
		47163	TN	Sullivan		108	3	0.0754	0.030	0.030	0.032	0.036	0.042	0.062	0.087	0.145	0.178	0.254	0.341
		48085	TX	Collin		108	3	0.2910	0.007	0.009	0.030	0.052	0.102	0.186	0.389	0.673	0.904	1.121	1.564
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2007-2009)																			
					060371405	24		0.8623	0.242	0.242	0.283	0.284	0.321	0.606	0.923	2.277	2.501	2.880	2.880
					290930016	36		0.7302	0.166	0.166	0.186	0.235	0.347	0.545	0.837	1.295	2.435	4.225	4.225
					290930021	36		0.7970	0.084	0.084	0.093	0.099	0.409	0.696	0.967	1.453	2.438	2.557	2.557
					290990004	36		1.1177	0.242	0.242	0.285	0.518	0.764	1.005	1.519	1.905	2.101	2.416	2.416
					290990015	34		1.3060	0.155	0.155	0.339	0.421	0.834	1.185	1.577	2.319	3.103	3.123	3.123
					290990021	33		0.7498	0.084	0.084	0.141	0.423	0.550	0.681	0.901	1.164	1.553	2.162	2.162

Table 3A-2. Distribution of 3-month moving average Pb-TSP concentrations (µg/m3) nationwide, source-oriented monitors, 2007-9. Sites listed in the bottom six rows of the table fall in the upper 90th percentile of the data pooled by site.

Year	Season	State/ County	State	County name	Site ID	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics																			
20079						1,737		0.2366	0.000	0.000	0.010	0.010	0.030	0.080	0.270	0.680	0.980	1.870	2.890
2007						507		0.2421	0.000	0.000	0.010	0.020	0.030	0.080	0.270	0.720	1.070	1.640	1.740
2008						612		0.2834	0.000	0.000	0.010	0.010	0.040	0.090	0.320	0.800	1.150	2.290	2.890
2009						618		0.1859	0.000	0.000	0.010	0.010	0.030	0.070	0.240	0.550	0.760	1.150	2.070
	Winter					417		0.2434	0.000	0.000	0.010	0.010	0.030	0.070	0.270	0.660	1.120	2.080	2.620
	Spring					436		0.2638	0.000	0.000	0.010	0.010	0.030	0.080	0.330	0.740	1.080	2.140	2.890
	Summer					448		0.2269	0.000	0.000	0.010	0.020	0.030	0.080	0.290	0.670	0.900	1.730	2.140
	Fall					436		0.2131	0.000	0.000	0.010	0.020	0.030	0.080	0.240	0.650	0.940	1.320	2.460
Nationwide statistics, pooled by site																			
20079						56		0.2386	0.000	0.000	0.011	0.017	0.032	0.099	0.333	0.733	0.930	1.332	1.332
2007						45		0.2435	0.003	0.003	0.016	0.019	0.033	0.084	0.277	0.687	0.831	1.524	1.524
2008						53		0.2903	0.000	0.000	0.010	0.022	0.034	0.119	0.383	0.806	1.301	1.641	1.641
2009						53		0.1848	0.000	0.000	0.009	0.014	0.029	0.075	0.229	0.617	0.855	0.863	0.863
	Winter					56		0.2482	0.000	0.000	0.010	0.015	0.041	0.104	0.316	0.683	1.150	1.308	1.308
	Spring					56		0.2736	0.000	0.000	0.010	0.014	0.032	0.096	0.369	0.820	1.320	1.679	1.679
	Summer					56		0.2243	0.000	0.000	0.010	0.012	0.034	0.093	0.287	0.707	0.900	1.232	1.232
	Fall					55		0.2132	0.000	0.000	0.011	0.017	0.032	0.085	0.341	0.592	0.960	1.164	1.164
Statistics for individual counties (2007-2009)																			
		01109	AL	Pike		44	2	0.5080	0.100	0.100	0.120	0.230	0.275	0.325	0.685	1.050	1.150	1.350	1.350
		06037	CA	Los Angeles		108	5	0.2594	0.010	0.010	0.010	0.010	0.045	0.075	0.185	0.710	1.260	2.450	2.490
		12057	FL	Hillsborough		48	2	0.2248	0.040	0.040	0.040	0.040	0.095	0.135	0.250	0.490	0.580	1.770	1.770
		17031	IL	Cook		144	4	0.0244	0.010	0.010	0.010	0.010	0.020	0.030	0.030	0.040	0.040	0.050	0.050
		17119	IL	Madison		36	1	0.1144	0.040	0.040	0.040	0.040	0.060	0.105	0.155	0.210	0.230	0.280	0.280
		17163	IL	Saint Clair		36	1	0.0250	0.010	0.010	0.010	0.020	0.020	0.020	0.030	0.040	0.040	0.050	0.050
		18035	IN	Delaware		72	2	0.3422	0.050	0.050	0.070	0.080	0.120	0.210	0.430	0.710	0.930	2.140	2.140
		18089	IN	Lake		36	1	0.0314	0.010	0.010	0.010	0.010	0.020	0.030	0.040	0.050	0.050	0.060	0.060
		18097	IN	Marion		69	2	0.0199	0.000	0.000	0.010	0.010	0.010	0.020	0.030	0.030	0.040	0.050	0.050
		27037	MN	Dakota		33	1	0.2742	0.100	0.100	0.120	0.140	0.210	0.240	0.350	0.400	0.470	0.570	0.570
		27163	MN	Washington		24	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
		29093	MO	Iron		144	4	0.4142	0.020	0.020	0.020	0.030	0.050	0.170	0.735	1.010	1.210	2.080	2.460
		29099	MO	Jefferson		280	10	0.6211	0.040	0.070	0.120	0.155	0.280	0.475	0.835	1.250	1.655	2.620	2.890
		29189	MO	Saint Louis		36	1	0.0383	0.010	0.010	0.010	0.010	0.010	0.050	0.050	0.050	0.050	0.060	0.060
		34023	NJ	Middlesex		12	1	0.0192	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.050	0.060	0.060	0.060
		36071	NY	Orange		102	3	0.0273	0.000	0.000	0.000	0.010	0.010	0.030	0.030	0.060	0.070	0.080	0.090
		39035	OH	Cuyahoga		102	3	0.0483	0.010	0.010	0.010	0.010	0.020	0.030	0.070	0.120	0.140	0.160	0.170
		39051	OH	Fulton		25	1	0.2420	0.050	0.050	0.050	0.080	0.130	0.240	0.320	0.450	0.520	0.570	0.570
		39091	OH	Logan		68	2	0.0809	0.030	0.030	0.030	0.030	0.050	0.070	0.090	0.160	0.190	0.260	0.260
		42007	PA	Beaver		32	1	0.1115	0.060	0.060	0.070	0.080	0.090	0.110	0.120	0.160	0.170	0.170	0.170
		42011	PA	Berks		108	3	0.1099	0.040	0.040	0.040	0.050	0.060	0.085	0.150	0.210	0.250	0.350	0.360
		47163	TN	Sullivan		102	3	0.0769	0.030	0.030	0.040	0.040	0.050	0.060	0.090	0.130	0.180	0.210	0.260
		48085	TX	Collin		108	3	0.2873	0.010	0.030	0.060	0.070	0.110	0.205	0.430	0.590	0.650	1.190	1.260
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2007-2009)																			
					060371405	24		0.9304	0.280	0.280	0.310	0.330	0.470	0.665	1.045	2.290	2.450	2.490	2.490
					290930016	36		0.7328	0.210	0.210	0.260	0.380	0.445	0.570	0.770	1.170	2.080	2.460	2.460
					290930021	36		0.8258	0.220	0.220	0.220	0.300	0.545	0.880	1.005	1.210	1.270	1.940	1.940
					290990004	36		1.1383	0.630	0.630	0.680	0.700	0.810	1.085	1.340	1.710	2.130	2.140	2.140
					290990015	34		1.3318	0.610	0.610	0.640	0.730	0.940	1.195	1.710	2.020	2.630	2.890	2.890
					290990021	33		0.7682	0.430	0.430	0.440	0.520	0.610	0.750	0.900	1.090	1.310	1.320	1.320

Table 3A-3. Distribution of annual 1-month site maxima TSP Pb concentrations (µg/m^3) nationwide, source-oriented monitors, 2007-2009.Sites listed in the bottom eight rows of the table fall in the upper 90th percentile of the data pooled by site.

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics														
2007-2009		56	0.7961	0.004	0.004	0.025	0.040	0.076	0.289	0.747	2.557	3.620	4.440	4.440
2007		48	0.5517	0.003	0.003	0.025	0.035	0.054	0.192	0.735	1.565	2.162	3.620	3.620
2008		53	0.7157	0.004	0.004	0.014	0.039	0.059	0.247	0.754	2.416	3.123	4.440	4.440
2009		53	0.3758	0.003	0.003	0.016	0.019	0.065	0.141	0.536	1.124	1.357	2.438	2.438
Annual site max 3-month means >= national 90th percentile (2007-2009)														
	180350009-2008		4.4400											
	290930016-2008		4.2252											
	120571066-2007		3.6200											
	290990015-2008		3.1228											
	060371405-2008		2.8800											
	290930021-2008		2.5566											
	180350009-2008		4.4400											
	290930016-2008		4.2252											

Table 3A-4. Distribution of annual 3-month site maxima Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2007-2009.Sites listed in the bottom nine rows of the table fall in the upper 90th percentile of the data pooled by site.

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics														
2007-2009		56	0.5409	0.000	0.000	0.020	0.030	0.060	0.215	0.590	1.940	2.460	2.890	2.890
2007		45	0.3616	0.010	0.010	0.030	0.030	0.050	0.130	0.520	1.210	1.350	1.740	1.740
2008		53	0.5177	0.000	0.000	0.010	0.030	0.050	0.170	0.530	1.770	2.460	2.890	2.890
2009		53	0.3123	0.000	0.000	0.010	0.020	0.040	0.110	0.390	0.940	1.240	2.070	2.070
Annual site max 3-month means >= national 90th percentile (2007-2009)														
	060371405-2008		2.4900											
	180350009-2008		2.1400											
	290930016-2008		2.4600											
	290930016-2009		2.0700											
	290930021-2009		1.9400											
	290990004-2008		2.1400											
	290990015-2008		2.8900											
	060371405-2008		2.4900											

Table 3A-5. One-month average Pb-TSP for individual county concentrations nationwide (µg/m^3), non-source-oriented monitors, 2007-2009

Stcou	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Statistics for individual counties (2007-2009)																
06025	CA	Imperial	33	1	0.0218	0.009	0.009	0.010	0.011	0.013	0.019	0.029	0.036	0.041	0.041	0.041
06037	CA	Los Angeles	117	5	0.0107	0.000	0.000	0.000	0.002	0.006	0.010	0.015	0.020	0.024	0.032	0.038
06065	CA	Riverside	48	2	0.0090	0.000	0.000	0.002	0.003	0.008	0.010	0.010	0.016	0.018	0.022	0.022
06071	CA	San Bernardino	47	2	0.0112	0.000	0.000	0.002	0.003	0.008	0.010	0.014	0.020	0.022	0.036	0.036
12103	FL	Pinellas	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
17031	IL	Cook	143	4	0.0144	0.010	0.010	0.010	0.010	0.010	0.014	0.017	0.020	0.024	0.028	0.032
17117	IL	Macoupin	36	1	0.0102	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.013	0.014	0.014
17119	IL	Madison	35	1	0.0187	0.010	0.010	0.010	0.010	0.010	0.015	0.022	0.030	0.038	0.066	0.066
17143	IL	Peoria	36	1	0.0113	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.014	0.015	0.018	0.018
18097	IN	Marion	35	1	0.0071	0.002	0.002	0.002	0.003	0.004	0.006	0.009	0.012	0.012	0.033	0.033
18163	IN	Vanderburgh	21	2	0.0048	0.001	0.001	0.002	0.002	0.004	0.005	0.005	0.006	0.010	0.010	0.010
25025	MA	Suffolk	24	1	0.0089	0.000	0.000	0.000	0.003	0.007	0.009	0.011	0.015	0.016	0.020	0.020
26163	MI	Wayne	24	1	0.0187	0.007	0.007	0.009	0.012	0.013	0.018	0.022	0.027	0.032	0.045	0.045
27037	MN	Dakota	129	4	0.0039	0.000	0.000	0.000	0.000	0.000	0.003	0.006	0.008	0.010	0.018	0.036
27053	MN	Hennepin	128	4	0.0034	0.000	0.000	0.000	0.000	0.002	0.003	0.005	0.008	0.008	0.010	0.010
27123	MN	Ramsey	71	3	0.0071	0.000	0.000	0.000	0.000	0.004	0.006	0.010	0.015	0.020	0.028	0.028
27137	MN	Saint Louis	70	2	0.0017	0.000	0.000	0.000	0.000	0.000	0.002	0.003	0.005	0.006	0.010	0.010
27163	MN	Washington	36	1	0.0035	0.000	0.000	0.000	0.000	0.003	0.004	0.004	0.006	0.006	0.008	0.008
36047	NY	Kings	36	1	0.0142	0.010	0.010	0.010	0.010	0.011	0.013	0.016	0.020	0.021	0.026	0.026
39017	OH	Butler	34	1	0.0052	0.000	0.000	0.000	0.003	0.004	0.005	0.007	0.008	0.009	0.009	0.009
39029	OH	Columbiana	107	3	0.0145	0.000	0.000	0.004	0.006	0.008	0.012	0.019	0.031	0.034	0.045	0.050
39035	OH	Cuyahoga	70	2	0.0138	0.004	0.004	0.006	0.006	0.009	0.013	0.016	0.022	0.028	0.041	0.041
39049	OH	Franklin	36	1	0.0088	0.004	0.004	0.004	0.005	0.007	0.008	0.011	0.014	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	43	2	0.0041	0.002	0.002	0.002	0.002	0.003	0.004	0.005	0.006	0.007	0.010	0.010
42003	PA	Allegheny	36	1	0.0067	0.000	0.000	0.000	0.000	0.000	0.004	0.012	0.016	0.021	0.053	0.053
42045	PA	Delaware	20	1	0.0400	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040
42129	PA	Westmoreland	36	1	0.0410	0.034	0.034	0.037	0.040	0.040	0.040	0.040	0.044	0.050	0.053	0.053
48061	TX	Cameron	35	1	0.0038	0.002	0.002	0.002	0.002	0.003	0.003	0.005	0.006	0.007	0.009	0.009
48141	TX	El Paso	22	2	0.0229	0.014	0.014	0.015	0.016	0.018	0.019	0.019	0.041	0.056	0.057	0.057
48201	TX	Harris	31	1	0.0054	0.003	0.003	0.004	0.004	0.005	0.005	0.006	0.007	0.009	0.010	0.010
48479	TX	Webb	31	1	0.0103	0.004	0.004	0.005	0.006	0.007	0.008	0.010	0.024	0.025	0.026	0.026

Table 3A-6. Three-month moving average Pb-TSP for individual county concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2007-2009

Stcou	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Statistics for individual counties (2007-2009)																
06025	CA	Imperial	33	1	0.0216	0.010	0.010	0.010	0.020	0.020	0.020	0.030	0.030	0.030	0.030	0.030
06037	CA	Los Angeles	117	5	0.0113	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.020	0.020	0.030	0.030
06065	CA	Riverside	48	2	0.0102	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.020
06071	CA	San Bernardino	47	2	0.0115	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.020	0.020	0.020
12103	FL	Pinellas	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
17031	IL	Cook	143	4	0.0139	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.020	0.020	0.020	0.020
17117	IL	Macoupin	36	1	0.0100	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
17119	IL	Madison	35	1	0.0182	0.010	0.010	0.010	0.010	0.010	0.020	0.020	0.030	0.040	0.040	0.040
17143	IL	Peoria	36	1	0.0100	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
18097	IN	Marion	35	1	0.0076	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.020	0.020	0.020
18163	IN	Vanderburgh	21	2	0.0037	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.010	0.010
25025	MA	Suffolk	24	1	0.0092	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.010	0.020	0.020	0.020
26163	MI	Wayne	24	1	0.0196	0.010	0.010	0.010	0.010	0.020	0.020	0.020	0.020	0.030	0.030	0.030
27037	MN	Dakota	129	4	0.0029	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.010	0.010
27053	MN	Hennepin	128	4	0.0024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.010
27123	MN	Ramsey	71	3	0.0071	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.020	0.020	0.020
27137	MN	Saint Louis	70	2	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
27163	MN	Washington	36	1	0.0011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.010
36047	NY	Kings	36	1	0.0128	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.020	0.020	0.020	0.020
39017	OH	Butler	34	1	0.0061	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.010	0.010	0.010
39029	OH	Columbiana	107	3	0.0151	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.020	0.030	0.030	0.030
39035	OH	Cuyahoga	70	2	0.0142	0.000	0.000	0.010	0.010	0.010	0.010	0.020	0.020	0.020	0.030	0.030
39049	OH	Franklin	36	1	0.0094	0.000	0.000	0.000	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
39143	OH	Sandusky	12	1	0.0060	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.010	0.010	0.010
39167	OH	Washington	43	2	0.0008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010
42003	PA	Allegheny	36	1	0.0058	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.020	0.030	0.030
42045	PA	Delaware	20	1	0.0400	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040
42129	PA	Westmoreland	36	1	0.0414	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.050	0.050	0.050	0.050
48061	TX	Cameron	35	1	0.0024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.010
48141	TX	El Paso	22	2	0.0256	0.020	0.020	0.020	0.020	0.020	0.020	0.030	0.040	0.040	0.040	0.040
48201	TX	Harris	31	1	0.0063	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.010	0.010	0.010
48479	TX	Webb	31	1	0.0100	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010

3.8.1. Intra-urban Variability

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 2007-2009 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), the 90th percentile of the absolute difference in concentrations (P90) in ppm, 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 3A-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 2007-2009 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 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 3A-1 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/yr (U.S. EPA, 2008b). 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/yr (U.S. EPA, 2008b). 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/yr (U.S. EPA, 2008b). 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 m from of a railroad. Monitor G was located on a rooftop approximately 20 m from an 8-lane arterial road, and monitor H was positioned at the curbside of a four-lane road roughly 650 m north of that road's junction with I-405. Monitor I was sited in a parking lot roughly 80 m from a

four-lane road, and monitor J was located approximately 130 m south of a 4-lane highway. Figure 3A-2 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 3A-1 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 3A-3. 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 $\mu g/m^3$) and summer (0.18 $\mu g/m^3$). Monitor C reported the highest concentrations in Los Angeles County, with a year-round mean of 0.68 $\mu g/m^3$. 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 $\mu g/m^3$, and the 75th percentile of year-round data exceeded the level of the NAAQS; in spring, the 70th percentile exceeded 0.15 $\mu g/m^3$. Monitor B reported the lowest values among the source-oriented monitors with an average annual concentration of 0.013 $\mu g/m^3$. 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 $\mu g/m^3$, and the 95th percentile of concentration was 0.17 $\mu g/m^3$.

The non-source-oriented monitors located at sites F-J all recorded low concentrations, with average values ranging from 0.004 to 0.018 $\mu g/m^3$ (Figure 3A-3). The highest average year-round concentrations were recorded at site F. The 95th percentiles at these sites ranged from 0.01 to 0.04 $\mu g/m^3$. 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 3A-7), illustrate that Pb has high intra-urban spatial variability. For the source-oriented monitors, the highest correlation $\rho = 0.57$, 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 correlation between source-oriented monitor B and non-source-oriented monitor F, with $\rho = 0.74$. 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 [$\rho = 0.37$ 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.

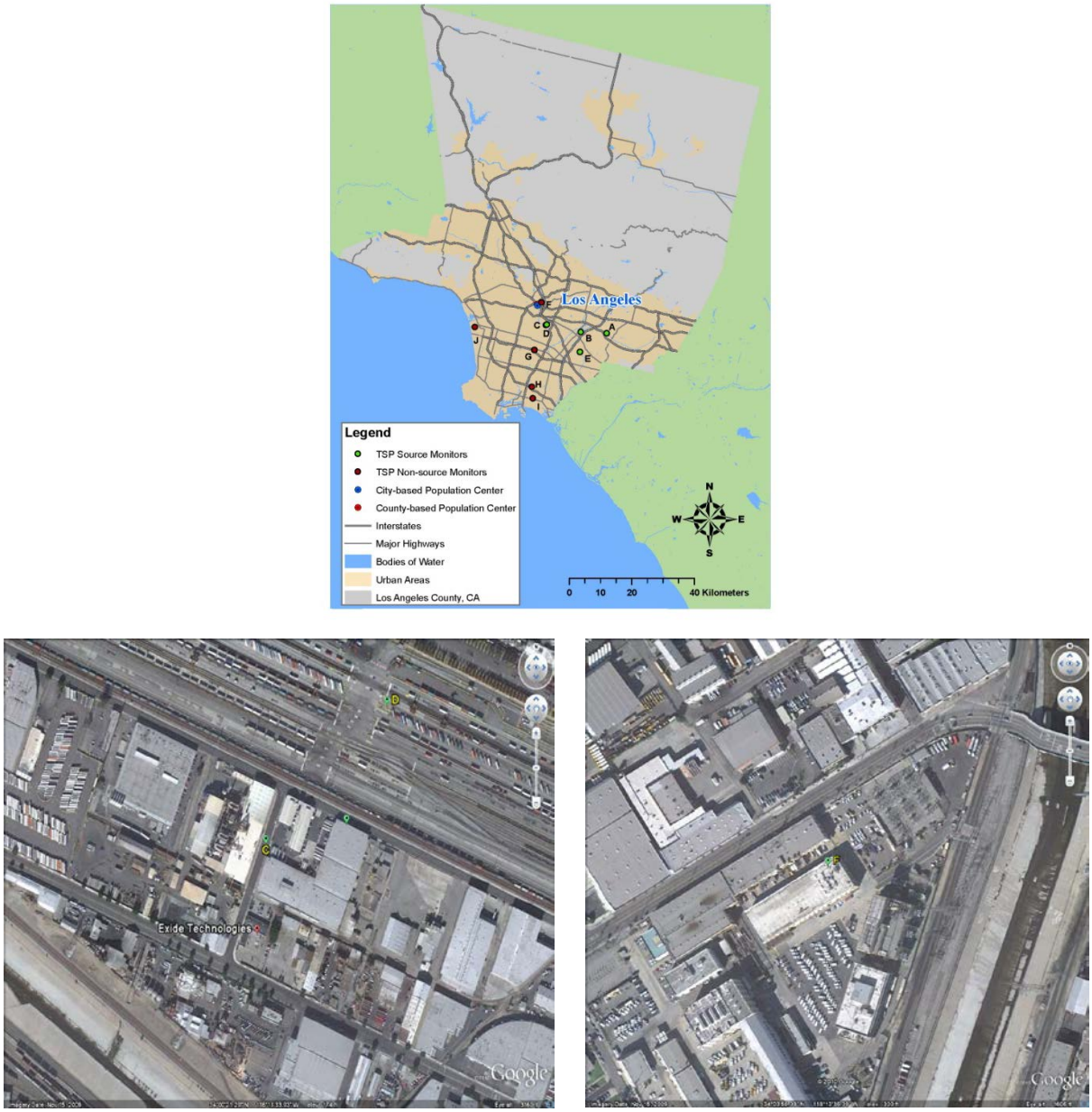
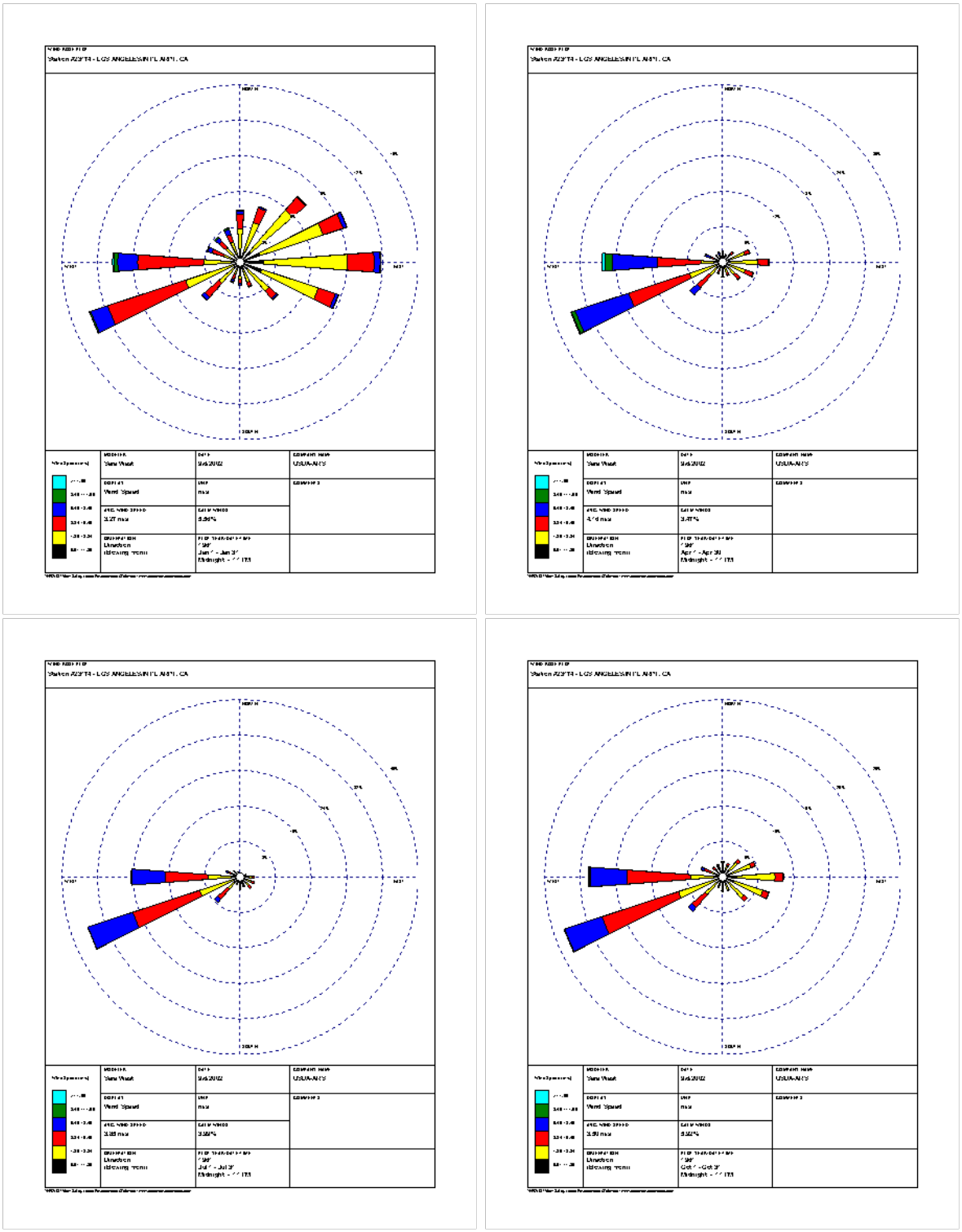


Figure 3A-1. Pb TSP monitor and source locations within Los Angeles County, CA (06-037), 2007-2009.Note that monitor locations are denoted by green markers, and source locations are denoted by red markers. Top: view of all Pb FRM monitors in Los Angeles County. Bottom left: Close up of the industrial site near monitors C and D. Bottom right: Close up of the populated area captured by monitor F.



Source: NRCS (2011).

Figure 3A-2. Wind roses for Los Angeles County, CA, from meteorological data at the Los Angeles International Airport, 1961-1990.Clockwise from top left: January, April, July, and October. Note that the wind percentages vary from month to month.

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

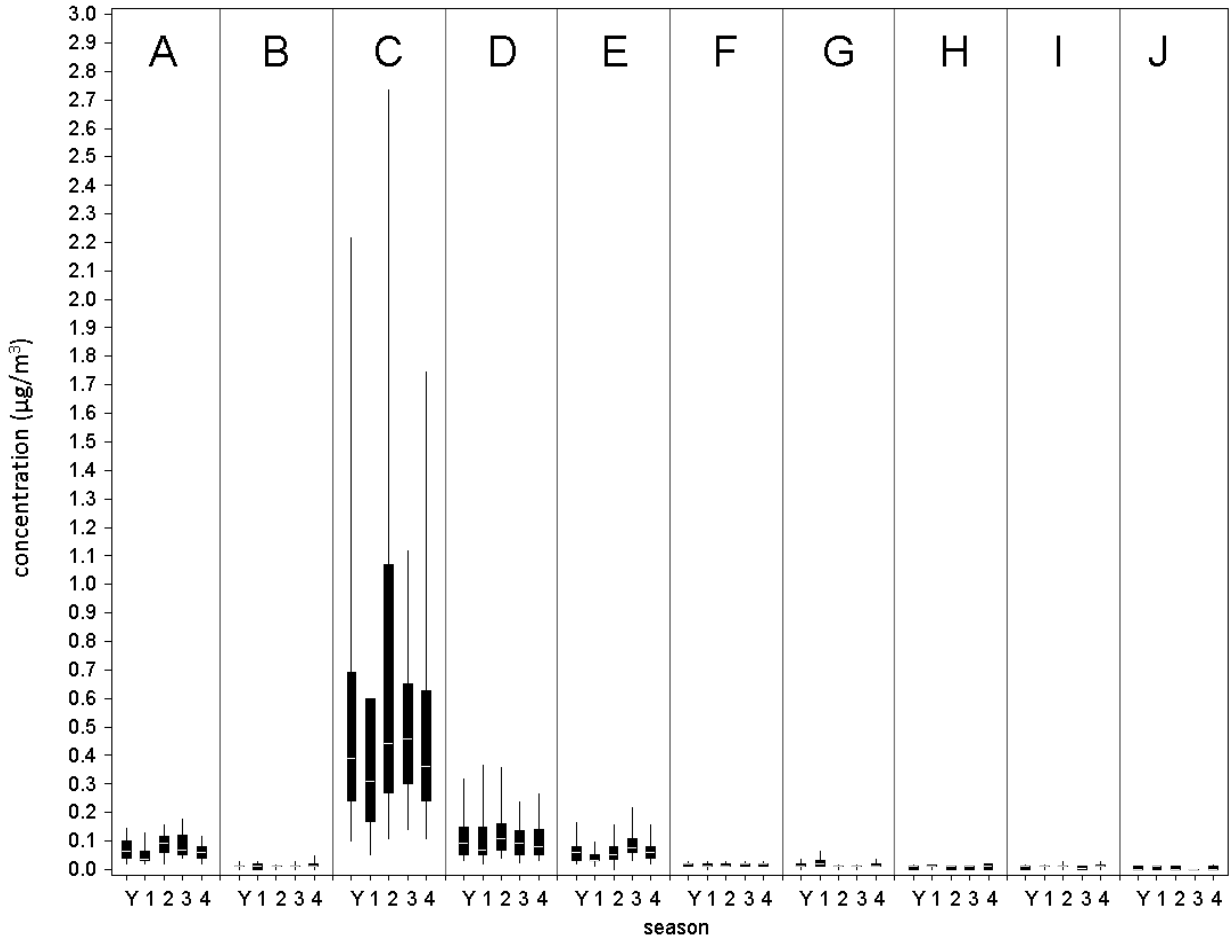


Figure 3A-3. Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Los Angeles County, CA (06-037), 2007-2009.

Table 3A-7. Correlations 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	1.00	0.16	0.10	0.08	0.27	-0.15	0.00	0.14	-0.02	-0.09
		0.00	0.08	0.49	0.10	0.10	0.08	0.06	0.08	0.08	0.08
		0.00	0.63	0.64	0.31	0.34	0.57	0.57	0.79	0.77	0.85
B	Source		1.00	0.05	0.05	0.07	0.74	0.12	0.28	0.11	0.10
			0.00	3.59	0.25	0.10	0.02	0.02	0.01	0.02	0.02
			0.00	0.96	0.84	0.71	0.46	0.48	0.61	0.60	0.81
C	Source			1.00	0.57	0.03	-0.08	0.26	0.28	0.20	0.13
				0.00	1.76	2.14	3.59	4.22	3.59	3.59	3.92
				0.00	0.68	0.77	0.95	0.96	0.98	0.98	0.99
D	Source				1.00	0.12	0.17	0.11	0.24	0.21	0.07
					0.00	0.17	0.24	0.25	0.25	0.25	0.25
					0.00	0.42	0.78	0.80	0.89	0.89	0.95
E	Source					1.00	0.13	0.06	0.24	0.07	0.18
						0.00	0.10	0.10	0.11	0.11	0.11
						0.00	0.61	0.64	0.78	0.79	0.90
F	Non-Source		Legend ρ P90 COD				1.00	0.02	0.19	0.09	0.09
							0.00	0.02	0.02	0.02	0.02
							0.00	0.39	0.61	0.58	0.82
G	Non-Source							1.00	0.65	0.39	0.38
								0.00	0.01	0.02	0.02
								0.00	0.54	0.61	0.85
H	Non-Source								1.00	0.51	0.40
									0.00	0.01	0.01
									0.00	0.55	0.77
I	Non-Source									1.00	0.37
										0.00	0.01
										0.00	0.78
J	Non-Source										1.00
											0.00
											0.00

Figure 3A-4 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 m north-northeast of the EnviroFocus Technologies battery recycling facility, which produced 1.3 tons/yr (U.S. EPA, 2008c), and monitor B was located 320 m southwest of the same facility. Monitor C was located next to a two-lane road in Pinellas Park, FL.

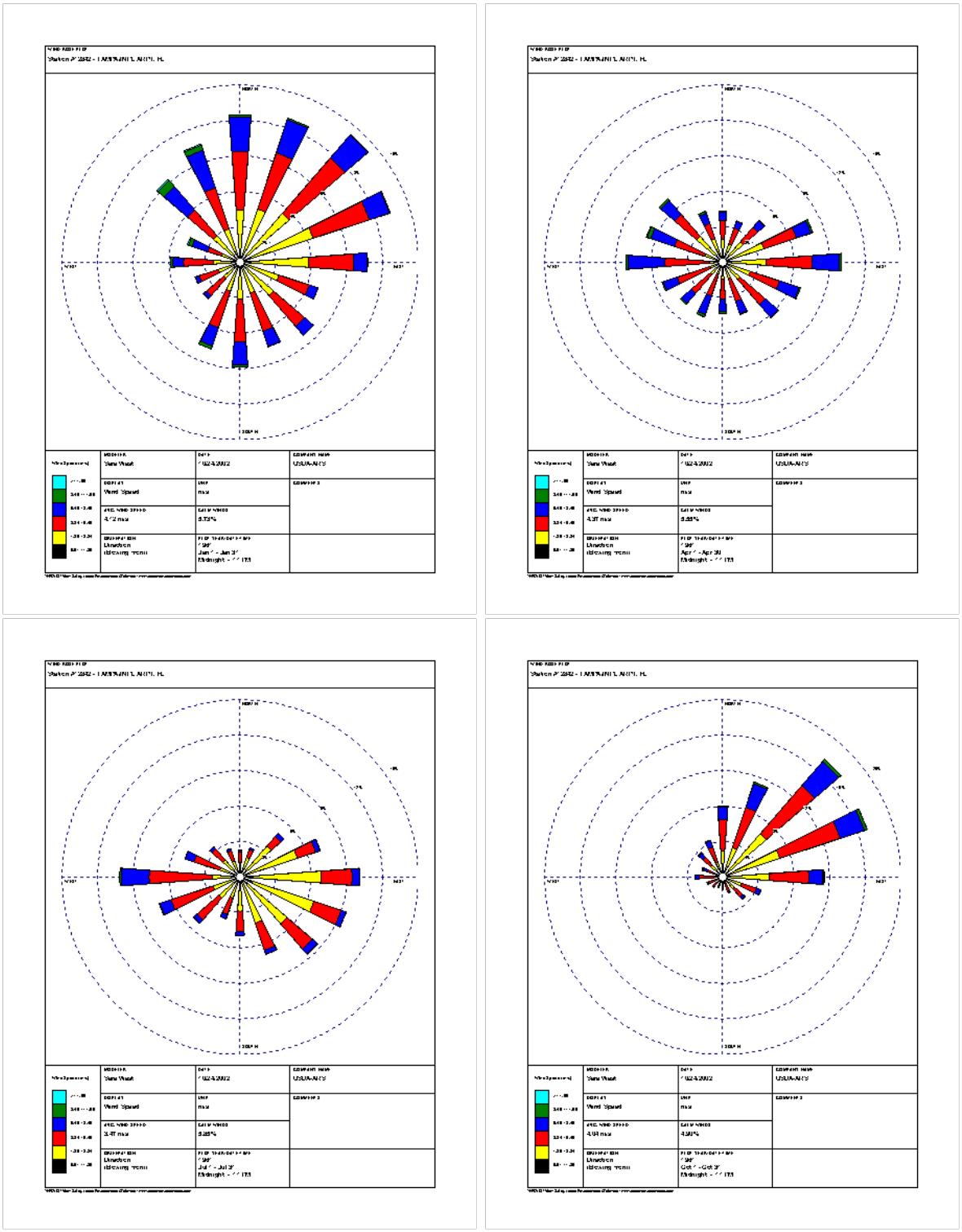
Figure 3A-5 displays seasonal wind roses for the Tampa-St. Petersburg metropolitan area. These wind roses suggest shifting wind directions throughout the winter, spring, and summer. During the winter,

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 3A-6. 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 3A-8, illustrate that Pb has high intra-urban spatial variability. The source oriented monitors were anticorrelated ($\rho = -0.08$). 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.

Figure 3A-4. Pb TSP monitor locations within Hillsborough and Pinellas Counties, FL (12-057 and 12-103), 2007-2009.Top: view of all Pb FRM monitors in Hillsborough and Pinellas Counties. Bottom: Close up of industrial site around monitors A and B.



Source: NRCS (2011).

Figure 3A-5. Wind roses for Hillsborough/Pinellas Counties, FL, obtained from meteorological data at Tampa International Airport, 1961-1990.Clockwise from top left: January, April, July, and October. Note that wind percentages vary from month to month.

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

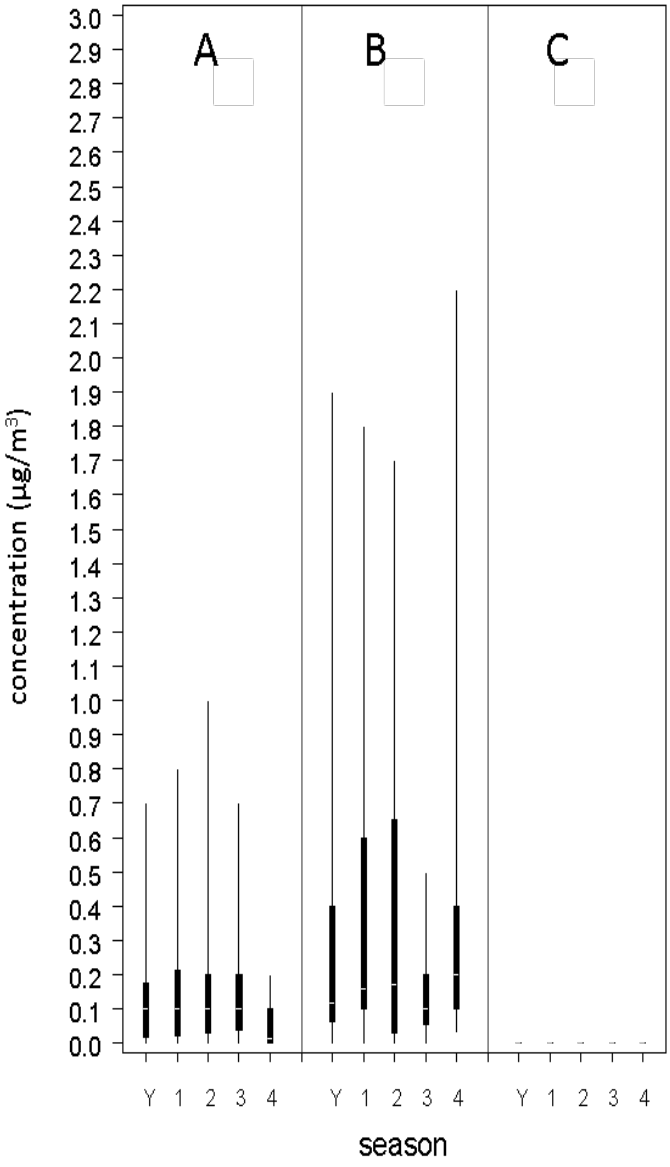


Figure 3A-6. Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Hillsborough and Pinellas Counties, FL (12-057 and 12-103), 2007-2009.

Table 3A-8. 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	1.00	-0.08	
		0.00	1.20	0.50
		0.00	0.71	1.00
B	Source		1.00	
			0.00	2.20
		Legend	0.00	1.00
C	Non-source	ρ		1.00
		P90		0.00
		COD		0.00

Figure 3A-7 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/yr (U.S. EPA, 2008a). Monitor A was also sited in the median of I-90/I-94. Monitor B was located on the northern roadside of I-290 and was within 10 km of 2 Pb sources (0.41 and 1.08 tons/yr) (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/yr 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 m west of 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 I-294 but not located on an arterial road; it was 9 km southeast of a 0.56 tons/yr source (U.S. EPA, 2008a). Monitor F was sited in the parking lot of a water pumping station, 100 m north of I-90 and 300 m northwest of the junction between I-90 and I-94. This site was 2 km north-northwest of a 0.10 tons/yr 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 m south of a rail line and over 300 m 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/yr (U.S. EPA, 2008a). Monitor H was sited on the grounds of the Northbrook Water Plant. I-94 curves around this site and was approximately 700 m from the monitor to the east and around to the north. Figure 3A-8 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 3A-9 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 3.2.2.5. 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 m from the closest interstate and 570 m from the closest runway at O'Hare International Airport. However, the average concentration at this site was 0.012 $\mu g/m^3$, and 85% of data were below the MDL. In contrast, 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 3A-9). 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 [$\rho = 0.26$]). 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 correlation occurred between monitors (D and H [$\rho =$

0.53]). 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 [$\rho = 0.46$]) 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 ($\rho = 0.36$ to 0.45), and the correlation between monitors (E and G) was $\rho = 0.40$. 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. 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.

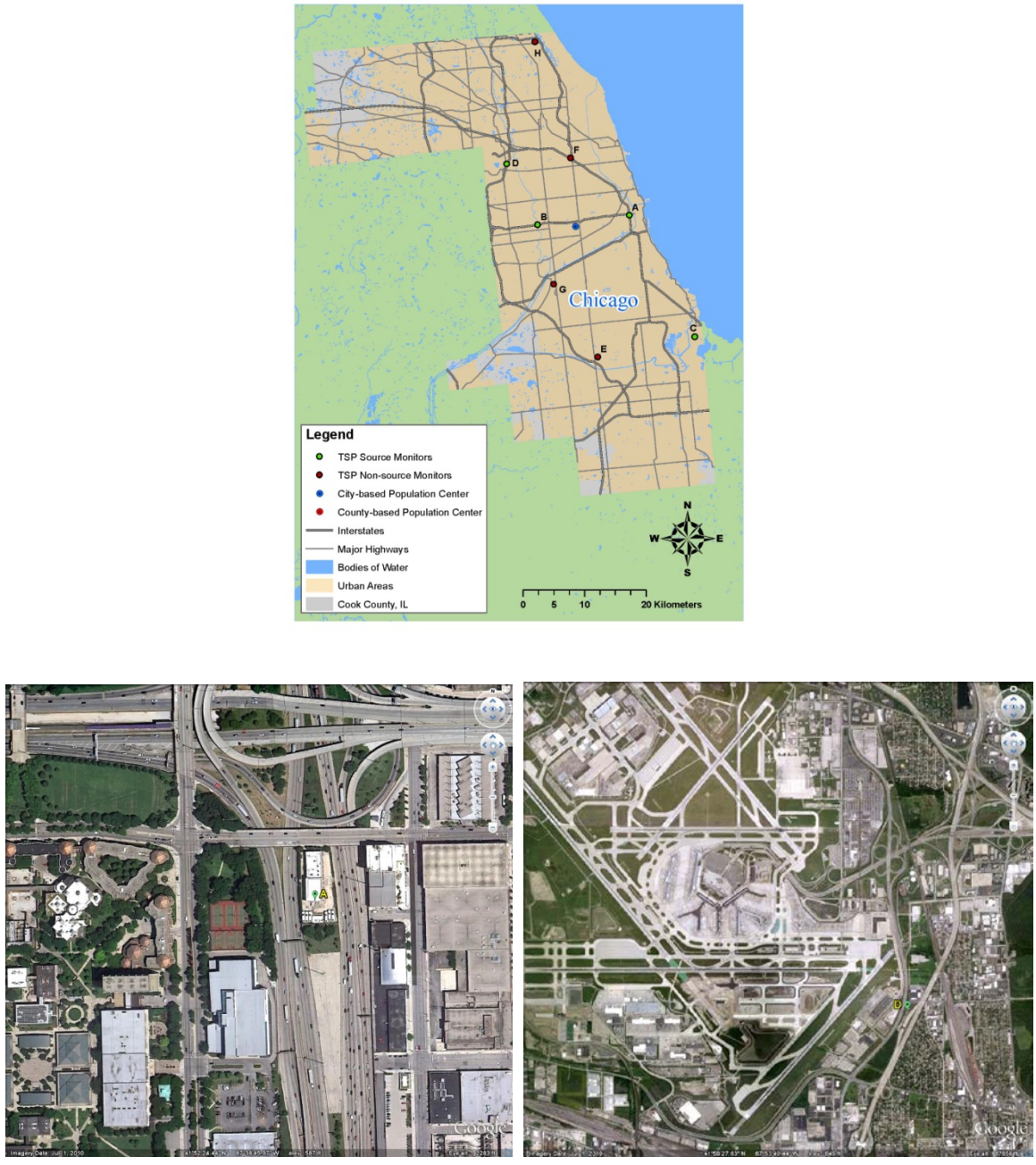
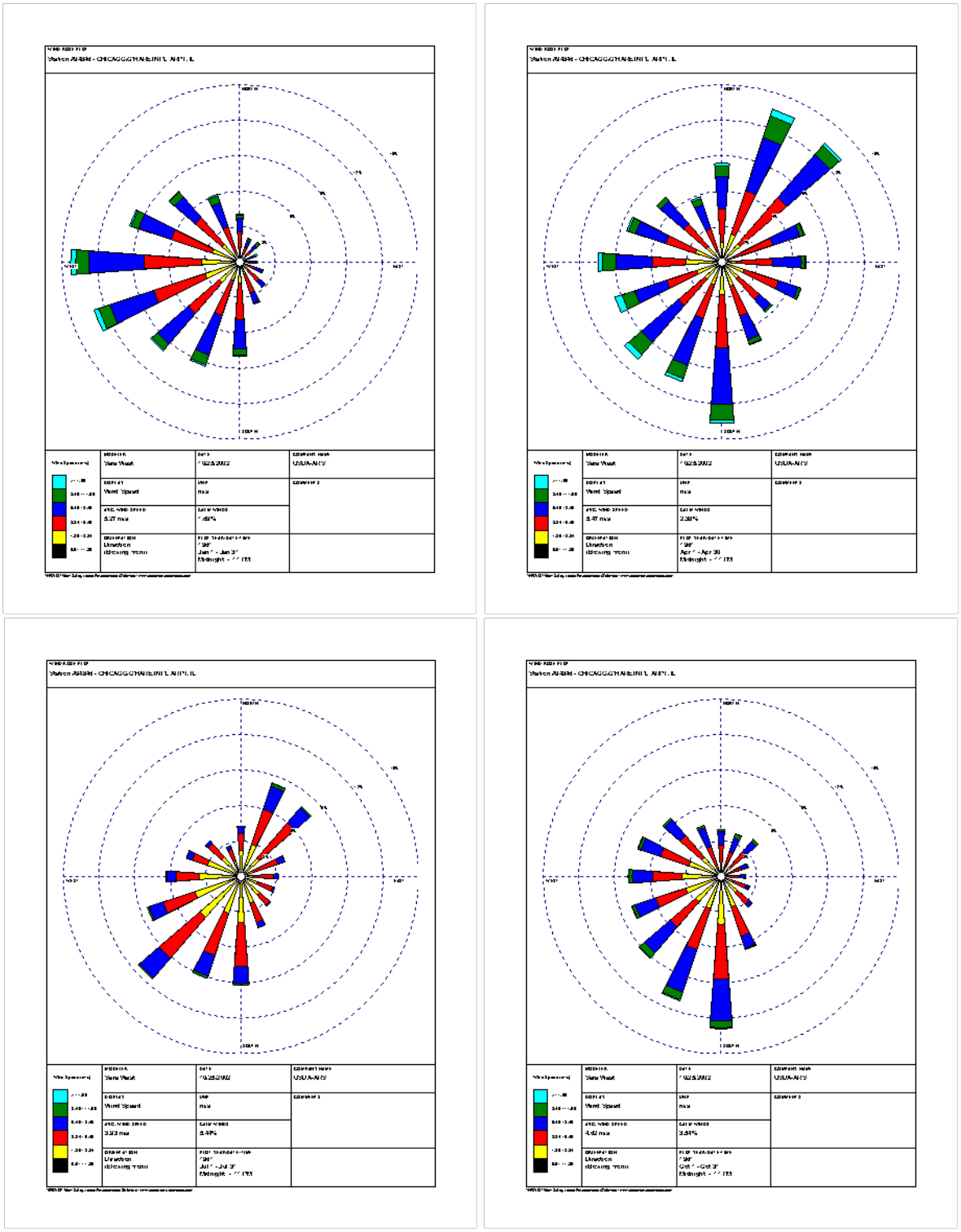


Figure 3A-7. Pb TSP Monitor locations within Cook County, IL (17-031), 2007-2009.Top: view of all Pb FRM monitors in Cook County. Bottom left: Close up of the high traffic site around monitor A. Bottom right: Close up of O'Hare International Airport adjacent to monitor D.



Source: NRCS (2011)

Figure 3A-8. Wind roses for Cook County, IL, obtained from meteorological data at O'Hare International Airport, 1961-1990.Clockwise from the top left: January, April, July, and October. Note that the wind percentages vary from month to month.

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

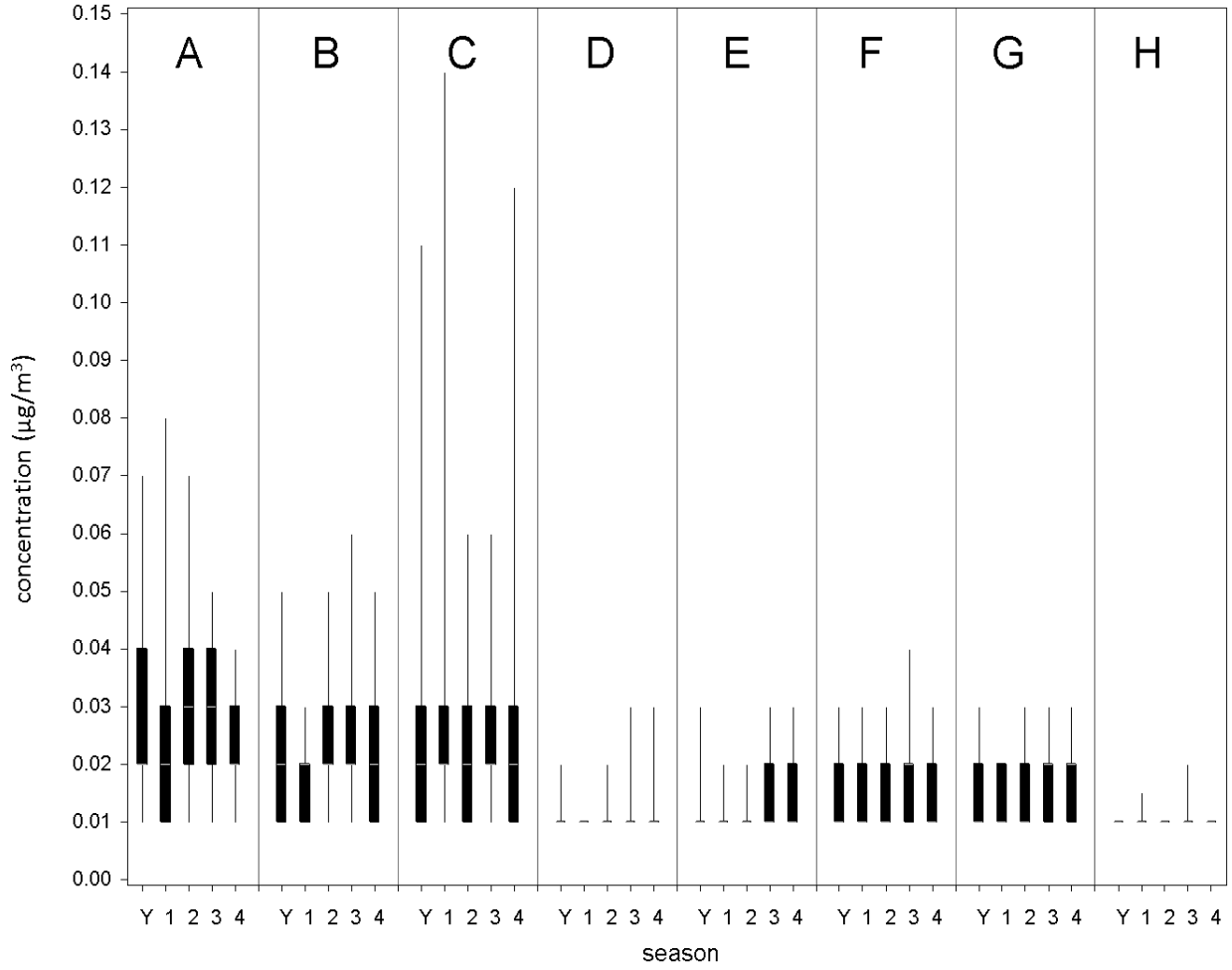


Figure 3A-9. Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Cook County, IL (17-031), 2007-2009.

Table 3A-9. 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	1.00	0.26	-0.01	0.08	0.06	0.32	0.18	0.06
		0.00	0.03	0.06	0.04	0.04	0.03	0.03	0.04
		0.00	0.29	0.38	0.43	0.41	0.36	0.36	0.45
B	Source		1.00	0.05	0.10	0.32	0.46	0.35	-0.01
			0.00	0.04	0.03	0.03	0.02	0.02	0.03
			0.00	0.33	0.36	0.34	0.29	0.30	0.40
C	Source			1.00	0.04	0.16	0.10	0.17	0.06
				0.00	0.05	0.05	0.04	0.05	0.05
				0.00	0.40	0.39	0.35	0.35	0.42
D	Source				1.00	0.21	0.37	0.07	0.53
					0.00	0.01	0.01	0.02	0.01
					0.00	0.19	0.24	0.28	0.15
E	Non-Source					1.00	0.36	0.40	0.07
						0.00	0.02	0.01	0.01
			Legend			0.00	0.24	0.24	0.20
F	Non-Source		ρ				1.00	0.41	0.45
			P90				0.00	0.01	0.02
			COD				0.00	0.24	0.26
G	Non-Source							1.00	0.05
								0.00	0.02
								0.00	0.27
H	Non-Source								1.00
									0.00
									0.00

Figure 3A-10 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 m west of the nearest building. Monitors A, B, C, D, F, G, and H were all located approximately 200 m west of the facility. Monitors D, E, and H were situated alongside service roads to the facility. Monitor I was sited 100 m north of the smelter, and monitor J was located approximately 600 m northwest of the facility. The Doe Run smelter was the only active primary smelter in the U.S. at the time of this review (2007-2009), and the facility was estimated to have emitted 41.1 tons Pb/yr (U.S. EPA, 2008d). Figure 3A-11 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 3A-12 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 emissions measured at the closer monitors were due to either fugitive emissions from the plant or, for the case where ground equipment or vehicles are operated nearby, that 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 3A-10). Correlations range from $\rho = -0.04$ to 0.96. High correlations ($\rho \geq 0.75$) 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 m of each other. For the highest correlation ($\rho = 0.96$, for monitors (E and F), monitor F is 250 m directly east of monitor E. Low correlation ($\rho \leq 0.25$) 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 pollutant to these sites concurrently with the signal recorded by the other monitors.

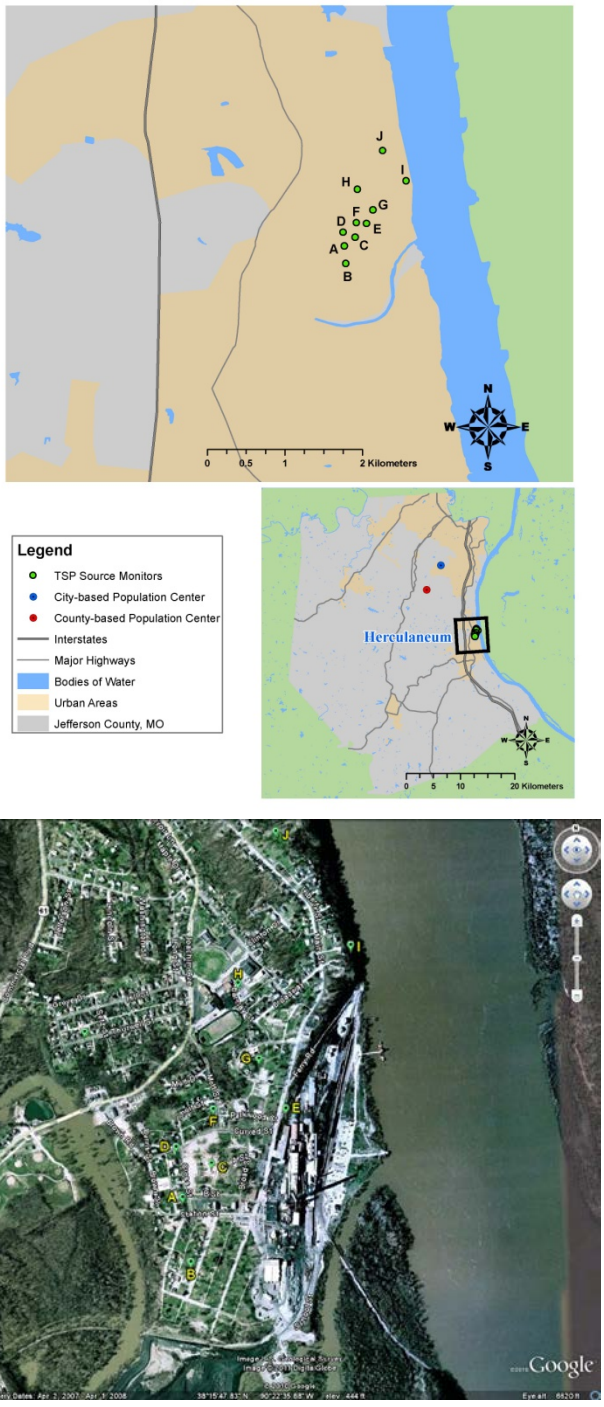
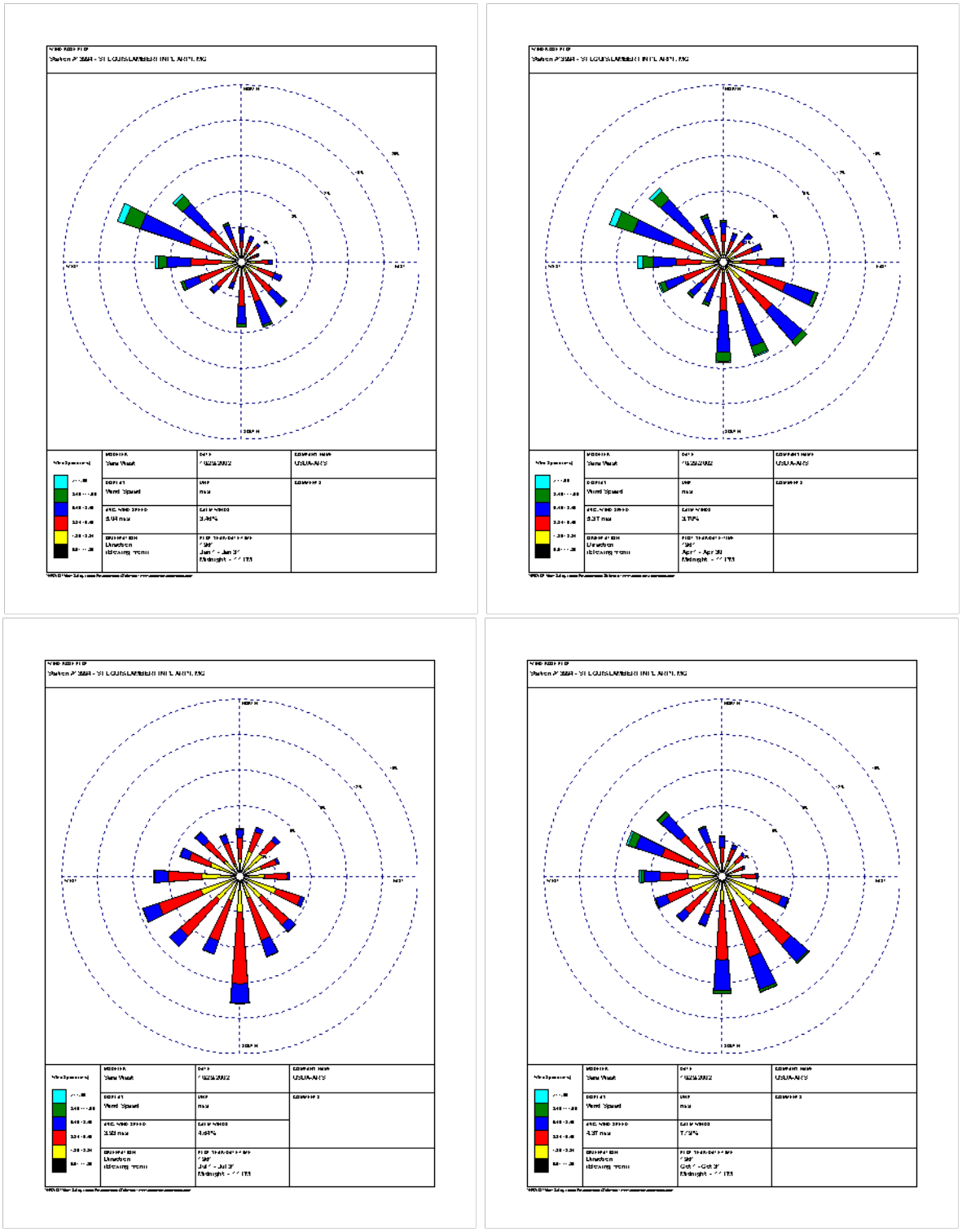


Figure 3A-10. Pb TSP Monitor locations within Jefferson County, MO (29-099), 2007-2009.Note that all monitors surround the Doe Run industrial facility. Top: Map view of all monitors in Jefferson County. Bottom: Satellite view of the monitors and the Doe Run facility.



Source: NRCS (2011)

Figure 3A-11. Wind roses for Jefferson County, MO, obtained from meteorological data at St. Louis/Lambert International Airport, 1961-1990.Clockwise from top left: January, April, July, and October. Note wind percentages vary from month to month.

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

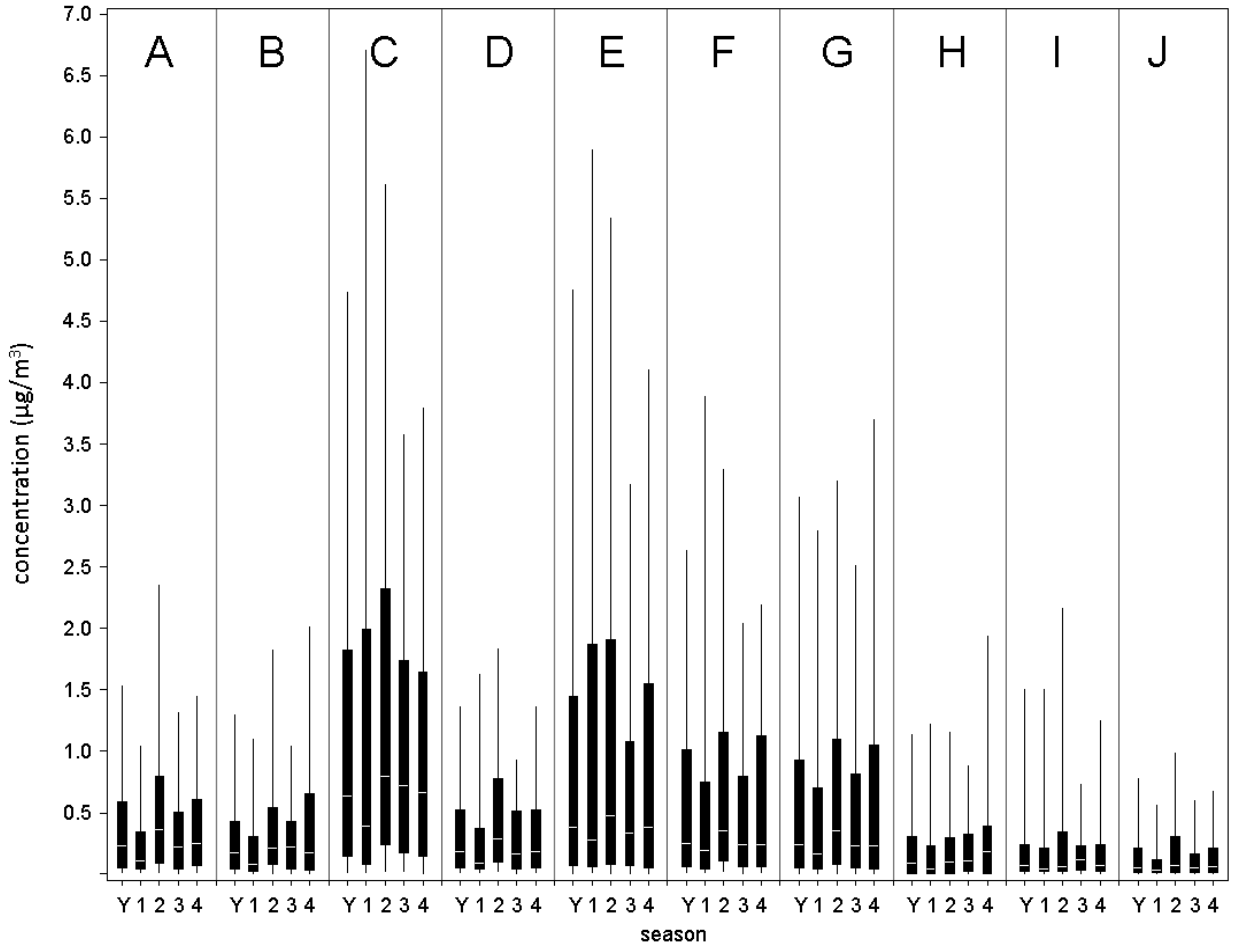


Figure 3A-12. Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Jefferson County, MO (29-099), 2007-2009.

Table 3A-10. 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	1.00	0.59	0.80	0.83	0.57	0.64	0.33	0.35	0.07	0.05
		0.00	0.71	1.55	0.42	1.93	1.14	1.41	0.74	0.92	0.78
		0.00	0.46	0.48	0.30	0.55	0.45	0.57	0.64	0.67	0.69
B	Source		1.00	0.53	0.43	0.10	0.14	0.07	0.22	0.10	0.09
			0.00	1.86	0.87	2.77	1.96	2.08	0.94	1.04	0.91
			0.00	0.58	0.51	0.69	0.62	0.68	0.68	0.65	0.65
C	Source			1.00	0.86	0.59	0.72	0.26	0.27	-0.04	0.04
				0.00	1.56	2.26	1.26	2.94	2.65	3.18	2.60
				0.00	0.50	0.50	0.46	0.60	0.74	0.73	0.73
D	Source				1.00	0.71	0.80	0.41	0.56	0.14	0.18
					0.00	1.83	1.02	1.38	0.76	0.88	0.70
					0.00	0.50	0.36	0.53	0.61	0.63	0.66
E	Source					1.00	0.96	0.54	0.46	0.06	0.16
						0.00	0.86	2.16	2.50	3.09	2.57
			Legend			0.00	0.35	0.49	0.66	0.70	0.72
F	Source		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							1.00	0.87	0.28	0.38
								0.00	1.53	2.10	2.08
								0.00	0.61	0.63	0.66
H	Source								1.00	0.20	0.30
									0.00	0.89	0.56
									0.00	0.67	0.65
I	Source									1.00	0.79
										0.00	0.62
										0.00	0.48
J	Source										1.00
											0.00
											0.00

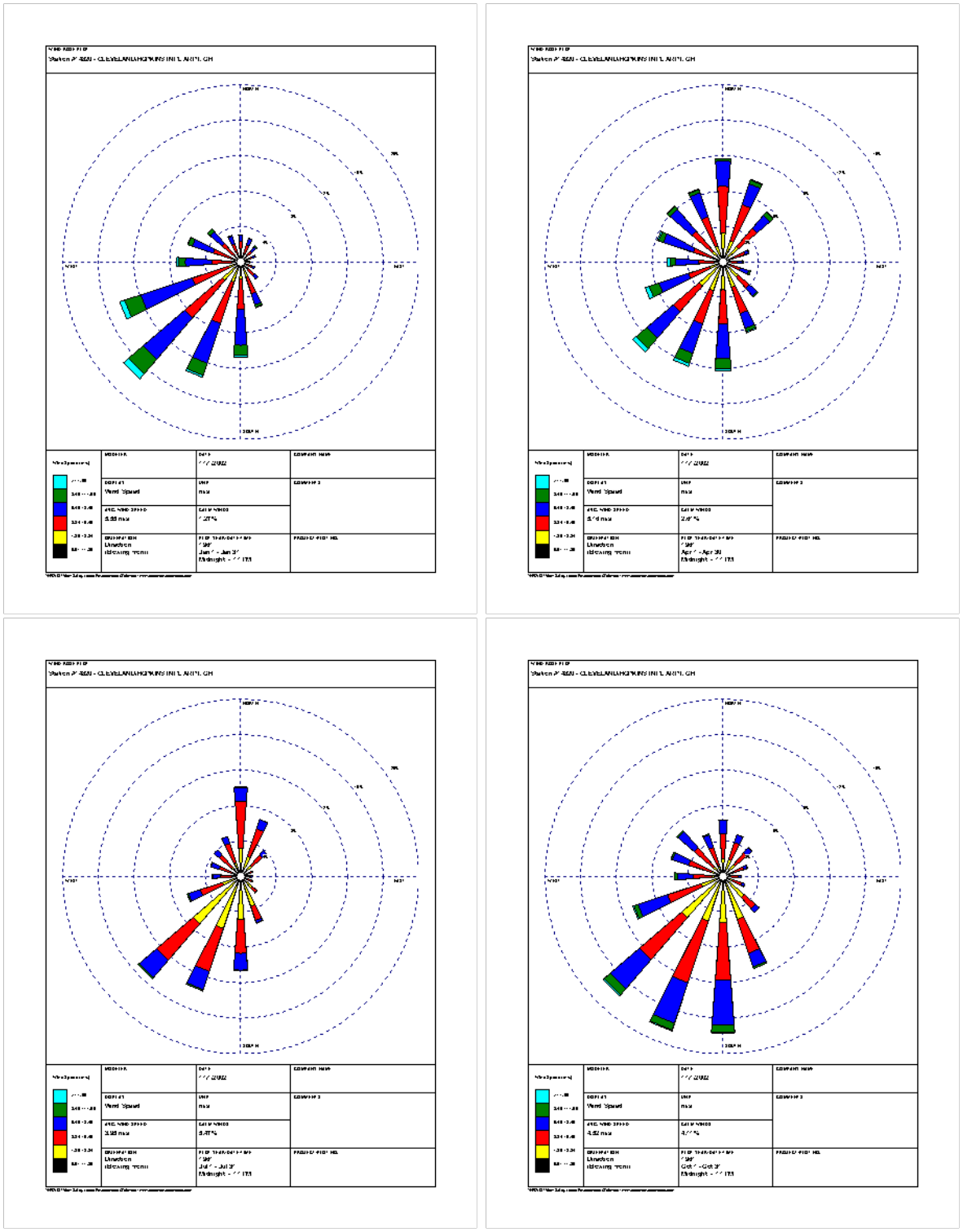
Figure 3A-13 illustrates Pb monitor locations with Cuyahoga County, OH. Five monitors are located within Cuyahoga County, three of which were designated by the Ohio EPA (OEPA) as source-oriented and the other two were non-source-oriented monitors. Monitors A, B, and C were all located within 1-10 km of six 0.1 tons/yr source facilities and one 0.2 tons/yr source (U.S. EPA, 2008e). Additionally, monitor B was located 30 m 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, 2008e). Monitor A was sited roughly 300 m south of the Ferro Corporation facility. Monitor C was located 2.2 km west-northwest of the 0.5 ton/yr Victory White Metal

Co. facility. Monitor C was also roughly 250 m southeast of I-490. Monitors D and E were designated as non-source-oriented monitors, although monitor D was just 600 m further from the Victory White Metal facility than was monitor C. Monitor D was sited on a residential street located 50 m north of I-490. Monitor E was located on the rooftop of a building within 20 m of a four-lane arterial road. Figure 3A-14 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 3A-15 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 $\mu g/m^3$). 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 $\mu g/m^3$. Concentration data from all other monitors were below the level of the NAAQS. For monitor A, the average concentration was 0.025 $\mu g/m^3$, and the median reached 0.04 $\mu g/m^3$ during the summer. Maximum concentration at this monitor was 0.07 $\mu g/m^3$. Concentrations at monitor C averaged 0.017 $\mu g/m^3$, and those at monitors D and E averaged 0.014 $\mu g/m^3$ and 0.013 $\mu g/m^3$, respectively. Maximum concentrations reached 0.04 $\mu g/m^3$ at all three monitors.

The level of spatial variability is illustrated by the intersampler correlations presented in Table 3A-11. Monitors A and B appear to be anticorrelated ($\rho = -0.13$). 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 well with each other ($\rho = 0.67$ 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.

Figure 3A-13. Pb TSP Monitor locations within Cuyahoga County, OH (39-035), 2007-2009.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 I-490.



Source: NRCS (2011)

Figure 3A-14. Wind roses for Cuyahoga County, OH, obtained from meteorological data at Cleveland/Hopkins International Airport, 1961-90.Clockwise from top left: Jan, April, July, and October. Note wind percentages vary from month to month.

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

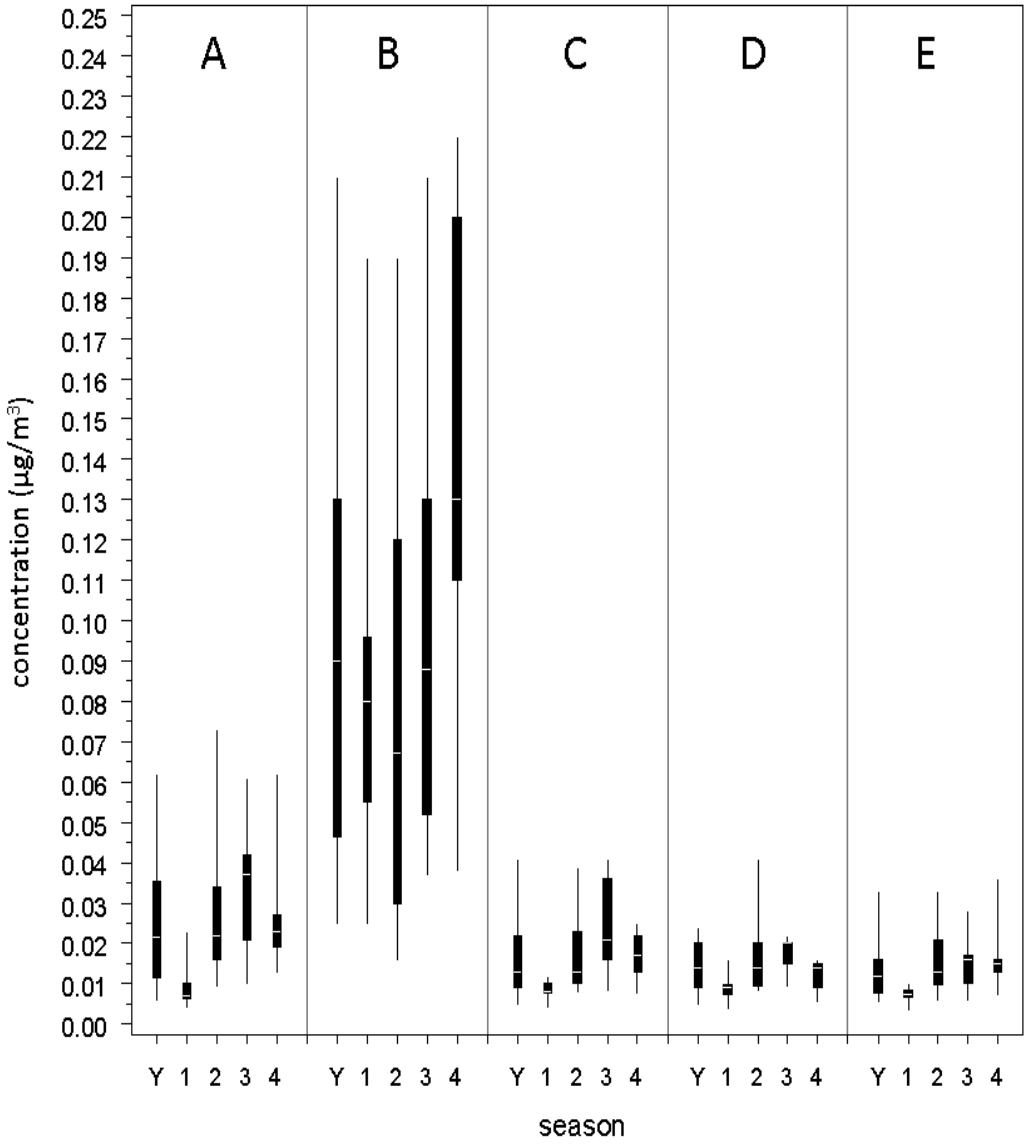


Figure 3A-15. Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Cuyahoga County, OH (39-035), 2007-2009.

Table 3A-11. 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	1.00	-0.13	0.24	0.19	0.21
		0.00	0.18	0.05	0.04	0.05
		0.00	0.64	0.33	0.35	0.37
B	Source		1.00	0.31	0.24	0.34
			0.00	0.18	0.19	0.19
			0.00	0.69	0.71	0.73
C	Source			1.00	0.77	0.67
				0.00	0.01	0.01
		Legend		0.00	0.17	0.18
D	Non-Source	ρ			1.00	0.67
		P90			0.00	0.01
		COD			0.00	0.17
E	Non-Source					1.00
						0.00
						0.00

Figure 3A-16 illustrates Pb monitor locations within Sullivan County, TN. Three source-oriented monitors were situated around an Exide Pb recycling facility emitting 0.78 tons/yr (U.S. EPA, 2008f). Monitors A and C are positioned along the facility's service road and are approximately 100 m and 200 m away from the facility, respectively. Monitor A is directly next to the road, and monitor C is roughly 15 m from the road. Monitor B is located in the facility's parking lot roughly 50 m 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 3A-17 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 3A-18 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. It was surprising that 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 C compared with those from monitor C are consistent with the southwestern winds.

Not surprisingly, the correlations of monitor A with monitors B and C were quite low (Table 3A-12). The correlation between monitors B and C was $\rho = 0.45$. 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.

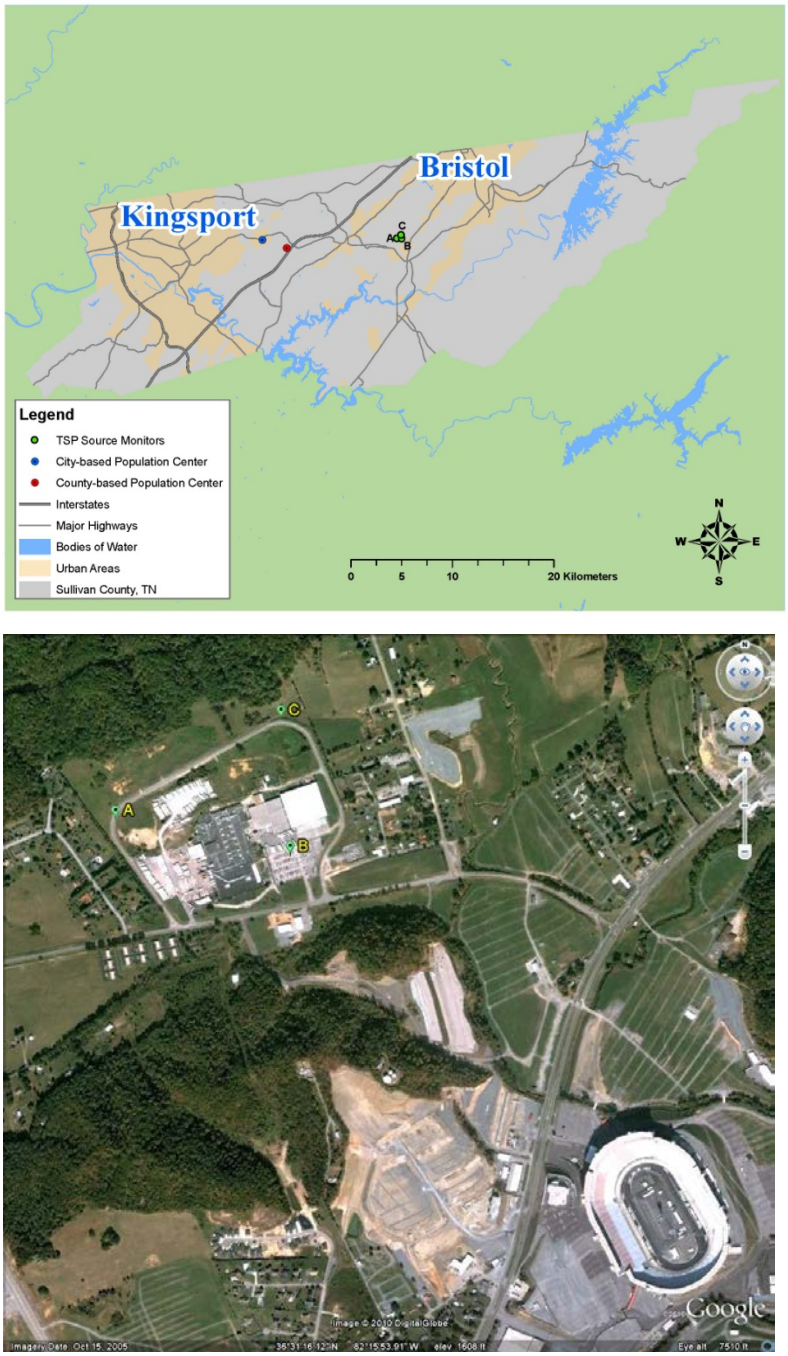
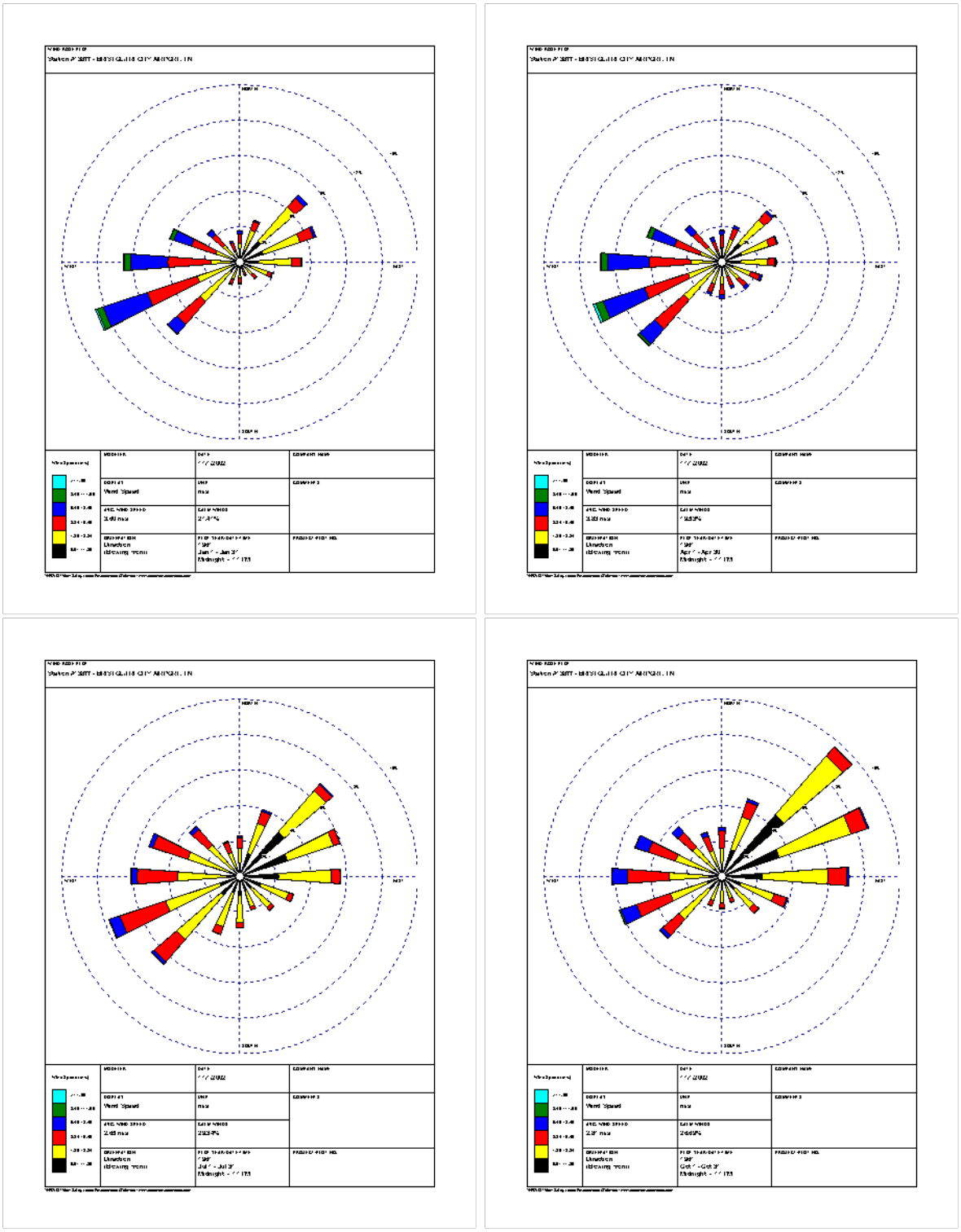


Figure 3A-16. Pb TSP Monitor locations within Sullivan County, TN (47-163), 2007-2009.Top: Map, bottom: Satellite image. Monitors A, B, and C surround the Exide Pb recycling facility. Just to the southeast is the Bristol motor speedway.



Source: NRCS (2011)

Figure 3A-17. Wind roses for Sullivan County, TN, obtained from meteorological data at Bristol/Tri City Airport, 1961-90.Clockwise from top left: January, April, July, and October. Note that the wind percentages vary from month to month.

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

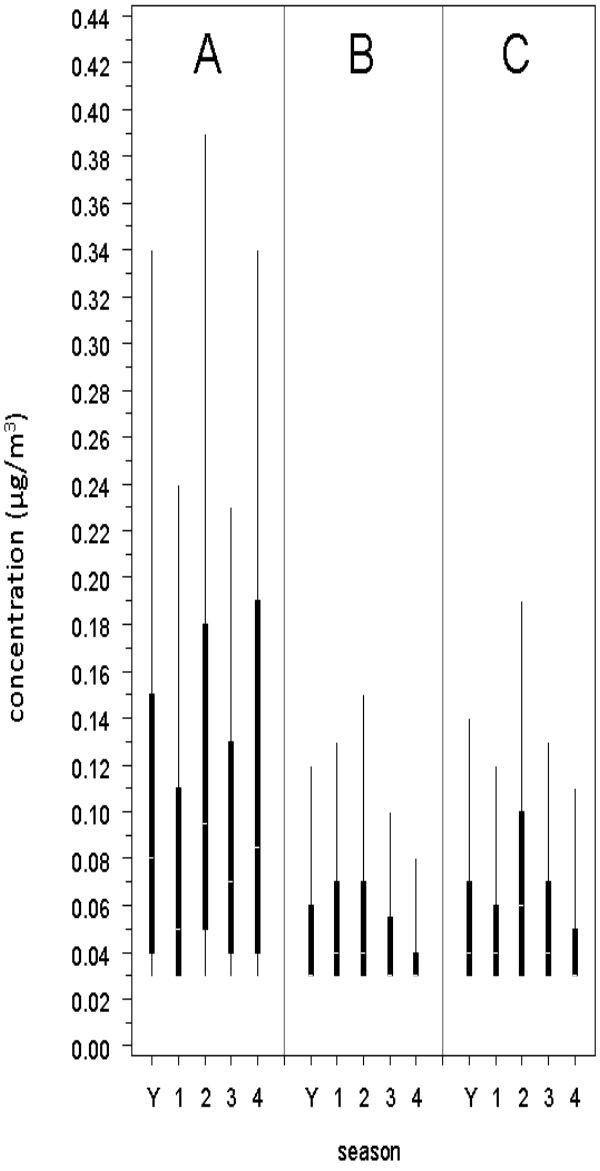


Figure 3A-18. Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Sullivan County, TN (47-163), 2007-2009.

Table 3A-12. Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Sullivan County, TN (47-163), 2007-2009

		A	B	C
		Source	Source	Source
A	Source	1.00	-0.04	0.13
		0.00	0.21	0.19
		0.00	0.47	0.43
B	Source		1.00	0.45
			0.00	0.06
		Legend	0.00	0.23
C	Source	ρ		1.00
		P90		0.00
		COD		0.00

3.8.2. Size Distribution of Pb-Bearing PM

Table 3A-13. Correlations and average of the concentration ratios for co-located monitors, TSP versus PM_{10}, TSP versus $PM_{2.5}$, and PM_{10} versus $PM_{2.5}$.Data are bolded for sites where the TSP, PM_{10}, and $PM_{2.5}$ monitors were co-located for at least one sampling year.

Site	County	Land Type	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio
			PM_{10}:TSP			$PM_{2.5}$:TSP			$PM_{2.5}$: PM_{10}		
010730023	Jefferson, AL	Urban and center city	2005-2006	0.87	0.80	2005-2006	0.74	0.84	2005-2006	0.81	1.02
040139997	Maricopa, AZ	Urban and center city							2006-2009	0.40	0.59
060011001	Alameda, CA	Suburban	1994-1998	0.00	2.31						
060130002	Contra Costa, CA	Suburban	1994-1998	0.27	2.60						
060130003	Contra Costa, CA	Suburban	1994-1997	0.31	1.04						
060190008	Fresno, CA	Suburban	1995-2001	0.92	0.92	2000-2009	0.58	0.66	2000-2001	0.83	1.16
060250005	Imperial, CA	Suburban	1996-2001	0.77	0.91	2002-2009	0.95	0.74			
060290014	Kern, CA	Urban and center city	1995-2000	0.92	0.76	2001-2009	0.22	0.72			
060371103	Los Angeles, CA	Urban and center city				2002-2009	0.22	0.28			
060374002	Los Angeles, CA	Suburban	1995-2000	0.72	0.39						
060550003	Napa, CA	Urban and center city	1994-1994	0.42	0.93						
060658001	Riverside, CA	Suburban	1995-1997	0.27	0.39	2001-2009	0.59	0.40			
060730003	San Diego, CA	Suburban				2001-2009	0.35	0.48			
060750005	San Francisco, CA	Urban and center city	1994-1998	0.29	0.98						
060771002	San Joaquin, CA	Urban and center city	1995-2000	0.73	0.82						
060850004	Santa Clara, CA	Urban and center city	1994-2000	0.32	0.95	2000-2002	0.63	0.63			
060850004	Santa Clara, CA	Urban and center city				2002-2009	0.11	0.78	2008-2009	0.76	1.41
060853001	Santa Clara, CA	Suburban	1994-1998	0.16	1.00						
060990002	Stanislaus, CA	Urban and center city	1995-1998	0.94	0.82						
060990005	Stanislaus, CA	Urban and center city	1998-2000	0.79	1.02						
061112002	Ventura, CA	Suburban				2001-2009	0.47	0.74			

Site	County	Land Type	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio
			PM_{10}:TSP			$PM_{2.5}$:TSP			$PM_{2.5}$: PM_{10}		
080010006	Adams, CO	Suburban				2001-2006	0.16	0.43			
080410011	El Paso, CO	Urban and center city				2001-2006	0.03	0.39			
080770017	Mesa, CO	Urban and center city							2004-2008	0.42	0.87
100032004	New Castle, DE	Urban and center city				2003-2007	0.47	0.44			
110010043	District of Columbia, DC	Urban and center city							2004-2009	0.47	0.73
120571075	Hillsborough, FL	Urban and center city				2001-2003	0.83	1.86			
120573002	Hillsborough, FL	Rural							2004-2009	0.66	1.55
121030026	Pinellas, FL	Suburban							2004-2009	0.56	1.89
130690002	Coffee, GA	Rural				2003-2009	0.82	1.46			
130890002	DeKalb, GA	Suburban							2003-2009	0.92	1.69
150032004	Honolulu, HI	Urban and center city				2003-2009	0.77	2.34			
170314201	Cook, IL	Suburban	2006-2009	0.53	0.39	2002-2009	0.25	0.36	2005-2009	0.65	1.01
180970078	Marion, IN	Suburban				2002-2009	0.75	0.78			
201731012	Sedgwick, KS	Suburban	1993-1997	0.63	0.33						
202090015	Wyandotte, KS	Urban and center city	1993-1997	0.66	0.55						
202090020	Wyandotte, KS	Urban and center city	1993-1997	0.99	0.56						
210430500	Carter, KY	Rural							2008-2009	0.22	1.39
211930003	Perry, KY	Suburban							2003-2008	0.76	1.47
220511001	Jefferson, LA	Suburban							2005-2006	0.96	0.79
220710010	Orleans, LA	Urban and center city							2005-2006	0.94	0.64
220710012	Orleans, LA	Urban and center city							2005-2006	0.91	0.53
220718105	Orleans, LA	Urban and center city							2005-2006	0.98	0.80
220718401	Orleans, LA	Urban and center city							2005-2006	0.89	0.45
220758400	Plaquemines, LA	Urban and center city							2005-2005	0.98	0.83
220870004	St. Bernard, LA	Suburban							2005-2006	0.94	0.78
220878103	St. Bernard, LA	Urban and center city							2005-2006	0.90	0.66
250250042	Suffolk, MA	Urban and center city				2009-2009	0.43	0.37	2003-2009	0.38	0.78
260770905	Kalamazoo, MI	Urban and center city	1993-1996	0.98	0.77						
260810020	Kent, MI	Urban and center city				2005-2007	0.58	0.70			
261130001	Missaukee, MI	Rural				2002-2007	0.73	1.38			
261250010	Oakland, MI	Urban and center city				2001-2002	0.40	1.60			
261390009	Ottawa, MI	Urban and center city	2000-2001	0.90	0.67						
261610008	Washtenaw, MI	Urban and center city				2003-2007	0.58	0.71			
261630001	Wayne, MI	Suburban				2001-2007	0.68	0.58			
261630019	Wayne, MI	Suburban				2001-2002	0.45	0.51			
261630033	Wayne, MI	Suburban	2003-2009	0.87	0.84	2002-2009	0.84	0.49	2003-2009	0.86	0.61
270530053	Hennepin, MN	Urban and center city	1996-2001	0.47	0.55						
270530963	Hennepin, MN	Urban and center city				2002-2009	0.64	0.42			
280458104	Hancock, MS	Suburban							2005-2006	0.93	1.51
280458105	Hancock, MS	Rural							2005-2006	0.99	1.02
280458201	Hancock, MS	Suburban							2005-2006	0.94	1.05
280470008	Harrison, MS	Rural							2005-2006	0.87	0.99
280478101	Harrison, MS	Suburban							2005-2006	0.98	0.99
280478102	Harrison, MS	Suburban							2005-2006	0.80	1.10
280478103	Harrison, MS	Suburban							2005-2006	0.93	1.09
280590006	Jackson, MS	Urban and center city							2005-2006	0.97	0.78
295100085	St. Louis City, MO	Urban and center city	2004-2004	0.96	1.10	2004-2004	0.26	1.08	2003-2009	0.76	0.76
330110020	Hillsborough, NH	Urban and center city							2002-2005	0.42	0.81

Site	County	Land Type	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio
			PM_{10}:TSP			$PM_{2.5}$:TSP			$PM_{2.5}$: PM_{10}		
330150014	Rockingham, NH	Urban and center city							2003-2005	0.80	0.72
350010023	Bernalillo, NM	Urban and center city				2004-2009	0.04	0.73			
360050110	Bronx, NY	Urban and center city							2007-2009	0.80	0.60
360551007	Monroe, NY	Urban and center city							2007-2009	0.77	1.30
360610062	New York, NY	Urban and center city							2005-2005	0.96	0.57
360632008	Niagara, NY	Suburban							2005-2005	0.74	0.84
401091037	Oklahoma, OK	Suburban				2009-2009	0.25	1.57			
401431127	Tulsa, OK	Urban and center city				2009-2009	0.34	0.74			
410390060	Lane, OR	Urban and center city				2002-2004	0.33	1.65			
410510246	Multnomah, OR	Urban and center city				2002-2003	0.90	0.90			
410290133	Jackson, OR	Urban and center city							2009-2009	0.54	1.10
410390060	Lane, OR	Urban and center city							2004-2009	0.76	2.15
410510246	Multnomah, OR	Urban and center city							2003-2006	0.98	0.99
410610119	Union, OR	Urban and center city							2004-2007	0.15	1.60
420450002	Delaware, PA	Urban and center city				2002-2008	0.04	0.11			
420710007	Lancaster, PA	Suburban				2004-2007	0.78	0.80			
421010004	Philadelphia, PA	Urban and center city				2000-2007	0.37	0.38			
421010055	Philadelphia, PA	Urban and center city				2005-2007	0.36	0.32			
421010136	Philadelphia, PA	Urban and center city				2004-2005	0.75	0.38			
440070022	Providence, RI	Urban and center city	2001-2002	0.78	0.54				2002-2009	1.00	0.70
440071010	Providence, RI	Suburban	2001-2002	0.98	0.71						
450250001	Chesterfield, SC	Rural				2002-2009	0.37	1.76	2004-2009	0.41	1.63
450790019	Richland, SC	Urban and center city				2001-2006	0.74	0.58			
470370023	Davidson, TN	Urban and center city				2003-2004	0.39	0.83			
482011034	Harris, TX	Urban and center city				2002-2005	0.41	0.40			
482011039	Harris, TX	Suburban							2000-2009	0.34	0.63
490110004	Davis, UT	Suburban							2003-2009	0.68	0.91
500070007	Chittenden, VT	Rural							2004-2009	0.89	0.97
510870014	Henrico, VA	Suburban				2004-2008	0.54	0.79	2008-2009	0.91	1.08
530330038	King, WA	Suburban				2001-2002	0.64	0.53			
530330080	King, WA	Urban and center city							2003-2009	0.91	0.81
530630016	Spokane, WA	Suburban							2005-2005	0.77	0.91
550270007	Dodge, WI	Rural				2004-2005	0.90	0.83	2005-2009	0.59	1.02

1

3.8.3. Lead Concentration in a Multipollutant Context

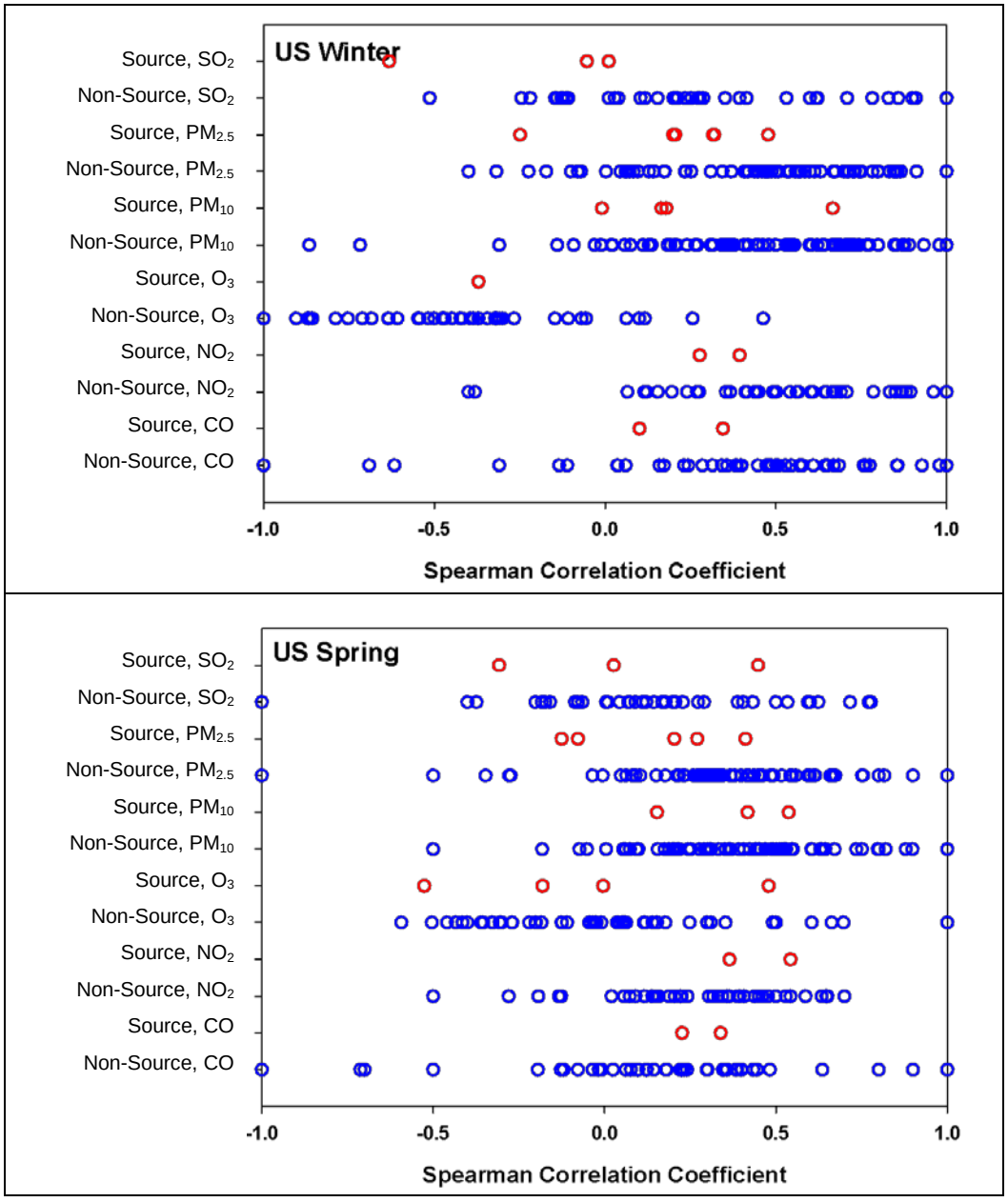


Figure 3A-19a. Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2007-2008. Top: winter; bottom: spring.

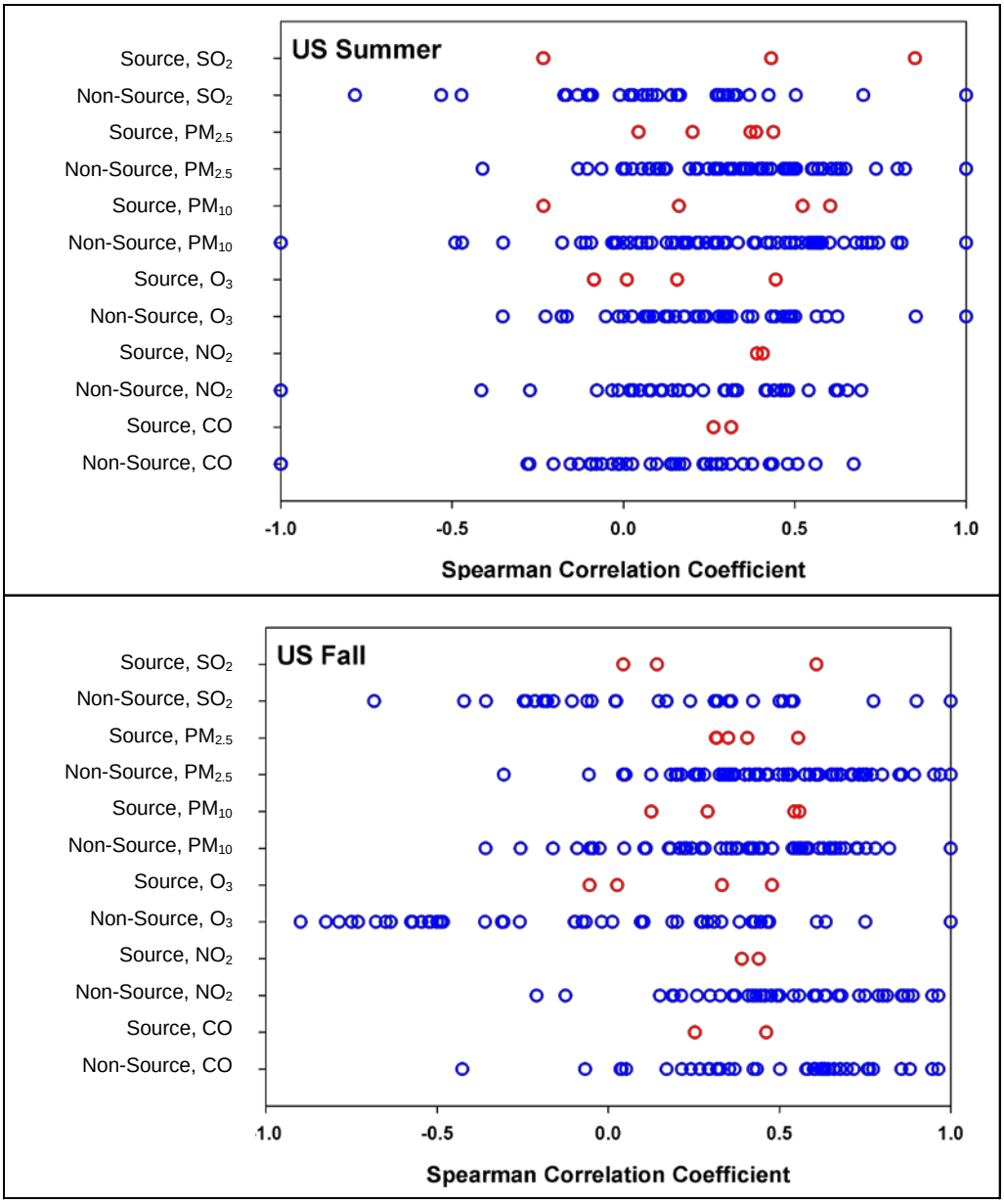


Figure 3A-19b. Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2007-2008. Top: summer; bottom: fall.

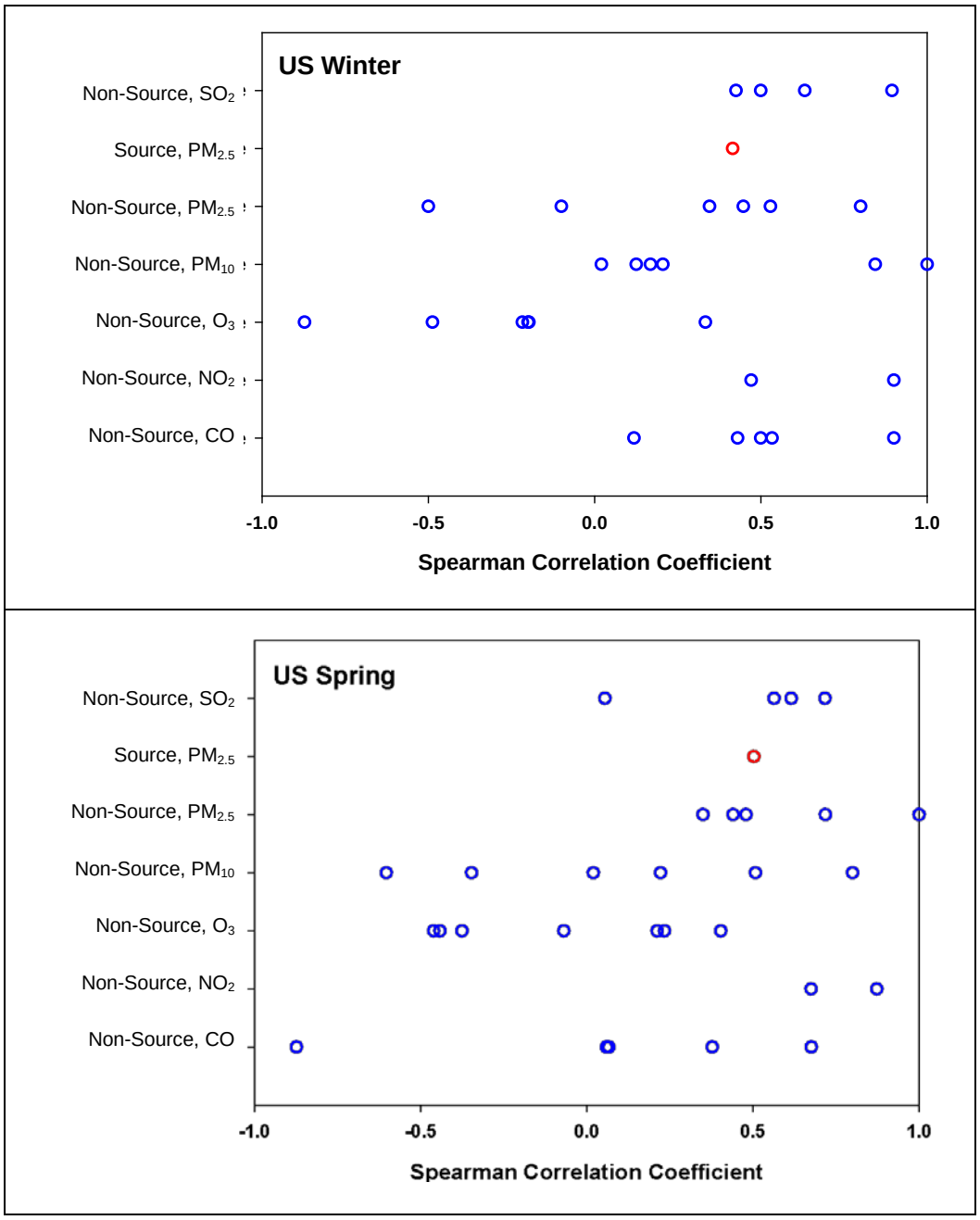


Figure 3A-20a. Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2009. Top: winter; bottom: spring.

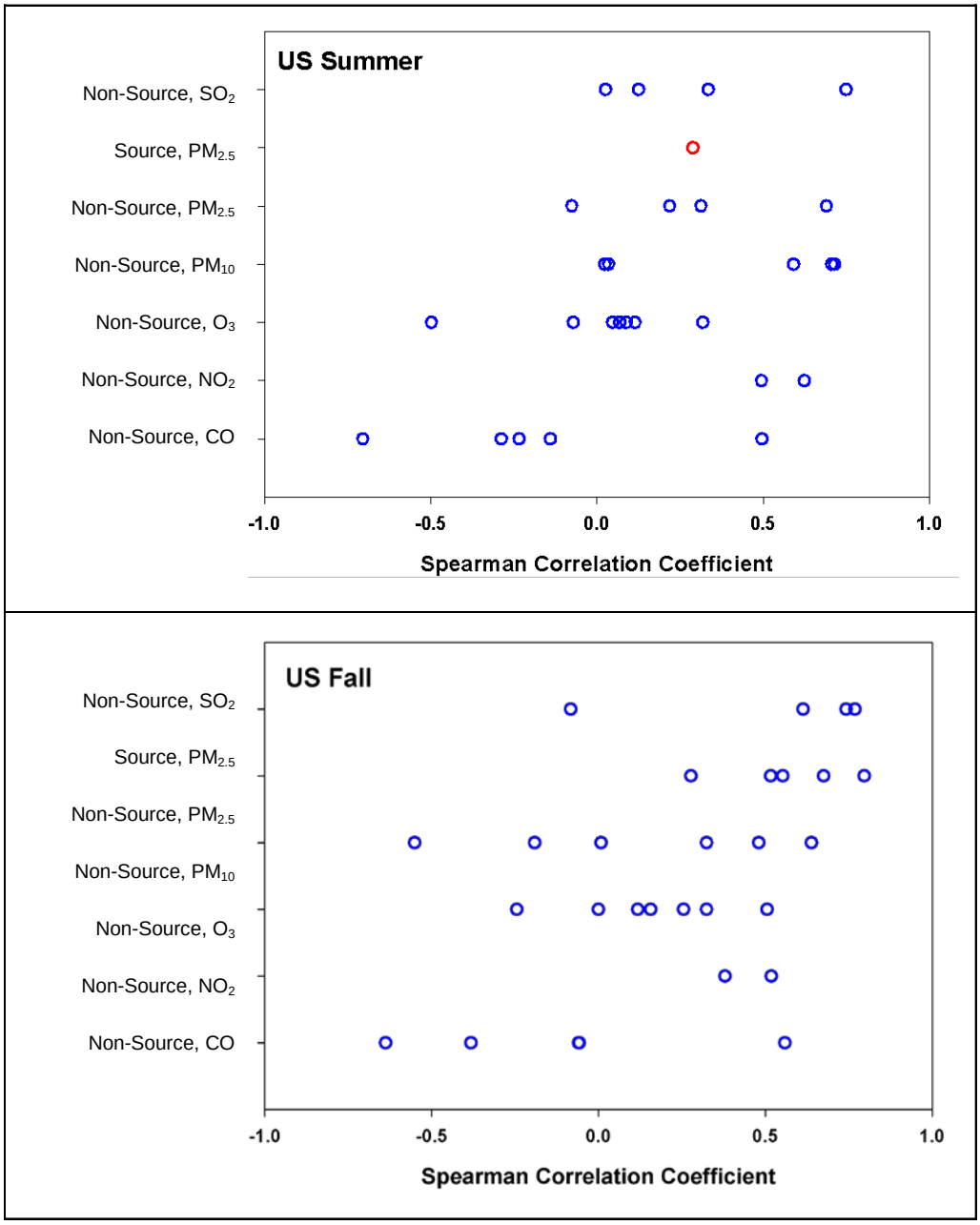


Figure 3A-20b. Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2009. Top: summer; bottom: fall.

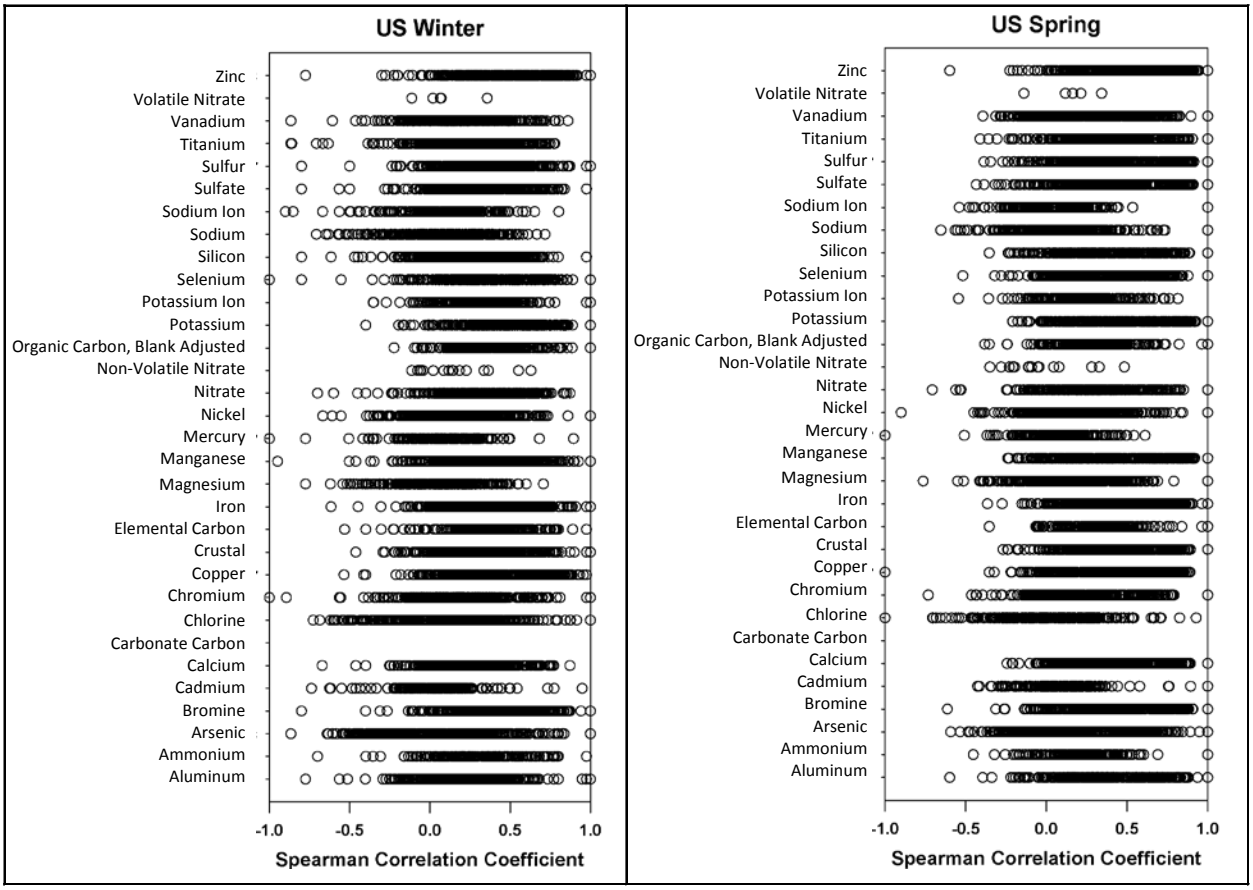


Figure 3A-21a. Seasonal correlations of monitored Pb-$PM_{2.5}$ concentration with copollutant concentrations, 2007-2009. Left: winter; right: spring.

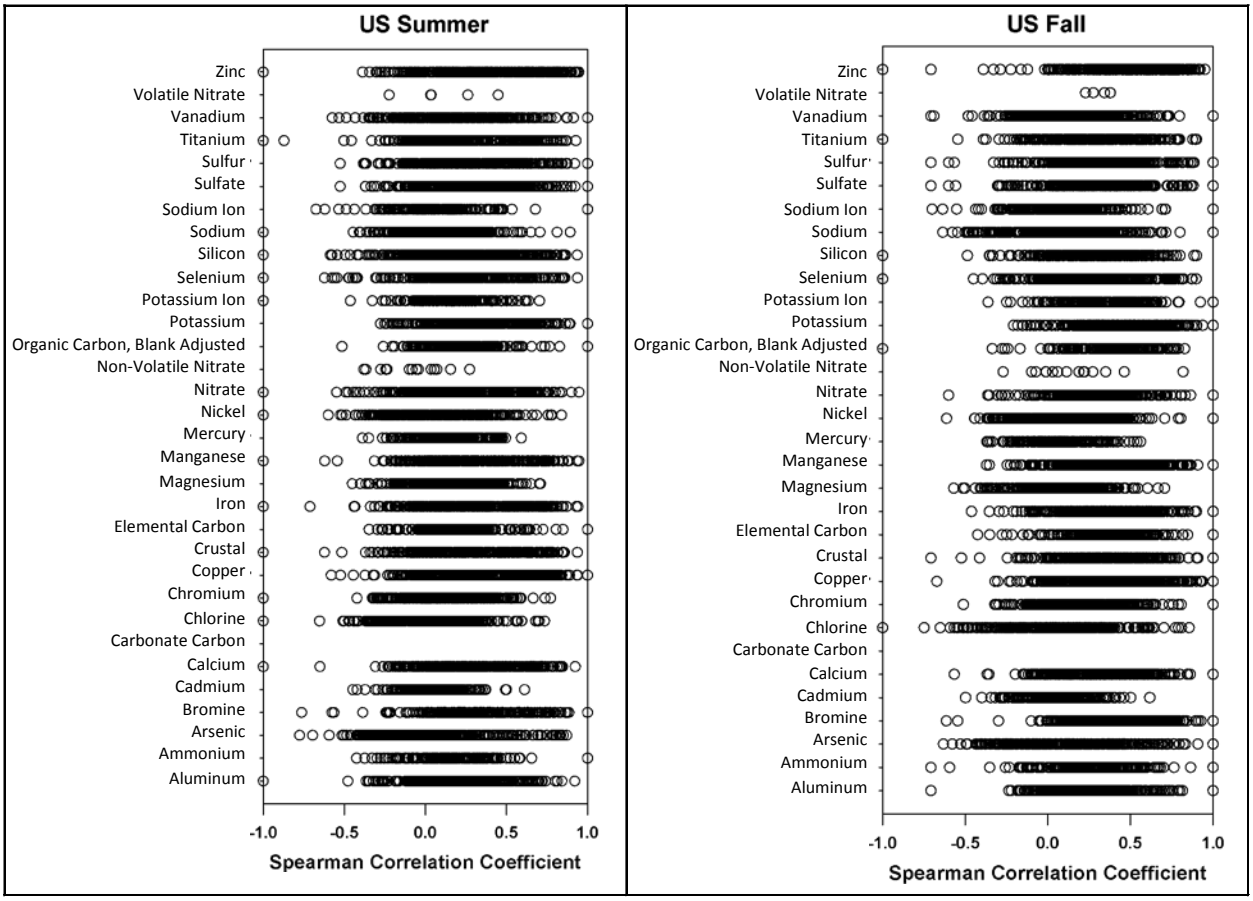


Figure 3A-21b. Seasonal correlations of monitored Pb-$PM_{2.5}$ concentration with copollutant concentrations, 2007-2009. Left: summer; right: fall.

Table 3A-14. 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		New Jersey		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	905-1592	1416-2231	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						
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

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Chapter 4. Exposure, Toxicokinetics, and Biomarkers

4.1. Exposure Assessment

The purpose of this section is to present recent studies that provide insight about human exposure to Pb through various pathways. The recent information provided here builds upon the conclusions of the 2006 Pb AQCD (2006), which found that air Pb concentrations and blood Pb levels have decreased substantially following the 1996 ban on Pb in on-road vehicle gasoline, the 1978 ban on Pb in household paints, and the 1986 and 1995 restrictions on uses of Pb solder. At the same time, 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, 2006) 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 ages 1-5 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. For the current review, Section 4.1. contains a description of studies related to pathways for human exposure to Pb.

4.1.1. Pathways for Lead Exposure

Pathways of Pb exposure are difficult to assess because Pb has multiple sources in the environment and passes through various environmental media. These issues are described in detail in Sections 3.2 and 3.3. Air-related pathways of Pb exposure are the focus of this ISA. Pb can be emitted to air or water. 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, and a fraction of that resuspended Pb may even originate from waters used to irrigate the soil. Additionally, Pb-bearing PM can be deposited from the air to soil or water through wet and dry deposition. In general, air-related pathways include those pathways where Pb passes through ambient air on its path from a source to human exposure. Air-related Pb exposures include inhalation and ingestion of Pb-contaminated food, water or other materials including dust and soil. Non-air-related exposures include ingestion of indoor Pb paint, Pb in diet as a result of inadvertent additions during food

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

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 Figure 4-1, 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 the Pb passed through the air compartment. When animals consume plant material exposed to Pb that has at some point passed through the air compartment, and when human diet includes animals and/or plants 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 4-1 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.

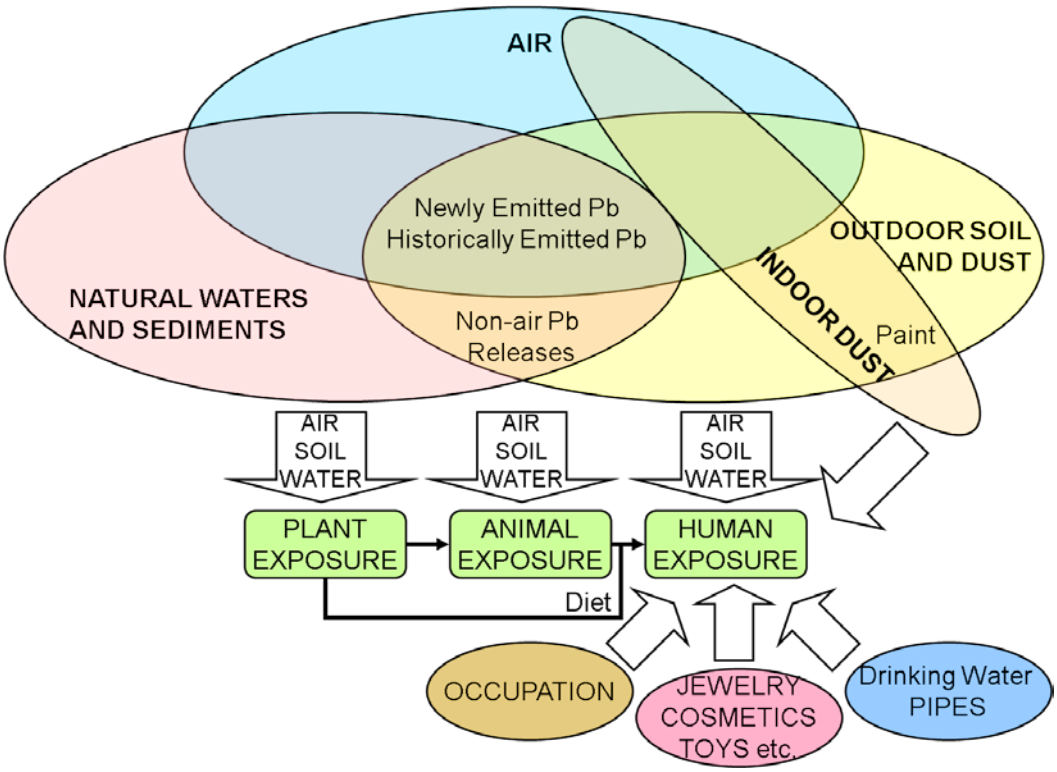


Figure 4-1. Conceptual model of multimedia Pb exposure.The Venn diagram is used to illustrate the passage of Pb through multiple environmental media compartments through which exposure can occur.

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 susceptibility factors (described in more detail in 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 (Table 4-1). It is plausible that local resuspension of Pb-containing dust due to human activity increased personal exposure concentrations of airborne Pb relative to indoor or outdoor air Pb concentrations. 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.

Table 4-1. Estimates of Pb measurements for EPA Region 5 from the NHEXAS study.

Medium[a]	n	Percentage measurable[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)
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 measurable, 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 measurable is the 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: Used with permission from Nature Publishing Group, Clayton et al. (1999)

4.1.1.1. Airborne Lead Exposure

Limited personal exposure monitoring data for airborne Pb were available for the 2006 AQCD (U.S. EPA, 2006). 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 shown to be moderately correlated with floor dust and residential yard soil Pb concentration (M. 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, homes built before 1950, and frequency of fireplace usage (Table 4-2).

Table 4-2. 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: Used with permission from Nature Publishing Group, Egeghy et al. (2005).

Some recent studies have measured personal exposure to particle-bound Pb along with other trace metals. Adgate et al. (2007) measured the concentrations of several trace elements in personal, indoor, and outdoor samples of $PM_{2.5}$ and found that average personal Pb-$PM_{2.5}$ concentration was roughly three times higher than outdoor Pb-$PM_{2.5}$ concentration and two times higher than indoor Pb-$PM_{2.5}$ concentration (Table 4-3). 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 roadwear on days with dry surfaces. In a pilot study in Windsor, Ontario, Rasmussen et al. (2007) observed that personal exposure to Pb measured using a $PM_{2.5}$

monitor was roughly 40% higher than outdoor Pb concentration and 150% higher than indoor Pb concentration. 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 Pb in PM varied by environment; it tended to be less than one, but the ratio varied from one microenvironment to another. The three studies that included personal samples recorded measurements that were consistently higher than indoor or outdoor levels. It is likely that a number of factors influenced the indoor-to-outdoor ratio of Pb in PM for these studies. These factors may have included seasonal air exchange, which can vary as a function of window opening or air conditioning usage, prevalence and strength of outdoor and indoor Pb sources, and size distribution of airborne Pb-bearing PM.

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 $PM_{2.5}$ ($r = 0.72$-0.85), but Pb was not always well-correlated with NO_2 ($r = 0.24$-0.75). In the case where Pb and NO_2 were well-correlated, it is possible that the Pb was traffic related from resuspended pulverized wheel weights or impurities in unleaded on-road gasoline. For the other two sites where the correlation between Pb and NO_2 was low, it is possible that they were less affected by traffic. Table 3A-14 in the Appendix illustrates that Pb exposures are typically well below the level of the NAAQS. The higher exposures occurred in a heavily industrialized area with an incinerator and several industrial facilities including metal processing, cement, petroleum refining, agriculture processing. Otherwise, exposures 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 and Fe. In the industrial area of Kocaeli, Pb comprised a greater proportion of the PM compared with other areas.

Table 4-3. Comparison of personal, indoor, and outdoor Pb-bearing 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
Rasmussen et al. (2007)	Windsor, Ontario, Canada	Med. Pb-$PM_{2.5}$ (mg/kg)	April, 2004	311	124	221
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

4.1.1.2. Exposure to Lead in Soil and Dust

The 2006 AQCD (U.S. EPA, 2006) lists indoor Pb dust infiltrated from outdoors as a potential source of exposure to Pb soil and dust. Outdoor soil Pb concentration may present a direct inhalation exposure, or it can be tracked into homes to result in exposure to resuspended Pb PM or to Pb dust during hand-to-mouth contact. A detailed description of studies of outdoor Pb concentration is provided in section 3.6.1. 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 4-4 presents indoor Pb levels for several studies.

Table 4-4. Measurements of indoor Pb dust from various studies.

Reference	Study Location	Metric (units)	Sample Site	Indoor Pb Concentration
Caravanos et al. (2006)	New York City, New York	Weekly dust loading (µg/m^2)	Glass plate	Median: 52
Khoder et al. (2010)	Giza, Egypt	Weekly dust loading (µg/m^2)	Glass plate	Median: 408
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, UK	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
Mielke et al. (2001)	New Orleans, Louisiana	Dust concentration (mg/kg)	Multiple locations within homes prepared for painting; sanded house	Range: <3-28,000
			Multiple locations within homes prepared for painting; scraped house	Range: 7-1,200
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		Vacuum	Median: 470 Max: 2,000
			Floor	Median: 290 Max: 4,600

Several studies have demonstrated the infiltration of Pb dust into buildings. For example, Caravanos et al. (2006) 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), 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 Giza, Egypt and found 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.

Residual Pb dust contamination following cleanup has been documented. For instance, Hunt et al. (2008) performed tests where moderately elevated soil Pb was tracked onto a tile test surface and then repeatedly cleaned with a moistened wipe and/or vacuumed until visual inspection of the tiles uncovered

no surface discoloration. The authors then used wet wipe samples to collect residual soil and estimate Pb deposition and concentration. Elevated Pb loadings and concentrations were observed even after multiple cleanings. Scanning electron microscopy (SEM) of the wipe samples revealed that most of the residual dust particles were in the range of 1-3 µm in area equivalent diameter. This indicates that Pb-bearing fine particles are not well captured by home cleaning. Johnson et al. (2009) surveyed the floors of 488 homes in Syracuse, NY census tracts and found that the variability in Pb dust within homes was greater than the variability between homes. A correlation between Pb dust loading on floors and the fraction of homes in census tracts that were renter-occupied (partial $R^2 = 0.48$; where total number of homes is the sum of owner-occupied, renter-occupied, and vacant homes) was also observed in this study. Yu et al. (2006) dissolved Pb dust, obtained from vacuuming carpet samples and found that Pb concentration in carpet ranged from 209 to 1,770 mg/kg dust.

Pb dust on floors, windowsills, and other accessible surfaces are potential exposure sources to small children who use touch to explore their environments. Gaitens et al. (2009) used National Health and Nutrition Examination Survey (NHANES) data from 1999 through 2004 to examine Pb dust in homes of children ages 12-60 months. The median value of Pb dust on floors was reported to be 1.7 µg/m^2 (mean: 4.4 µg/m^2), with floors that were not smooth and cleanable having a median Pb dust value of 5.6 µg/m^2 (mean: 16 µg/m^2). Floor Pb dust level was modeled against several survey covariates and was significantly associated ($p < 0.05$) with floor surface condition, windowsill Pb dust loading, race and ethnicity, poverty-to-income ratio, year of home construction, presence of smokers in the home, and year of survey. It was nearly significantly associated ($p = 0.056$) with renovations made to pre-1950 homes. Median Pb dust on smooth windowsills was 25 µg/m^2 (mean: 190 µg/m^2). When windowsills were not smooth, the median Pb dust level was 55 µg/m^2 (mean: 480 µg/m^2). Windowsill Pb dust level was also found to be significantly associated ($p < 0.05$) with race and ethnicity, year of home construction, window surface condition, presence of smokers in the home, deterioration of indoor paint, and year of survey. It was nearly significantly associated ($p = 0.076$) with deterioration of outdoor paint when homes were built prior to 1950. Dust Pb levels were found by Egeghy et al. (2005) to be significantly associated with the log-transform of soil Pb levels, cement content in the home entryway, indoor pesticide use, frequency of fireplace usage, number of hours in which windows were open, and homes built before 1950 (Table 4-2).

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, while the other was hand-scraped. Immediately after sanding, Pb dust samples ranged from <3 to 28,000 mg/kg at the sanded house. Pb dust samples from the scraped house ranged from 7 to 1,200 mg/kg. Pb in dust or paint samples was not quantified.

Dust Pb concentrations have also been reported for homes in the vicinity of historical metals mining and smelting sources. For example, Spalinger et al. (2007) measured Pb dust in homes in a 34 km^2 area surrounding a designated Superfund site where formerly a Pb and Zn smelter operated at Bunker Hill, ID. 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 a maximum of 830 mg/kg, and Pb concentration in floor dust had a median of 95 mg/kg and a maximum of 1,300 mg/kg. The median Pb dust loading rate was measured to be 40 µg/m^2 per day. Among the background homes, median vacuum and floor mat Pb dust samples were 3 and 2.5 times higher, respectively, when comparing homes built before 1960 with those built after 1960. Deposition rate of Pb dust was 5 times higher in the older homes. In contrast, Pb in vacuum dust and floor mats for the towns contained within the Bunker Hill Superfund site had a median of 470 mg/kg with a maximum of 2,000 mg/kg and a median of 290 mg/kg with a maximum of 4,600 mg/kg, respectively. The median Pb loading rate for these towns was 300 µg/m^2 per day, and the maximum Pb dust loading rate was 51,000 µg/m^2 per day. These results suggest that those living in close proximity to an industrial site are at much greater risk of exposure to Pb dust compared to the general population.

4.1.1.3. Dietary Lead Exposure

This subsection covers several dietary Pb exposures from a diverse set of sources. Included among those are drinking water, fish and meat, pesticides via vegetables, urban gardening, dietary supplements, tobacco, cultural food sources, and breastfeeding.

Drinking Water

Differences in sources and transport of drinking water may cause variation in Pb levels. 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 that have higher Pb concentrations due to leaching from the glass, the median Pb concentration was 8.5 ng/L (range $\leq$ 1 to 761 ng/L). This was significantly less than the EU, Health Canada and WHO drinking water maximum admissible concentration of 10 µg/L. It is now recognized that environmental nanoparticles (NPs) (~1-100 nm) can play a key role in determining the chemical characteristics of engineered 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. Edwards and Dudi (2004) observed a red-brown particle-bound Pb in Washington, DC 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.

Corrosion byproducts can lead to Pb exposures 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 would have implications for V release into drinking water. The stability of vanadinite in the presence of monochloramine is also not known, and this 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 potentially occurring after using acidic alum as a disinfection agent. High Pb concentrations in Washington, DC drinking water were attributed to leaching of Pb from Pb-bearing pipes promoted by breakdown products of disinfection agents (Edwards & 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 fluorosilic acid and ammonia, the Pb concentration spiked to 900 ppb and increased further over time. Similarly, Lasheen et al. (2008) observed leaching from pipes in Egypt. In this study, the authors tested polyvinyl chloride (PVC), polypropylene (PP), and galvanized iron pipes and observed leaching from both the PVC and PP pipes when exposed to an acid of pH = 6, with PVC having greatest amount of leaching. Exposure to basic solutions actually resulted in reduction of Pb concentration in the drinking water.

Miranda et al. (2007) modeled blood Pb levels among children living in Wayne County, NC as a function of household age, use of chloramines and other covariates. It was found that blood Pb level was significantly associated with the year the home was built ($p < 0.001$), use of chloramines ($p < 0.001$), and

the interaction between these two variables ($p < 0.001$). When year in which the home was built was broken into categories for the independent variables and interaction terms, Miranda et al. (2007) found that significance increased with the age of the home, based on the assumption that older homes will have more Pb pipes and Pb solder connecting the pipes. However, the study did not control for the presence of Pb paint in the dwellings, so it is difficult to distinguish the effect of Pb pipes from the presence of paint from that variable.

Several chemical mechanisms may contribute to release of Pb during use of chloramine disinfection agents. Edwards and Dudi (2004) hypothesized that Pb leaching through chloramines exposure through the breakdown of brass alloys and solder containing Pb. They also proposed that chloramines may trigger nitrification and hence cause decreasing pH, alkalinity and dissolved oxygen that lead to corrosion after observing that nitrification also leads to increased Pb concentrations in water. However, Zhang et al. (2009) 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 chloramines 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

Dietary Pb has the potential to emanate from soil Pb used for agricultural purposes. For example, Jin et al. (2005) tested 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. Fernandez et al. (2007; 2010; 2008) 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 Pb were distributed bimodally 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 change the acidity of the soil and hence the bioaccessibility of the Pb within 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).

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% prior to its application. Chen et al. (2008) observed that the median concentration of Pb in California crop soil samples was 16.2 mg/kg (range: 6.0-62.2 mg/kg). Factor analysis suggested that the soil was enriched with Pb in crop soils in the Oxnard/Ventura region. Chen et al. (2008) further observed that in three of the seven California agricultural regions sampled, concentrations of Pb increased following addition of fertilizer, but sub-proportionally to the increase in 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 observed a median 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) observed that the amount of Pb applied via fertilizers was only 2.6% of that from atmospheric deposition. Although Pb in on-road vehicle gasoline has been phased out in the U.S., this remains a relevant issue in the U.S. because some imported crops that are produced in countries that still use Pb antiknock agents in on-road gasoline. For example, high concentrations of Pb have been found in chocolate from beans grown in Nigeria, where leaded gasoline is legal. Rankin et al. (2005) observed that the ratios of ^{207}Pb to ^{206}Pb and ^{208}Pb to ^{207}Pb were found to be 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 was observed to have 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. Likewise, it is possible that resuspended Pb that originated from legacy mobile and industrial sources could deposit on crops.

Uptake of Pb by plants growing in contaminated soil has been 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 *Cichorium*

intybus [chicory], *Cynodon dactylon* [Bermuda grass], *Amaranthus blitoides* [matweed or mat amaranth], and *Silybum marianum* [milk thistle]) 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 were demonstrated to have 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, measured as the percent of Pb mass in the shoots compared to the Pb mass within the entire plant, 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) compiled bioaccumulation data for plant groupings from various references; these data are reproduced in Table 4-5. Based on this review, grasses had the highest uptake, followed by leafy vegetables and root crops grown in sandy soils; these references also suggested high transfer from roots to shoots among root crops, with shoots having roughly four times higher Pb bioaccumulation than roots.

Table 4-5. 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%	2500%
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%	2500%
		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%	1600%
		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: Used with permission from Elsevier Publishers, Vandenhove et al. (2009).

Findings from Pb uptake studies have implications for urban gardening if urban soils may be contaminated with Pb, as described in Section 4.1.1.2. For instance, Clark et al. (2006) tested the soil in 103 urban gardens in two Boston neighborhoods. They found that Pb-based paint contributed 40-80% of measured Pb in the urban garden soil samples, with the rest coming from historical gasoline emissions. Furthermore, Clark et al. (2006) estimated that Pb consumption from urban gardens can be responsible for up to 25% of exposure to Pb in 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.

In addition to uptake of Pb through the roots of a plant, deposition of airborne Pb-bearing PM can also contribute to human dietary Pb exposures, as described in the 2006 Pb AQCD (2006). In a recent study, 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 m from the smelter had concentrations that were 10-20 times lower, which is consistent with findings described in Section 3.3 that deposition of Pb containing material drops off with distance from a source.

Fish

Accumulation in fish could also lead to human exposure to Pb. Ghosh et al. (2007) demonstrated in laboratory experiments that exposure to Pb in water can lead to linearly increasing accumulation in fish. Several studies have documented the potential for human 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 Pb concentrations ranged from 580 to 6,500 mg/kg. In total, 65% of those surveyed 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 Pb content varied with respect to species of fish but were found to be elevated in some species. Pb concentrations in fish were higher in areas close to mining activities. Similarly, Besser et al. (2008) observed higher levels of blood Pb in fish close to mining activities in southeastern Missouri. In a related study of fish species in the same region of Missouri, blood Pb levels in fish were found to be 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., 2007), and elevated blood Pb levels in fish 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. There has also been evidence of elevated Pb concentration within large game from mining areas (Reglero et al., 2009).

4.1.1.4. 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. 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, reported to have 75% below 2.5 µm diameter, was not reported.

Operations involving PM 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 µg/m^3, with a maximum daily value of 210 µg/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 4.5.1. Nwajei and Iwegbue (2007) measured Pb contamination in sawdust; such contamination has been reported to occur when trees are grown in soil contaminated with Pb (Andrews et al., 1989). Sawdust samples from fifteen locations in Nigerian sawmills were reported to have Pb concentrations ranging from 2.0 to 250 mg/kg.

4.1.1.5. Exposure to Lead from Consumer Products

Pb is present in varying amounts in several consumer products including alternative medicines, candies, cosmetics, pottery, tobacco, toys, and vitamins (Table 4-6). 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 (Levin et al., 2008; Miodovnik & Landrigan, 2009).

Table 4-6. 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.) -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/day	Kim et al (2009)
	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, Charma 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)
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.-55 mg/kg	Omolaoye et al (2010a)
Pottery	Foods prepared in Pb-glazed pottery	Mexico	N.D.-3,100 mg/kg	Villalobos et al (2009)
Tobacco	Smokeless Tobacco	United Kingdom	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

4.2. Kinetics

This section summarizes the empirical bases for our understanding of 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, 2006). 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 4.3, Pb biokinetics is described from the context of model predictions.

4.2.1. Absorption

The focus of the following sections within absorption is on inhalation and ingestion because these are the major exposure routes of Pb in humans. The 2006 AQCD also presented dermal absorption of inorganic and organic Pb compounds, which is generally considered to be much less than by inhalation or ingestion. No recent literature has advanced our knowledge of dermal absorption of Pb beyond that which was included in the 2006 AQCD. No additional information provides evidence of dermal absorption being a major exposure route of environmental Pb.

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. 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+}).

4.2.1.1. 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 & 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 (Chamberlain et al., 1978; Morrow et al., 1980; M. B. 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 & 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 attempted to quantify the bioavailability of Pb in atmospheric PM, although different laboratory techniques are used throughout the literature, as described in Section 3.4. Unlike the bioavailability methods described in Section 4.2.1.2, many of these in vitro assays have not been validated with in vivo models. Therefore, any in vitro method can only be a simplistic surrogate of the complex uptake of Pb from the gastrointestinal (GI) tract. Despite this limitation, the studies mentioned below are the only ones to evaluate atmospheric Pb.

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 bioavailability of heavy metals including Pb. The rationale was that inhaled PM 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 bioavailability 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 bioavailability of Pb 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 PM smaller than 10 µm were 8,800 and 7,800 mg/kg for the two different locations. The bioavailability was based on ammonium acetate extractability and it was found that, within the fine and nano-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 bioavailable 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 PM, with percent bioavailability decreasing with increasing PM size. This indicated that Pb was potentially most bioavailable in the nano-size range.

A recent study by Barrett et al. (2010) investigated the solid phase speciation of Pb in urban road dust in Manchester, UK, 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 bioavailable if ingested compared with PbO or Pb-acetate because they are less soluble.

Organic Lead

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 AQCD, 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 organic Pb is relevant to some aviation fuel exposures (i.e., piston-engine aircraft). Mahaffey (1977) estimated that 40% of inhaled Pb is bioavailable to adults. Chamberlain et al. (1975) suggested that 35% of inhaled combustion products of tetraethyl ^{203}Pb fuel 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 at a concentration of 0.001 μg/m^3 could produce a 1 μg/dL increment in blood Pb.

4.2.1.2. 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 and iron 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 (Pocock et al., 1983; J. Sherlock et al., 1982; J. C. Sherlock et al., 1984; J. C. Sherlock & Quinn, 1986). 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 (Heard & Chamberlain, 1982; James et al., 1985; Maddaloni et al., 1998; M. B. Rabinowitz et al., 1980; Watson et al., 1986). 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 (Blake et al., 1983; Heard & Chamberlain, 1982; James et al., 1985; Maddaloni et al., 1998; M. B. Rabinowitz et al., 1980). Reported fed:fasted ratios for soluble Pb absorption in adults range from 0.04 to 0.2 (Blake et al., 1983; Heard & Chamberlain, 1982; James et al., 1985; M. B. 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 (Alexander et al., 1974; Ziegler et al., 1978). Experimental studies provide further evidence for greater absorption of Pb from the gut in young animals compared to adult animals (Aungst et al., 1981; Forbes & Reina, 1972; Kostial et al., 1978; Pounds et al., 1978). 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).

Nutritional deficiencies have also been linked to Pb absorption in the GI tract, particularly in children. Children who are iron-deficient have higher blood Pb concentrations than similarly exposed iron-replete children, suggesting that iron deficiency may result in higher Pb absorption or, possibly, other changes in Pb biokinetics that contribute to altered blood Pb concentrations (Mahaffey & Annest, 1986; Marcus & Schwartz, 1987; Schell et al., 2004). Studies conducted in animal models have provided direct evidence for interactions between iron deficiency and increased Pb absorption, perhaps by enhancing binding of Pb to iron-binding proteins in the intestine (Bannon et al., 2003; Barton, Conrad, Nuby, et al., 1978; Morrison & Quarterman, 1987).

The effects of iron nutritional status on blood Pb include changes in blood Pb concentrations in association with genetic variation in genes involved in iron 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 calcium 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 calcium carbonate (0.2 g calcium carbonate) than when the Pb was ingested without additional calcium (Blake & Mann, 1983; Heard & Chamberlain, 1982). A similar effect of calcium occurs in rats (Barton, Conrad, Harrison, et al., 1978). Similarly, an inverse relationship was observed between dietary calcium intake and blood Pb concentration in children, suggesting that children who are calcium-deficient may absorb more Pb than calcium-replete children (Elias et al., 2007; Mahaffey et al., 1986; Schell et al., 2004; Ziegler et al., 1978). These observations suggest that calcium and Pb share and may compete for common binding and transport mechanisms in the small intestine which are regulated in response to dietary calcium and calcium body stores (Bronner et al., 1986; Fullmer & Rosen, 1990). However, animal studies have also shown that multiple aspects of Pb

toxicokinetics are affected by calcium nutritional status. For example, feeding rats a calcium deficient diet is associated with increased Pb absorption, decreased whole body Pb clearance, and increased volume of distribution of Pb (Aungst & Fung, 1985). These studies suggest that associations between calcium nutrition and blood Pb that have been observed in human populations may not be solely attributable to effects of calcium nutrition on Pb absorption. Other potential mechanisms by which calcium nutrition may affect blood Pb and Pb biokinetics include effects on bone mineral metabolism and renal function.

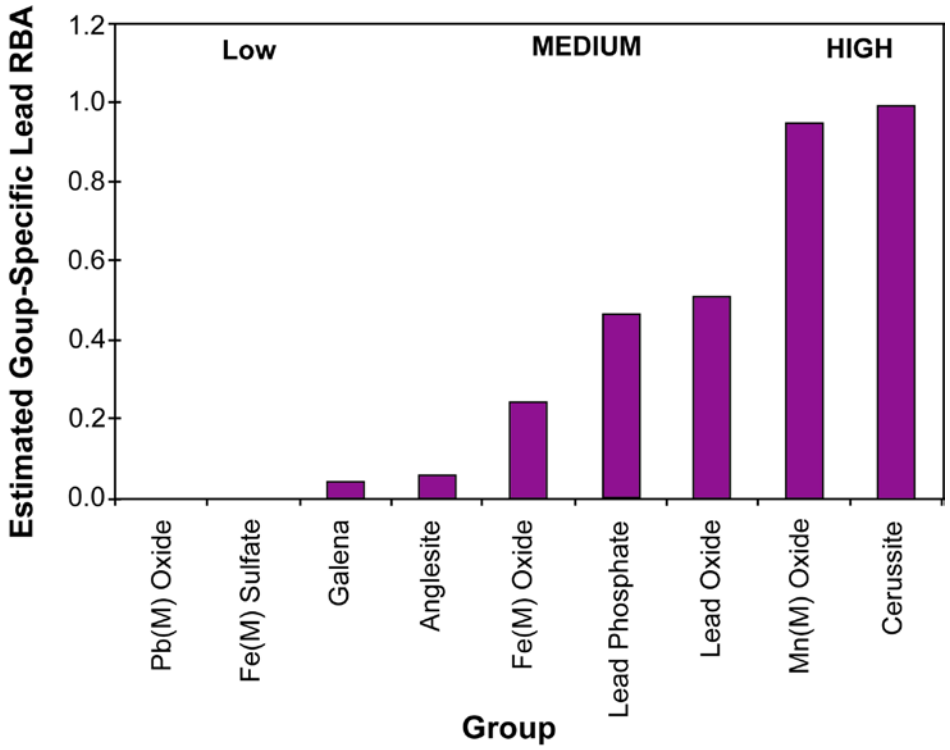
Blood Pb concentrations in young children have also been shown to increase in association with lower dietary Zn levels (Schell et al., 2004). Mechanisms for how Zn affects blood Pb concentration, i.e., whether it involves changes in absorption or changes in distribution and/or elimination of Pb, have 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. Relative bioavailability (RBA) of Pb in soils and dust has been most extensively studied in animal models. Relative bioavailability is the ratio of the absorbed fraction (AF) of ingested dose of soil Pb to that of a water-soluble form of Pb (e.g., Pb-acetate) that is considered to be completely bioaccessible (e.g., RBA = $AF_{Soil\ Pb}/AF_{Pb\text{-}acetate}$). In typical studies, the absorbed fraction of the Pb dose is estimated based measurements of Pb measured in blood and/or other tissues (e.g., kidney, liver, bone) after dosing . Gastric function of swine is thought to be sufficiently similar to that of humans to justify use of swine as a model for assessing RBA of Pb in soils (Casteel et al., 1997; Casteel et al., 2006; Juhasz et al., 2009; U.S. EPA, 2007a; Weis & 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 bioavailability of soluble Pb (e.g., Pb-acetate) in swine, which is more similar to humans than rats (D. M. Smith et al., 2009). Relative bioavailability 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 (Barltrop & Meek, 1979; Healy et al., 1992).

Collectively, published studies conducted in swine have provided 39 estimates of Pb RBA for 37 different soil or "soil-like" test materials (Bannon et al., 2009; Casteel et al., 2006; Marschner et al., 2006; D. M. Smith et al., 2009). The mean of RBA estimates from 25 soils is 49% (± 29[SD]), median is 51%, and 5th to 95th percentile range is 12 to -89%. RBA estimates for soils collected from 8 firing ranges were approximately 100% (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 (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 (14-40%, n = 3), as did a sample from a mine tailings pile (RBA = 6%), 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 4-2 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 that 100% (Freeman et al., 1996; Freeman et al., 1992; Freeman et al., 1994; D. M. Smith et al., 2008, 2009).



Source: Casteel et al. (2006).

Figure 4-2. Estimated relative bioavailability (RBA, compared to Pb-acetate) of ingested Pb in mineral groups, based on results from juvenile swine assays.

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 4-1):

$$RBA = (0.878 \bullet IVBA) - 0.028$$

Equation 4-1

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, 2007b). A review of soil Pb RBA estimates made using the IVBA assay described above and Equation 4-1 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 57% (SD 15), median was 63%, and 5th to 95th percentile range was 34 to 71%.

Equation 4-1 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 of in vitro assays applied to the same soil test materials have found considerable variability in IVBA estimates (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). 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, 2007b).

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 has been reported to be approximately 72% (95% CI: 44, 98) in juvenile swine, suggesting that paint Pb dust maybe highly bioavailable (Casteel et al., 2006). The same material yielded a bioaccessibility value (based on IVBA assay) of 75% (Drexler & Brattin, 2007), which corresponds to a predicted RBA of 63%, based on Equation 4-1. A review of indoor Pb RBA estimates made using the IVBA assay and Equation 4-1 identified 100 estimates of Pb RBA in dusts obtained from two hazardous waste sites. Mean Pb RBAs for the Herculaneum site were 47% (SD 7, 10 samples) for indoor dust and 69% (SD 3, 12 samples) for soil. At the Omaha site, mean Pb RBAs were 73% (SD 10, 90 samples) for indoor dust and 70% (SD 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 64.8% (SD 8.2, 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 would be that a validated IVBA methodology for soil would perform well for predicting RBA of interior dust, this has not actually been

experimentally confirmed. Factors that may affect in vitro 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 "bioavailability" of Pb exposure samples are derived from less validated in vitro methods. Roussel et al. (2010) estimated that 63% of soil Pb is bioavailable in the stomach, while 39% is bioavailable in the intestines, using different acidities of solutions to simulate acids in the digestive system. Yu et al. (2006) dissolved Pb dust, obtained from vacuuming carpet samples, into simulated gastric and intestinal acids. 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% bioavailability 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.

4.2.2. Distribution

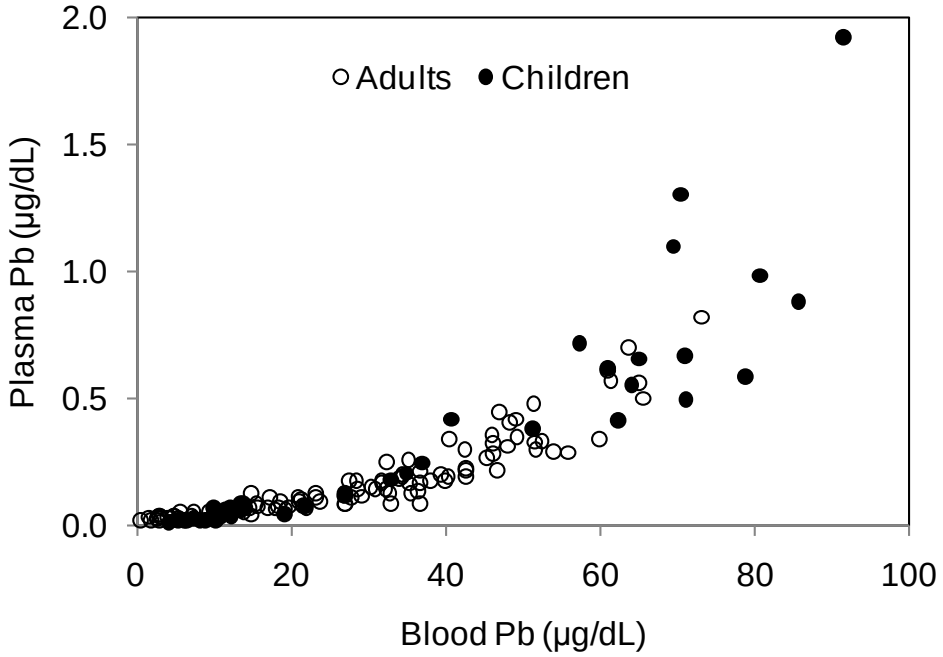
A simple conceptual representation of Pb distribution is that it contains a fast turnover pool, comprising mainly soft tissue, and a slow pool, comprising mainly skeletal tissues (M. B. Rabinowitz et al., 1976). The highest soft tissue concentrations in adults occur in liver and kidney cortex (Barry,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

4.2.2.1. Blood

Blood comprises ~1% of total Pb body burden. Pb in blood is found primarily (>99%) in the RBCs (Bergdahl, Grubb, et al., 1997; Bergdahl et al., 1998; Bergdahl et al., 1999; Hernandez-Avila et al., 1998; Manton et al., 2001; Schutz et al., 1996; D. Smith et al., 2002). δ-aminolevulinic acid dehydratase (ALAD) is the primary binding ligand for Pb in erythrocytes (Bergdahl, Grubb, et al., 1997; Bergdahl et al., 1998; Sakai et al., 1982; Xie et al., 1998). 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(s) having a molecular weight <10 kDa (Bergdahl, Grubb, et al., 1997; Bergdahl et al., 1996; Bergdahl et al., 1998). 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., 1996; Bergdahl et al., 1998; Sakai et al., 1982). Pb binding to ALAD is saturable; the binding capacity has been 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).

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 (Barbosa, Ramires, et al., 2006; Bergdahl, Schutz, et al., 1997; Bergdahl et al., 1998; Bergdahl et al., 1999; DeSilva, 1981; C. Jin et al., 2008; Kang et al., 2009; Manton et al., 2001; D. Smith et al., 2002). An example of this is shown in Figure 4-3. 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 at approximately 40 µg/dL blood, 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.

Typically, at blood Pb concentrations <100 µg/dL, only a small fraction (<1%) of blood Pb is found in plasma (DeSilva, 1981; Manton & Cook, 1984; Marcus, 1985). However, as previously noted, plasma Pb may be the more biologically labile and toxicologically active 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 & Lee, 1980). Pb in serum that is not bound to protein exists largely as complexes with low molecular weight sulfhydryl compounds (e.g., cysteine, homocysteine) and other ligands (Al-Modhefer et al., 1991).

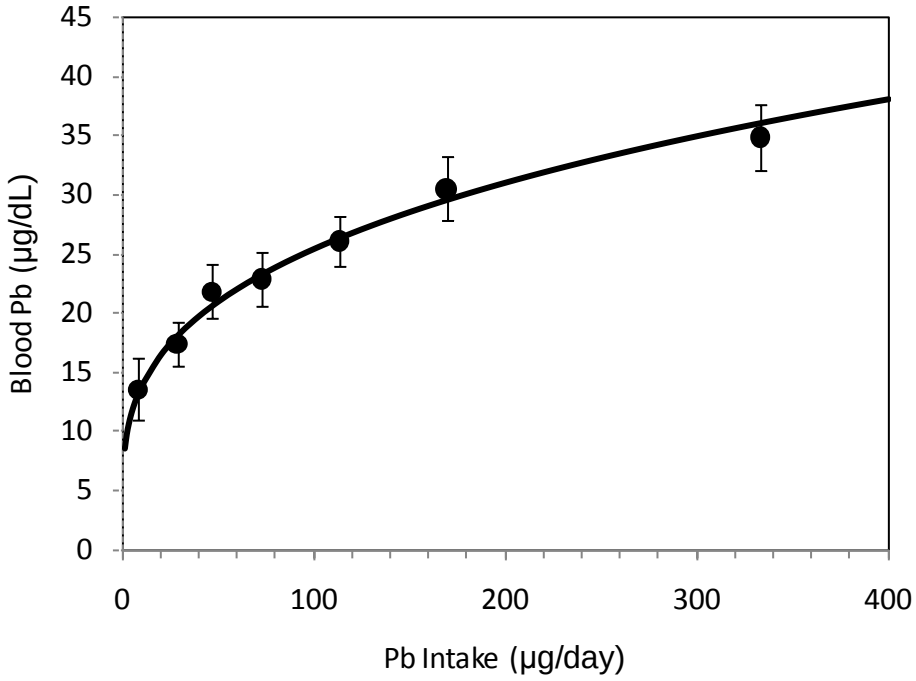


Source: Adapted, with permission from Elsevier Publishing and the Finland Institute of Occupational Health, from Bergdahl et al. (1997; 1999).

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

As shown in Figure 4-3, 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 (Lacey et al., 1985; Ryu et al., 1983; J. C. Sherlock & Quinn, 1986). As shown in Figure 4-4, the relationship becomes pseudo-linear at relatively low daily Pb intakes (i.e., <10 µg/day/kg) and at blood Pb concentrations <25 µg/dL.



Source: Adapted, with permission from Taylor & Francis Publishing, from Sherlock and Quinn (1986).

Figure 4-4. Relationship between Pb intake and blood Pb concentration in infants (n = 105, age 13 weeks, formula-fed). Data represent mean and standard errors for intake; the line is the regression model (blood Pb = 3.9 + 2.43 (Pb intake [µg/week]$^{1/3}$).

Figure 4-5 shows the predicted relationship between quasi-steady state blood and plasma Pb concentrations in a 4-year old child using the ICRP model (ICRP, 1994; Leggett, 1993; Pounds & Leggett, 1998). 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 this 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 could potentially contribute to non-linearity in dose-response relationships in studies in which blood Pb is the used as the internal dose metric.

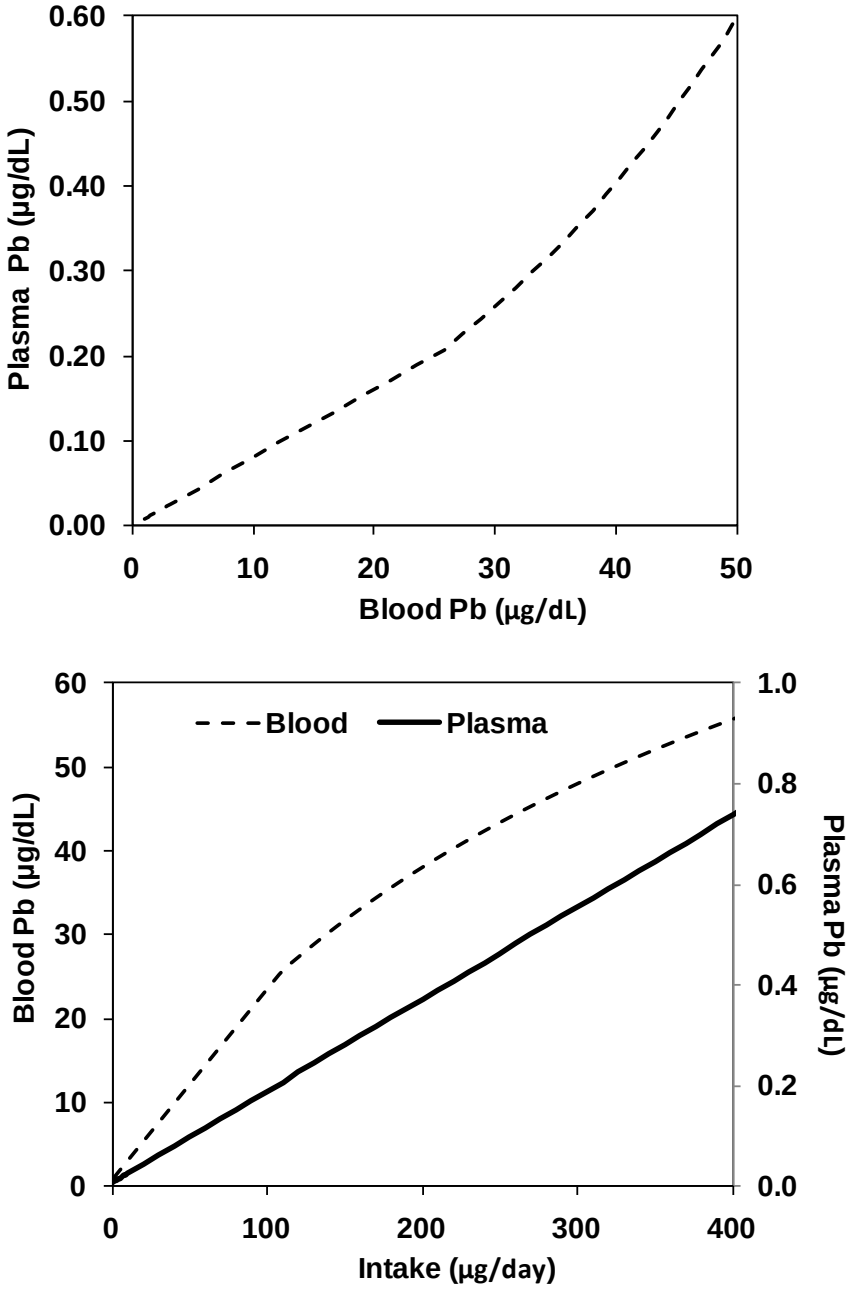


Figure 4-5. Simulation of quasi-steady state blood and plasma Pb concentrations in a child (age 4 years) associated with varying Pb ingestion rates. Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

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 urinary Pb excretion and plasma Pb concentration (Section 4.2.3) (Bergdahl, Schutz, et al., 1997; Besser et al., 2008).

4.2.2.2. Bone

The dominant compartment for Pb in the body is in bone. In human adults, 94% of the total body burden of Pb is found in the bones, whereas bone Pb accounts for 73% of the body burden in children (Barry, 1975). Bone is comprised of two main types, cortical (or compact) and trabecular (or spongy or calcellous). The proportion of cortical to trabecular bone in the human body varies by age, but on average is about 80 to 20 (ICRP, 1973; Leggett, 1993; O'Flaherty, 1998).

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" 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 (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 & 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 & 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 (Barry, 1975; Barry & Connolly, 1981; Gross et al., 1975; Park, Mukherjee, et al., 2009; Schroeder & 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 (M. B. 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%) (ICRP, 1973; Leggett, 1993; O'Flaherty, 1995). 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.

A key factor affecting Pb uptake into bone is the fraction of bone surface in trabecular and cortical bone adjacent to active bone marrow. Of the total bone surface against red marrow, 76% is trabecular and 24% is cortical endosteal (Salmon et al., 1999). The fraction of total bone marrow that is red and active decreases from 100% at birth to about 32% in adulthood (Cristy, 1981). However, bone marrow has much lower Pb concentrations than bone matrix (Skerfving et al., 1983).

4.2.2.3. Soft Tissues

Most of the Pb in soft tissue is in liver and kidney (Barry, 1975; Gerhardsson et al., 1986; Gerhardsson et al., 1995; Gross et al., 1975; Oldereid et al., 1993). 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 & 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 & 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 (D. R. 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 (Eaton et al., 1980; Waalkes & Klaassen, 1985).

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 (Azar et al., 1973; Casteel et al., 1997; Casteel et al., 2006; D. M. Smith et al., 2008). In contrast to Pb in bone, which accumulates Pb with continued exposure in adulthood, concentrations in soft tissues (e.g., liver and kidney) are relatively constant in adults (Barry, 1975; Treble & Thompson, 1997), reflecting a faster turnover of Pb in soft tissue relative to bone.

4.2.2.4. Fetus

Evidence for maternal-to-fetal transfer of Pb in humans is derived from cord blood to maternal blood Pb ratios. These ratios range from about 0.6 to 1.0 at the time of delivery (Carbone et al., 1998; Goyer, 1990; Graziano et al., 1990; B. Gulson, Jameson, et al., 1998; Kordas et al., 2009; Manton, 1985). In addition, the similarity of isotopic ratios in maternal blood and in blood and urine of newly-born infants provide further evidence for placental transfer of Pb to the fetus (B. Gulson et al., 1999).

Transplacental transfer of Pb may be facilitated by an increase in the plasma/blood Pb concentration ratio during pregnancy (Lamadrid-Figueroa et al., 2006; Montenegro et al., 2008). 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 (Franklin et al., 1997; O'Flaherty, 1998).

4.2.2.5. Organic Lead

Information on the distribution of Pb in humans following exposures to organic Pb is extremely limited. However, as reported in the 2006 AQCD, 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).

4.2.3. Elimination

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

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

Approximately 30% of Pb excretion during the first 20 days after exposure is due to urinary and fecal losses (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 (Bergdahl, Schutz, et al., 1997) (i.e., glomerular filtration rate × plasma Pb concentration), and both relationships are linear. While the relationship between urinary Pb excretion and plasma Pb concentration has been shown to be linear, the plasma Pb relationship to blood Pb concentration is curvilinear (as described in Section 4.2.2.1 and demonstrated in Figure 4-5). This 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 4.2.7).

Estimates of urinary filtration of Pb from serum (or plasma) range from 13-22 L/day, with a mean of 18 L/day (Araki et al., 1986; Chamberlain et al., 1978; Manton & Cook, 1984; Manton & Malloy, 1983), 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 (Vander et al., 1977; Victery et al., 1979). 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 (Araki et al., 1990; Berger et al., 1990; Chamberlain et al., 1978; Koster et al., 1989; Manton & Malloy, 1983; M. B. Rabinowitz et al., 1973; Ryu et al., 1983) (Diamond, 1992), 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 (Booker et al., 1969; Chamberlain et al., 1978; 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 Lead

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 who were exposed to tetraethyl Pb have shown that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb (Turlakiewicz & Chmielnicka, 1985; Vural & Duydu, 1995; W. Zhang et al., 1994).

4.3. Lead Biomarkers

This section describes the biological measurements of Pb and their interpretation as indicators of exposure or body burden. The focus is on blood Pb and bone Pb and the interplay between them, as these are the most commonly measured biomarkers in recent epidemiologic and toxicological studies.

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 we have for synthesizing our 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 our understanding of Pb biokinetics.

Numerous mechanistic models of Pb biokinetics in humans have been proposed, and these are described in the 2006 Pb AQCD (U.S. EPA, 2006) and in the supporting literature cited in that report. In this section, for simplicity and for internal consistency, we have limited the discussion to predictions from a single model, the ICRP Pb biokinetics model (ICRP, 1994; Leggett, 1993; Pounds & Leggett, 1998). This model was originally developed for the purpose of supporting radiation dosimetry predictions; however, it has also been applied in Pb risk assessment (Abrahams et al., 2006; Khoury & Diamond, 2003; Lorenzana et al., 2005). Portions of the model have been incorporated into an All Ages Lead Model (AALM) that is being developed by EPA (2005).

4.3.1. Bone Lead 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, because it has more bone mass and may afford better measurement precision than the calcaneus. Bone analysis methods have included 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). 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, 2006).

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. (2011) 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 and a high correlation with results obtained from K-shell methods (intraclass correlation = 0.65).

Bone Pb measurements are typically expressed in units of Pb/g bone mineral. This 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 (Hu et al., 2007; Theppeang et al., 2008).

4.3.2. Blood Lead 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 & 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 & 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, it has not received widespread use in epidemiologic studies.

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 ALAD2 alleles can be codominantly 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, noninstitutionalized 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 AQCD document reports 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 (Astrin et al., 1987; Bergdahl, Schutz, et al., 1997; H.-S. Kim et al., 2004; Pérez-Bravo et al., 2004; C. M. Smith et al., 1995; Wetmur, 1994; Wetmur, Lehnert, et al., 1991). 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 would be consistent with the structural differences that result in greater electronegativity of ALAD1 compared to ALAD2 (Wetmur, 1994; Wetmur, Kaya, et al., 1991), 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). Both analyses reported the greatest differences for ALAD2 compared to ALAD1 in highly exposed adults and little difference among environmentally-exposed adults; large differences were also observed for children at low exposures. However, there were 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, with blood Pb levels being higher in individuals with ALAD2 alleles (Miyaki et al., 2009; Shaik & 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 (Montenegro et al., 2006; Rabstein et al., 2008; Wananukul et al., 2006) or lower blood Pb levels for individuals with ALAD 1-2/2-2 (Chia et al., 2006).

Analyses of serial blood Pb concentrations measured in longitudinal epidemiologic studies have found relatively strong correlations (e.g., r = 0.5-0.8) between individual blood Pb concentrations measured after 6-12 months of age (Dietrich et al., 1993; McMichael et al., 1988; Otto et al., 1985; M. Rabinowitz et al., 1984; Schnaas et al., 2000). 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 is illustrated in Figure 4-6. Two hypothetical children are simulated. Child A has a relatively constant Pb intake from birth, whereas Child B has the same long-term Pb intake as Child A,

but with a 1-year elevated intake beginning at age 24 months (Figure 4-6, upper panel). 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 4-6, 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.

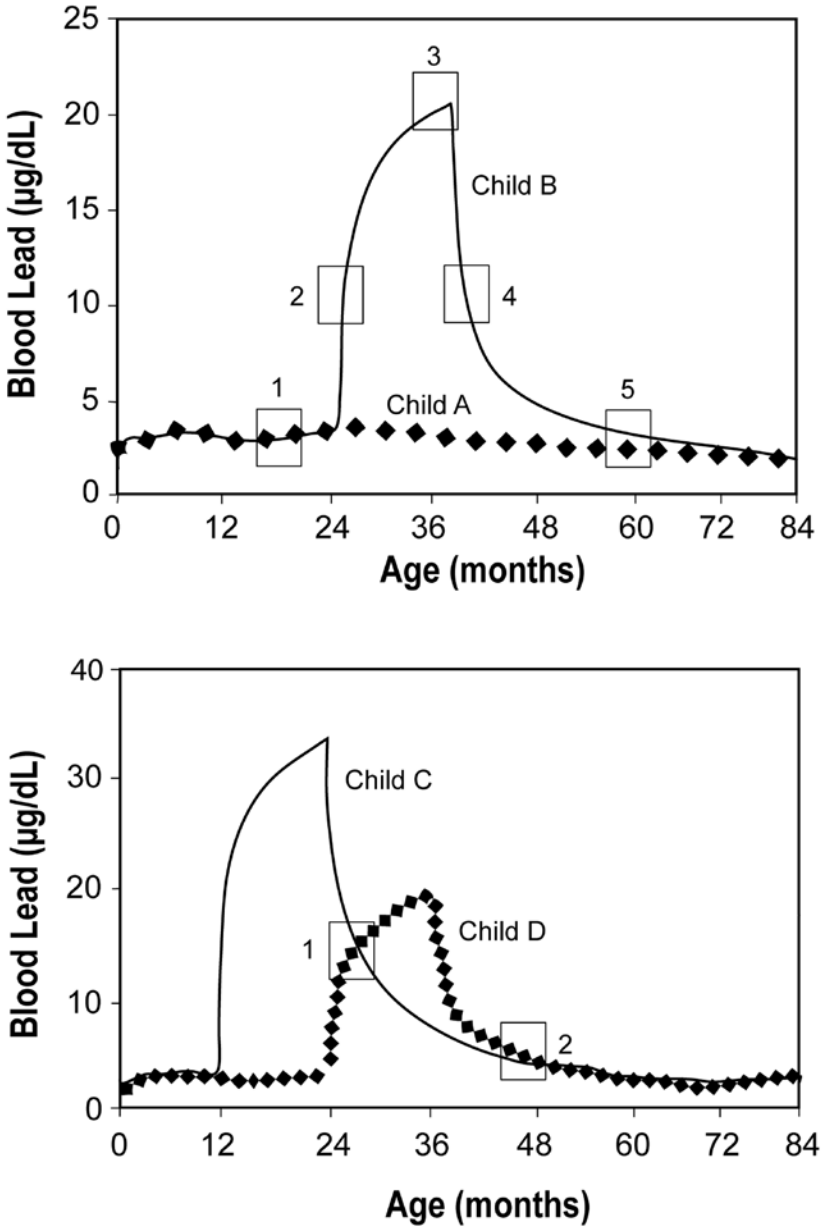


Figure 4-6. Simulation of temporal relationships between Pb exposure and blood Pb concentration in children.Child A and Child B have a relatively constant basal Pb intake (µg/day/kg body weight) from birth; Child B experiences 1-year elevated intake beginning at age 24 months (upper panel). Blood Pb 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 scenarios for these samples are very different. As shown in the example of Child C and Child D, two samples can provide information about the magnitude of change in exposure, but not necessarily the temporal history of the change (lower panel). Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

4.3.3. Urine Lead 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 4.2.3) 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 (Araki et al., 1990; Fukui et al., 1999). 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 exposure history of the previous few months; thus, 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. 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 six 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 4-7).

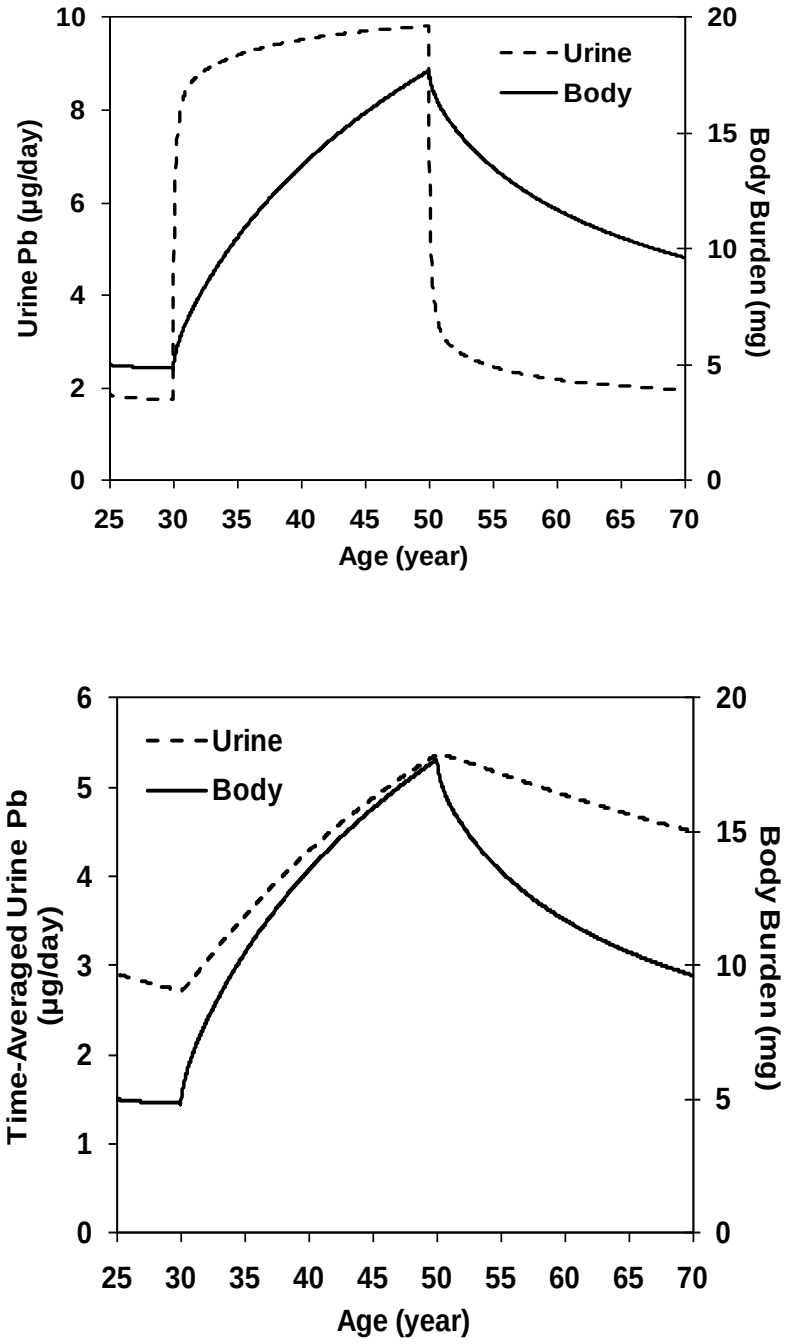


Figure 4-7. Simulation of relationship between urinary Pb excretion and body burden in adults.A change in Pb uptake results in a relatively rapid change in urinary excretion of Pb, to a new quasi-steady state, and a relatively small change in body burden (upper panel). The long-term average urinary Pb excretion more closely tracks the pattern of change in body burden (lower panel). Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

4.3.4. Lead in Other Potential Biomarkers

There was extensive discussion in the 2006 Pb AQCD 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 as biomarkers of exposure or body burden, 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.

4.3.4.1. 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 (B. L. Gulson et al., 1997; M. B. Rabinowitz et al., 1993). Limited studies have demonstrated moderate-to-high correlations between tooth Pb levels and blood Pb levels (M. B. Rabinowitz, 1995; M. B. 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 (M. B. Rabinowitz et al., 1993). This 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) was able to discern 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., (Fosse et al., 1995; Tvinnereim et al., 1997). 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.

4.3.4.2. Hair

The 2006 Pb AQCD 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; M. B. Rabinowitz et al., 1976) and has been explored as a possibly noninvasive approach for estimating Pb body burden (Gerhardsson et al., 1995; Wilhelm et al., 1989; Wilhelm et al., 2002). 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 low levels (<10-12 µg/dL) (Campbell & Toribara, 2001; G. Drasch et al., 1997; Esteban et al., 1999; J. L. Rodrigues et al., 2008). 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, an empirical basis for interpreting hair Pb measurements in terms of body burden or exposure has not been firmly established.

4.3.4.3. Saliva

A growing body of literature on the utility of measurements of salivary Pb has developed since the completion of the 2006 AQCD (U.S. EPA, 2006). Earlier reports suggested a relatively strong correlation between salivary Pb concentration and blood Pb concentration (Brodeur et al., 1983; Omokhodion & Crockford, 1991; P'an, 1981); however, more recent assessments have shown relatively weak or inconsistent associations (Barbosa, Heloisa, et al., 2006; Costa de Almeida et al., 2009; 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. (2006) 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.

4.3.4.4. Serum δ-ALA and ALAD

The association between blood Pb and blood ALAD activity and serum δ-aminolevulinc acid (δ-ALA) levels was recognized decades ago as having potential use as a biomarker of Pb exposure (Hernberg et al., 1970; Mitchell et al., 1977). 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., 2004; Opler et al., 2008).

4.3.5. Relationship between Lead in Blood and Lead in Bone

The kinetics of elimination of Pb from the body reflects the existence of fast and slow pools of Pb in the body. The dominant phase of Pb kinetics in the blood, exhibited shortly after a change in exposure occurs, has a half-life of ~20-30 days (Leggett, 1993). A slower phase becomes evident with longer observation periods following a decrease in exposure. 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.

Bone Pb stores can contribute 40-70% to blood Pb (B. Gulson et al., 1995; Manton, 1985; D. R. Smith et al., 1996). Bone Pb burdens in adults are slowly lost by diffusion (heteroionic exchange) as well as by resorption (O'Flaherty, 1995). Half-times for the release of Pb in bone is dependent on age and intensity of exposure. Slow bone volume compartments are much more labile in infants and children than in adults as reflected by half-times for movement 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). Children who have been removed from a relatively brief exposure to elevated environmental Pb 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). 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 Pb dose. 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; Kehoe, 1987; O'Flaherty et al., 1982; D. R. Smith et al., 1996), even for exposures that occurred during childhood (F. E. 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.

There is a stronger relationship between patella Pb and blood Pb than tibia Pb and blood Pb (Hernandez-Avila et al., 1996; Hu et al., 1996; Hu et al., 1998; Park, Mukherjee, et al., 2009). 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 relationships 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.

4.3.5.1. Children

As mentioned in Section 4.2.2.2, 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 4-8 shows a 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 cessation of 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 in to the bone mineral matrix.

Empirical evidence in support of this comes from longitudinal studies in which relatively high correlations ($r = 0.85$) were found between concurrent ($r = 0.75$) or lifetime average blood Pb concentrations ($r = 0.85$) and tibia bone Pb concentrations (measured by XRF) in a sample of children in which average blood Pb concentrations exceeded 20 µg/dL; the correlations was much weaker ($r < 0.15$) among children who had average blood Pb concentration <10 µg/dL (Wasserman et al., 1994).

Two alternative blood Pb metrics depicted in Figure 4-8 include the time-averaged and time-integrated blood Pb concentrations. Both the time-averaged and time-integrated blood Pb metrics display rates of change in response to the exposure event that more closely approximate the slower kinetics of bone Pb and body burden, than the kinetics of blood Pb concentration, with notable differences. The time-averaged blood Pb concentration increases during the exposure event and decays following the event, consistent with the changing body burden. The time-integrated blood Pb concentration (conceptually identical to cumulative blood lead index [CBLI] used in epidemiologic studies) 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 exposure, the time-integrated blood Pb and body burden diverge. This 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 cessation of 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 is illustrated in the hypothetical simulations of an exposure event experienced by a child (Figure 4-9). This pattern is similar for adults.

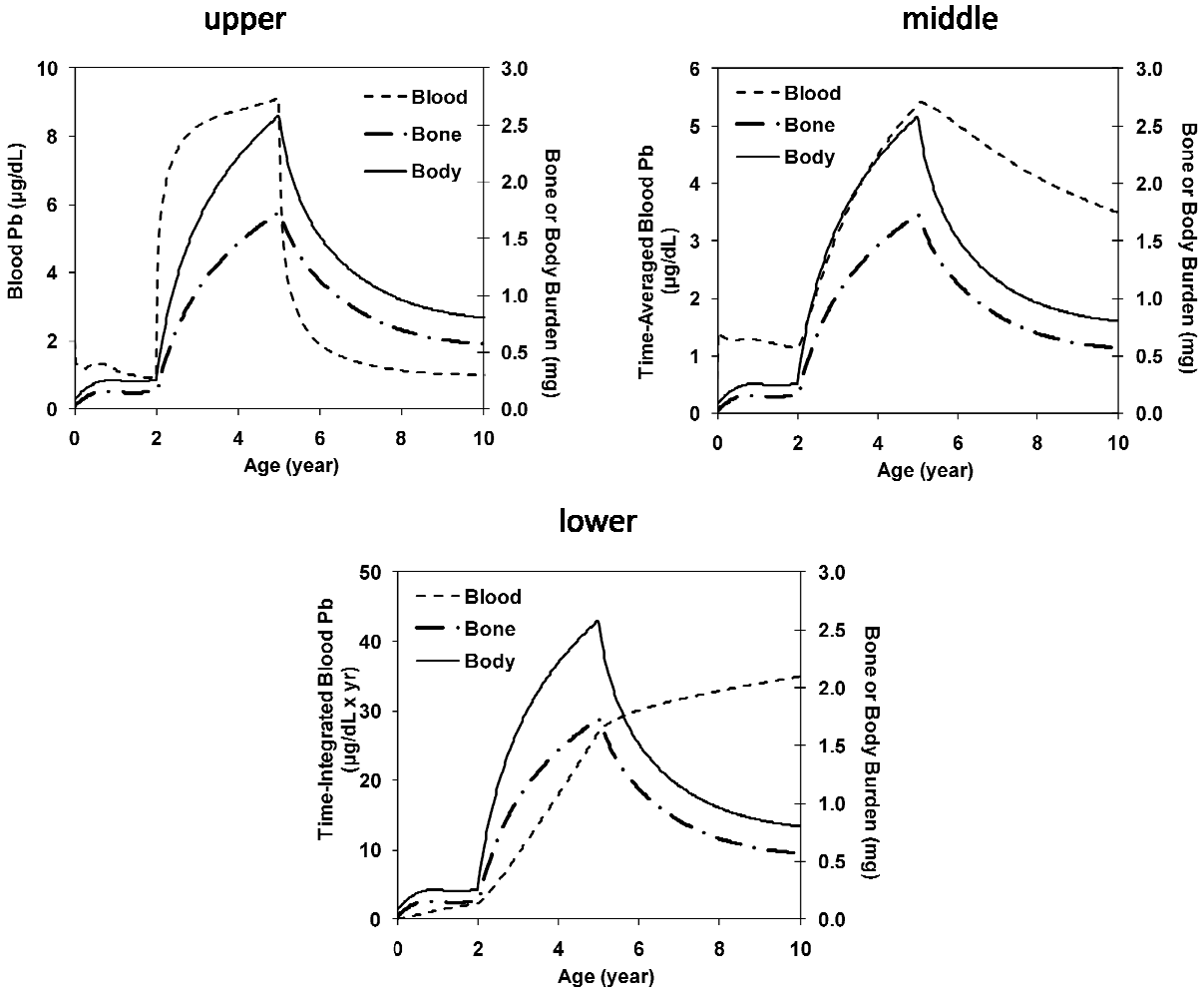


Figure 4-8. Simulation of relationship between blood Pb concentration and body burden in children, with a constant Pb intake from age 2 to 5.Blood Pb concentration is thought to parallel body burden more closely in children than in adults, due to more rapid turnover of bone and bone-Pb stores in children (upper panel). Nevertheless, the time-averaged blood Pb concentration more closely tracks the pattern of change in body burden (middle panel). The time-integrated blood Pb concentration increases over time (lower panel). Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

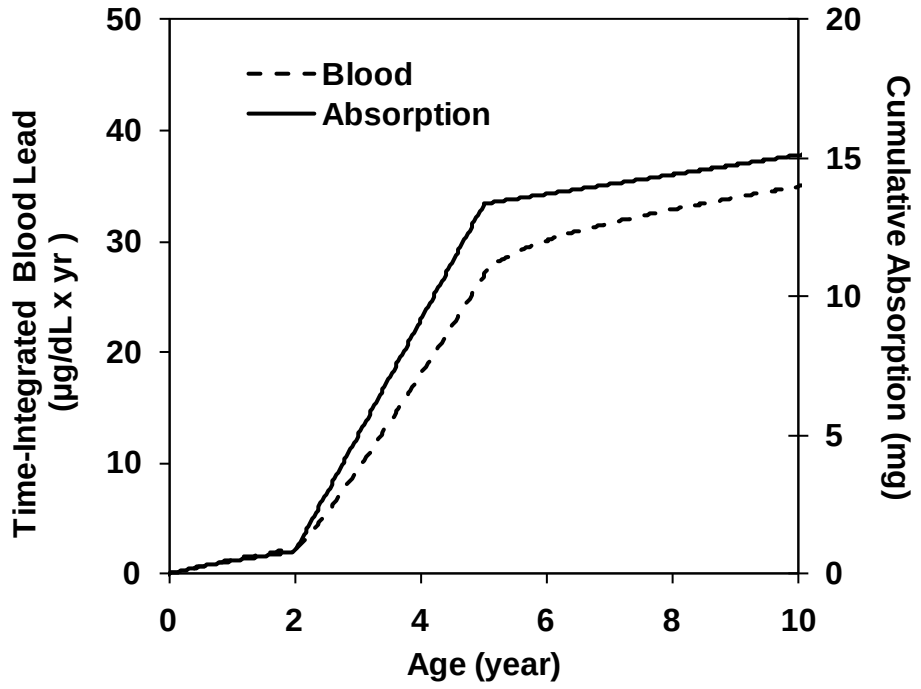


Figure 4-9. Simulation of relationship between time-integrated blood Pb concentration and cumulative Pb absorption in children. The simulations include a 3-year period of elevated Pb intake during ages 2-5 years. The time-integrated blood Pb concentration closely parallels cumulative Pb absorption. Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

4.3.5.2. 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 4-10 which depicts a hypothetical simulation of an exposure event consisting of a 20-year period of daily ingestion of Pb in an adult. The exposure event shown in the simulations gives rise to a relatively rapid increase in blood Pb concentration, to a new quasi-steady state, achieved in ~75-100 days (i.e., approximately 3-4 times the blood elimination half-life). In contrast, the body burden continues to increase during this period. Following cessation of the exposure, blood Pb concentration declines relatively rapidly compared to the slower decline in body burden. Careful examination of the simulation shown in Figure 4-10 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. In the adult simulation shown in Figure 4-10, the initial phase of elimination (the first 5 years following cessation of exposure at 50 years) has a half-time of approximately 14 years; however, the half-time increases to approximately 60 years during the period 5-30 years after cessation of exposure. These model predictions are consistent with the slow elimination of Pb from blood and elimination half-times of several decades for bone Pb (e.g., 16-98 years) that have been estimated from observations made on Pb workers (Fleming et al., 1997; Gerhardsson et al., 1995). Based on this hypothetical simulation, a blood Pb concentration measured 1 year following cessation of a period of increased Pb uptake would show little or no appreciable change from prior to the exposure event, whereas, the body burden would remain elevated. This illustrates 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.

One important potential implication of the profoundly different kinetics of Pb in blood and bone is that, for a constant Pb exposure, bone Pb will increase with increasing duration of exposure and, therefore, with age. In contrast, blood Pb 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 effects of bone Pb were more pronounced 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).

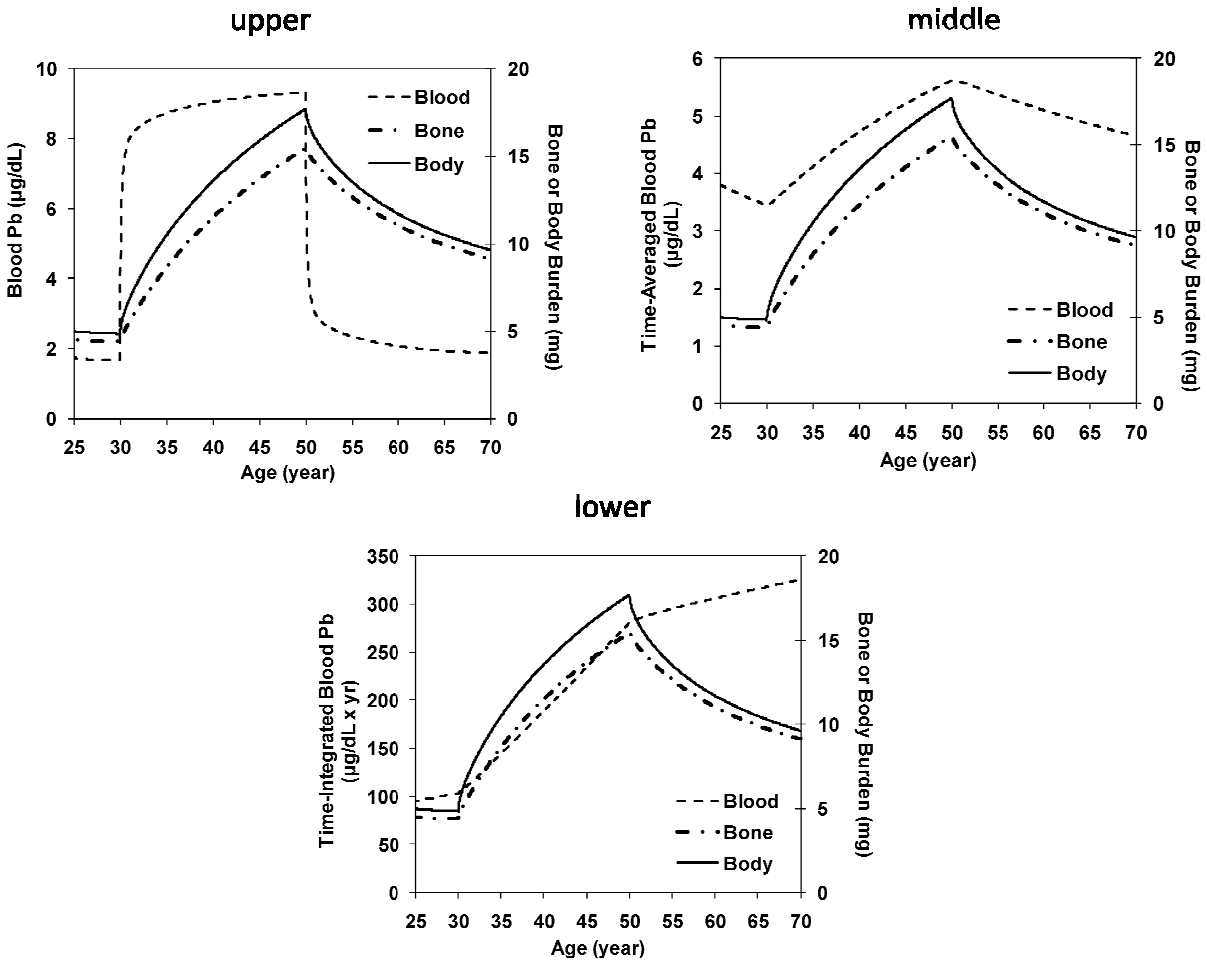


Figure 4-10. Simulation of relationship between blood Pb concentration, bone Pb and body burden in adults.A constant baseline intake results in a quasi-steady state blood Pb concentration and body burden (upper panel). A change in Pb uptake gives rise to a relatively rapid change in blood Pb, to a new quasi-steady state, and a slower change in body burden. The long-term time-averaged blood Pb concentration more closely tracks the slower pattern of change in body burden (middle panel). 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).

Tibia bone Pb has been shown to be 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., 2007). 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 has been 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 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, Mukherjee, et al., 2009).

Mobilization of Lead from Bone in Adulthood

Potential mobilization of Pb from the skeleton increases this contribution of bone Pb to blood Pb, which occurs at times of physiological stress associated with enhanced bone remodeling such as during pregnancy and lactation (Hertz-Picciotto et al., 2000; Manton, 1985; Silbergeld, 1991), menopause or in the elderly (Silbergeld et al., 1988), extended bed rest (Markowitz & 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 (Franklin et al., 1997; B. Gulson et al., 1999; B. Gulson et al., 2003; B. L. 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 (B. Gulson et al., 2004; B. L. Gulson et al., 1997; Hertz-Picciotto et al., 2000; Lagerkvist et al., 1996; Lamadrid-Figueroa et al., 2006; Rothenberg et al., 1994; Schuhmacher et al., 1996). The U-shaped relationship reflects the relatively higher impact of hemodilution in the second trimester versus the rate of bone Pb resorption accompanying calcium 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. (1998) 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. 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. (2004) 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 calcium 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 (1200 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 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 (B. 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, 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 remobilization from bone stores from historic exposures as opposed to contemporaneous exposures.

Lactation can affect 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. (2004; 2006) 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 (Ettinger et al., 2006; B. Gulson, Jameson, et al., 1998; Koyashiki et al., 2010). 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).

The Pb content in some bones (i.e., mid femur and pelvic bone) plateau at middle age and then decreases at older ages (G. A. 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 (B. 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) (Garrido Latorre et al., 2003; Popovic et al., 2005).

Studies of the effect of hormone replacement therapy on bone Pb mobilization have yielded conflicting results (Berkowitz et al., 2004; Garrido Latorre et al., 2003; Korrick et al., 2002; Popovic et al., 2005; 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).

4.3.6. Relationship Between Lead in Blood and Lead in Soft Tissues

Figure 4-11 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.

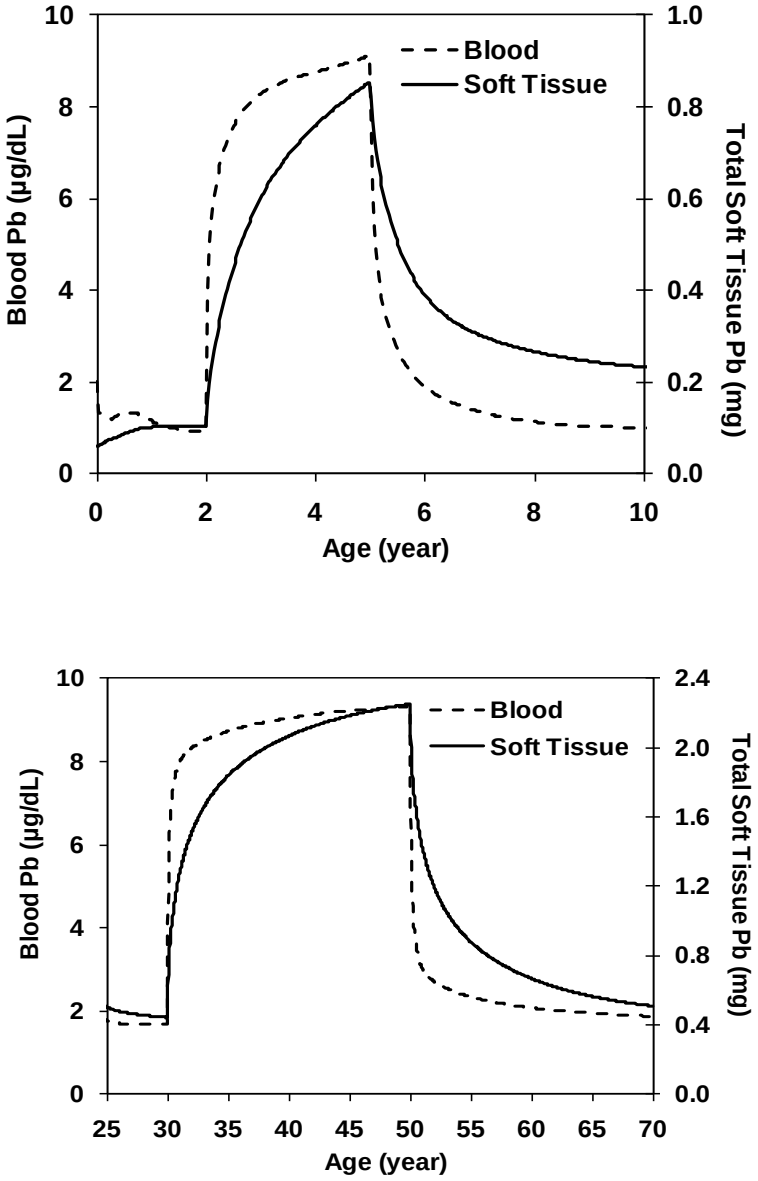


Figure 4-11. Simulation of blood and soft tissue (including brain) Pb in children and adults who experience a period of increased Pb intake. Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

Information on Pb levels in human brain are limited to autopsy data and the simulation of brain Pb shown in Figure 4-12 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 in shown Figure 4-12. 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. This is reflected in the simulation as slower brain Pb accumulation in children. 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.

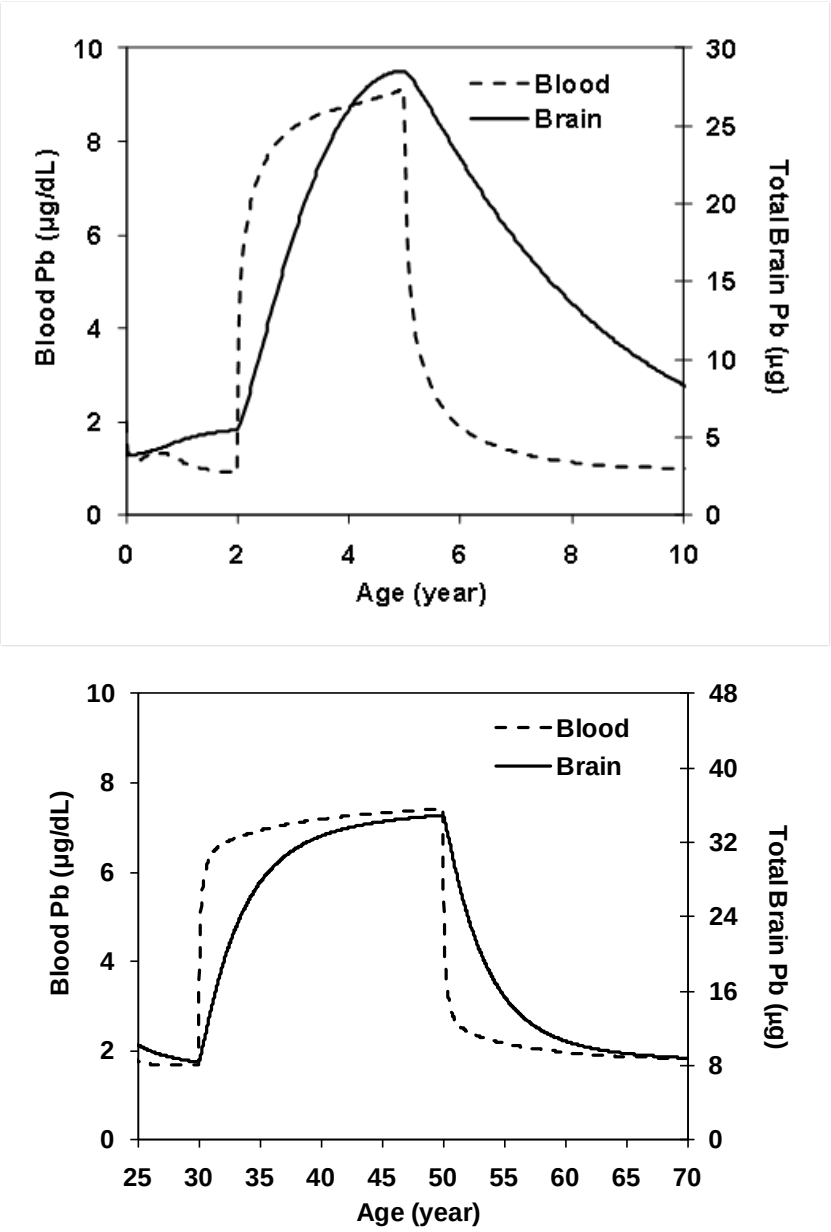


Figure 4-12. Simulation of blood and brain Pb in children and adults who experience a period of increased Pb intake.Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

4.3.7. Relationship Between Lead in Blood and Lead in Urine

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 4-13 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. At low blood Pb concentrations (<25 µg/dL), urinary Pb excretion is predicted to closely parallel plasma Pb concentration for any given blood Pb level (Figure 4-13, 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 (i.e., not provoked by administration of chelating agents) will quickly decrease while bone Pb will remain elevated (Figure 4-13, lower panel).

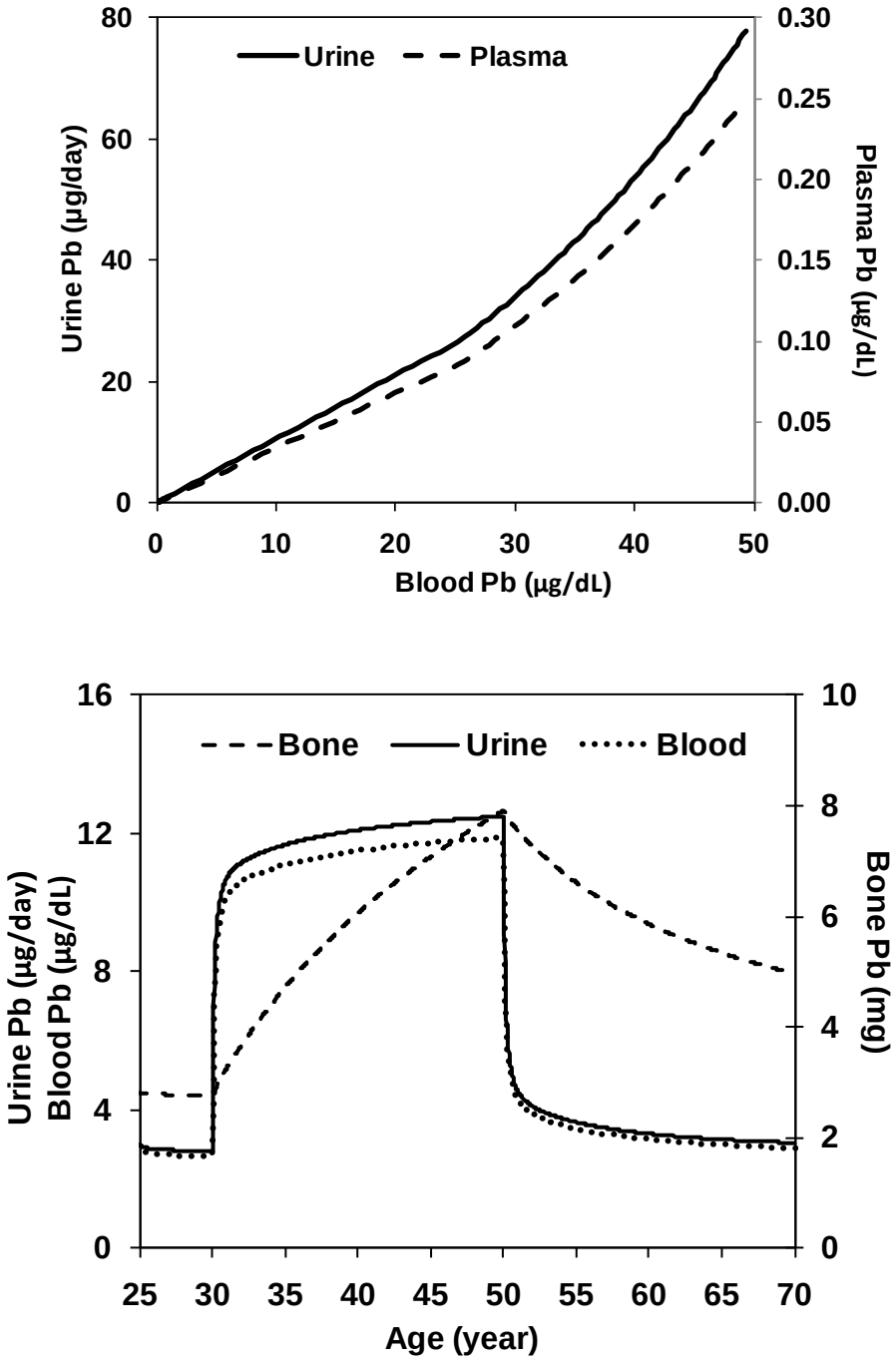


Figure 4-13. Top panel: Predicted relationship between plasma Pb concentration and urinary Pb excretion in an adult (age 40 years). Lower panel: Simulation of blood Pb, bone Pb and urinary excretion of Pb in an adult who experiences a period of increased Pb intake. Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

4.4. Observational Studies of Lead Exposure

4.4.1. Lead in Blood

Overall, trends in blood Pb levels have been decreasing among U.S. residents over the past twenty years. Blood Pb concentrations in the U.S. general population have been monitored in the NHANES. Analyses of these data have shown a progressive downward trend in blood Pb concentrations during the period 1976-2008, with the most dramatic declines coincident with the phase out of leaded gasoline (Brody et al., 1994; Pirkle et al., 1994; Pirkle et al., 1998; J. Schwartz & Pitcher, 1989). The temporal trend for the period 1988-2008 is shown in Figure 4-14. Summary statistics from the most recent publically available data (1999-2008) are presented in Table 4-7 (CDC, 2011). The geometric mean Pb concentration among children 1-5 years of age, based on the sample collected during the period 2007-2008, was 1.51 µg/dL (95% CI: 1.37, 1.66), which was a slight increase from the previous year (1.46 µg/dL, 95% CI: 1.36, 1.57). Figure 4-15 uses NHANES data to illustrate the distribution of blood Pb levels among U.S. children aged 12-60 months. The median blood Pb in this age group was 1.4 µg/dL with a 95th percentile value of 4.1 µg/dL (NCHS, 2010). For 2005-2008, 95% of childhood blood Pb levels were less than 5 µg/dL.

When data were aggregated for years 1999-2004, Pb concentrations 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., 2009). Figure 4-16 demonstrates the change in percent of children with various blood Pb levels by race/ethnicity from 1988-1991 and 1999-2004. When these data were aggregated for the years 1988-2004, residence in older housing, poverty, age, and being non-Hispanic black were significant risk factors for higher Pb levels (Jones et al., 2009). The geometric mean blood Pb concentration among adults ≥ 20 years of age was 1.38 µg/dL (95% CI: 1.31, 1.46) based on the sample collected during the period 2007-2008 (CDC, 2011). Based on these same data, the geometric mean for all males was 1.47 µg/dL (95% CI: 1.39, 1.56), and for females was 1.11 µg/dL (95% CI: 1.06, 1.16).

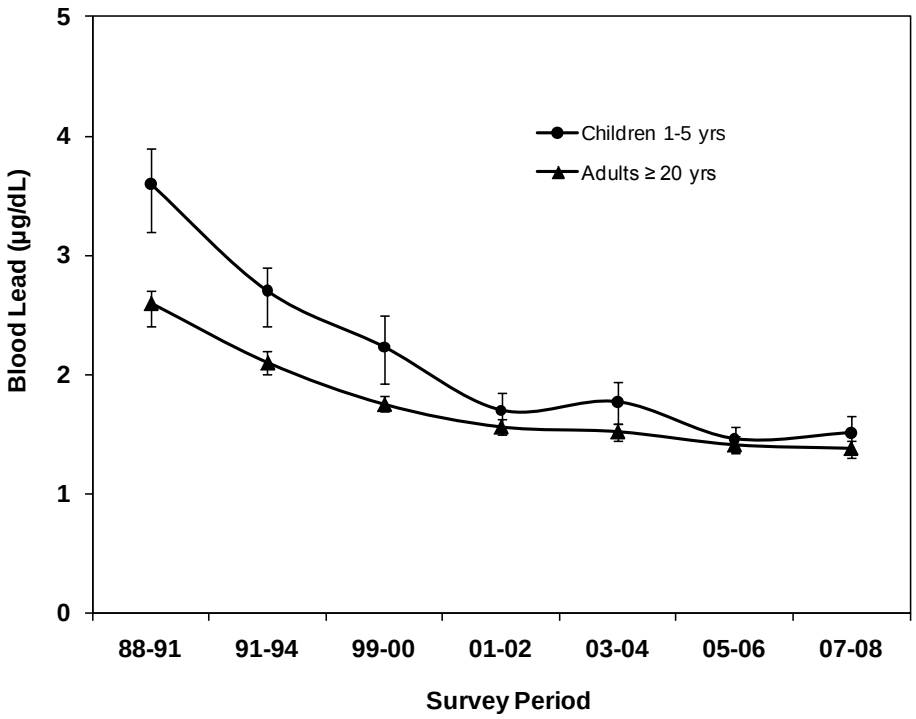
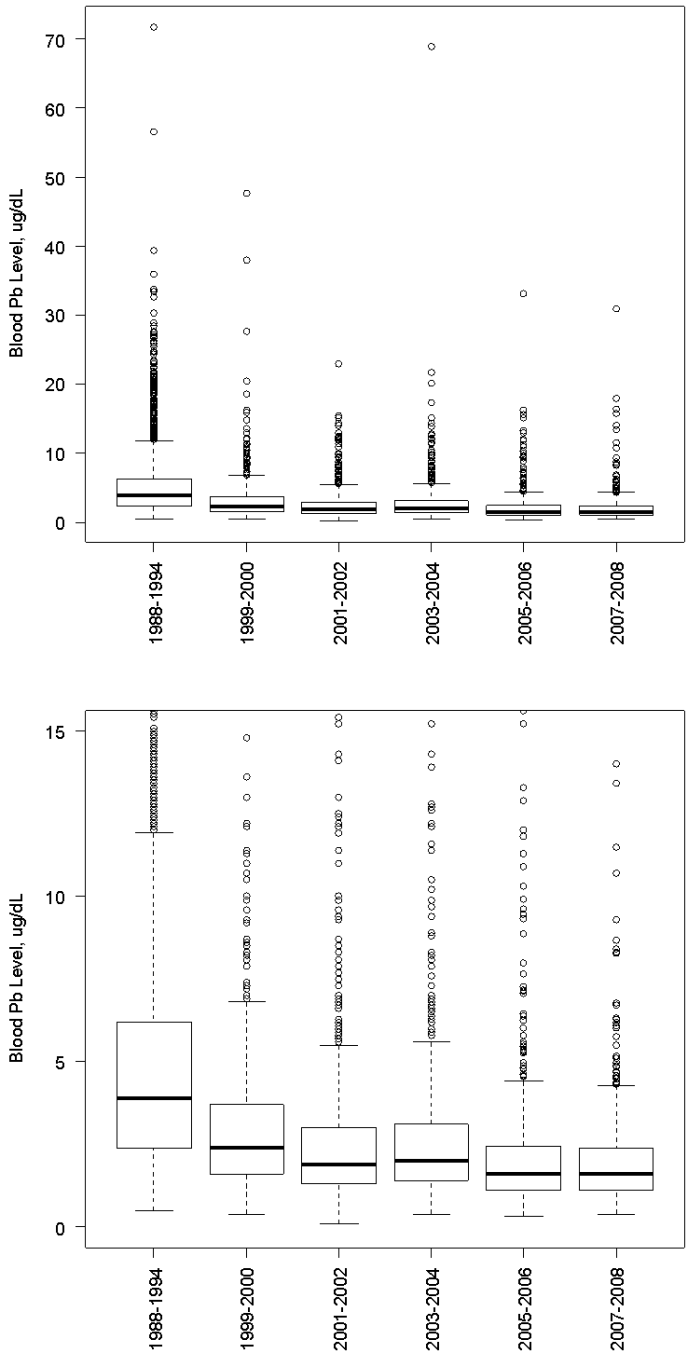


Figure 4-14. Temporal trend in blood Pb concentration.Shown are geometric means and 95% CIs based on data from NHANES III Phase 1 (Brody et al., 1994; Pirkle et al., 1994); NHANES III Phase 2 (Pirkle et al., 1998); and NHANES IV (CDC, 2011). Data for adults during the period 1988-1994 are for ages 20-49 years, and ≥ 20 years for the period 1999-2008.

Table 4-7. Blood Pb concentrations in the U.S. population

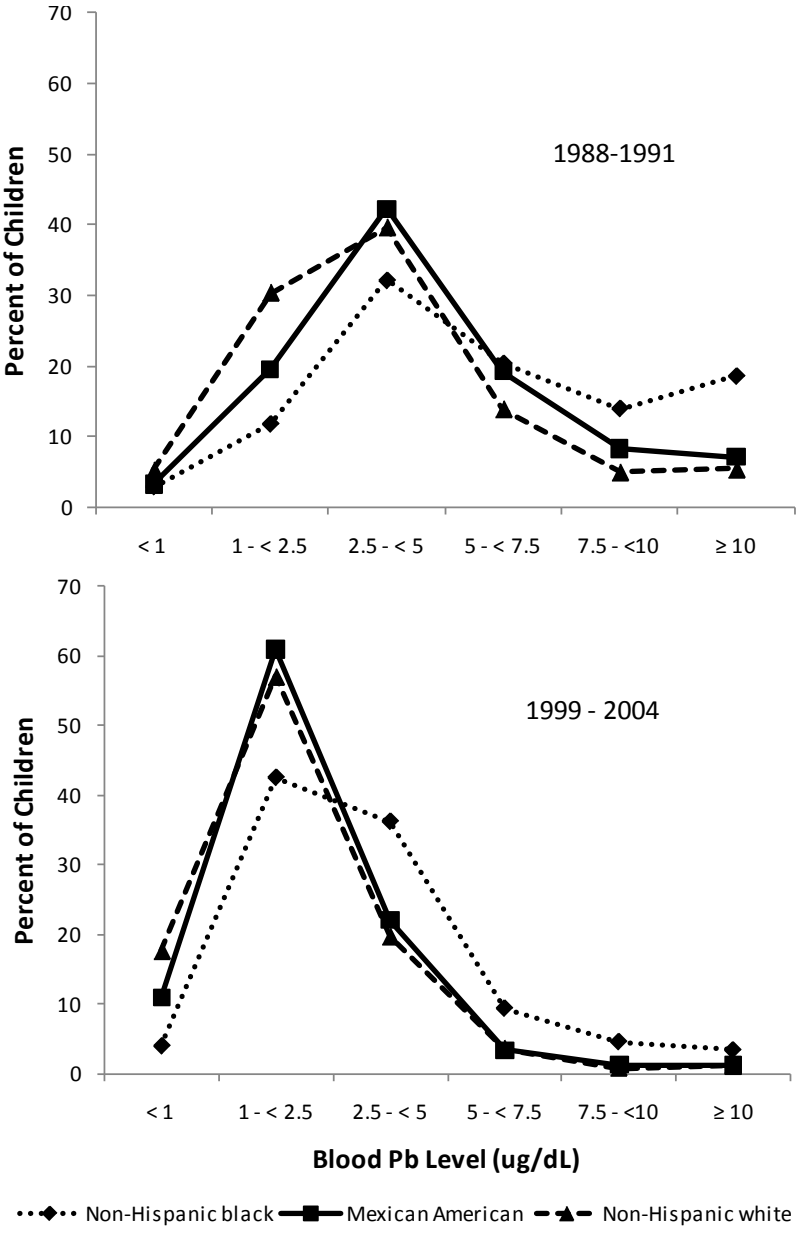
Survey Stratum	Period	Geometric Mean (µg/dL)	95% Confidence Interval	Number of Subjects
All	1999-2000	1.66	1.60, 1.72	7970
	2001-2002	1.45	1.39, 1.51	8945
	2003-2004	1.43	1.36, 1.50	8373
	2005-2006	1.29	1.23, 1.36	8407
	2007-2008	1.27	1.21, 1.34	8266
1-5 yr	1999-2000	2.23	1.96, 2.53	723
	2001-2002	1.70	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
6-11 yr	1999-2000	1.51	1.36, 1.66	905
	2001-2002	1.25	1.14, 1.36	1044
	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	1011
12-19 yr	1999-2000	1.10	1.04, 1.17	2135
	2001-2002	0.94	0.90, 0.99	2231
	2003-2004	0.95	0.88, 1.02	2081
	2005-2006	0.80	0.75, 0.85	1996
	2007-2008	0.80	0.74, 0.86	1074
≥ 20 yr	1999-2000	1.75	1.68, 1.81	4207
	2001-2002	1.56	1.49, 1.62	4772
	2003-2004	1.52	1.45, 1.60	4525
	2005-2006	1.41	1.34, 1.48	4509
	2007-2008	1.38	1.31, 1.46	5364
Males	1999-2000	2.01	1.93, 2.09	3913
	2001-2002	1.78	1.71, 1.86	4339
	2003-2004	1.69	1.62, 1.75	4132
	2005-2006	1.52	1.42, 1.62	4092
	2007-2008	1.47	1.39, 1.56	4147
Females	1999-2000	1.37	1.32, 1.43	4057
	2001-2002	1.19	1.14, 1.25	4606
	2003-2004	1.22	1.14, 1.31	4241
	2005-2006	1.11	1.05, 1.17	4315
	2007-2008	1.11	1.06, 1.16	4119
Mexican - Americans	1999-2000	1.83	1.75, 1.91	2742
	2001-2002	1.46	1.34, 1.60	2268
	2003-2004	1.55	1.43, 1.69	2085
	2005-2006	1.29	1.21, 1.38	2236
	2007-2008	1.25	1.15, 1.36	1712
Non-Hispanic blacks	1999-2000	1.87	1.75, 2.00	1842
	2001-2002	1.65	1.52, 1.80	2219
	2003-2004	1.69	1.52, 1.89	2293
	2005-2006	1.39	1.26, 1.53	2193
	2007-2008	1.39	1.30, 1.48	1746
Non-Hispanic whites	1999-2000	1.62	1.55, 1.69	2716
	2001-2002	1.43	1.37, 1.48	3806
	2003-2004	1.37	1.32, 1.43	3478
	2005-2006	1.28	1.19, 1.37	3310
	2007-2008	1.24	1.16, 1.33	3461

Source: Adapted from data from the NHANES (CDC, 2011)



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

Figure 4-15. Box plots of blood Pb levels among U.S. children (1-5 years old) from the NHANES survey, 1988-2008.Top: all data. Bottom: data for subjects having blood Pb levels less than 15 µg/dL.



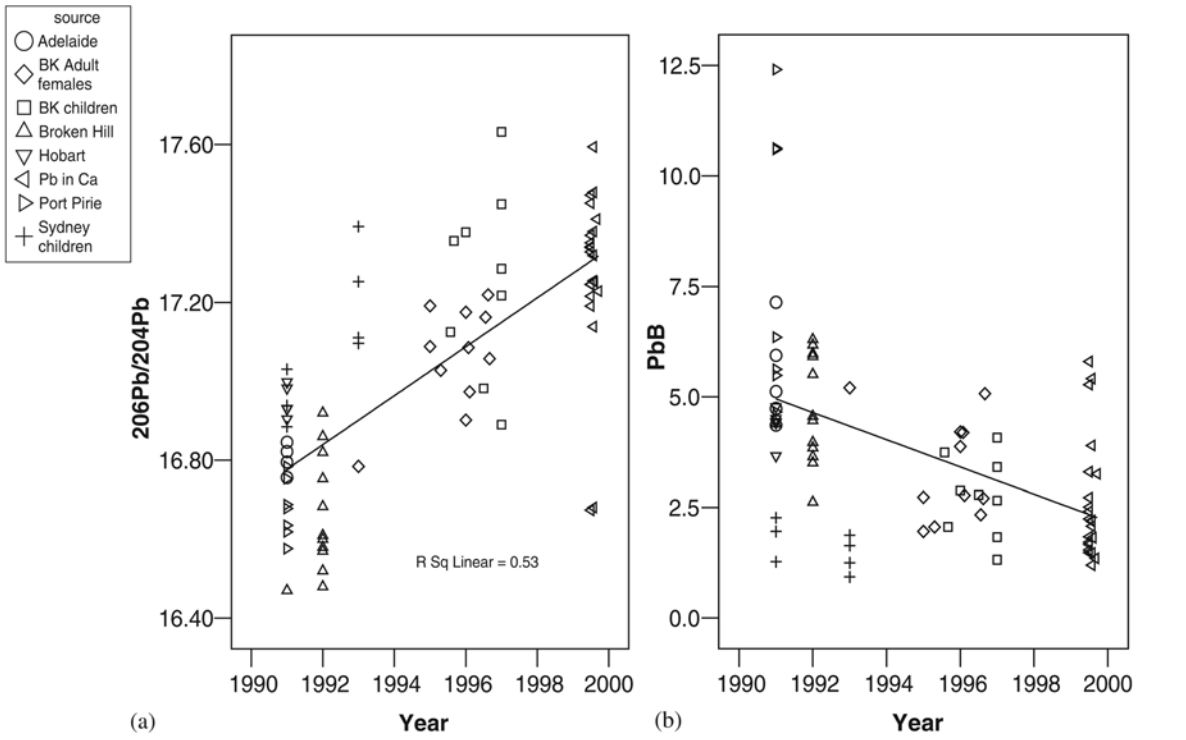
Data used with permission from the American Academy of Pediatrics, Jones et al. (2009)

Figure 4-16. 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).

Several studies have shown seasonal variation in blood Pb concentrations in children (B. Gulson et al., 2008; D. L. Johnson et al., 1996; Laidlaw et al., 2005). Seasonal variation in blood Pb concentration was also evident in individual children when repeated blood Pb measurements were made over a 5-year

period (B. Gulson et al., 2008). Meteorological factors appear to contribute to blood Pb seasonality. Laidlaw et al. (2005) analyzed the temporal relationships between child blood Pb concentrations and various atmospheric variables in three cites (Indianapolis, IN; Syracuse, NY; New Orleans, LA). Blood Pb data was obtained from public health screening programs conducted in the three cities during 1998-2003. Blood Pb samples were dominated by children <5 years of age and age distribution varied across the three cities. The number of blood Pb measurements included in the analyses were as follows: Indianapolis, 15,969; Syracuse, 14,457 (D. Johnson & Bretsch, 2002; D. L. Johnson et al., 1996); New Orleans, 2,295 (Mielke et al., 2007). The temporal variation in blood Pb concentrations in each city were 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 ($p < 0.00001$). This analysis provides a possible explanation for the seasonal patterns of blood Pb concentrations in children that involves weather-dependent entrainment and air transport of surface dusts.

Trends in blood Pb levels have been accompanied by changes in Pb isotope ratios within blood Pb. Isotopic ratios, described in Sections 3.2 and 3.3 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 (B. Gulson et al., 2006a; B. Gulson et al., 2008; Ranft et al., 2006, 2008). 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 various study populations in Australia during the period 1990-2000 (Figure 4-17). 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.



Source: Used with permission from Academic Press, Gulson et al. (2006a)

Figure 4-17. a) Trends in $^{206}Pb/^{204}Pb$ isotope ratio in blood Pb and b) trends in blood Pb levels among Australian study populations during the period 1990-2000.

4.4.2. Lead in Bone

An extensive national database (i.e., NHANES) is available for blood Pb concentrations in children and adults, as described in Section 4.4.1. Bone Pb concentrations are less well characterized. Tables 4-8 and 4-9 are compilations of data from epidemiologic studies that provided bone Pb concentrations 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 4-8). 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 4-9 for occupationally exposed individuals were also generally higher compared to non-occupationally exposed individuals (Table 4-8).

Table 4-8. Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations

Reference	Cohort	Age (yrs)	N	Location	Study Period	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Bandeen-Roche et al. (2009)	Baltimore Memory Study cohort	50-70	1140	Baltimore, MD	2001-2005	Cumulative	Tibia	Mean±SD Tibia: 18.8 ± 11.6	Not reported
Bellinger et al. (1994)	Not reported	5-8 (recruited) 19-20 (follow-up)	79	Boston, MA	1989-1990	Cumulative	Tibia Patella	Mean (Range): Tibia: 5.4 (3-16) Patella: 9.2 (4-18)	High exposure: >24 Low exposure: <8.7
Cheng et al. (2001)	Normative Aging Study cohort	Mean±SD: Normotensive : 65.49 ± 7.17 Borderline hypertension: 68.3 ± 7.79 Definite hypertension: 67.93 ± 6.79	833 males	Boston, MA	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)	Participants from Henry Ford Health System (HFHS)	≥ 50 Mean: 69.9	121 cases 414 controls	Southeastern Michigan	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)	Normative Aging Study	Not reported	471 elderly males	Greater Boston area, MA	6/1991-12/1994	Not reported	Tibia Patella	Mean±SD: Tibia: 21.6 ±12.0 Patella: 31.7 ± 18.3	Not reported
Glass et al.,(2009)	Baltimore Memory Study	Mean: 59.4 Range: 50-70	1,001	Baltimore, MD	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

Reference	Cohort	Age (yrs)	N	Location	Study Period	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Hsieh et al. (2009)	Not reported	Mean: Control: 46.06	18 controls	Not reported	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. (1996) (As reported in Navas-Acien et al., (2008))	Normative Aging Study	48-92 Mean ± SD: 66.6 ± 7.2	590 males	Boston, MA	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
Jain et al. (2007)	VA-Normative Aging Study	Not reported	837 males	Greater Boston, MA	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: Noncases: -3-126 Cases: -5-75 Patella: Noncases: -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)
Kamel et al. (2002); Kamel et al. (2005); Kamel et al. (2008)	Not reported	30-80	256 controls (Bone samples collected from 41 controls)	New England (Boston, MA)	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. (2009)	1982 Pb Occupational Study	Control mean: 55	51 controls	Eastern Pennsylvania	1982-2004	Control group for occupational exposure group	Tibia	Median (IQR) Tibia Control: 12 (-8-32)	Not reported

Reference	Cohort	Age (yrs)	N	Location	Study Period	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Korrick et al. (1999) (As reported in from Navas-Acien et al., (2008)	Nurses' Health Study	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	284 females (89 cases; 195 controls)	Boston, MA	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
Lee et al. (2001) (As reported in Navas-Acien et al., (2008))	Not reported	22.0-60.2 Mean ± SD: Controls: 34.5 ± 9.1	135 controls	Republic of Korea	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)	Baltimore Memory Study	50-70 Mean: 59.4	964	Baltimore, MD	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)	Not reported	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	194 male youth cases 146 male youth controls	Allegheny County, PA (cases); Pittsburgh, PA (controls)	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 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))	Not reported	Median: 41.5	19 male controls	Not reported	Not reported	Control group for occupational exposure group	Finger bone	Median (range) Finger Bone Controls: 4 (-19-18)	Not reported

Reference	Cohort	Age (yrs)	N	Location	Study Period	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Park et al. (2006)	Normative Aging Study	Mean: 72.9 ± 6.5	413 males	Greater Boston, MA	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. (2009)	Normative Aging Study	Mean: 67.3 ± 7.2	613 males	Greater Boston, MA	8/1991 - 12/1995	Not reported	Tibia Patella	Median (IQR) Tibia: 19 (14-27) Patella: 26 (18-37)	Table 1 distributes tibia and patella Pb by genotype; Table 2 distributes tibia and patella Pb by number of gene variants
Park et al. (2010)	VA Normative Aging Study cohort	Mean: 64.9 (at bone Pb measurement)	448 males	Eastern Massachusetts	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 provides age-adjusted mean bone Pb levels (age, race, education, smoking [pack-yr], occupational noise, noise notch, BMI, hypertension, diabetes)
Payton et al. (1998)	VA Normative Aging Study cohort	Mean: 66.8	141 males	Boston, MA	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)	Normative Aging Study cohort	Mean: 66.9	513 male cases	Boston, MA	1991-1996	Cumulative	Tibia Patella	Mean ± SD Tibia: 21.5 ± 13.4 Patella: 31.5 ± 19.3	Not reported
Rajan et al. (2007)	VA Normative Aging Study Cohort	Mean: 67.5 (at bone scan)	1075 males	Boston, MA	1991-2002	Not reported	Tibia Patella	Mean ± SD Tibia: 22.1 ± 13.8 Patella: 31.4 ± 19.6	Not reported

Reference	Cohort	Age (yrs)	N	Location	Study Period	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Rajan et al. (2008)	VA Normative Aging Study Cohort	≥ 45	720 males	Boston, MA	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
Rhodes et al. (2003)	VA Normative Aging Study Cohort	Mean: 67.1	526 males	Boston, MA	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)	Not reported	30-60	68 males	Belgium	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. (2002) (As reported in Navas-Acien et al., (2008))	Not reported	15-44 Mean ± SD: 31.0 ± 7.7	720 females	Los Angeles, CA	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)	Baltimore Memory Study cohort	Mean: 59.39	985	Baltimore, MD	Not reported	Not reported	Tibia	Mean ± SD: Tibia: 18.7 ± 11.2	Not reported

Reference	Cohort	Age (yrs)	N	Location	Study Period	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)	Not reported	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)	257 cases 276 controls	Silver Valley, ID; Spokane, WA	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)	Not reported	Mean: 61.5	47	Rochester, NY	Not reported	Cumulative	Tibia Calcaneus	Mean ± SD Tibia: 2.0 ± 5.2 Calcaneus: 6.1 ± 8.5	Not reported
Wasserman et al. (2003)	Yugoslavia Prospective Study of Environmental Pb Exposure	10-12	167 children	Kosovska, Mitrovica, Kosovo, Yugoslavia; Pristina, Kosovo, Yugoslavia	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 distributes tibia Pb by sex, ethnicity, address at birth relative to factory, and maternal education

Reference	Cohort	Age (yrs)	N	Location	Study Period	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Weisskopf et al. (2004), as reported in Shih et al. 194225 (2007)	Normative Aging Study	Mean ± SD: 67.4 ± 6.6	466 males	Boston, MA	1991-2002	Environmental	Tibia Patella	Median (IQR) Tibia: 19 (12,26) Patella: 23 (15, 35)	Tibia IQR: 14 Patella IQR: 20 Table 3 shows mean Pb levels across categorical variables (yr of education, smoking status, computer experience, first language English)
Weisskopf et al. (2007)	VA Normative Aging Study cohort	Mean: Lowest Patella quintile: 73.2 Highest Patella quintile: 80.7	31 males	Boston, MA	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
Weisskopf et al. (2007)	VA Normative Aging Study cohort	Mean: 68.7	1,089 males	Boston, MA	1993-2001	Concurrent and cumulative	Tibia Patella	Median (IQR) Tibia: 20 (13-28) Patella: 25 (17-37)	Table 1 shows the 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)	Normative Aging Study (95% white)	Mean ± SD (at Patella baseline) Tertile 1: 65.2 ± 7.1 Tertile 2: 66.5 ± 6.5 Tertile 3: 70.2 ± 7.2	868 males	Greater Boston area, MA	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)	BUMC, BWH, BIDMC, HVMA, Normative Aging Study (NAS), Harvard Cooperative Program on Aging (HCPOA)	Mean: Cases: 66.5 Controls: 69.4	330 cases 308 controls	Boston, MA	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

Reference	Cohort	Age (yrs)	N	Location	Study Period	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Weuve et al. (2006)	VA Normative Aging Study cohort	≥ 45	720 males	Boston, MA	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 shows distribution of mean Pb bio-marker levels by characteristics of participants (age, education, computer experience, smoking status, alcohol consumption, tertile of cal-cium intake, tertile of physical activity, diabetes)
Weuve et al. (2009)	Nurses' Health Study cohort	47-74	587 females	Boston, MA	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. 194225 (2007)	Normative Aging Study	Mean ± SD: 68.2 ± 6.9	736 males	Boston, MA	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 4-9. Epidemiologic studies that provide bone Pb measurements for occupationally exposed populations

Reference	Cohort	Age (years)	Number of Subjects	Location	Study Period	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))	Canada Lead Study	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	80 males	Canada	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. (2007)	Not reported	Mean: 39.7	61	Northern Canada	Not reported	Occupational (primary Pb smelter workers)	Tibia	Mean: Tibia: 38.6	Not reported
Caffo et al. (2008)	Not reported	Mean: 60.39	513 males	Delaware and New Jersey, US	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)	Not reported	Mean: 43.4	652	Korea	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
Glenn et al. (2003), as reported in Navas-Acien et al., (2008)	Not reported	40-70 Mean: 55.8 (baseline)	496 males	Eastern U.S.	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)	Not reported	0-36.2 (baseline) Mean ± SD: 41.4 ± 9.5 (baseline)	575 (76% male; 24% female)	South Korea	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

Reference	Cohort	Age (years)	Number of Subjects	Location	Study Period	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Hanninen et al. (1998), as reported in Shih et al., (2007)	Not reported	Mean±SD: Male: 43 Female: 48 BPb(max) ≤ 2.4 µmol/L: 41.7 ± 9.3 BPb (max) >2.4 µmol/L: 46.6 ± 6.2	54 (43 males, 11 females)	Helsinki, Finland	Not reported	Occupational (Pb acid battery factory workers)	Tibia Calcaneus	Mean±SD: Tibia: BPb (max) ≤ 2.4 µmol/L: 19.8 ± 13.7 BPb (max) >2.4 µmol/L: 35.3 ± 16.6 Calcaneus: BPb (max) ≤ 2.4 µmol/L: 78.6 ± 62.4 BPb (max) >2.4 µmol/L: 100.4 ± 43.1	Not reported
Hsieh et al. 2009 (2009)	Not reported	Mean: Cases: 45.71 Controls: 46.06	22 cases 18 controls	Location NR	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)	Not reported	30-80	109 cases 256 controls (Bone samples collected from 104 cases and 41 controls)	New England (Boston, MA)	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. (2009)	1982 Pb Occupational Study cohort	Mean: Cases: 54 Controls: 55	83 cases 51 controls	Eastern Pennsylvania	1982-2004	Occupational (Pb battery plant workers)	Tibia	Median (IQR) Tibia Cases: 57 (20-86) Controls: 12 (-8-32)	Not reported
Osterberg et al. (1997), as reported in Shih et al. (2007)	Not reported	Median: 41.5	38 male cases 19 male controls	Not reported	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

Reference	Cohort	Age (years)	Number of Subjects	Location	Study Period	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Roels et al. (1994)	Not reported	30-60	76 male cases 68 male controls	Belgium	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 (2000) et al., as reported in Shih et al., (2007)	U.S. Organolead Study	Mean ± SD: Cases: 55.6 ± 7.4 Controls: 58.6 ± 7.0	535 male cases 118 male controls	Eastern U.S.	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
Schwartz et al. (2000), as reported in Navas-Acien et al.(2008)	Not reported	41.7-73.7 (Combined) Mean ± SD: Combined: 57.6 ± 7.6 Hypertensive: 60.2 ± 6.9 Nonhypertensive: 56.6 ± 7.5	543 males	Eastern U.S.	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. (2001)	Not reported	Mean: Exposed: 40.4 Control: 34.5	803 cases 135 controls	South Korea	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)	Not reported	Mean at 1st visit: 41.4	576	South Korea	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

Reference	Cohort	Age (years)	Number of Subjects	Location	Study Period	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)	U.S. Organolead Study	40-70 (in 1995) 38% ≥ 60 yrs Mean: 58	534 males	Eastern U.S.	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)	Not reported	Mean: 56.1	532 males	Eastern U.S.	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)	Not reported	Mean ± SD: 43.3 ± 9.8	652	South Korea	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

4.4.3. Lead in Urine

Urine Pb concentrations in the U.S. general population have been monitored in the NHANES. Data from the most recent survey (CDC, 2011) are shown in Table 4-10. 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 4-10. Urine Pb concentrations in the U.S. population

Survey Stratum	Period	Geometric Mean (µg/g CR)	95% Confidence Interval	Number of Subjects
All	1999-2000	0.721	0.700, 0.742	2465
	2001-2002	0.639	0.603, 0.677	2689
	2003-2004	0.632	0.603, 0.662	2558
	2005-2006	0.546	0.502, 0.573	2576
	2007-2008	0.515	0.483, 0.549	2627
6-11 yr	1999-2000	1.170	0.975, 1.41	340
	2001-2002	0.918	0.841, 1.00	368
	2003-2004	0.926	0.812, 1.06	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	1406
	2001-2002	0.658	0.617, 0.703	1559
	2003-2004	0.641	0.606, 0.679	1543
	2005-2006	0.573	0.548, 0.600	1520
	2007-2008	0.546	0.513, 0.580	1857
Males	1999-2000	0.720	0.679, 0.763	1227
	2001-2002	0.639	0.607, 0.673	1334
	2003-2004	0.615	0.588, 0.644	1281
	2005-2006	0.551	0.522, 0.582	1271
	2007-2008	0.502	0.471, 0.534	1327
Females	1999-2000	0.722	0.681, 0.765	1238
	2001-2002	0.639	0.594, 0.688	1355
	2003-2004	0.648	0.601, 0.698	1277
	2005-2006	0.541	0.507, 0.577	1305
	2007-2008	0.527	0.489, 0.568	1300
Mexican - Americans	1999-2000	0.940	0.876, 1.01	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	1132
	2003-2004	0.623	0.592, 0.655	1074
	2005-2006	0.541	0.500, 0.585	1041
	2007-2008	0.506	0.466, 0.550	1095

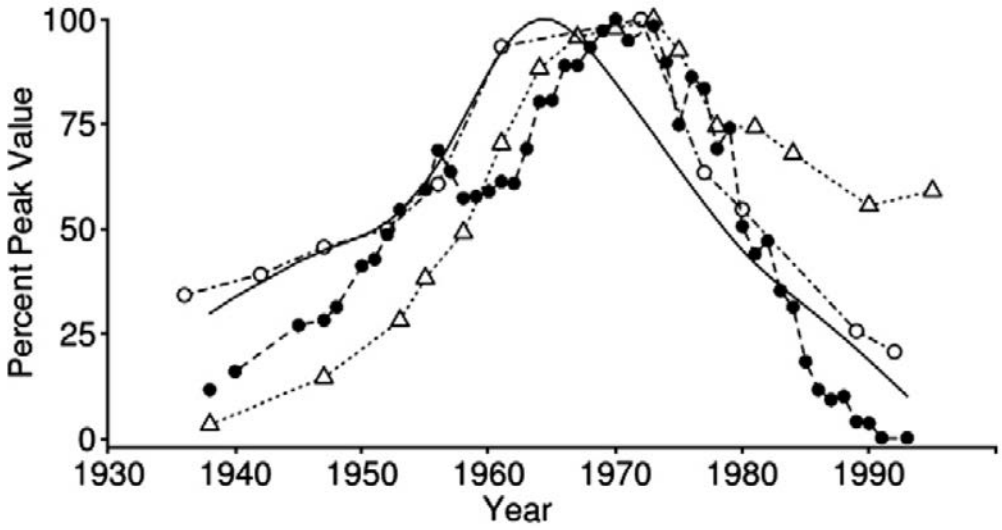
Values are µg Pb/g creatinine

Source: Based on data from the NHANES (CDC, 2011)

4.4.4. Lead 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 ($p < 0.00001$); Figure 4-18. 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 ($p < 0.0002$).



Source: Used with permission from Elsevier Publishing, Robbins et al. (2010).

Figure 4-18. Comparison of tooth enamel50% formation (solid line) with newly obtained Pb sediment Lake Erie cores (open triangles), 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.

4.5. Empirical Models of Lead Exposure-Blood Lead 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 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, which is often necessary for regulatory applications of the models, can be problematic.

4.5.1. Air Lead-Blood Lead Relationships

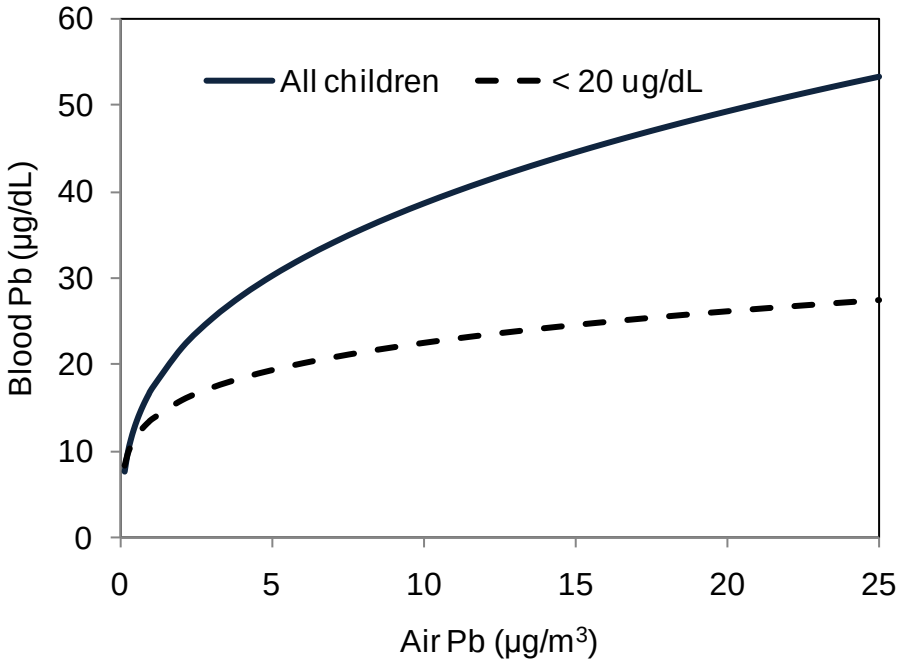
The 2006 AQCD and its 1986 predecessor (U.S. EPA, 1986, 2006) described epidemiological studies of relationships between air Pb and blood Pb. Much of the pertinent earlier literature (e.g., prior to 1984) was summarized by Brunekreef (1984). Based on meta-analysis of 18 studies of urban or industrial-urban populations, 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; Figure 4-19). This corresponds to an increase of 6.3 µg/dL blood Pb for an increase in air Pb concentration from 0.15 to1.5 µg/m^3. When the analysis was limited to children whose blood Pb concentrations were <20 µg/dL, the regression coefficient was 0.2159 (R^2=0.33), which corresponds to an increase of 3.2 µg/dL blood Pb for an increase in air Pb from 0.15 to1.5 µg/m^3. Blood Pb-air Pb slopes are presented for recent studies in the following paragraphs. These data are summarized in Table 4-11.

Table 4-11. Summary of estimated slopes for blood Pb to air Pb relationships in humans

Reference	Study Methods	Model Description	Blood Pb–Air Pb Slope (μg/dL/μg/m^3)
Children Populations			
Brunekreef et al. (1984)	Location: Various countries Years: 1974-1983 Subjects: Children (varying age ranges) Analysis: Meta analysis of 18 studies	Model: Log-Log Blood Pb: 5-41 μg/dL (mean range for studies) Air Pb: 0.2-10 μg/m^3 (mean range for studies)	All children: 18[a], 6.1[b] Children <20 μg/dl: 13[a], 3.0[b]
Hayes et al. (1994)	Location: Chicago, IL Years: 1974-1988 Subjects: 0.5-6 yr (9,604 blood Pb measurements) Analysis: Regression of blood Pb screening and quarterly average air Pb	Model: Log-Log Blood Pb: 12-30 μg/dL (annual GM range) Air Pb: 0.5-1.2 μg/m^3 (annual GM range)	24[a], 5.7[b]
Hilts et al. (2003)	Location: Trail, BC Years: 1989-2001 Subjects: 0.5-6 yr (292-536 blood Pb measurements/yr) 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 median range) Air Pb: 0.03-1.1 μg/m^3 (annual median range)	6.5
Ranft et al. (2008)	Location: Germany Years: 1983-2000 Subjects: 6-11 yr (n = 843) Analysis: Pooled regression 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[c]
Schnaas et al. (2004)	Location: Mexico City Years: 1987-2002 Subjects: 0.5-10 yr (n = 321) Analysis: Regression of longitudinal blood Pb measurements and annual average air Pb data	Model: Log-Log Blood Pb: 5-12 μg/dL (annual GM range) Air Pb: 0.7-2.8 μg/m^3 (annual mean range)	4.8[a], 1.1[b]
Schwartz and Pitcher (1989), U.S. EPA (1986)	Location: US Years: 1976-1980 Subjects: 0.5-7 yr (n = 7,000) Analysis: NHANES blood Pb, gasoline consumption data and Pb concentrations in gasoline	Model: Linear Blood Pb: 11-18 μg/dL (mean range) Air Pb: 0.36-1.22 μg/m^3 (annual maximum quarterly mean)	9.3
Schwartz and Pitcher (1989), U.S. EPA (1986)	Location: Chicago, IL Years: 1976-1980 Subjects: 0-5 yr (n = 7,000) Analysis: Chicago blood Pb screening, gasoline consumption data, and Pb concentrations in gasoline	Model: Linear Blood Pb: 18-27 μg/dL (mean range) Air Pb: 0.36-1.22 μg/m^3 (annual maximum quarterly mean)	7.7
Tripathi et al. (2001)	Location: Mumbai, India Years: 1984-1996 Subjects: 6-10 yr (n = 544) Analysis: Regression of blood Pb and air Pb data	Model: Linear Blood Pb: 8.6-14.4 μg/dL (regional GM range) Air Pb: 0.11-1.18 μg/m^3 (regional GM range)	3.6
Adult Populations			
Rodrigues et al. (2010)	Location: New England, U.S. Years: 1994-1995 Subjects: Adult bridge painters (n-84, 1 female) Analysis: Regression analysis of blood Pb and air Pb data (personal monitors) collected during work performing various job-related tasks	Model: Log-log Blood Pb: 16.1 μg/dL (GM, 1,7 GSD) Air Pb: 58 μg/m^3 (GM, 2.8 GSD)	5.4[a], 4.2[b]

[a]At an air concentration of 0.15 μg/m^3
[b]At an air concentration of 1 μg/m^3
[c]For a change in air Pb concentration from 0.025 to 0.465 μg/m^3

GM, geometric mean; GSD, geometric standard deviation



Data provided from Brunekreef (1984).

Figure 4-19. Predicted relationship between air Pb and blood Pb based on a meta analysis of 18 studies. The regression model is: ln[µg/dL blood Pb] = 0.3485·ln[µg/m^3 air Pb] + 2.85 for all children 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.

4.5.1.1. Children

Hilts et al. (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 (n: 292-536 per year) who lived within 4 km from the smelter. 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 4-12. 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). 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 include aggressive public education and exposure intervention programs (Hilts, 1996; Hilts et al., 1998). Therefore, the coefficients shown in Table 4-12 are likely to overestimate the influence of air, dust, or soil Pb on blood Pb concentrations at this site.

Table 4-12. Environmental Pb levels and blood Pb levels in children in Trail, British Columbia

Date	1996	1999	2001	Regression Coefficient (µg/dL per µg/m^3)
Blood Pb (µg/dL)	11.5	5.9	4.7	NA
Air Pb (µg/m^3)	1.1	0.3	0.03	6.5 ± 0.52 (R^2=0.99, p=0.050)
Soil Pb (mg/kg)	844	756	750	0.068 ± 0.008 (R^2=0.99, p=0.069)
Interior Dust Pb (mg/kg)	758	583	580	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 averages; values for blood Pb are annual geometric means. Regression coefficients are for simple linear regression of each exposure variable on blood Pb.

Source: Data from Hilts et al. (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. Pb was measured in PM_{10} samples collected 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. 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 95th percentile value. In a multivariate linear regression model (R^2 = 0.586) 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 varied depending on the sampling data and ranged from 1.41 to 2.89, with most recent data (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)/5th-95th 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. 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 0.6 µg/dL per mg/kg soil. 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. The Ranft et al. (2008) model is log-linear, with the natural logarithm of blood Pb being a function of linear increase in air Pb. This results an upward curvature of the blood Pb-air Pb relationship (i.e., in linear scale, the blood Pb-air Pb slope increases with increasing air Pb concentration). By comparison, log-log models predict an increase in the blood Pb-air Pb slope with decreasing air Pb concentration, whereas linear models predict a constant blood Pb-air Pb slope across all air Pb concentrations.

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 in PM_{10} (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 and were assigned the corresponding annual average air Pb concentrations for appropriate air monitoring zones. Associations between blood Pb concentration, air Pb concentration and other variables (e.g., age, year of birth, family use of glazed pottery) were evaluated using multivariate regression models. The regression model ($r^2 = 0.96$) predicted blood Pb-air Pb slopes that decreased with year of birth. The largest slope occurred in the cohort born in 1987, who experienced the largest decline in air Pb (from 2.8 to <0.1 µg/m^3); the predicted slope for this group of children was 0.213 (95% CI: 0.114-0.312) ln [µg/dL blood] per ln[µg/m^3 air]. This slope corresponds to an increase of 2.1 µg/dL blood Pb for an increase in air Pb from 0.15 to1.5 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, 1986). 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. children 6 months to 7 years of age (n = 9,996) during 1976-1980, and the City of Chicago blood Pb screening program provided approximately 7,000 blood Pb measurements in black children during 1976-1980. Gasoline Pb consumption was estimated as the product of monthly gasoline sales in the U.S. and quarterly estimates of Pb concentrations in gasoline reported to U.S. EPA. Based on the NHANES blood Pb data for white children, the regression coefficient was 2.14 µg/dL blood per 100 metric tons of gasoline Pb/day (SE=0.19, p=0.0000); results for black children were essentially identical. Based on the Chicago blood Pb data the regression coefficient was 16.12 (µg/dL per 1,000 metric tons gasoline Pb/quarter (SE=1.37, p=0.0001), which is roughly equivalent to 1.79 µg/dL blood per 100 metric tons of

gasoline Pb/day. U.S. EPA (1986) reported data on gasoline Pb consumption (sales) and ambient Pb levels in the U.S. during the period 1976-1984 (Table 4-13). Based on these data, air Pb concentrations decreased in association with gasoline Pb consumption. The linear regression coefficient for the air Pb decrease was 0.23 µg/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 7.8 µg/dL per µg/m^3, based on the NHANES and Chicago data, respectively (e.g., 2.14/0.23 = 9.3).

Table 4-13. U.S. gasoline Pb consumption and air Pb levels

Date	Total Gasoline Pb (103 metric tons/yr)	Total Gasoline Pb (102 metric tons/day)[a]	Air Pb (µg/m^3)
1976	171.4	4.70	1.22
1977	168.9	4.63	1.20
1978	153	4.19	1.13
1979	129	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	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.

Source: U.S. EPA (1986).

Tripathi et al. (2001) reported child blood Pb and air Pb trends for the city and suburbs of Mumbai, India over the period 1984-1996. 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, residential soil, interior dust, and blood for three sampling periods are summarized in Table 4-14. Based on these data, blood Pb increased 3.6 µg/dL per 1 µg/m^3 air Pb (based on linear regression with air or soil 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. The regression estimates were based on group mean blood Pb estimates for the 13 sampling locations, rather than on the individual blood Pb estimates, which included repeated measures on an unreported fraction of the sample.

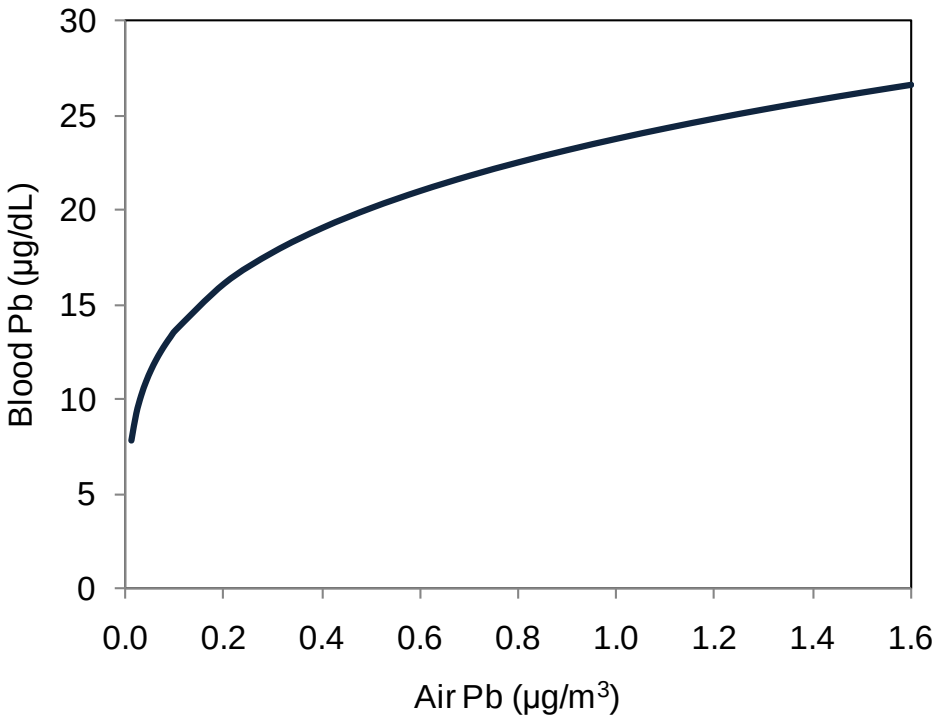
Table 4-14. Air Pb levels and blood Pb levels in children in Mumbai, India

	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 6 years) and quarterly average air Pb concentrations measured at 12 monitoring stations in Cook County, IL. 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 4-20. This corresponds to an increase of 11.1 µg/dL blood Pb for an increase in air Pb from 0.15 to1.5 µg/m^3.



Modified from Hayes et al. (1994).

Figure 4-20. Predicted relationship between air Pb and blood Pb based on data from Chicago, IL (1974–1988).The regression model is: ln[µg/dL blood Pb] = 0.24·ln[µg/m^3 air Pb] + 3.17.

4.5.1.2. Adults

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 (1 female) who were observed during a 2-week period in 1994 or 1995. Subjects wore personal inhalable PM samplers designed to capture PM smaller than 100 µm, while performing various job-related tasks. The geometric mean air Pb concentration for the 2-week period was 58 µg/m^3 (GSD 2.8), with a maximum daily value of 210 µg/m^3. The Pb concentrations reported were corrected by the National Institute for Occupational Safety and Health (NIOSH) respirator protection factors, which were not reported by the authors. Hand wipe samples were collected at the mid-shift break and at the end of the shift (after the subjects had reportedly cleaned up for the day; 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). Associations between exposure variables and blood Pb concentrations were explored with multivariate regression models (Table 4-15). When the model excluded hand-wipe data (not all participants who wore the personal air samplers agreed to provide hand-wipes), 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 1.3-fold increase in blood Pb concentration for a 10-fold increase in air Pb concentration.

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 1.12-fold increase in blood Pb concentration per 10-fold increase in air Pb concentration.

Table 4-15. Significant predictors of blood Pb concentration in bridge painters

Parameters	Blood Pb (Air Only)		Blood Pb (Air and Hand Wipe)	
	β(SE)	p-value	β(SE)	p-value
Intercept	1.90 (0.24)	<0.0001	2.12 (0.44)	0.0007
Time of blood Pb (end vs start of study)	0.16 (0.04)	<0.0001	–0.31 (0.11)	0.005
Mean air Pb (μg/m^3)	0.11 (0.05)	0.03	0.05 (0.07)	0.45
Hand wipe at break (μg Pb)	—		0.007 (0.06)	0.91
Hand wipe at break * time of blood Pb	—		0.07 (0.01)	<0.0001
Months on bridge painting crews	0.001 (0.0004)	0.03	0.001 (0.0006)	0.04
Education				
≤ High school	0.38 (0.10)	0.0002	0.29 (0.13)	0.03
> High school	Reference		Reference	
Respirator fit test				
No	–0.14 (0.14)	0.32	–0.13 (0.21)	0.53
Yes	Reference		Reference	
Respirator fit test * time of blood Pb				
No	0.18 (0.06)	0.003	0.17 (0.07)	0.01
Yes	Reference		Reference	
Smoke on site				
No	0.14 (0.09)	0.14	0.15 (0.10)	0.14
Yes	Reference		Reference	
Smoke on site * time of blood Pb				
No	–0.15 (0.05)	0.002	–0.11 (0.04)	0.009
Yes	Reference		Reference	
Personal hygiene index				
Low	0.27 (0.11)	0.02	0.29 (0.12)	0.02
High	Reference		Reference	
Site-level variables				
Containment facility				
Poor	–0.59 (0.18)	0.001	–0.57 (0.22)	0.01
Good	Reference		Reference	

Air Pb, hand wipe, and blood Pb levels are natural log-transformed.
Blood Pb concentration in units of μg/dL.

Source: Data from Rodrigues et al. (2010).

4.5.2. Environmental Lead-Blood Lead 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 (Abadin & Wheeler, 1997; Bowers et al., 1994; Carlisle & Wade, 1992; Maddaloni et al., 2005; Stern, 1994, 1996; U.S. EPA, 2003). 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:

$$PbB = E \cdot ISF$$

Equation 4-2

$$PbB = E \cdot AF \cdot USF$$

Equation 4-3

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 a 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 AQCD (U.S. EPA, 2006).

Previous studies included in the 2006 AQCD (U.S. EPA, 2006) 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 (Bornschein et al., 1985; Lanphear & Roghmann, 1997; U.S. EPA, 1996b).

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 4-16). 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 4-16. Linear model relating environmental Pb exposure and blood Pb concentration in children[a]

Variables	Overall p-value	Levels	Estimate (SE)	p-Value
Intercept	0.172		- 0.517 (0.373	0.172
Age (in yr)	< 0.001	Age	2.620 (0.628)	< 0.001
		Age2	–1.353 (0.354)	< 0.001
		Age3	0.273 (0.083)	0.002
		Age4	–0.019 (0.007)	0.008
Yr 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
		United States[b]	0.000	-
		Mexico	0.353 (0.097)	< 0.001
		Elsewhere	0.154 (0.121	0.209
Floor surface/condition × log floor PbD	< 0.001	Intercept for missing	0.178 (0.094)	0.065
		Not smooth and cleanable	0.386 (0.089)	< 0.001
		Smooth and cleanable or carpeted	0.205 (0.032)	< 0.001
Floor surface/condition × log floor PbD)^2		Not smooth and cleanable	0.023 (0.015)	0.124
		Smooth and cleanable or carpeted	0.027 (0.008)	0.001
Floor surface/condition × (log floor PbD)^3		Uncarpeted not smooth and cleanable	–0.020 (0.014)	0.159
		Smooth and cleanable or carpeted	–0.009 (0.004)	0.012
Log windowsill PbD	0.002	Intercept for missing	0.053 (0.040	0.186
		Slope	0.041 (0.011	< 0.001
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)	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]n = 2,155 (age 10-60 mo); R^2 = 40%
[b]Includes the 50 states and the District of Columbia

Source: Dixon et al. (2009).

Mielke et al. (2007) analyzed data on blood Pb and soil Pb concentration collected as part of a universal blood Pb screening program in New Orleans (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 non-parametric permutation methods. The resulting best-fit model (R^2=0.528) was:

$$\mathbf{PbB = 2.038 + (0.172 \bullet PbS^{0.5})}$$

Equation 4-4

where PbB is the median blood Pb concentration and PbS is the median soil Pb concentration. The resulting curvilinear relationship predicts a twofold increase in blood Pb concentration for an increase in soil Pb concentration from 100 to 1000 ppm (Figure 4-21).

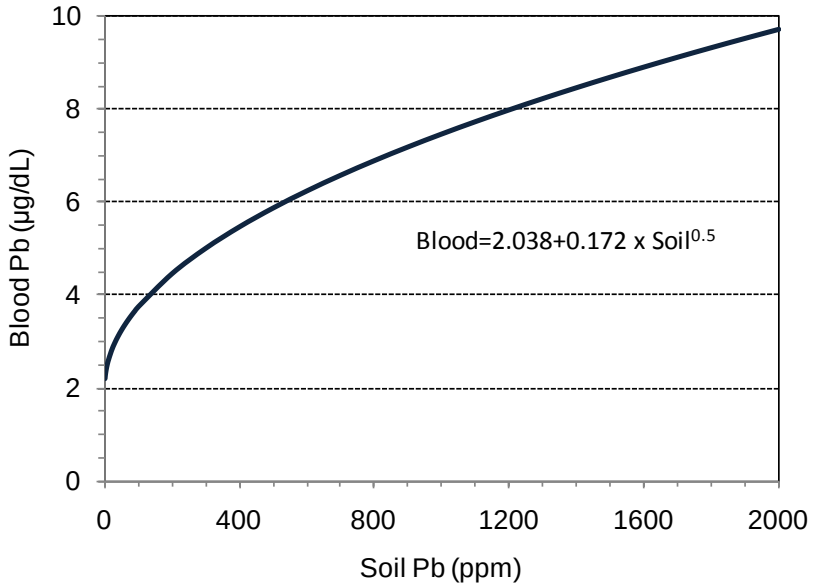


Figure 4-21. Predicted relationship between soil Pb concentration and blood Pb concentration in children based data collected in the New Orleans child Pb screening program (2000-2005) (Mielke et al., 2007). The data set included 55,551 blood Pb measurements for children 0-6 years of age and 5,467 soil Pb measurements. Blood Pb and soil Pb concentrations were matched at the level of census tracts.

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. (2007).

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 1000 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, a former Pb mining and smelting site, was provided in the 2006 AQCD. 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 4-17. 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 4-17). 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.

Table 4-17. 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., hand-to-mouth activity, 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.

4.6. Biokinetic Models of Lead Exposure-Blood Lead 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). They 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. They 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, they 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 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, large uncertainty can be expected to remain about many, or even most, 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 is the *Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in Childre*n (U.S. EPA, 1994) and models that simulate Pb biokinetics in humans from birth through adulthood (Leggett, 1993; O'Flaherty, 1993, 1995). 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, 2006).

4.7. Summary

4.7.1. Exposure

Exposure data considered in this assessment build upon the conclusions of the 2006 AQCD for Pb (2006), which found air Pb concentrations in the U.S. and associated biomarkers of exposure to Pb have decreased substantially following the ban 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. Air-related pathways of Pb exposure are the focus of this assessment. Pb can be emitted to air, water, or soil. In addition to primary emission of particle-bound or gaseous Pb to the atmosphere, Pb can be suspended to the air from disturbance of soil or dust, and a fraction of that suspended Pb may even originate from waters used to irrigate the soil. Pb-bearing PM can be deposited from the air to soil or water through wet and dry deposition. In general, air-related pathways include those pathways where Pb passes through ambient air on its path from a source to human exposure. Air-related Pb exposures include inhalation and ingestion of Pb-contaminated food, water or other materials including dust and soil. Non-air-related Pb exposures may 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. 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.

Section 4.1 presents data illustrating potential exposure mechanisms. Several studies suggested that soil can act as a reservoir for Pb emissions from industrial or and other activities. Exposure to soil contaminated with deposited Pb can occur through hand-to-mouth contact as well as inhalation of resuspended Pb-bearing PM. In general, soil Pb concentrations tended to be higher within inner-city communities compared with neighborhoods surrounding city outskirts. Infiltration of Pb dust has been demonstrated, and Pb dust has been shown to persist in indoor environments even after repeated cleanings. Measurements of particle-bound Pb exposures reported in this assessment have shown that personal exposure concentrations for Pb are typically higher than indoor or outdoor ambient Pb concentrations. These findings may be related to local resuspension with body movement.

4.7.2. Kinetics

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 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. 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. As blood Pb increases and the higher affinity binding sites for Pb in RBCs become saturated at approximately 40 µg/dL blood, 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 compartment (bone) and a smaller fast compartment (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 & Wittmers, 1992). 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.

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 to 20. 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 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 to the plasma (e.g., cortical $t_{1/2}$ = 0.23 years at birth, 3.7 years at 15 years of age, and 23 years in adults; trabecular $t_{1/2}$ = 0.23 years at birth, 2.0 years at 15 years of age, and 3.8 years in adults) (Leggett, 1993). Due to the more rapid turnover of bone mineral in children, changes in blood Pb concentration are thought to more closely parallel changes in total body burden. However, some Pb accumulated in bone during childhood does persist into later life. Potential mobilization of Pb from the skeleton could occur in adults at times of physiological stress associated with enhanced bone remodeling such as during pregnancy and lactation, menopause or in the elderly, extended bed rest, hyperparathyroidism, and weightlessness. Regardless of age, however, similar blood Pb concentrations in two individuals (or populations) do not necessarily translate to similar body burdens or similar exposure histories.

The kinetics of elimination of Pb from the body reflects the existence of fast and slow pools of Pb in the body. The dominant phase of Pb kinetics in the blood, exhibited shortly after a change in exposure occurs, has an elimination 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 may become evident with longer observation periods following a decrease in exposure due to the gradual redistribution of Pb among other compartments via the blood. Therefore, a single blood Pb concentration may reflect the near-term or longer-term history of the individual to varying degrees, depending on the relative contributions of internal (e.g., bone) and external sources of Pb to blood Pb, which in turn will depend on the exposure history and possibly age-related and individual-specific (e.g., pregnancy, lactation) characteristics of bone turnover. In general,

higher blood Pb concentrations can be interpreted as indicating higher exposures (or Pb uptakes); however, they do not necessarily predict higher body burdens, especially in adults.

4.7.3. Lead Biomarkers

Observational studies using biomarkers of Pb are included in Section 4.4. The median blood Pb level for the entire U.S. population is 1.2 μg/dL and the 95th percentile blood Pb level was 3.7 μg/dL, based on the 2007-2008 NHANES data (NCHS, 2010). Among children aged 1-5 years, the median and 95th percentiles were slightly higher at 1.4 μg/dL and 4.1 μg/dL, respectively. Overall, trends in blood Pb levels have been decreasing among U.S. children and adults over the past twenty years. Concurrent changes in isotopic ratios of blood Pb samples reflect changes in source composition over the past several decades. Recent studies have observed a relationship between blood Pb and soil Pb concentration. Additionally, studies have shown that blood Pb is also associated with non-air-related, non-policy-relevant exposure to Pb paints in older homes, Pb released into drinking water, and occupational work with materials containing Pb.

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 variables that may affect bone remodeling (e.g., nutritional status, pregnancy, menopause). Blood Pb in adults is typically more an index of recent exposures than body burden, whereas bone Pb is an index of cumulative exposure and body burden. In children, 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 some physiological circumstances (e.g., osteoporosis), bone Pb may contribute to blood Pb in adults. The disparity between blood Pb and body burden may have important implications for the interpretation of blood Pb measurements in some epidemiology studies. Conceptually, measurement of long-term Pb body burden (i.e., based on tibia Pb) may be the appropriate metric if the effects of Pb on a particular outcome are lasting and cumulative. However, if the effects of Pb on the outcome represent the acute effects of current exposure, then blood Pb may be the preferred metric. In the absence of clear evidence as to whether a particular outcome is an acute effect of recent Pb dose or a chronic effect of cumulative Pb exposure, both blood and bone metrics should be considered.

Cross-sectional studies that sample blood Pb once generally provide an index of recent exposures. In contrast, cross-sectional studies of bone Pb and longitudinal samples of blood Pb concentrations over time provide 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.

The concentration of Pb in urine is a function of the urinary Pb excretion and the urine flow rate. Urine flow rate requires collection of a timed urine sample, which is often problematic in epidemiologic studies. Collection of un-timed ("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). Urinary Pb concentration reflects, mainly, the exposure history of the previous few months; thus, a single urinary Pb measurement cannot distinguish between a long-term low level of exposure or a higher acute exposure. 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 better reflect body burden.

4.7.4. Air Lead-Blood Lead Relationships

The 1986 Pb AQCD described epidemiological studies of relationships between air Pb and blood Pb. Much of the pertinent earlier literature described in the AQCD was drawn from a meta-analysis by Brunekreef (1984). In addition to the meta-analysis of Brunekreef (1984), seven more recent studies have provided data from which estimates of the blood Pb-air Pb slope can be derived for children (Table 4-11). The range of estimates from these seven studies is 1-9 µg/dL per µg/m^3, which encompasses the estimate from the Brunekreef (1984) meta-analysis of (3-6 µg/dL per µg/m^3). The Schnaas et al. (2004) had a particularly strong experimental design in that is the only longitudinal study in which blood Pb concentration was monitored repeatedly in individual children from age 6 months to 10 years. For children who experienced the largest declines in air Pb (i.e., from 2.8 to <0.1 µg/m^3), the predicted blood Pb-air Pb slope (adjusted for age, year of birth, SES, and use of glazed pottery) was 0.213 ln[µg/dL blood] per ln[µg/m^3 air]. The cross-sectional study done by Ranft et al. (2008) attempted to account for potential co-variates that influence blood Pb (e.g., soil Pb concentration, gender, environmental tobacco smoke, fossil heating system and parental education). It is the only study that reported a logarithmic blood Pb-linear air Pb relationship, which results in an upward curvature of the blood Pb-air Pb relationship (i.e., the blood Pb-air Pb slope increases with increasing air Pb concentration). In other studies (or based on other studies), the blood Pb-air Pb relationship was either log-log (Brunekreef, 1984; Hayes et al., 1994; Schnaas et al., 2004), which predicts an increase in the blood Pb-air Pb slope with decreasing air Pb concentration or linear (Hilts, 2003; J. Schwartz & Pitcher, 1989; Tripathi et al., 2001), which predicts a constant blood Pb-air Pb slope across all air Pb concentrations. These differences may simply reflect model selection by the investigators; alternative models are not reported in these studies. Because air Pb

contributes to Pb in soil and indoor dusts, adjustment for the correlated co-variates such as soil Pb would introduce a downward bias in the slope estimate.

Chapter 4. References

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Chapter 5. Integrated Health Effects of LeadExposure

5.1. Introduction

This chapter reviews, summarizes, and integrates the evidence for the broad spectrum of health effects associated with exposure to Pb. The chapter begins (Section 5.2) with a discussion of the evidence for the modes of action that mediate the health effects of Pb, including those common to all health effects evaluated in the ISA and those specific to particular endpoints. Subsequent sections consist of assessments of the epidemiologic and toxicological evidence for the effects of Pb exposure on major health effect categories such as neurological effects (Section 5.3), cardiovascular effects (Section 5.4), renal effects (Section 5.5), immune effects (Section 5.6), effects on heme synthesis and red blood cell function (Section 5.7), and reproductive effects and birth outcomes (Section 5.8). Section 5.9 provides reviews of the evidence for Pb effects on health outcomes for which a fewer number of studies are available, including those related to the hepatic system (Section 5.9.1), gastrointestinal system (Section 5.9.2), endocrine system (Section 5.9.3), bone and teeth (Section 5.9.4), ocular health (Section 5.9.5), and respiratory system (Section 5.9.6). Chapter 5 concludes with a discussion of the evidence for Pb effects on cancer (Section 5.10).

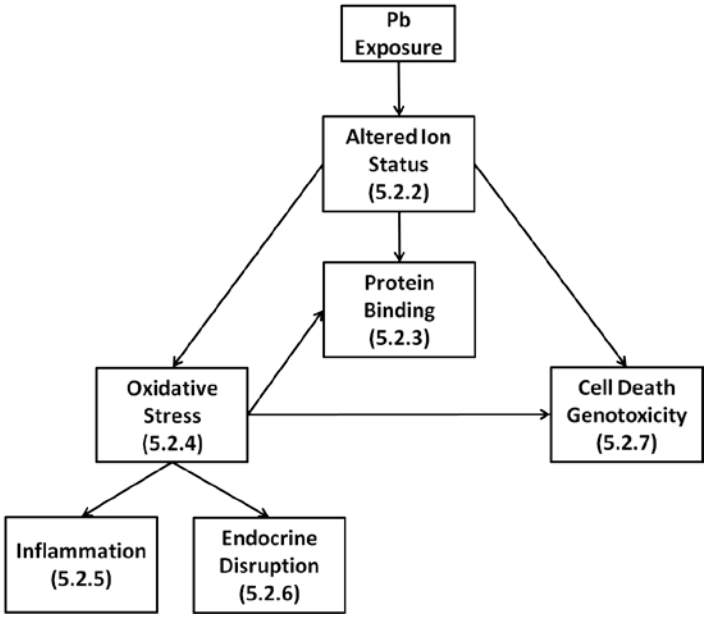
Individual sections for major health effect categories (e.g., neurological, cardiovascular, renal) include a brief summary of conclusions from the 2006 Pb AQCD and an evaluation of recent evidence that is intended to build upon evidence from previous reviews. Within each of these sections, results are organized by endpoint (e.g., cognitive function, behavior, neurodegenerative diseases) then by specific scientific discipline (i.e., epidemiology, toxicology). Each major section (e.g., neurological, cardiovascular, renal effects) concludes with an integrated summary of the findings and a conclusion regarding causality. Based upon the framework described in Chapter 1, a determination of causality is made for a broad health effect category, such as neurological effects, with coherence and biological plausibility being based on evidence available across disciplines and also across the suite of related health endpoints.

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

5.2. Modes of Action

5.2.1. Introduction

The diverse health effects of Pb are dependent on multiple factors, including the concentration and duration of exposure, the particular Pb compounds constituting the exposure, and which tissues are targeted. A mode of action is the sequence of key events (i.e., empirically observable precursor steps) that cumulatively result in the formation of negative health outcomes. Although the toxic effects of Pb 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 5-1). The following section draws information from all of the health effects sections in the current document and identifies the major modes of action operating at the molecular, cellular, and tissue/organ level. In turn, individual health effect sections bridge these effects to toxicities observed on the organismal level. 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 that information with older studies that represent the current state of the science on the possible modes of action of Pb.



Note: The sections where these MOAs are discussed are indicated in parentheses.

Figure 5-1. Schematic representation of the relationships between the various MOAs by which Pb exerts its toxic effects.

5.2.2. Altered Ion Status

Physiologically-relevant metal ions (e.g., Ca, 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 as structural elements in the proper maintenance of tertiary protein conformations (Garza et al., 2006). It is through disruption of these biological functions that Pb effects its toxic action, ultimately adversely affecting such tightly regulated processes as cell signaling, intracellular ion homeostasis, ion transport, energy metabolism, and enzymatic function.

5.2.2.1. Disruption of Ca^{2+} Homeostasis

Calcium 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 (Long et al., 1992; Wiemann et al., 1999). Bone cells also are unique in that they exist in a microenvironment that is high in both Ca^{2+} and Pb concentrations, potentially increasing their relative susceptibility to Pb-induced toxicity (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 disrupts 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., 1989; Schanne et al., 1997). Long et al. (1992) observed that exposure of osteoblasts to either 400 ng parathyroid hormone (PTH)/ml culture for 1 hour or 25 µM Pb for 20 hours increased $[Ca^{2+}]_i$. Pretreatment of Pb-exposed cells with PTH increased $[Ca^{2+}]_i$ above concentrations observed in either single exposure, indicating that Pb may disrupt bone cell's ability to respond to normal hormonal control. A similar additive increase in $[Ca^{2+}]_i$ was also observed when bone cells were co-treated with epidermal growth factor (EGF) and Pb versus Pb alone (Long & 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 the 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 (CRAC) influx of Ca^{2+} independent of any inhibitory effect on Ca^{2+}- ATPases.

Ca^{2+} homeostasis has also been shown be to disturbed in erythrocytes due to Pb exposure (Quintanar-Escorza et al., 2010; Quintanar-Escorza et al., 2007; Shin et al., 2007). In blood samples taken from Pb-exposed workers (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 (blood Pb level = 9.9 ± 2 µg/dL) (Quintanar-Escorza et al., 2007). The increase in $[Ca^{2+}]_i$ was associated with higher osmotic fragility and modifications in erythrocyte shape. When erythrocytes from 10 healthy volunteers were exposed to Pb at concentrations of 0.2 to 6.0 µM for 24 or 120 hours, dose-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 demonstrated duration-related increases at 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, Ca^{2+} homeostasis has been observed in platelets and white blood cells. Dowd and Gupta (1991) observed statistically significant increases in $[Ca^{2+}]_i$ in human platelets exposed to as little as 1 µM Pb for 3.5 hours. The observed increase in Ca^{2+} levels was attributed to increased influx of external Ca^{2+}, possibly through receptor-operated Ca^{2+} channels. In mouse splenic lymphocytes exposed to Pb, $[Ca^{2+}]_i$ was increased at exposure levels as low as 1 µM when incubated for 10 minutes or greater (S. Li et al., 2008). These increases in Ca^{2+} 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 in lymphocytes. In rat tail arteries exposed to 1.2 µM Pb acetate for 1 hour, intracellular stores of Ca^{2+} increased over controls, possible through increased transmembrane influx of Ca^{2+} (Piccinini et al., 1977).

Exposure of the microsomal fraction of rat brain cells to as little as 0.25 µM Pb for 2 minutes resulted in increased release of Ca^{2+} into the media (Pentyala et al., 2010). Further, Pb exposure also decreased the activity of the microsomal Ca^{2+}-ATPase, thus decreasing the sequestration of Ca^{2+} into microsomes. The results of this study suggest that 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 100 nM 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.

5.2.2.2. Disruption of Ion Transport Mechanisms

As described above, deregulation of Ca^{2+} homeostasis results in negative effects in multiple organ systems. Under normal conditions, cytosolic concentrations of free Ca^{2+} are maintained at low levels (0.1 µM) by extrusion and internal compartmentalization processes (Huel et al., 2008). An important component of the maintenance of Ca^{2+} homeostasis is transmembrane transport of Ca ions via Ca^{2+}-ATPase and voltage-sensitive gates (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 the effects of Pb exposure on Na^+-K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPases 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 (blood Pb = 55.6 ± 6.3 µg/dL) (Kharoubi, Slimani, Aoues, et al., 2008) and in erythrocytes of rats exposed to 0.2% Pb in drinking water for 5 weeks (blood Pb = 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 synaptosomes isolated from rats exposed to 200 mg/L Pb in drinking water for 3 months (blood Pb = 378 µg/dL) (Rafalowska et al., 1996) or 15 mg/kg Pb injected intraperitoneally for 7 days (blood Pb = 112.5 µg/dL) (Struzynska, Dabrowska-Bouta, et al., 1997). The activity of Ca^{2+}-ATPase in the sarcoplasmic reticulum of rabbits exposed to 0.01 µmol/L Pb was similarly decreased (Hechtenberg & Beyersmann, 1991). The inhibitory effect of Pb was diminished in the presence of high MgATP concentrations. The activity of generic ATPase was reported to be altered in the testes of rats exposed to 300 mg/L Pb acetate gestationally, and in drinking water after weaning to the age of 6, 8, 10, or 12 weeks (H. T. Liu et al., 2008). In 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 P_0 generation (Eder et al., 1990). However, the F_1 generation of Pb-depleted rats displayed decreased activities in both enzymes (meaning that animals with higher exposure to Pb, i.e., "control" animals, had higher enzymatic activities). A similar increase in the activity Na^+-K^+-ATPase was observed in rats exposed to 20 mg/kg Pb intraperitoneally for 14 consecutive days (Jehan & Motlag, 1995). Co-exposure of Pb with zinc and copper greatly reduced the increase in ATPase activity observed. 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 (blood Pb = 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. (1999) observed that 10-50 mM 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 (Calderon-Salinas, Quintanar-Escorza, et al., 1999) 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 mM. 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 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 the activity of Na^{+}-K^{+}-ATPase was decreased in erythrocyte ghosts exposed to concentrations as low as 10 µg/dL Pb; activity of Ca^{2+}- Mg^{2+}-ATPase was less sensitive to Pb exposure and Mg^{2+}-ATPase activity was not affected.

In a study investigating ATPase activities in occupationally-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 - 42.07 ± 12.01 µg/dL) compared to nonexposed controls (blood Pb = 12.34 ± 2.44 (males) and 16.85 ± 6.01 µg/dL (females)). Increased membrane concentrations of Ca^{2+} and magnesium 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. Huel et al. (2008) found that newborn hair and cord blood Pb levels (1.22 ± 1.41 µg/g and 3.54 µg/dL) were negatively associated with Ca^{2+}-ATPase activity in plasma membranes of erythrocytes isolated from cord blood; newborn hair Pb levels were more strongly associated with cord Ca^{2+} pump activity than cord blood Pb

Pb has also been shown to disrupt cation transport mechanisms through direct action on voltage-sensitive cation channels. Audesirk and Audesirk (1991, 1993) demonstrated that extracellular free Pb inhibits the action of multiple voltage-sensitive 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, and IC_{50} values as low as 0.03 µM for L-type channels in cultured hippocampal neurons. Sun and Suszkiw (1995) confirmed the inhibitory action of extracellular Pb on Ca^{2+} channels, demonstrating an IC_{50} value of 0.3 µM in adrenal chromaffin cells. The observed disruption of the 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, 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} value of 0.2 nM, possibly through blocking the intracellular Ca^{2+} binding domain, or through Ca^{2+} dependent dephosphorylation of the channel (L. R. Sun & Suszkiw, 1995).

Pb also disrupts the action of Ca^{2+}-dependent potassium channels. Alvarez et al. (1986) observed that Pb promoted the efflux of potassium from inside-out erythrocyte vesicles in a dose-dependent manner at concentrations of 1-300 µM, either through action on a Mg modulatory site or through direct interaction with the Ca^{2+} binding site. Fehlau et al. (1989) also demonstrated Pb-induced activation of the potassium channel in erythrocytes. However, Pb only activated the potassium channels at concentrations below 10 µM; higher concentrations of Pb completely inhibited the channel's activity, indicating the modulation of potassium permeability is due to alterations in channel gating. Silken et al. (2001) observed that Pb activated potassium channels in erythrocytes from the marine teleost *Scorpaena porcus* in a dose-dependent manner after a 20-minute incubation; minor loss of potassium was seen at Pb concentrations of 1-2 µM, whereas exposure to 20-50 µM Pb resulted in approximately 70% potassium loss. Competitive and inhibitory binding assays suggest that Pb directly activates potassium 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-sensitive channels in in vitro experiments (Cooper & Manalis, 1984; J. Suszkiw et al., 1984). However, concentrations of Pb as low as 5 µM were also observed to actually increase the spontaneous release of neurotransmitters in these same experiments. Subsequent research by other groups confirmed 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, 1995) reported that Pb exposure induced the release of norepinephrine (NE) from bovine adrenal chromaffin cells, and was considerably more potent at doing so than Ca^{2+} ($K_{0.5}$ of 4.6 nM for Pb versus 2.4 µM for Ca^{2+}). Activation of protein kinase C (PKC) was observed to enhance the Pb-induced release of NE. Westrink and Vijverber (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 30 nM in permeabilized cells. The suppression of Ca^{2+}-induced evoked release of neurotransmitters combined with the ability of Pb to enhance spontaneous releases could result in higher noise in the synaptic transmission of nerve impulses in Pb-exposed animals. In rats exposed to Pb at concentrations of 0.1-1.0% in drinking water beginning at GD15-16 and continuing to 120 days postnatal, decreases in total potassium-stimulated hippocampal GABA release were seen at exposure levels of 0.1-0.5% (blood Pb = 26.8 ± 1.3 - 61.8 ± 2.9 µg/dL) (Lasley & Gilbert, 2002). Maximal effects were observed at 0.2% Pb in drinking water, but effects were less evident at 0.5%,

and were absent at 1.0%. In the absence of Ca^{2+}, potassium-induced release was increased in the two highest exposure concentrations, suggesting a Pb-induced enhancement of evoked release of GABA. The authors suggest that this pattern of response indicates that Pb is a potent suppressor of evoked release at low concentrations, but a Ca^{2+} mimic in regard to independently evoking exocytosis and release at higher concentrations (Lasley & 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-sensitive Ca^{2+} channels.

5.2.2.3. Displacement of Metal Ions and Perturbed Protein Function

The binding of metal ions to proteins causes specific changes in protein shape, and the specific cellular function of many proteins may be altered by conformational changes (Kirberger & 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 (Garza et al., 2006; Kirberger & Yang, 2008). 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 (Garza et al., 2006; Kirberger & Yang, 2008). However, due to differences in its physical properties, Pb induces abnormal conformational changes when it binds to proteins (Bitto et al., 2006; Garza et al., 2006; Kirberger & Yang, 2008; 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 protein kinase C (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 the cation, as well as 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 picomolar concentrations of Pb for 5 minutes directly activated PKC purified from rat brains. The activation of PKC by Pb was more potent than Ca^{2+}-dependent activation by five orders of magnitude. Long et al. (1994) confirmed these findings, reporting that Pb had a K_{act} 4800 times smaller than Ca^{2+} (55 pM versus 25 µM, following a 3 minute exposure). However, Ca^{2+} had a higher maximal activation of PKC than 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 Ca^{2+}, but not subsequent sites. Tomsig

and Suszkiw (1995) further demonstrated the ability of Pb to activate PKC at picomolar concentrations in adrenal chromaffin cells incubated with 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 greater than one nanomolar.

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. This suggests that Pb redistributes PKC at micromolar 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 100 nM (Belloni-Olivi et al., 1996). The observation that neither Ca^{2+} nor diacylgycerol was increased due to 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 picomolar concentrations of Pb (Chai & Webb, 1988; Watts et al., 1995) and human erythrocytes from Pb-exposed workers (blood Pb = 5.4 to 69.3 µg/dL) (K.-Y. Hwang et al., 2002), and decreased activity in mouse macrophages and the rat brain cortex at micromolar concentrations (Lison et al., 1990; Murakami et al., 1993).

Calmodulin is another important protein essential for proper Ca^{2+}-dependent cell signaling. Calmodulin contains an "EF-hand" Ca^{2+} binding domain, and 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 (Saimi & Kung, 2002; Vetter & Leclerc, 2003). Haberman 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 100 nM, EC_{50} = 0.5-1.0 µM), stimulation of brain membrane phosphorylation at Pb concentrations greater than 400 nM after 1 minute incubation, and increased binding of calmodulin to brain membranes at Pb concentrations greater than 1 µM after 10 minutes incubation. Haberman et al. (1983) reported the affinity of Pb for calmodulin's Ca^{2+}-binding sites 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+} at binding calmodulin (IC_{50} = 11 and 26 µM, respectively). Both studies indicated that Pb was much more effective at binding calmodulin than any other metal cation investigated (e.g., mercury, cadmium, iron). 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 400-550 pM (threshold = 100 pM) and 450-500 nM (threshold = 100 nM), 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 430 pM [threshold = 300 pM versus EC_{50} 1200 nM (threshold = 200 nM; 50-minute

incubation]). When incubated with physiological concentrations of Ca^{2+}, Pb induced phosphodiesterase activity at concentrations as low as 50 pM. Pb activated calcineurin, a phosphatase with widespread distribution in the brain and immune system, at threshold concentrations as low as 20 pM in the presence of Ca^{2+} (incubation time = 30 minutes), but inhibited its activity at concentrations greater than 200 pM (Kern & Audesirk, 2000). Thus, picomolar 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-dose (<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 1000-times more tightly than Ca^{2+} (K_d = 1.6 ± 0.42 nM versus 0.007 mM, respectively), and analysis with NMR indicated 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 confirmed that Pb bound osteocalcin approximately 10,000-times more tightly than Ca^{2+} (K_d = 0.085 µM versus 1.25 mM, 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 seen with Ca^{2+}. Additionally, Pb can displace Ca^{2+} in numerous other Ca^{2+}-binding proteins important in muscle contractions, renal Ca^{2+} transport and neurotransmission, including troponin C, parvalbumin, CaBP I and II, phospholipase A_2, and syntapotagmin I, at concentrations as low as the nanomolar range (Bouton et al., 2001; Osterode & 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 zinc-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, Grubb, et al., 1997), and exposure to Pb results in inhibition of the enzyme in the erythrocytes of Pb-exposed workers and adolescents (blood Pb level >10 µg/dL) (Ademuyiwa, Ugbaja, Ojo, et al., 2005; Ahamed et al., 2006), in human erythrocytes exposed to Pb for 60 minutes (K_i = 0.07 pM) (Simons, 1995), and in rats exposed to 25 mg/kg Pb once a week for 4 weeks (blood Pb level = 6.56 ± 0.98 µg/dL) (M. K. Lee et al., 2005). Further experiments indicated that

lower concentrations of zinc result in greater inhibition of enzyme activity by Pb, suggesting a competitive inhibition between zinc and Pb at a single site (Simons, 1995).

Zinc-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; Hanas et al., 1999; M. Huang et al., 2004; Zawia et al., 1998) (G. R. Reddy & Zawia, 2000). Pb was found to form tight complexes with the cysteine residues in GATA proteins ($\beta^{Pb} = 6.4 \times 10^{9}\ M^{-1}$ for single zinc fingers and $\beta_2^{Pb2} = 6.4 \times 10^{19}\ M^{-2}$), and was able to displace bound zinc from the protein under physiologically relevant conditions (Ghering et al., 2005). Once Pb was bound to GATA proteins, they displayed decreased ability to bind to DNA (Pb concentrations $\geq$ 1.25 µM) and activate transcription (Pb concentration = 1 M). Pb also binds to the zinc domain of TFIIIA, inhibiting its ability to bind DNA at concentrations as low as 10 µM (Hanas et al., 1999; M. Huang et al., 2004). Huang et al. (2004) also reported that exposure to Pb caused the dissociation of TFIIIA-DNA adducts and that NMR spectroscopy indicated that altered TFIIIA activity is 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 0.2% Pb acetate via lactation, resulting in a shift in DNA-binding towards early development (i.e., the first week following birth) (G. R. Reddy & 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 5.2.7.5). Further mechanistic research utilizing a synthetic peptide containing a zinc finger motif demonstrated that Pb can bind the histidine and cysteine residues of the zinc finger motif, thus displacing zinc and resulting in an increase in the DNA-binding efficiency of the synthetic peptide (Razmiafshari et al., 2001; Razmiafshari & Zawia, 2000). However, in DNA-binding assays utilizing recombinant Sp1 (which has three zinc finger motifs, opposed to only one in the synthetic peptide), incubation with as little as 37 µM Pb resulted in the abolishment of Sp1's DNA-binding capabilities (Razmiafshari & 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 of rats exposed to 0.1% Pb in drinking water for 9 months (blood Pb = 7.01 ± 1.64 µg/dL) and in human erythrocyte lysates exposed to 100 nM Pb for as little as 5 minutes (Baranowska-Bosiacka et al., 2009), and cGMP phosphodiesterase at picomolar concentrations in homogenized bovine retinas (D. Srivastava et al., 1995). Pb was also reported to inhibit pyrimidine 5'-nucleotidase through competitive inhibition of magnesium binding, resulting in conformational changes and improper amino acid positioning in the active site (Bitto et al., 2006).

In summary, Pb has the ability to displace metal cations from the active sites of multiple enzymes and proteins, and thus to alter the functions of those proteins. 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 5-1 provides a list of enzymes and proteins whose function may be perturbed by Pb exposure.

Table 5-1. 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	Metal Cation; Reference
Enzymes	Aminolevulinic acid dehydratase	↓	Zn; Simons (1995)
	Ferrochelatase	↓	Fe (2Fe-2S Cluster); Crooks (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; Leseney (1999)
	Pyrimidine 5'-nucleotidase	↓	Mg, Ca; Bitto et al. (2006), Amici (1997), Paglia and Valentine (1975)
	Erythrocyte phosphoribosyltransferase	↓	Mg (Mn, Ca, Co, Ni, Zn); Deng et al. (2010), Arnold and Kelley (1978)
Ion Channels/ Transport	ATPase	↓↑	Ca, Mg, Na-K; Technische Universitat Braunschweig (2011)
	Mitochondrial transmembrane pore	↑	Ca; He et al. (2000)
	Calcium-dependent potassium channel	↑	Ca; Silkin et al. (2001), Alvarez et al. (1986)
Signal Transduction	Protein kinase C	↓↑	Ca; Garza et al. (2006)
	Calmodulin	↑	Ca; 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)

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

5.2.2.4. Mitochondrial Abnormality

Alterations in mitochondrial function, including disruptions in ion transport, ultrastructural changes, altered energy metabolism, and perturbed enzyme activities due to Pb intoxication are well documented in the scientific literature. Exposure of rats to Pb in feed (1% Pb for 4, 6, 8, 10, 12, or 20 weeks) or drinking water (300 ppm for 8 weeks, 500 ppm for 7 months, or 1% for 9 months) resulted in gross ultrastructural changes in renal tubule 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 (Goyer, 1968; Goyer et al., 1968; Marchlewicz et al., 2009; Navarro-Moreno et al., 2009; L. Wang et al., 2010).

Transmembrane mitochondrial ion transport mechanisms are perturbed by exposure to Pb. Pb inhibits the uptake of Ca^{2+} into mitochondria (Parr & 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 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 (L. Wang, H. Wang, et al., 2009), and retinal rod photoreceptor cells incubated with 10 nM to 10 µM for 15 minutes (L. H. He et al., 2000). Further research indicated that observed Pb-induced mitochondrial swelling and decreased membrane potential is the result of the opening of a mitochondrial transmembrane pore (MTP), possibly by directly binding to the metal (Ca^{2+})-binding site on the matrix side of the pore (Bragadin et al., 2007; L. H. He et al., 2000). Opening of the MTP is the first step of the mitochondrial-regulated apoptotic cascade pathway in many cells (Lidsky & Schneider, 2003; Rana, 2008). He et al. (2000) additionally observed cytochrome c release from mitochondria, and caspase-9 and -3 activation following exposure of 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 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 1% 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 in 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). Statistically significant Pb-induced decreases in ATP and adenylate energy charge were observed concurrently with increases in ADP, AMP, and adenosine in rats exposed to 1% Pb in drinking water for 9 months (Marchlewicz et al., 2009) and cellular ATP levels were decreased in differentiated PC-12 cells incubated with as little as 1 µM Pb for 48 hours (Prins et al., 2010). The observed decrease in cellular ATP levels in Prins et al. (2010) were correlated with a Pb-induced decrease in the expression of the voltage-dependent anion channel (VDAC), which maintains cellular ATP levels in neurons. 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 coupled and uncoupled respiratory oxygen use in mitochondria, and that Pb prevented pyruvate, but not malate, uptake. Mitochondrial levels of ATP were diminished after 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. Contrary to the above findings, Rafalowska et al. (1996) reported that, although ATP levels did decrease, chronic exposure to Pb did not inhibit oxidative phosphorylation in the synaptosomes of rats exposed to 200 mg/L Pb in water for 3 months. Similar effects with regard to the activity of the mitochondrial oxidative chain were observed in rats injected with 15 mg/kg Pb i.p. daily for seven days, as reported by Struzynksa et al. (1997), 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 to 30 µg/dL Pb (Dowd et al., 1990; Grabowska & Guminska, 1996). 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 controls exposed to lower concentrations of Pb (blood Pb level = 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 & 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 1% Pb in drinking water for four weeks (Baranowska-Bosiacka & Hlynczak, 2004). In erythrocytes, nucleotides are synthesized via salvage pathways such as the adenine pathway, which requires adenine phosphoribosyltransferase (APRT). The activity of this enzyme is inhibited by exposure to Pb in human and rat erythrocytes (see above for dose and duration) (Baranowska-Bosiacka et al., 2009).

Disruptions in erythrocyte energy metabolism have been observed in workers 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). Morita et al. (1997) evaluated the effect of Pb on NAD synthetase in the erythrocytes of Pb-exposed workers (blood Pb = 34.6 ± 20.7 µg/dL) and observed an apparent dose-dependent decrease in NAD synthetase activity with increased blood Pb. The blood Pb associated with 50% inhibition of NAD synthetase, which requires a magnesium cation for activity (Hara et al., 2003), was 43 µg/dL.

Altered Heme Synthesis

Exposure to Pb is known to inhibit two key steps in the synthesis of heme: porphobilinogen synthase (i.e., δ-aminolevulinic acid dehydratase), a cytoplasmic enzyme requiring zinc for enzymatic activity that condenses two molecules of aminolevulinic acid into porphobilinogen, and ferrochelatase, a mitochondrial iron-sulfur containing enzyme that incorporates Fe^{2+} into protoporphyrin IX to create

heme. Farant and Wigfield (1987, 1990) 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 for thiol groups at its active site. Taketami 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 zinc protoporphyrin (ZPP). While not directly measuring the activity of ferrochelatase, numerous studies have shown that blood Pb levels are statistically significantly associated with increased erythrocyte ZPP levels in humans (average blood Pb ranging from 21.92 to 53.63 µg/dL) (Ademuyiwa, Ugbaja, Ojo, et al., 2005; Counter et al., 2007; Mohammad et al., 2008; Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006) and animals (blood Pb = 24.7 µg/dL) (Rendón-Ramirez et al., 2007).

5.2.3. Protein Binding

Pb is able to bind to proteins within cells through interactions with side group moieties (e.g., thiol residues) and can potentially disrupt cellular function (Sections 5.2.2.3 and 5.2.2.4). However, some proteins are also able to bind Pb and protect against its toxic effects through sequestration. The ability of Pb to bind proteins was first reported by Blackmon (1936): Pb intoxication was observed to induce the formation of intranuclear inclusion bodies in the liver and kidney. Further research into the composition of intranuclear inclusion bodies and the identification of specific Pb-binding proteins has been conducted since that time.

5.2.3.1. 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 1% 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 (Choie & Richter, 1972; Fowler et al., 1980; Goyer, Leonard, et al., 1970; Goyer, May, et al., 1970; McLachlin et al., 1980; Navarro-Moreno et al., 2009; Oskarsson & Fowler, 1985). 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 (Deveci, 2006; Navarro-Moreno et al., 2009). Intranuclear and cytoplasmic inclusions have also been found in organs other than the kidney, including liver, lung, and glial cells (Goyer & Rhyne, 1973; J. Singh et al., 1999). Pb found within nuclei has also been shown to bind to the nuclear membrane and histone fractions (Sabbioni & 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 kDa protein that migrates as a single band on acrylamide gel electrophoresis. In contrast with Moore and Goyer's findings, Shelton and Egle (1982) identified a 32 kDa protein with an isoelectric point of 6.3 from the kidneys of rats exposed to 1% Pb acetate in feed or 0.75% 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 formation of intranuclear inclusion bodies required protein synthesis (Choie et al., 1975; McLachlin et al., 1980). 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 & 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 & 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 postnatal exposure of rats to 0.2-1.0% Pb in drinking water increased the levels of another brain protein, glial fibrillary acidic protein (GFAP), in developing astrocytes and that this increase may be indicative of a demand for astrocytes to sequester Pb.

5.2.3.2. Cytosolic Lead Binding Proteins

Numerous studies have also identified cytosolic Pb-binding proteins. Two binding proteins, with molecular weights of 11.5 and 63 kDa, were identified by (Oskarsson et al., 1982) in the kidney postmitochondrial cytosolic fraction after injection with 50 mg Pb. The two proteins were also found in the brain, but not the liver or lung. Mistry et al. (1985) observed three proteins (MW = 11.5, 63, and >200 kDa) in rat kidney cytosol, and that 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 zinc cation to ALAD (Goering & Fowler, 1984, 1985). Cadmium and zinc, but not Ca^{2+} or Fe, prevent the binding of Pb to the 63 and 11.5 kDa cytosolic proteins, which agrees with previous observations that cadmium is able to reduce total kidney Pb and prevent intranuclear inclusion bodies (Mahaffey et al., 1981; Mahaffey & Fowler, 1977; Mistry et al., 1986). 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) (Fowler & DuVal, 1991; D. R. Smith et al., 1998).

Cytosolic Pb-binding proteins distinct from kidney proteins have also been identified in the brain of exposed rats and human brain homogenates exposed in vitro (DuVal & Fowler, 1989; Goering et al.,

1986; Quintanilla-Vega et al., 1995). One protein (MW = 12 kDa) was shown to alleviate hepatic ALAD inhibition due to Pb exposure through competitive binding with Pb and donation of zinc to ALAD. Cytosolic Pb-binding proteins have been shown to be high in glutamic acid, aspartic acid, and cysteine residues (DuVal & Fowler, 1989; Fowler et al., 1993). Some evidence exists that cytosolic Pb-binding proteins directly target Pb and compartmentalize intracellular Pb as protective measure against toxicity (Y. Qian et al., 2000; Y. Qian et al., 2005).

5.2.3.3. Erythrocytic Lead Binding Proteins

The majority (94%) of Pb in whole blood is found in erythrocytes (Ong & Lee, 1980a). Originally, the major Pb-binding protein in erythrocytes was identified as hemoglobin (B. S. Cohen et al., 2000; Lolin & O'Gorman, 1988; Ong & Lee, 1980a, 1980b; Raghavan & Gonick, 1977). However, Bergdahl et al. (1997) 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, increases after exposure in both exposed humans (blood Pb = 30-75 µg/dL) and rats (Pb exposure = 25 mM in drinking water) (Boudene et al., 1984; Fujita et al., 1981; Fujita et al., 1982).

ALAD is a polymorphic gene with three isoforms: 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 (Astrin et al., 1987; H.-S. Kim et al., 2004; Pérez-Bravo et al., 2004; Scinicariello et al., 2007; C. M. Smith, Hu, et al., 1995; Wetmur, 1994; Wetmur et al., 1991; Y. Zhao et al., 2007). Some newer 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 (Y. Chen et al., 2008; Chia et al., 2007; Chia et al., 2006; E. F. Krieg, Jr. et al., 2009; Scinicariello et al., 2010; Wananukul et al., 2006).

The ALAD-2 protein binds Pb more tightly than the ALAD-1form: in 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, Grubb, et al., 1997). 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 (B. S. Schwartz, Lee, Stewart, Sithisarankul, et al., 1997), decreased levels of zinc protoporphyrin (H.-S. Kim et al., 2004; Scinicariello et al., 2007), lower cortical bone Pb (C. M. Smith, Wang, et al., 1995), and lower amounts of DMSA-chelatable Pb (B. S. Schwartz et al., 2000; B. S. Schwartz, Lee, Stewart, Ahn, et al., 1997; Scinicariello et al., 2007). However, the findings that ALAD-2 polymorphisms reduced the bioavailability of Pb are somewhat equivocal. Wu et al. (2003) observed that ALAD-2 carriers had lower blood Pb level (5.8 ± 4.2 µg/dL) than carriers of the ALAD-1 gene (blood Pb level = 6.2 ± 4.1 µg/dL), and that ALAD-2 carriers demonstrated decreased

renal function at lower patellar Pb concentrations than those observed to decrease renal function in ALAD-1 carriers. This potentially indicates that ALAD-2 carriers have enhanced Pb bioavailability. Weaver et al. (2003) observed that ALAD-2 polymorphisms were associated with higher DMSA-chelatable Pb concentrations, when normalized to creatinine levels. Further, Montenegro et al. (2006) observed among individuals with ALAD 1-1 or ALAD 1-2/2-2 genotypes a significant increase in the amount of Pb found in the plasma (0.44 µg/L versus 0.89 µg/L, respectively) and in the % plasma/blood ratio (0.48% versus 1.45%, respectively). This suggests that the increased plasma levels of Pb in subjects with ALAD 1-2/2-2 genotypes increases the probability of adverse health effects in these individuals.

ALAD's capacity for binding Pb has been estimated at 85 µg/dL in erythrocytes and 40 µg/dL in whole blood (Bergdahl et al., 1998). The 45 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 likely would 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.

Studies also observed the presence of an inducible, low-molecular weight (approximately 10 kDa) Pb-binding protein in workers occupationally exposed to Pb (Gonick et al., 1985; Raghavan et al., 1980, 1981; Raghavan & Gonick, 1977). The presence of this low molecular weight protein seemed to have a protective effect as workers that exhibited toxicity at low blood Pb concentrations were observed to have lowered expression of this protein or low levels of Pb bound to it (Raghavan et al., 1980, 1981). The presence of low molecular weight Pb-binding proteins in exposed workers was confirmed by Lolin and O'Gorman (1988) and Church et al. (1993a, 1993b). 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 ALAD's Pb-binding capacity identified by Bergdahl et al. (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, 1993b) observed the presence of a 6-7 kDa protein in the blood of 2 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 worker. The reported protein was rich in cysteine residues and tentatively identified as metallothionein.

5.2.3.4. Metallothionein

Metallothionein is a low-molecular metal-binding protein, most often zinc or copper, that is rich in cysteine residues and plays an important role in the protection against heavy metal toxicity, trace element homeostasis, and scavenging free radicals (J. Yu et al., 2009). Exposure to Pb acetate induces the production of Pb- and Zn-metallothionein in mice exposed via i.p. or i.v. injection at 30 mg/kg (Maitani et

al., 1986), in mice exposed via i.p. injection at 300 µmol/kg (J. Yu et al., 2009), or in rats exposed 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 mg/L) failed to induce metallothionein in the kidneys or intestines (Jamieson et al., 2007; L. Wang, D. W. Chen, et al., 2009). Goering and Fowler (1987a, 1987b) observed that pretreatment of rats with zinc before injection with Pb resulted in Pb and zinc co-eluting with zinc-thionein, and that zinc-thionein I and II were able to bind Pb in vitro (Goering & Fowler, 1987a, 1987b). Further, Goering and Fowler (1987a) found that kidney and liver zinc-thionein decreased binding of Pb to liver ALAD and was able to donate zinc 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 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 zinc-metallothionein reduced Pb-induced membrane leakage and loss of potassium 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 the wild-type mice (Qu et al., 2002). 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 mitigation of Pb-induced toxic effects, and that those with polymorphisms in metallothionein coding genes may be at greater susceptibility to Pb. In support of this theory, Chen et al. (2010) observed that Pb-exposed workers with a mutant metallothionein allele had higher blood Pb levels than carriers of the normal allele (24.17 and 21.27 versus 17.03 µg/dL), and were more susceptible to Pb toxicity.

5.2.4. 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 ($OH^\cdot$), and hydrogen peroxide (H_2O_2), that can attack and denature functional/structural molecules and, thereby, promote tissue damage, cytotoxicity, and dysfunction. Pb 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 pathologies (Gonick et al., 1997; Khalil-Manesh, Gonick, Cohen, Bergamaschi, et al., 1992; Khalil-Manesh et al., 1994; Salawu et al., 2009; Sandhir & Gill, 1995; Shan et al., 2009; Vaziri, 2008b). 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, nicotinamide adenine dinucleotide phosphate (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 proteins, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Interestingly, Pb exposure in many species of plants, invertebrates, and vertebrates discussed in the Ecological Effects of Pb results in upregulation of antioxidant enzymes and increased lipid peroxidation (Chapter 7).

5.2.4.1. 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-ALA dehydratase (δ-ALAD) both identified as targets (Bergdahl, Grubb, et al., 1997). 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 is 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 picomolar concentrations (Simons, 1995). Additionally, low blood Pb levels (7 µg/dL) have been found to inhibit the activity of δ-ALAD in humans with a threshold value around 5 µg/dL (Ahamed et al., 2005; Sakai & Morita, 1996). A significant negative correlation (r = -0.6) was found between blood Pb levels in adolescents (4-20 µg/dL) and blood δ-ALA 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 complexes (Hermes-Lima et al., 1991; Monteiro et al., 1991). The autoxidation of δ-ALA produces $O_2^{\cdot-}$, $OH^{\cdot}$, H_2O_2, and an ALA radical (Monteiro et al., 1989; Monteiro et al., 1986).

5.2.4.2. Membrane and Lipid Peroxidation

A large number of studies in humans and experimental animals have found 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 (Bechara et al., 1993; Oteiza et al., 1995). Additionally, Pb has the capacity to stimulate ferrous ion initiated membrane lipid peroxidation serving as a catalyst for these events (Adonaylo & Oteiza, 1999; Quinlan et al., 1988). Increased lipid peroxidation

measured as TBARS from liposomes, microsomes, and erythrocytes was shown after Pb (Pb(NO3)2) exposure for ≤ 2 hours at concentrations as low as 10 µM in vitro (Aruoma et al., 1989; 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 H removal easier (Yiin & Lin, 1995). After Pb exposure (4-12 µg/dL, 24 hours, in vitro), 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. In the absence of Fe^{2+}, Pb does not 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 (AA, 20:4) as a percentage of total fatty acids, and decreased the relative proportion of shorter chain fatty acids (linoleic acid, 18:2) (Lawton & 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 AA. 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 & 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. Occupational Pb exposure resulting in elevated blood Pb levels (>8 µg/dL) in various countries provides evidence of lipid peroxidation, including increased plasma malondialdehyde levels (Dogru et al., 2008; Ergurhan-Ilhan et al., 2008; D. A. Khan et al., 2008; Mohammad et al., 2008; Patil, Bhagwat, Patil, Dongre, Ambekar, & Das, 2006; Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006; Quintanar-Escorza et al., 2007). One study found a correlation between the MDA levels and blood Pb levels even in the unexposed workers who had lower (i.e., <12 µg/dl) blood Pb levels (Quintanar-Escorza et al., 2007). Other studies found increased evidence of lipid peroxidation among the general population, including children, with elevated blood Pb levels (>10 µg/dL) (Ahamed et al., 2008; Ahamed et al., 2006; Y. P. Jin et al., 2006). Ahamed et al. (2006) found a significant positive correlation (r = 0.7) between blood Pb levels between 4-20 µg/dL in adolescents and blood MDA level. Similar results have been shown after Pb exposure in animal studies (Adegbesan & Adenuga, 2007; M. K. Lee et al., 2005; Pandya et al., 2010; D. Y. Yu et al., 2008). An enhanced rate of lipid peroxidation has been found in Pb treated (50 µg, 1-4 hours) rat brain homogenates (Rehman et al., 1995) 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.

Interestingly, studies in many species of plants, invertebrates, and vertebrates exhibit increased lipid peroxidation (Chapter 7.4.4). The increase in lipid peroxidation following Pb exposure observed across species and kingdoms demonstrate an evolutionarily conserved oxidative response following Pb exposure.

5.2.4.3. 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 contributing 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.

5.2.4.4. Antioxidant Enzyme Disruption

Oxidative stress will 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 it could also be a consequence of increased oxidative damage by Pb.

Studies of the effects of Pb on the activities of SOD and CAT give divergent results. These metalloproteins 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 iron ions to function (Putnam et al., 2000). SOD exists in multiple isoforms that require copper and zinc (SOD1 and SOD3) (Antonyuk et al., 2009) or manganese (SOD2) (Borgstahl et al., 1992). A number of studies have found decreased activity of these enzymes (Ergurhan-Ilhan et al., 2008; Mohammad et al., 2008; Pandya et al., 2010; Patil, Bhagwat, Patil, Dongre, Ambekar, & Das, 2006; Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006; D. Y. Yu et al., 2008), whereas others observe increased activity (Ahamed et al., 2008; M. K. Lee et al., 2005). Pb exposure (500 ppm, 1, 4, and 8 weeks) in rats showed that organ SOD and CAT responded

differently depending on the dose and tissue investigated. Activity of SOD and CAT varied based on the brain region analyzed and time of exposure (Bennet et al., 2007). Another study found that the brain had consistently decreased SOD activity, irrespective of dose in prenatally exposed animals (0.3 and 3.0 mg/l, 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 SOD and CAT protein 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 and decrease levels of GSH. Acute administration of Pb (0.1 µM in vitro, 18 µg/dL human) results in decreased blood and organ GSH and cysteine content, which may be due to increased GSH efflux from tissues (Ahamed et al., 2008; Ahamed et al., 2009; Ahamed et al., 2005; Chetty et al., 2005; Flora et al., 2007; Nakagawa, 1989, 1991; Pandya et al., 2010). Chronic Pb exposure elicits 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 (Corongiu & Milia, 1982; Daggett et al., 1998; J. M. Hsu, 1981). However, other studies have found that chronic Pb exposure resulting in blood Pb level between 6.6 and 22 µg/dL, causes the depletion of GSH (Ercal et al., 1996; M. K. Lee et al., 2005; Mohammad et al., 2008). Thus, the time of exposure is important to consider when measuring GSH levels.

Glutathione reductase (GR) is able to reduce GSSG back to GSH. Therefore, an increased GSSG/GSH ratio is considered indicative of oxidative stress. Pb exposure has been shown to increase the GSSG/GSH ratio (Ercal et al., 1996; Mohammad et al., 2008; Sandhir & Gill, 1995), even at blood Pb level below 10 µg/dL in children (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 have found decreased (Bokara et al., 2009; Sandhir & Gill, 1995; Sandhir et al., 1994), increased (Howard, 1974; Sobekova et al., 2009), and no change (J. M. 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).

GSH is used as a cofactor for peroxide reduction and detoxification of xenobiotics by the enzymes GPx and GST. GPx requires selenium for peroxide decomposition (Rotruck et al., 1973), whereas GST functions via a sulfhydryl group. By reducing the uptake of selenium, depleting cellular GSH, and disrupting protein thiols, Pb is able to decrease the activity of GPx and GST (M. K. Lee et al., 2005; Nakagawa, 1991; Schrauzer, 1987; D. Y. Yu et al., 2008). Similar to other antioxidant enzymes, compensatory upregulation of these enzymes is described after Pb treatment (Bokara et al., 2009;

Conterato et al., 2007; Daggett et al., 1998; Ergurhan-Ilhan et al., 2008). However, other studies have shown that these enzymes may not be able to compensate for the increased Pb-induced ROS, further contributing to the oxidative environment (Farmand et al., 2005).

Recently, γ-glutamyltranferase (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 (D. H. Lee et al., 2004). Occupational Pb exposure (blood Pb level, 29.1 µg/dL) results in increased serum GGT levels (D. A. Khan et al., 2008). Interestingly, low blood Pb levels found in a sample of the U.S. population (NHANES III) were positively associated with serum GGT levels showing a dose-response relationship at levels <7 µg/dL (D. H. Lee et al., 2006).

5.2.4.5. Nitric Oxide Signaling

˙NO, also known as endothelium-derived relaxing factor, is a potent endogenous signaling molecule involved in vasodilation. Acute and chronic Pb exposure decreases the biologically active ˙NO, not through reduction in ˙NO-production capacity (Vaziri & Ding, 2001; Vaziri, Ding, et al., 1999), but as a result of inactivation and sequestration of ˙NO by ROS (Malvezzi et al., 2001; Vaziri, Liang, et al., 1999). 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, Liang, et al., 1999). ˙NO is also produced by macrophages in the defense against certain infectious agents, including bacteria. Studies have found that Pb exposure can significantly reduce production of ˙NO in immune cells (J.-E. Lee et al., 2001; Pineda-Zavaleta et al., 2004; L. Tian & Lawrence, 1995), possibly leading to reduced host resistance (L. Tian & 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), that require a heme prosthetic group and a zinc cation for enzymatic activity (Messerschmidt et al., 2001). Paradoxically, the reduction in ˙NO availability in vascular tissue following Pb exposure is accompanied by significant upregulation in NOS isotypes (Gonick et al., 1997; Vaziri & Ding, 2001; Vaziri, Ding, et al., 1999). A direct inhibitory action of Pb on NOS enzymatic activity has been rejected (Vaziri, Ding, et al., 1999). Instead, the upregulation of NOS occurs as compensation for the decreased ˙NO resulting from ROS inactivation (Vaziri & Ding, 2001; Vaziri et al., 2005; Vaziri & 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 (GTP). Soluble guanylate cyclase is a heterodimer requiring one molecule of heme for enzymatic activity (Boerrigter & Burnett, 2009). In vascular smooth muscle cells (VSMC), sGC serves as the ˙NO receptor. Marked reduction in plasma concentrations and urinary excretion of cGMP is observed after Pb exposure (5 ppm for 30 days and 0.01% for 12 months) (Khalil-Manesh, Gonick, Weiler, et al., 1993; M. Marques et al., 2001). In addition, Pb exposure downregulated the protein abundance of sGC in vascular tissue (Courtois et al., 2003; Farmand et al., 2005; M. Marques et al., 2001). This downregulation in sGC was prevented by antioxidant therapy suggesting that oxidative stress also plays a role in Pb-induced downregulation of sGC (M. Marques et al., 2001).

5.2.5. Inflammation

Misregulated inflammation represents one of the major effects of Pb-induced immunotoxicity. 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., selenium) accompany this immunomodulation (Chetty et al., 2005).

Traditional immune mediated inflammation can be seen with bronchial hyper-responsiveness, asthma, and respiratory infections after 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 (Carmignani et al., 2000; Mudipalli, 2007). Pb spheres implanted in the brains of rats produced neutrophil-driven inflammation with apoptosis and indications of neurodegeneration (Kibayashi et al., 2010). Pb also induces renal tubulointerstitial inflammation (18 µg/dL or 100 ppm for 14 weeks) (Ramesh et al., 2001; Rodriguez-Iturbe et al., 2005), which has been coupled with activation of the redox sensitive nuclear transcription factor kappa B (NFκB) and lymphocyte and macrophage infiltration (23-27 µg/dL, 100 ppm for 14 weeks) (Bravo et al., 2007). These events could be in response to the oxidative environment developed from Pb, since Pb-induced inflammation and NFκB activation can be ameliorated by antioxidant therapy (Rodriguez-Iturbe et al., 2004).

Inflammation can be mediated by the production of chemical messengers such as prostaglandins (PG). Pb exposure has been reported to increase arachidonic acid (AA) metabolism, thus elevating the production of PGE_2, PGF_2, and thromboxane in humans (48 µg/dL) and cell models (0.01 µM, 48 h) (Cardenas et al., 1993; Chetty et al., 2005; Flohe et al., 2002; Knowles & Donaldson, 1997; J. J. Lee &

Battles, 1994). Dietary Pb supplementation (500 ppm, 19 days) can increase the percentage of cell membrane AA, the precursor of cyclooxygenase and lipoxygenase metabolism to PGs and leukotrienes (Knowles & Donaldson, 1990). Additionally, Pb (1 µM, 20 µg/dL) may promote the release of AA via activation of phospholipase A_2, as shown in isolated VSMC (Dorman & Freeman, 2002).

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

5.2.5.1. Cytokine Production

There are three modes of major effects of Pb on immune cytokine production. First, Pb can act on macrophages to elevate the production of pro-inflammatory cytokines such as TNF-α and IL-6 (S. Chen et al., 1999; Y.-J. Cheng et al., 2006; Dentener et al., 1989). This can result in local tissue damage during the course of immune responses affecting such targets as the liver. Second, when Pb acts on dendritic cells, it skews the ratio of IL-12/IL-10 such that Th1 responses are suppressed and Th2 responses are promoted (S. Chen et al., 2004; Miller et al., 1998). Third, when acquired immune responses occur following exposure to Pb, Th1 lymphocyte production of cytokines is suppressed (e.g. IFN-γ) (Heo et al., 1996; Lynes et al., 2006). In contrast, Th2 cytokines such as IL-4, IL-5, and IL-6 are elevated (D. Gao et al., 2007; D. Kim & Lawrence, 2000). The combination of these three modes of cytokine changes induced by Pb creates a hyperinflammatory state among innate immune cells and acquired immunity is skewed toward Th2 responses.

Iavicoli et al. (2006) reported below-background blood Pb concentrations produced significant changes in cytokine levels. At the lowest dietary Pb concentration (0.8 µg/dL), IL-2 and IFN-γ were elevated over the controls, indicating an enhanced Th1 response. However, as the dietary and blood Pb concentrations increased (resulting in blood Pb level 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 extremely low Pb exposures. TGF-β production is also altered by Pb exposure to cells (1 µM, 3 days) (Zuscik et al., 2007). IL-2 is one of the more variable cytokines relative 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 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 after Pb exposure and increased IL-6 was found in the forebrain (Struzynska et al., 2007).

Consistent with animal studies, epidemiologic studies have also demonstrated Pb-associated decreases in Th1-type cytokines and increases in Th2-type cytokines. Among adults without occupational Pb exposures in Incheon, Korea with blood Pb levels ranging from 0.337 to 10.47 µg/dL, Kim et al. (2007) found associations of blood Pb with serum levels of TNF-α and IL-6 that were larger among men with blood Pb levels ≥ 2.51 µg/dL (median). In models that adjusted for age, sex, BMI, and smoking status, a 1 µg/dL increase in blood Pb was associated with a 23% increase (95% CI: 4,55) in log of TNF-α and a 26% increase in log of IL-6 (95% CI: 0,55).

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, analysis 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; Yucesoy et al., 1997a). The exception was the study of male foundry workers, pottery workers, and unexposed workers by Valentino et al. (2007). Multiple regression analyses were performed with age, BMI, smoking, and alcohol consumption included as covariates. Although effect estimates were not provided, statistically significant associations were observed between blood Pb and IL-10 and TNF-α, with R^2 values of 0.249 and 0.235, respectively. Exposed workers also had higher levels of most Th2 cytokines (IL-2, IL-6, IL-10, and TNF-α) and lower levels of the Th1 cytokine IFN-γ. Levels of IL-2, IL-6, and IL-10 showed an increasing trend 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). Excluded from this study were exposed workers from the highest end of the distribution of blood Pb levels in DiLorenzo et al. (2006). Mean TNF-α levels showed a monotonic increase from the low to high blood Pb group, which was suggestive of a concentration-dependent relationship. 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 statistically significant lower serum levels of the Th1 cytokines, IL-1β and IFN-γ, in workers compared with controls; however levels of the Th2 cytokines, IL-2 and TNF-α levels, were similar between groups. Overall, exposure to Pb increases the production of pro-inflammatory cytokines, skews the ratio of Th1 and Th2 cytokines to favor Th2 responses, and suppresses lymphocyte cytokine production.

5.2.6. Endocrine Disruption

5.2.6.1. Hypothalamic-Pituitary-Gonadal Axis

Pb is a potent endocrine disrupting chemical that causes reproductive and developmental effects at moderate levels of exposure in both male and female animal models. 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. Chronic Pb exposure has been shown to reduce serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and estradiol (Biswas & Ghosh, 2006; Dearth et al., 2002; E. F. Krieg, Jr., 2007; Ronis et al., 1998; Rubio et al., 2006; Sokol & Berman, 1991). This is likely through the inhibition of LH secretion and the reduction in the expression of the steroidogenic acute regulatory protein (StAR) (B. M. Huang et al., 2002; B. M. Huang & Liu, 2004; Ronis et al., 1996; V. Srivastava et al., 2004). StAR is the rate-limiting step essential in maintaining gonadotropin-stimulated steroidogenesis, which results in the formation of testosterone and estradiol. Pb (prenatal exposure resulting in blood Pb level 3 µg/dL) decreases basal StAR synthesis, but not gonadotropin-stimulated StAR synthesis, suggesting that Pb may not directly affect ovarian responsiveness to gonadotropin stimulation (V. Srivastava et al., 2004). Instead, Pb may act on 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, which 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) is able to decrease the number of estrogen receptors found in the uterus and receptor binding affinity (Wiebe & 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 is able to counteract a number of the endocrine disrupting effects of Pb, including apoptosis and decreased sperm motility and production (P. C. Hsu, Liu, et al., 1998; Madhavi et al., 2007; Rubio et al., 2006; Salawu et al., 2009; Shan et al., 2009; C. H. Wang et al., 2006). Direct generation of ROS in epididymal spermatozoa was observed after Pb exposure (i.p. 20 or 50 mg/kg, 6

wk) (P. C. Hsu, Hsu, et al., 1998). In addition, lipid peroxidation in the seminal plasma was significantly increased in a group of Pb-exposed workers with blood Pb >40 µg/dL (A. 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). Pb exposed humans (>4 µg/dL), animals (14 µg/dL), and gonadal cells (0.05 mg/mL) show a decrease in plasma IGF-1 (Dearth et al., 2002; Huseman et al., 1992; Kolesarova et al., 2010; Pine et al., 2006), which may be the result of decreased translation or secretion of IGF-1 (Dearth et al., 2002). 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. 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., 1987; Huseman et al., 1992). 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 52 pM, 30 minutes) (Lau et al., 1991). GH secretion may also be altered from decreased testosterone, a result of Pb exposure.

5.2.6.2. Hypothalamic-Pituitary-Thyroid Axis

The effects of Pb on the hypothalamic-pituitary-thyroid (HPT) axis are mixed. Pb exposure impacts a variety of players in the thyroid hormone system. A number of human studies have shown a negative association between elevated blood Pb and thyroxine (T_4) and free T_4 levels without alteration in triiodothyronine (T_3), suggesting that long-term Pb exposure may depress thyroid function (Dundar et al., 2006; Robins et al., 1983; Tuppurainen et al., 1988). However, animal studies on thyroid hormones have shown mixed results. Pb exposed cows 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 accompanied by increased lipid peroxidation (Chaurasia et al., 1998; Chaurasia & Kar, 1997). Decreased serum T_3 and increased lipid peroxidation were both restored by vitamin E treatment, suggesting the disruption of thyroid hormone homeostasis could be a result of altered membrane architecture and oxidative stress (Chaurasia & 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 subjects (Abdelouahab et al., 2008; Gustafson et al., 1989; Lopez et al., 2000; B. Singh et al., 2000), which would result in increased T_4 levels. Overall, results on the effects of Pb on the HPT axis are inconclusive.

5.2.7. 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, 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 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) are discussed below.

5.2.7.1. DNA Damage

A number of studies in human populations have observed positive associations between Pb exposure and DNA damage, as measured as DNA strand breaks. Most of these associations have been observed in occupationally-exposed populations (Danadevi et al., 2003; de Restrepo et al., 2000; Fracasso et al., 2002; Grover et al., 2010; Hengstler et al., 2003; Minozzo et al., 2010; Palus et al., 2003; Shaik & Jamil, 2009). It is important to note that occupationally-exposed workers have very high blood Pb levels, and in one study (de Restrepo et al., 2000) the association between blood Pb and DNA damage was only observed in workers with blood Pb greater than 120 µg/dL. Also, the studies were 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 Paulus et al. (2003) and Minozzo et al. (2010) observed no correlation (mean blood Pb = 50.4 (range = 28.2-65.5) and 59.43 ± 28.34 µg/dL, respectively). Lastly, workers occupationally-exposed to Pb are also potentially exposed to other genotoxic materials, making it difficult to rule out confounding co-exposures. For example, Hengstler et al. (2003) examined workers exposed to Pb, cadmium, and cobalt and observed that neither blood or air Pb (4.4 (IQR: 2.84-13.6) µg/dL; 3.0 (IQR: 1.6-50.0) µg/m^3) was 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 cadmium in air and blood and cobalt in air. Two studies were found that investigated Pb-induced DNA damage resulting from nonoccupational exposures. Mendez-Gomez (2008) observed that children living at close and intermediate distances to a Pb smelter had blood Pb levels of 19.5 (11.3-49.2) or 28.6 (11.4-47.5) µg/dL, compared to blood Pb level of 4.6 (0.1-8.7) µg/dL for children living distant to the smelter. DNA damage was significantly increased in children living nearest the smelter, compared to the intermediate distant children, but was not significantly different from children living farthest away from the smelter. Multivariate analysis (which

considered the children's urinary As levels, highest in children farthest from the smelter), revealed no significant associations between DNA damage and blood Pb. Further, DNA repair ability was also observed to be nonrelated to blood Pb. Alternatively, Yanez et al. (2003) observed that children living close to a mining complex (blood Pb level= 11.6, range = 3.0 to 19.5 µg/dL) did have increased levels of DNA damage compared to control children that lived further away from the mining facility (blood Pb level = 8.3 (3.0-25.0) µg/dL).

In mice given 0.7 to 89.6 mg/kg Pb nitrate 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 dose (K. D. Devi et al., 2000). The three highest doses had responses which were similar in magnitude and were actually lower than the responses to lower doses tested. In mice exposed to Pb (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 over a period of 4 weeks, although variability was high in all groups, and the magnitude of the DNA damage was characterized as weak and did not increase with increasing durations of exposure. Xu et al. (2008) exposed mice to 10-100 mg/kg Pb acetate via gavage for four weeks and observed a dose-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 Pb-exposed rats (blood Pb level = 19.5 and 21.9 µg/dL, respectively) compared to control rats (Nava-Hernandez et al., 2009). The level of DNA damage was not dose-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 0.5 or 1% Pb nitrate in drinking water for 60 days. Interestingly, although the results were not statistically significant and highly variable, 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 HeLa cells when exposed to 500 µM for 20-25 hours or 100 µM for 0.5-4 hours (Hartwig et al., 1990; R. D. Snyder & Lachmann, 1989). Pb nitrate, administered to lymphoma cells at 1-10 mM for 6 hours, did not result in any DNA-protein crosslinks (M. Costa et al., 1996). Pb acetate was observed by Wozniack and Blasiak (2003) to result in DNA single and double strand breaks in primary human lymphocytes exposed 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 dose group may be a result of increased crosslinking in this group. Shaik et al. (2006) also observed DNA damage in human lymphocytes exposed to 2.1-3.3 mM Pb nitrate for 2 hours. Although there was a dose-dependent increase in DNA damage from

2.1 to 3.3 mM, no statistics were reported and no unexposed control group was included making it difficult to interpret these results. Gastaldo et al. (2009) observed that exposure of human endothelial cells to 1-1000 µM Pb nitrate for 24 hours resulted in a dose-dependent increase in DNA double strand breaks.

Studies in animal cell lines 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 mM, respectively) did not induce single or double DNA strand breaks or DNA-protein or DNA-DNA crosslinks in CHV79 cells exposed to Pb acetate. However, both Xu et al. (2006) and Kermani et al. (2008) reported Pb acetate induced DNA damage in 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 dose-dependent manner in hepatoma cells exposed to 50-5000 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 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 to 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 to 100 µM for 24 hours. Exposure to Pb inhibited nonhomologous end joining (NHEJ) repair, but increased two other repair pathways, MRE11-dependent and Rad51-related repair (Gastaldo et al., 2007). Interestingly, 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 (J. P. Li et al., 2008), though this result is difficult to interpret. Roy and Rossman (1992) observed an increase in UV-induced mutagenicity when CHV79 cells were co-exposed to 400 µM Pb acetate (a nonmutagenic dose 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 dose-dependent accumulation of apurinic/apyrimidinic site incision activity, indicating that DNA repair was adversely affected (McNeill et al., 2007).

5.2.7.2. 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 oxxupational Pb exposures or a number of other heavy metals and organic contaminants. Blood Pb (range 78.2-251.0 nM) was statistically significantly positively associated with HPRT mutation frequency in the total population. Also, women with high blood Pb (i.e.,

greater than the population median, not reported) demonstrated a greater mutation frequency compared to women with lower blood Pb.

Pb-induced mutagenicity was investigated in four studies using human cell cultures. Ye (1993) exposed human keratinocytes to 0.1-100 µM/mL Pb acetate for 2-24 hours. This study did not measure HPRT mutations directly, but rather measured the amount of tritium 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 500-2000 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) (J. P. Li et al., 2008; C. Y. Wang et al., 2008). 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 the findings from human cell line studies, although differences in mutagenicity may be reflective of specific Pb compounds used. Pb acetate was observed to be nonmutagenic (HPRT assay) in Chinese hamster V79 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 1700 µM for 5 days (N. K. Roy & 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 observation of mutagenicity in the second study is complicated by the concurrent observation of severe cytotoxicity at the same dose. Pb nitrate was alternatively found to be nonmutagenic in CHV79 cells (gpt assay) exposed to 0.5-2000 µM for 5 days (N. K. Roy & Rossman, 1992), or mutagenic in the same cell line (HPRT assay) exposed to 50-5000 µM for 5 days (Zelikoff et al., 1988). However, mutagenicity was only observed at 500 µM, and was higher than that observed at higher doses. Pb sulfate was also observed to be mutagenic in CHV79 cells (HPRT assay) exposed to 100-1000 µM for 24 hours, but as with Pb nitrate, it was not dose-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 dose-dependent mutagenicity in the gpt assay in Chinese hamster ovary cells exposed to 0.1-1 µM Pb chloride for one hour (Ariza et al., 1998; Ariza & Williams, 1996, 1999).

5.2.7.3. Clastogenicity

Clastogenicity is the ability of a compound to induce chromosomal damage, and is commonly observed as sister chromatid exchange, micronuclei formation, or incidence of chromosomal aberrations

(i.e., breaks or gaps in chromosomes). The potential for Pb to be clastogenic has been investigated in numerous studies as described below.

Sister Chromatid Exchange

An association between blood Pb (mean blood Pb = 10.48 - 86.9 µg/dL) and sister chromatid exchange (SCE) was observed in a number of occupational studies (Anwar & Kamal, 1988; Bilban, 1998; Duydu et al., 2005; Duydu et al., 2001; X. P. Huang et al., 1988; Palus et al., 2003; Pinto et al., 2000; Wiwanitkit et al., 2008). However, there are numerous methodological issues that limit firm conclusions from being drawn, most notably that occupational co-exposures to other genotoxic materials were possible, although some studies excluded workers with exposures to known mutagens (X. P. Huang et al., 1988; Pinto et al., 2000). In most studies that attempted to investigate the dose-response relationship in workers, no association was observed between increasing blood Pb levels and the incidence of SCE (Duydu et al., 2001; Palus et al., 2003; 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), although the association was only statistically significant 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 incidence of SCE (T. Rajah & Ahuja, 1995; T. T. Rajah & Ahuja, 1996). However, these two studies may have suffered from limited statistical power to observe an effect as they only included very small Pb exposed populations. Mielzynska et al. (2006) investigated the incidence of SCE in children exposed to Pb and PAHs in Poland. Children had an average blood Pb concentration of 7.69 µg/dL and 7.87 SCEs/cell. Male children had higher blood Pb concentrations compared to females, but lower numbers of SCEs. No control population was included in this study, and thus interpretation of these findings is difficult.

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

Only two studies were found that investigated SCE formation in human cell lines due to Pb exposure. Statistically significant, dose-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 (Ustundag & Duydu, 2007). Melatonin and N-acetylcysteine were reported to ameliorate these effects, indicating Pb may induce SCE 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.).

Studies investigating SCE in rodent cells were more equivocal than those in human cells. Pb sulfate, acetate, and nitrate were found to not induce SCE in Chinese hamster V79 cells (Hartwig et al., 1990; Zelikoff et al., 1988). Both of these studies only examined 25-30 cells per concentration, reducing their ability to detect Pb-induced SCE. 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, dose-dependent increase in SCE in Chinese hamster ovary cells. Lin et al. (1994) also observed a dose-dependent increase in SCE in Chinese hamster cells exposed to 3-30 µM Pb nitrate for 2 hours.

Micronucleus Formation

Micronucleus formation in Pb-exposed workers was investigated in numerous occupational studies (Bilban, 1998; Grover et al., 2010; M. I. Khan et al., 2010; Minozzo et al., 2004; Minozzo et al., 2010; Palus et al., 2003; Pinto et al., 2000; Shaik & Jamil, 2009; Vaglenov et al., 1998; Vaglenov et al., 2001). The workers in the occupational studies generally had high blood Pb levels (>20 µg/dL) making comparisons to the general population difficult, although Pinto et al. (2000) observed increased micronuclei in exposed workers with an average blood Pb concentration of only 10.48 µg/dL. Studies that analyzed workers according to the magnitude of their blood Pb levels reported no correlation between Pb exposure and the observation of micronuclei (Minozzo et al., 2004; Minozzo et al., 2010; 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. Only one study was found that investigated micronucleus formation in a nonworker population; Mielzynska et al. (2006) examined associations of blood Pb and urinary PAH metabolite levels with micronuclei formation in children in Poland. Children, with an average blood Pb concentration of 7.69 µg/dL, were observed to have 4.44 micronucleated cells per 1000 cells analyzed. Although no control group was included in this study, a statistically significant positive correlation was observed between blood Pb concentrations and micronuclei frequency, and children with blood Pb greater than 10 µg/dL had significantly more micronucleated cells than children with blood Pb 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 140, 250, or 500 g/kg Pb acetate once per week for 10 weeks resulted in statistically significantly increased numbers of micronucleated

polychromatic erythrocytes (PCEs) compared to controls. Similarly, Alghazal et al. (2008) exposed rats to 100 mg/L Pb acetate daily for 125 days and observed statistically significant increases in micronucleated PCEs in both sexes. Tapisso et al. (2009) exposed rats to 21.5 mg/kg Pb acetate (1/10th the LD50) via i.p. injection on alternating days for 11 or 21 days, for a total of 5 or 10 exposures. Formation of micronuclei in the bone marrow of exposed rats was increased over 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) exposed mice to 10 or 20 mg/kg Pb nitrate i.p. and observed a dose-dependent increase in micronuclei, whereas Jagetia and Aruna (1998) observed an increase in micronuclei in mice exposed to 0.625-80 mg/kg Pb nitrate i.p., though the increase was not dose-dependent. Mice exposed to 1 g/L Pb acetate via a more environmentally relevant route of exposure, drinking water, for 90 days had statistically significant increases in micronucleated PCEs (C. C. Marques et al., 2006).

Three studies were found that reported increased micronucleus formation in human cell lines treated with Pb. Dose-dependent micronucleus formation was observed in human lymphocytes when exposed to either 1, 5, 10, or 50 µM Pb nitrate or 3 or 5 ppm Pb chloride (Turkez et al.; Ustundag & Duydu, 2007). Gastaldo et al. (2007) also observed a dose-dependent increase in micronuclei in human endothelial cells exposed to 1-1000 µM Pb nitrate for 24 hours. Only two animal cell culture studies investigating micronuclei formation were found. One study observed that micronuclei were not induced in Chinese hamster cells exposed to 3-30 µM Pb nitrate for 2 hours (R. H. Lin et al., 1994), whereas the other observed that Pb acetate induced a dose-dependent increase in Chinese hamster cells when administered at 0.05-10 µM for 18 hours (Bonacker et al., 2005).

Chromosomal Aberrations

Chromosomal aberrations (e.g., chromosome breaks, nucleoplasmic bridges, di- and acentric chromosomes, and rings) were examined in a number of occupational studies (Bilban, 1998; De et al., 1995; Grover et al., 2010; X. P. Huang et al., 1988; Pinto et al., 2000; Shaik & Jamil, 2009). Methodological limitations outlined in previous sections, including potential for occupational co-exposure to genotoxic substances and generally high blood Pb levels (>20 µg/dL) that are difficult to interpret in the context of the general population, also pertain to the present findings. No correlation was observed between increasing blood Pb levels and incidence of chromosomal aberrations, although an association was observed between duration of exposure and chromosomal damage (Grover et al., 2010; Pinto et al., 2000). Two studies reported no association between occupational exposure to Pb and chromosomal aberrations (Andreae, 1983; Anwar & Kamal, 1988). Smejkalova (1990) observed chromosomal damage and aberrations in children living in a heavily Pb-contaminated area of Czechoslovakia and an area with less contamination, although the difference between the two was not statistically significant. Although blood Pb levels were statistically significantly higher in the Pb-contaminated children, they were

generally comparable (low 30s versus high 20s µg/dL, respectively), indicating there may not be enough of an exposure difference to detect a significant difference in aberration rates.

The majority of animal studies investigating Pb-induced genotoxicity focused on the ability of Pb to produce chromosomal damage. Fahmy (1999) exposed mice to 25-400 mg/kg Pb acetate 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 dose-dependent manner after both exposure regimens. Pb nitrate was also observed to produce dose-dependent chromosomal damage in mice exposed i.p. to a single dose of 5, 10, or 20 mg/kg (Dhir, Sharma, et al., 1992). In a similar experiment, Dhir et al. (1990) exposed mice to 10, 20, or 40 mg/kg Pb nitrate and saw an increase in chromosomal aberrations, although there was no dose-response as the response was similar in all doses tested. Nayak et al. (1989) exposed pregnant mice to 100-200 mg/kg Pb nitrate 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 12.5-75 mg/kg Pb nitrate, but there was no dose-response reported and few cells were analyzed (Nayak, Ray, & Persaud, 1989). In rats given 2.5 mg/100 g Pb acetate 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 suffer from the use of a route of exposure that may not be relevant to human environmental exposures. However, studies utilizing drinking water or dietary exposures also observed increases in chromosomal damage. Aboul-Ela (2002) exposed mice to 200 or 400 mg/kg Pb acetate by gavage for 5 days and reported that chromosomal damage was present in the bone marrow cells and spermatocytes of animals exposed to both doses. Dhir et al. (1992) also observed a dose-dependent increase in chromosomal damage in mice exposed via gavage, albeit at much lower doses: 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 0.5% Pb acetate in feed, and observed an increase in abnormal cells and frequency of chromosomal damage.

Only three studies were found that investigated the ability of Pb to induce chromosomal damage in human cell lines and all three reported negative findings. Wise et al. (2004; 2005) observed that Pb glutamate was not mutagenic in human lung cells exposed to 250-2,000 µM for 24 hours. Shaik et al. (2006) observed that Pb nitrate did not increase chromosomal aberrations in primary lymphocytes (obtained from healthy volunteers) when incubated with 1.2 or 2 mM for 2 hours. Four 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 500-2000 µM for 24 hours (J. P. Wise, Sr. et al., 1994), 3-30 µM for 2 hours (R. H. Lin et al., 1994), or 0.05-1 µM for 3-12 hours (Cai & 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).

5.2.7.4. 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.

DNA Methylation

A single i.v. injection of 75 µmol/kg Pb nitrate 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. Two additional studies in humans observed that DNA methylation patterns in adults and cord blood were inversely correlated with bone Pb levels (Pilsner et al., 2009; R. O. Wright et al., 2010). Changes in DNA methylation patterns could potentially lead to dysregulation of gene expression and altered tissue differentiation.

Mitogenesis

Only a few studies have investigated the potential epigenetic effects of Pb exposure in human populations. One such epigenetic effect investigated was mitogenesis that induces cells to proliferate when they should not. Three studies (Minozzo et al., 2004; Minozzo et al., 2010; T. Rajah & Ahuja, 1995) were found that reported that Pb reduced mitogenesis in Pb-exposed workers (blood Pb = 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 exposed to 100 µM/kg via i.v. injection (Columbano et al., 1987; Columbano et al., 1990; Coni et al., 1992; Ledda-Columbano et al., 1992; Nakajima et al., 1995). 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 and NF-κB, TNF-α, and iNOS in liver cells in exposed animals at 12.5 mg/kg and 100 µM/kg, respectively (Y.-J. Cheng et al., 2002; Menegazzi et al., 1997). Only one study was found that observed a mitogenic effect after inhalation: exposure to 0.01M Pb acetate for 4 weeks resulted in increased cellular proliferation in the lungs (Fortoul et al., 2005).

A great amount of 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 & Tonner, 1995) and primary oligodendrocyte progenitor cells (1 µM for 24 hours) (W. Deng & Poretz, 2002), and had no observable effects on growth in glioma cells (0.01-10 µM for 12-72 hours) (M. Y. Liu et al., 2000). Pb glutamate had no effect on cell growth in human lung cells, but did increase the mitotic index (250-1,000 µM for 24 hours) (S. S. 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 dose-dependent inhibition of DNA synthesis reported in hepatoma cells (1-100 µM for 72 hours) (Heiman & 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, chloride, oxide, and sulfate were all observed to inhibit cell growth (10 µM -1 mM for 1-7 days and 0.078-320 µM for 48 hours, respectively) (Apostoli et al., 2000; Iavicoli et al., 2001). 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 mesenchymal stem cells when administered at 0-100 µM for 48 hours (Kermani et al., 2008). Pb nitrate was alternatively reported to increase (R. H. Lin et al., 1994) and decrease (Cai & Arenaz, 1998) the mitotic index in Chinese hamster ovary cells exposed to 1µM 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 the untreated control cells.

5.2.7.5. Gene Expression

Two animal studies have investigated the ability of Pb to alter gene expression in regard to phase I and II metabolizing enzymes. Suzuki et al. (1996) exposed rats to 100 µg/kg Pb acetate or nitrate via i.p. injection and observed an induction of glutathione transferase P (GST-P) with both Pb compounds. The induction of GST-P by Pb was observed to operate on the transcriptional level and to be dependent on the direct activation of the cis-element GPEI enhancer. Degawa et al. (1993) reported that i.v. exposure to 20, 50, or 100 µmol/kg Pb nitrate selectively inhibited CYP1A2 levels. Pb was shown to not inhibit CYP1A2 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 10-100 µM Pb nitrate for 24 hours increased the amount of Cyp1A1 mRNA while not influencing the activity of the enzyme. NAD(P)H:quinone oxidoreductase and glutathione S-transferase Ya activities and mRNA levels were

increased after exposure to Pb. Incubation of primary human bronchial epithelial cells with 500 µg/L Pb acetate 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).

One additional animal study provides 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 0.2% Pb acetate via lactation from PND1-20, the expression of proteolipid protein (PLP), a major structural constituent of myelin, was statistically significantly elevated at PND20, compared to controls. The expression of another structural element of myelin, myelin basic protein (MBP), 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.

5.2.7.6. Apoptosis

Occupational exposure to Pb and induction of apoptosis was investigated in three studies. One study directly reported that exposure to Pb increased apoptosis compared to nonexposed controls (Minozzo et al., 2010), whereas the other two reported that two early indicators of apoptosis, karyorrhexis and karyolysis, were elevated in exposed workers (Grover et al., 2010; M. I. Khan et al., 2010). 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 to Pb acetate and rat alveolar macrophages exposed to Pb nitrate (Iavicoli et al., 2001; Shabani & Rabbani, 2000). Observation of Pb-induced apoptosis may represent the dysregulation of genetically-controlled cell processes and tissue homeostasis.

5.2.8. Summary

The diverse health effects of Pb are mediated through multiple, interconnected modes of action. Each of the modes of action discussed has the potential to contribute to the development of a number of Pb-induced health effects (Table 5-2). Evidence for the majority of these modes of action is observed at low blood Pb level in humans and animals, between 2 and 5 µg/dL, and at doses as low as the picomolar range in animals and cells. Dose captures Pb exposure concentration in invitro systems or in animal studies when no blood Pb level was reported. These observable effect levels are limited by the data and methods available 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.

Table 5-2. Related health effects resulting from the MOAs of Pb and the lowest level eliciting the MOA reported as blood Pb level and dose delivered

MOA	Related Health Effects (Section) ISA	Lowest Level at which MOA Observed		Studies
		Blood Pb	Dose	
Altered Ion Status	All Heath Effects of Pb	3.5 µg/dL	50 pM	Huel et al. (2008) Kern et al. (2000)
Protein Binding	Renal (5.5), Effects on Heme Synthesis and Red Blood Cell Funtion (5.7)	6.4 µg/dL	50 µM	Chen et al. (2010) Klann and Shelton (1989)
Oxidative Stress	All Heath Effects of Pb	5-10 µg/dL	10 µM	Quinlan et al. (1988) Ahamed et al. (2006) Yiin and Lin (1995)
Inflammation	Neurological (5.3), Cardiovascular (5.4), Renal (5.5), Immune (5.6), Respiratory (5.6.4), Cancer (5.10), Hepatic (5.9.1)	3 µg/dL	0.01µM	Kim et al. (2007) Chetty et al. (2005)
Endocrine Disruption	Reproductive Effects and Birth Outcomes (5.8), Bone and Teeth (5.9.4), Endocrine System (5.9.3)	2 µg/dL	20 ppm	Krieg (2007) Wiebe and Barr (1988)
Cell Death/Genotoxicity	Cancer (5.10), Reproductive Effects and Birth Outcomes (5.8), Bone and Teeth (5.9.4)	3.1 µg/dL	50 nM	Van et al. (2004) Bonacker et al. (2005)

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 5-1). Pb will interfere with endogenous Ca^{2+} homeostasis, necessary as a cell signal carrier mediating normal cellular functions. $[Ca^{2+}]_i$ have been shown to increase 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-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 PKC or calmodulin. Disruption of ion transport not only leads to altered Ca^{2+} homeostasis, it can also result in perturbed neurotransmitter function. Pb-exposure decreases evoked release of neurotransmitters, while simultaneously increasing spontaneous release of neurotransmitters through Ca^{2+} mimicry. Pb is able to displace metal ions, such as Zn, Mg, and Ca^{2+}, from proteins due to the flexible coordination number of Pb and multiple ligand binding ability, leading to abnormal conformational changes to proteins and altered protein function. Evidence for this metal ion displacement and protein pertubation has been shown at picomolar 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 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. 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 a 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 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, 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 will 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 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.

In a number of organ systems Pb-induced oxidative stress is accompanied by misregulated inflammation. Pb exposure will 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 production of related cytokines.

Pb is a potent endocrine disrupting chemical. Pb will disrupt the HPG axis evidenced 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 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 are mixed and require further investigation.

Genotoxicity and cell death has been investigated after Pb exposure in humans, animals, and cell models. High level Pb exposure to humans leads to increased DNA damage, however lower blood Pb levels have caused 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 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 (>10 µg/dL), supported by Pb-induced effects in both animal and cell studies. 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 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 following Pb exposure.

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

5.3. Neurological Effects

5.3.1. Introduction

The central nervous system undergoes rapid differentiation in utero and in early life, which makes the developing fetus and infant particularly susceptible to neurological effects associated with environmental exposures (Landrigan et al., 1999; Rice & Barone, 2000). Based on evidence from diverse prospective and cross-sectional studies focusing primarily on effects associated with Pb exposure levels less than 10 µg/dL, 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, 2006). Several individual studies observed inverse associations between blood Pb levels and IQ that persisted at blood Pb levels in the range of 2-8 µg/dL (Lanphear et al., 2000; J. Schwartz, 1994). This association was substantiated in a pooled analysis of children, 5 to 10 years of age, 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). The 2006 AQCD described associations of blood Pb with a broad range of additional, related neurodevelopmental endpoints, including academic achievement and performance, motor skills, mood, and antisocial and delinquent behavior (U.S. EPA, 2006).

Toxicological studies not only provided coherence with similarly consistent findings for Pb-induced impairments in learning, behavior and attention, and sensory acuities, but also provided biological plausibility by characterizing mechanisms for Pb-induced neurological effects. In particular, toxicological evidence for Pb exposure interfering with neuronal metabolism at the cellular and histological level (e.g., synaptic architecture during development, neurotransmitter release, glia, neurite outgrowth, the blood brain barrier, and oxidative stress), provided biological plausibility for blood Pb

levels of children being associated with deficits in multiple functional domains such as cognitive function, motor function, memory, mood, and behavior. Additional biological plausibility was provided by observations of associations of childhood blood Pb levels with decreased neuronal density and neuronal loss measured in adulthood, as assessed by magnetic resonance imaging techniques (Cecil et al., 2005; Meng et al., 2005; Trope et al., 2001; Trope et al., 1998; Yuan et al., 2006).

A key finding across several epidemiologic studies of children was a larger estimated effect for a given incremental increase in blood Pb levels on neurocognitive deficits in children with lower blood Pb levels compared with children with higher blood-Pb levels (Bellinger & Needleman, 2003; Canfield, Henderson, et al., 2003; Kordas et al., 2006; Lanphear et al., 2005; Rothenberg & Rothenberg, 2005; Tellez-Rojo et al., 2006). Among these studies were two analyses of the pooled cohort data, both of which demonstrated a supralinear relationship between blood Pb level and IQ with a steeper negative slope observed at blood Pb levels <10 µg/dL (Lanphear et al., 2005; Rothenberg & Rothenberg, 2005). Consistent with epidemiologic findings, toxicological studies also observed nonlinear Pb exposure-response relationships for outcomes such as behavioral responses, neuronal activation, and dopamine release.

Another area of focus included the comparison of various lifestages of Pb exposure in terms of risk of neurodevelopmental deficits. Toxicological studies demonstrated that in utero with or without early postnatal exposure to Pb was the most sensitive window for Pb-dependent neurological effects. Epidemiologic studies observed neurocognitive deficits in association with prenatal, peak childhood, cumulative childhood, and concurrent blood Pb levels. Among studies of children that examined multiple lifestages of exposure, several found that concurrent blood Pb was associated with the largest decrement in IQ (Baghurst et al., 1992; Canfield, Henderson, et al., 2003; A. Chen et al., 2005; Lanphear et al., 2005), with some studies finding that the magnitude of association increased with age (Dietrich, Berger, Succop, et al., 1993; Factor-Litvak et al., 1999; Ris et al., 2004; G. A. Wasserman et al., 1994). Several studies of children compared effect estimates for Pb levels measured in blood, deciduous tooth, or tibial bone and found that compared with blood Pb levels, tooth or bone Pb levels were associated with an equal or larger magnitude of neurodevelopmental deficits (Bellinger et al., 1991; Greene & Ernhart, 1993; Needleman et al., 1979; G. A. Wasserman et al., 2003). These findings pointed to an effect of cumulative childhood exposure. A common limitation of epidemiologic studies of children was the high correlation among Pb exposure metrics at different ages, making it difficult to distinguish among effects of Pb exposure at different ages (Lanphear et al., 2005) and to ascertain which developmental periods of Pb exposure were associated with the greatest risk of neurodevelopmental decrements. The issue of persistence of the neurological effects of Pb exposure also was considered, with some evidence suggesting that the associations of blood Pb levels with neurodevelopmental outcomes persisted into adolescence and young adulthood in the absence of marked reductions in blood Pb level (Needleman et al., 1990; Ris et al., 2004; Tong et al., 1996).

In epidemiologic studies of adults, neurological effects (e.g., impaired memory, attention, reaction time, visuomotor tasks and reasoning, alterations in visual or brainstem evoked potentials, postural sway, and nerve conduction) were mostly clearly indicated in occupationally-exposed workers in association with blood Pb levels in the range of 14 to 40 μg/dL (Baker et al., 1979; Cantarow & Trumper, 1944). Studies of environmentally-exposed adults produced mixed findings; however, bone Pb levels (Weisskopf et al., 2004; R. O. Wright et al., 2003) were associated with decrements in neurocognitive function more so than were blood Pb levels (E. F. Krieg, Jr et al., 2005; Nordberg et al., 2000). These findings suggested that cumulative Pb exposures, including higher past exposures, may be important contributors to neurological effects in adults.In addition to cognitive function, blood and bone Pb levels also were associated with greater self-reported anxiety and depression scores among environmentally-exposed adults (Rhodes et al., 2003). These findings in adults were strongly supported by parallel findings in female and male rodents for Pb-induced depression and emotional changes, respectively. Studies of adults also reported associations of blood Pb with risk of amyotrophic lateral sclerosis (ALS) and essential tremor, although the body of literature was smaller and evidence was less consistent than that for cognitive function. Whereas toxicological studies demonstrated Pb-induced neurodegeneration and formation of neurofibrilary tangles commonly associated with Alzheimer's disease pathophysiology, blood Pb level generally was not associated with Alzheimer's disease in adults.

As discussed throughout this section, recent epidemiologic and toxicological studies continue to demonstrate associations between exposure to or biomarkers of Pb and neurological effects and expand upon the previous body of evidence by demonstrating similar effects (e.g., cognitive function, impairments in behavior) at lower blood Pb levels (1-5 μg/dL). Whereas previous evidence was inconsistent, new studies in children report positive associations between blood Pb levels and attention deficit hyperactivity disorder (ADHD). New toxicological studies expand evidence for prenatal and postnatal Pb exposure effects on learning, memory, and attention and provide insight into the contribution of stress to this paradigm. New or expanded areas of toxicological research related to Pb exposure include mood disorders, neurofibrilary tangle formation, and adult dementia after early life Pb exposures. Historically important areas of toxicological research are further expanded with recent publications of Pb-dependent effects on neurotransmitters, synapses, glia, neurite outgrowth, the blood brain barrier, and oxidative stress. The data detailed in the subsequent sections continue to enhance the understanding of neurological and neurobehavioral outcomes associated with Pb exposure.

5.3.2. Neurocognitive Function and Learning

5.3.2.1. Epidemiologic Studies of Cognitive Function in Children

Full-scale IQ in Children

Several longitudinal cohort studies were initiated in the 1980s in order to address limitations of cross-sectional studies, including establishing a temporal association between exposure and outcome, examining persistence of neurocognitive deficits to older ages, and comparing risk estimates among blood Pb levels at different lifestages. Moreover, cooperation among investigators to adopt similar assessment protocols facilitated pooled and meta-analyses and comparison of results across populations that differed in blood Pb levels, race/ethnicity, and SES. Individual cohort studies in diverse populations were consistent in demonstrating that blood Pb levels in the range of 5 to 10 µg/dL (Bellinger et al., 1987; Bellinger & Needleman, 2003; Bellinger et al., 1991; Canfield, Henderson, et al., 2003; J. Schwartz, 1994; Tellez-Rojo et al., 2006), were associated with losses in full-scale IQ (FSIQ) pointsin children. These findings were substantiated in a pooled analysis of seven prospective studies (Boston, MA; Cincinnati, OH; Rochester, NY; Cleveland, OH; Mexico City, Mexico; Port Pirie, Australia; and Kosovo, Yugoslavia) by Lanphear et al. (2005) as well as multiple meta-analyses that included both cross-sectional and prospective studies (Needleman & Gatsonis, 1990; Pocock et al., 1994; J. Schwartz, 1994) (Figure 5-2 and Table 5-3).

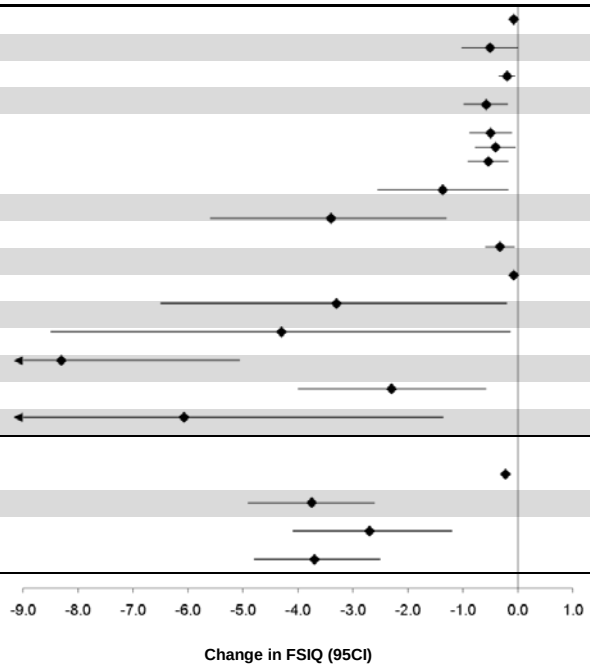
The analysis pooling data from seven prospective studies included 1333 children with mean (5th-95th percentile) blood Pb levels of 12.4 µg/dL (4.1-34.8 µg/dL) (Lanphear et al., 2005). In multivariate models that adjusted for study site, maternal IQ, Home Observation for the Measurement of Environment (HOME) inventory, birth weight, and maternal education, IQ measured at schoolage (mean 6.9 years) was inversely associated with concurrent, peak, average lifetime, and early childhood blood Pb levels, with the largest decrement in IQ estimated for concurrent blood Pb levels (-0.23 points [95% CI: -0.32, -0.14] at a blood Pb level of 1 µg/dL).

In Lanphear et al. (2005), various models were investigated to characterize the shape of the blood Pb dose-response relationship. Consistent with findings from individual studies, Lanphear et al. (2005) found that a log-linear model best fit the data, with a greater decrease in IQ estimated for an increase in concurrent blood Pb from <1-10 µg/dL (6.2 points [95% CI: 3.8, 8.6]) than an increase from 10 to 20 µg/dL (1.9 points [95% CI: 1.2, 2.6]). Sensitivity analyses, in which one study was successively excluded, revealed that no single study was responsible for driving the results. Although HOME score was not available in the Rochester study, exclusion of that cohort's data resulted in a less negative effect estimate. However, the change was less than 3%.

Studies published since the 2006 Pb AQCD continue to demonstrate associations between increasing blood Pb level and decrements in FSIQ (Figure 5-2 and Table 5-3). Whereas most studies demonstrated decrements in FSIQ in association with blood Pb levels ranging from 5 to10 µg/dL, Kim et al. (2009) was particularly noteworthy for demonstrating an association in a population with lower blood Pb levels (mean 1.73 µg/dL, range 0.42-4.91 µg/dL). Children ages 8 to 11 years of age in Korea were tested using the Korean Educational Development Institute-WISC, which assesses vocabulary, arithmetic, picture arrangement, and block design. In a linear regression analysis adjusted for age, sex, maternal and paternal education, yearly income, prenatal smoking, postnatal environmental tobacco smoke exposure, birth weight, and maternal age at birth, a 1 µg/dL increase in blood Pb level was associated with a -1.03 point decrease (95% CI: -1.71, -0.36) in FSIQ. Increasing blood Pb levels also was associated in performance and verbal IQ (PIQ and VIQ, respectively). Although several important confounders were considered, there was no direct assessment of the home environment and the primary caregiver's IQ in this study which are notable limitations.

Kim et al. (2009) also examined effect modification of the blood Pb-FSIQ relationship by blood manganese (Mn) levels. The mean (range) blood Mn level was 14.3 µg/dL (5.3-29.02), respectively. Blood Pb and Mn levels were not correlated ($r = -0.03$, $p = 0.64$). To examine effect modification, children were divided into two groups: blood Mn level above and below the median (14 µg/dL). Multivariate linear regression models predicting FSIQ, VIQ, and PIQ used concurrently measured blood Pb level as the predictor variable in the low and high Mn groups. The associations for blood Pb level with FSIQ and VIQ were larger in magnitude for children in the high Mn group (e.g., -5.3 FSIQ points [95% CI: -10.1, -5.3] per 1 µg/dL increase in blood Pb level), compared with children in the low Mn group (e.g., -4.0 FSIQ points [95% CI: -9.9, 1.80] per 1 µg/dL increase in blood Pb level) (Figure 5-3).

Reference	Exposure Period	Mean (SD) Blood Pb (µg/dL)	FSIQ Age (Years)
Kim et al. (2009)	Concurrent	1.73 (0.80)	8-11
Walkowiak et al. (1998)	Concurrent	4.7 (2.3)	6
Chiodo et al. (2004)	Concurrent	5.4 (3.3)	7.5
Bellinger et al. (1992)	Early childhood	6.5 (4.9)	10
Min et al. (2009)	Concurrent	7.0	4
			9
			11
Canfield et al. (2003)	Cumulative	7.4 (4.3)	3 or 5
Schnaas et al. (2006)	Prenatal	7.8	6-10
Dietrich et al. (1993)	Concurrent	NR	6.5
Ris et al. (2004)	Early childhood	NR	15-17
Baghurst et al. (1992)	Early childhood	21.7 (25^{th}-50^{th})	7-8
Tong et al. (1996)	Cumulative	17.8 (5.8)	11-3
Factor-Litvak et al. (1999)	Cumulative	30 and 8[a]	7
Wasserman et al. (2003)	Cumulative	31.6 and 6.3[a]	10-12
Surkan et al. (2007)	Concurrent	5-10 vs. 1-2[b]	6-10
Pooled/Meta-Analyses			
Lanphear et al. (2005)	Concurrent	6.9 (1.2)	4.8-10
Schwartz et al. (1994)	Early childhood	6.5-23	School age
Pocock et al. (1994)	Early childhood	6.8-21.2	5-14
	Concurrent	7.4-23.7	5-14



Note: In general, studies are presented in ascending order of mean blood Pb level, followed by a study analyzing blood Pb level as a categorical variable and then by pooled/meta-analyses. Effect estimates are standardized to a 1 µg/dL increase in blood Pb. The various tests used to measure IQ are scored on a similar scale (approximately 40-160). [a]Effect estimate represents the loss in FSIQ points in children with blood Pb levels 5-10 µg/dL, with children with blood Pb levels 1-2 µg/dL serving as the reference group. [b]These values represent the mean blood Pb levels in the two groups from different cities.

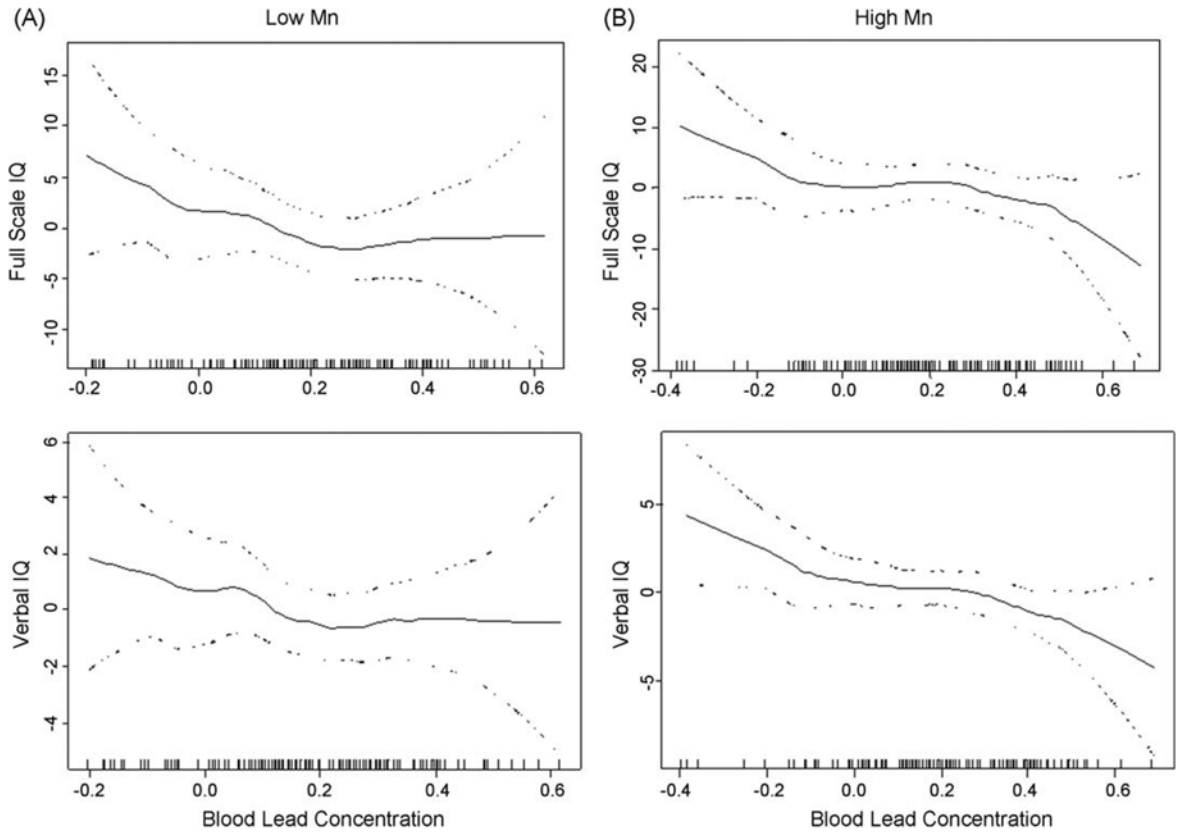
Figure 5-2. Associations of blood Pb levels with full-scale IQ (FSIQ) among children.

Table 5-3. Additional characteristics and quantitative results for studies presented in Figure 5-2

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	FSIQ Assessment	Effect Estimate (95% CI)[a]
Kim et al. (2009)	279 children in Seoul, Seongnam, Ulsan, and Yeoncheon, Korea, ages 8-11 yr in April-December 2007	Concurrent mean (SD): 1.73 (0.80)	Log linear regression model adjusted for age, sex, maternal education, paternal education, yearly income, maternal smoking during pregnancy, indirect smoking after birth, birth weight, maternal age	KEDI-WISC at ages 8-11 yr	-1.03 (-1.71, -0.36)
Walkowiak et al. (1998)	384 children in East Germany, age 6 yr in 1994	Concurrent (age 6 yr) mean (SD): 4.7 (2.3)	Log linear regression model adjusted for city, visual acuity, contrast sensitivity, parental education, sex, breastfeeding, height, nationality	WISC verbal and block design summed index	-0.51 (-1.03, 0.01)
Chiodo et al. (2004)	246 African-American children Detroit, MI	Concurrent (age 7.5 yr) mean (SD): 5.4 (3.3)	Regression model adjusted for SES, education, number of children <18 yr, HOME score, maternal vocabulary test score, sex, parity, family environment scale	WISC-III at age 7.5 yr	-0.20 (-0.35, -0.05)[b]
Bellinger et al. (1992)	148 children in the Boston, MA area followed from birth (1979-1981) to age 15-17 yr	Early childhood (age 2 yr) mean (SD): 6.5 (4.9)	Linear regression model adjusted for HOME score (age 10 and 5), child stress, race, maternal IQ, SES, sex, birth order, marital status	WISC-R at age 10 yr	-0.58 (-0.99, -0.18)
Min et al. (2009)	267 primarily African-American children in the Cleveland, OH area followed from birth (1994-1996) to age 11 yr. Children were exposed prenatally to multiple drugs.	Concurrent mean (range): 7.0 (1.3-23.8)	Linear regression model adjusted for HOME score, cargiver's vocabulary test, sex, parity, maternal marital status, head circumference at birth	WISC-R at age 4 yr WISC-R at age 9 yr WISC-R at age 11 yr	-0.50 (-0.89, -0.11) -0.41 (-0.78, -0.04) -0.54 (-0.91, -0.17)
Canfield et al. (2003)	172 children born 1994-1995 in Rochester, NY followed from infancy to age 3-5 yr	Lifetime avg (3 or 5 yr). mean (SD): 7.4 (4.3)	Mixed effects models adjusted for sex, maternal race, parental smoking, child iron status, maternal income, maternal IQ, HOME score	Stanford-Binet at age 3 or 5 yr.	-1.37 (-2.56, -0.17)
Schnaas et al. (2006)	175 children in Mexico City, Mexico followed from birth (1987-1992) followed until age 10-15 yr.	Prenatal (3rd trimester) geometric mean (5-95th): 7.8 (2.5-24.5)	Linear mixed effects regression model adjusted for sex, SES, maternal IQ, HOME score, birth weight, postnatal blood Pb, random slope for subject	WISC-R at ages 6-10 yr	-3.4 (-5.6, -1.3)
Dietrich et al. (1993)	253 children in Cincinnati, OH followed from birth (1979-1985) to age 20-23 yr	Concurrent	Linear regression model	WISC-R at age 6.5 yr	-0.33 (-0.60, -0.06)
Ris et al. (2004)	195 children in Cincinnati, OH followed from birth (1979-1985) to age 20-23 yr	Early childhood (age 6.5 yr)	Linear regression model adjusted for maternal IQ, sex, and average total HOME score	WISC-III indices at age 15-17 yr factored into Learning/IQ	-0.08 (-0.16, 0.004)
Baghurst et al. (1992)	494 children in Port Pirie, Australia followed from birth (1979-1982) to age 11-13 yr.	Early childhood (avg of age 0-3 yr) 25-50th: 17.4, 50-75th: 21.7	Log linear regression model adjusted for sex, birth weight, birth order, feeding method, breastfeeding duration, parental education, maternal age, parental smoking, SES, quality of home environment, maternal IQ, parents living together	WISC-R at age 7-8 yr	-3.3 (-6.5, -0.2)

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	FSIQ Assessment	Effect Estimate (95% CI)[a]
Tong et al. (1996)	375 children in Port Pirie, Australia followed from birth (1979-1982) to age 11-13 yr.	Cumulative 0-7 yr mean (SD): 17.8 (5.8)	Regression model adjusted for sex, age, schoolgrade, parental occupational prestige, HOME score, maternal IQ, family functioning score, parental smoking, marital status, parental education, maternal age, birth weight, birth order, feeding method, breastfeeding duration, family size, life events, prolonged absences from school	WISC-R at age 11-13 yr	-4.3 (-8.5, -0.14)
Factor-Litvak et al. (1999)	577 children in Kosovo, Yugoslavia followed from birth (1985-1986) to age 10-12 yr	Cumulative (4-7 yr) mean[c]: 30 (K. Mitrovica, 8 (Pristina)	Log linear regression model adjusted for HOME score, ethnic group, maternal age, birth weight, maternal Raven's score, maternal education, birth order, sibship size, sex	WISC-R at age 7 yr	-8.3 (-11.4, -5.05)
Wasserman et al. (2003)	290 children in Kosovo, Yugoslavia followed from birth (1985-1986) to age 10-12 yr	Lifetime avg mean: 31.6 (K. Mitrovica, 6.3 (Pristina)	Generalized estimating equations with log-transformed blood Pb adjusted for age, sex, sibship size, birth weight, language spoken in home, HOME score, maternal age, maternal education, maternal Raven score	WISC-III at ages 10-12 yr	-2.3 (-4.0, -0.58)
Surkan et al. (2007)	389 children ages 6-10 yr. from Boston, MA and Farmington, ME	Concurrent mean (range): 2.2 (1-10)	Linear regression model adjusted for caregiver IQ, child age, SES, race, birth weight.	WISC-III at ages 6-10 yr	-6.07 (-10.7, -1.36), blood Pb = 5-10 vs. 1-2
Pooled/Meta-analyses					
Lanphear et al. (2005)	1333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts	Concurrent mean (SD): 6.9 (1.2)	Log linear regression model adjusted for HOME score, birth weight, maternal IQ, maternal education	FSIQ measured at ages 4.8-10 yr	-0.23 (-0.32, -0.14)
Schwartz et al. (1994)	Meta-analysis of 7 studies with sample sizes 75-579 children	Early childhood (2-3 yr) range in study means: 6.5-23	Meta-analysis of combining effect estimates from individual studies	FSIQ measured at schoolage	-3.75 (-4.91, -2.60)
Pocock et al. (1994)	Meta-analysis of 5 prospective (over 1,100 children and 14 cross-sectional studies (3,499 children)	Prospective studies mean at age 2 yr: 6.8-21.2 Cross-sectional means: 7.4-23.7	Meta-analysis of combining effect estimates from individual studies	FSIQ measured at ages 5-14 Prospective studies Cross-sectional studies	-2.7 (-4.1, -1.2) -3.7 (-4.8, -2.5)

[a]Effect estimates are standardized to a 1 µg/dL increase in blood Pb level.
[b]95% CI was constructed using a standard error that was estimated for a p-value of 0.01. Authors specified a p-value of <0.01.
[c]Quantitative data not presented. Means estimated from a figure.



Source: Used with permission from Elsevier Science, Kim et al. (2009).

Figure 5-3. Effect modification of association between concurrent blood Pb level and IQ by blood Mn level.High and low Mn refers to levels above and below the median of 14 µg/dL, respectively.

Min and colleagues (2009) examined the relationship between Pb exposure assessed at age 4 years and children's IQ and academic achievement at 4, 9, and 11 years of age in a sample of 278 urban children originally enrolled in a prospective study on the effects of prenatal poly-drug exposure (determined by assay of infant meconium or urine, maternal urine, or maternal self-report). The study population was primarily African-American (86%) and low SES (98%); 39% of mothers had not finished high school, and 14% were married at the time of enrollment. The mean blood Pb level at age 4 years was 7.0 µg/dL (SD 4.1, range 1.3-23.8); 36% had blood Pb levels <5 µg/dL, and 19% had levels >10 µg/dL. The researchers utilized restricted cubic spline functions for blood Pb level to test for a nonlinear relationship between blood Pb levels and FSIQ. Although the cubic spline term did not attain statistical significance, analyses suggested a steeper slope at lower Pb levels (up to 7 µg/dL) These findings were consistent with those from the pooled analysis (Lanphear et al., 2005) and other studies (Tellez-Rojo et al., 2006).

Also similar to previous studies with repeated assessments of cognitive function over time, Min et al. (2009) found that the association between concurrent blood Pb level and FSIQ persisted with

increasing age. The magnitude of the inverse association between blood Pb level and FSIQ was consistent at ages 4, 9 and 11 years. A 1 µg/dL increase in blood Pb level was associated with a loss in IQ points of 0.50, 0.41, and 0.54 at ages 4, 9, and 11 years, respectively (Figure 5-2 and Table 5-3). The findings of this study also indicated that specific cognitive domains may be more sensitive to Pb exposure at different stages of development. Non-verbal reasoning decrements assessed using the Wechsler Preschool and Primary Scales of Intelligence-Revised (WPPSI-R) Performance IQ (PIQ) and Wechsler Intelligence Scales (WISC)-IV Perceptual Reasoning Index were consistently associated with increasing blood Pb level even at younger ages while verbal decrements did not become apparent until assessments at 11 years of age. Lower reading scores were associated with increased Pb exposure at 9 and 11 years while math scores were not affected until age 11 years. An important consideration that may limit the generalizability of these findings is the high prevalence of prenatal exposure to cocaine (51% of subjects) and alcohol (77% of subjects). However, accounting for prenatal drug exposure did not attenuate or modify (i.e., no interaction effects) the negative associations between blood Pb level and cognitive outcomes.

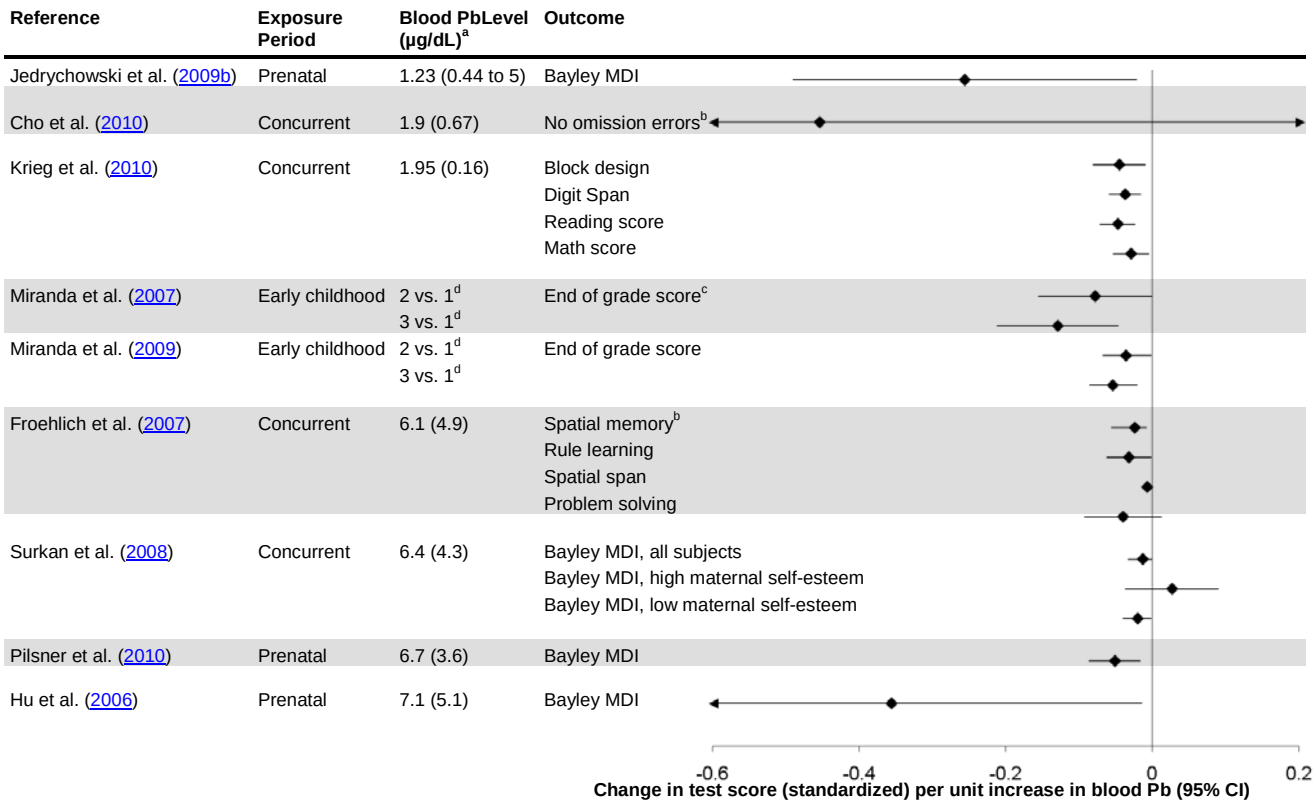
Surkan et al. (2007) used data originally collected for the New England Children's Amalgam Trial (NECAT), a study of 6 to 10 year old English-speaking children from urban Boston, Massachusetts and rural Farmington, Maine designed to assess the effect of amalgam dental fillings on children's neurodevelopment. At baseline (prior to placement of amalgam fillings), blood Pb levels were measured, and children were administered an extensive battery of neuropsychological tests including tests of memory, learning, visual-motor ability, reading, reaction time. In analyses that excluded 3 children with blood Pb level >10 µg/dL, children with blood Pb levels of 5 to 10 µg/dL had significantly lower WISC-III Full FSIQ scores (-6.07 points (95% CI: -10.7, -1.36]) compared with children who had levels of 1 to 2 µg/dL (referent group), adjusting for age, race/ethnicity (Black or Hispanic vs. non-Hispanic white), birth weight, SES, and primary caregiver IQ.

Specific Indices of Cognitive Function in Children

In addition to FSIQ, an index of global cognitive function, blood Pb levels also were associated with specific cognitive abilities, including attention, executive function, language, memory and learning, and visuospatial processing in previous studies of children and adolescents (Bellinger et al., 1991; Bellinger & Stiles, 1993; Canfield, Kreher, et al., 2003; Chiodo et al., 2004; Dietrich et al., 1991; Dietrich et al., 1992; Kordas et al., 2006; Lanphear et al., 2000; Needleman et al., 1979; Ris et al., 2004; Tellez-Rojo et al., 2006). Studies often find associations with several endpoints, and because several tests of neurocognitive function are interrelated, it is difficult to ascribe the effects of Pb exposure to a specific domain of neurocognitive function. For example, in U.S. representative analysis of NHANES III (1988-1994) data, which included 4853 children ages 6-16 years with a geometric mean blood Pb level of 1.9 µg/dL, Lanphear et al. (2000) found that a 1 µg/dL increase in blood Pb levels was associated with

decreases in arithmetic (-0.70 points [95% CI: -1.0, -0.37]), reading (-0.99 points [95% CI: -1.4, -0.62]), block design (-0.10 points [95% CI: -0.18, -0.02]), and digit span (-0.05 [95% CI: -0.09, -0.01]) subtests. Finding that blood Pb levels are associated with a spectrum of neurocognitive indices provides biological plausibility for associations observed between blood Pb levels and IQ. Furthermore, these tests of attention, learning, and memory in humans have parallel tests in animals, and compared with evidence for IQ, evidence for these specific tests may improve understanding of the coherence between findings in humans and animals (Rice, 1996).

Studies published since the 2006 Pb AQCD continue to observe associations between increasing blood Pb level and decrements in these specific indices of cognitive function. Compared with studies of IQ, studies of specific cognitive indices consistently find associations at lower blood Pb levels (population means: 1.2 to 7 µg/dL and quantities of blood Pb levels as low as 2 µg/dL) (Figure 5-4 and Table 5-4). Recent studies of cognitive function also expanded on previous evidence by providing information on effect modification by genetic, nutritional, and caregiving quality (E. F. Krieg, Jr. et al., 2010; Pilsner et al., 2010; Solon et al., 2008; Surkan et al., 2008).



Note: Test scores were standardized to their standard deviation to facilitate comparisons among tests with different scales. Studies are presented in ascending order of blood Pb level. Effect estimates are standardized to a 1 µg/dL increase in blood Pb. MDI = Mental Developmental Index. [a]Blood Pb level refers to the study mean (SD), unless otherwise specified. [b]Standard error was estimated from p-value. Effect estimate represents association of blood Pb level with number of correct answers on test. [c]Standard error was estimated from p-value. [d]Effect estimate compares test scores of children in different categories of blood Pb concentration.

Figure 5-4. Associations of blood Pb levels with standardized scores for specific indices of cognitive function in children.

Table 5-4. Additional characteristics and quantitative results for studies presented in Figure 5-4

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Cognitive Index	Effect Estimate (95% CI)[a]
Jedrychowski et al. (2009b)	444 children born 2001-2004 followed prenatally to age 36 mo Krakow, Poland	Prenatal (cord blood) geometric mean (range): 1.29 (0.44-5)	Linear regression model adjusted for maternal education, birth order, prenatal ETS, sex	Bayley MDI assessed at age 36 mo	-0.26 (-0.49, -0.02)
Cho et al. (2010)	667 children ages 8-11 yr in 2008 Five Korean cities	Concurrent mean (range): 1.9 (0.53-6.16)	Log linear regression model adjusted for age, sex, parental education, maternal IQ, child IQ, birth weight, urinary cotinine	No omission errors[b] No comission errors[b] Word reading score[c] using KEDI-WISC at ages 8-11 yr	-0.45 (-3.02, 2.06) -1.80 (-4.33, 0.26) -1.37 (-3.77, 1.03)

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Cognitive Index	Effect Estimate (95% CI)[a]
Krieg et al. (2010)	842 children ages 12-16 yr U.S. NHANES III (1991-1994)	Concurrent mean (95% CI): 1.95 (1.63, 2.27)	Log linear regression model adjusted for sex, caregiver education, family income, race-ethnicity, test language	Block design (WISC-R) Digit span (WISC-R) Reading score (WRAT-R) Math score (WRAT-R) assessed at ages 12-16 yr	-0.05 (-0.08, -0.009) -0.04 (-0.06, -0.02) -0.05 (-0.07, -0.02) -0.03 (-0.05, -0.004)
Miranda et al. (2007)	8603 4th grade children, 2000-2004 7 counties in central North Carolina	Early childhood (ages 0-5 yr.) range: 1-≥10	Linear regression model adjusted for sex, race, free/reduced–price lunch, parental education, daily computer use, charter school, age of blood Pb screening, school system	4th grade end-of-grade score[c]	-0.08 (-0.16, 0), blood Pb 2 µg/dL vs. 1 µg/dL[d] -0.13 (-0.21, -0.05), blood Pb 3 µg/dL vs. 1 µg/dL[d]
Miranda et al. (2009)	57,678 4th grade children, 2001-2005 All 100 North Carolina counties	Early childhood (ages 9-36 mos.) mean (range): 4.8 (1-16)	Linear regression model adjusted for race, sex, parental education, free/reduced-price lunch, charter school, school system	4th grade end-of-grade score	-0.04 (-0.07, -0.001), blood Pb 2 µg/dL vs. 1 µg/dL[d] -0.05 (-0.09, -0.02) , blood Pb 3 µg/dL vs. 1 µg/dL[d]
Froelich et al. (2007)	174 children age 5 yr Rochester, NY	Concurrent mean (SD): 6.1 (4.9)	Linear regression model adjusted for income(spatial memory); NICU, sex (rule learning); HOME score, maternal IQ, race (spatial span); or maternal IQ, transferrin saturation (problem solving)	Spatial memory[p] Rule learning and reversal Spatial span Problem solving using CANTAB at age 5 yr	-0.02 (-0.06. -0.008) -0.03 (-0.06, -0.001) -0.007 (-0.01, 0) -0.04 (-0.09, 0.01)
Surkan et al. (2008)	309 children ages 12-36 mo during 1996-2001 or 2004-2005 Mexico City, Mexico	Concurrent mean (SD): 6.4 (4.3)	Linear mixed effects regression model adjusted for sex, maternal age, maternal IQ, maternal education, parity, alcohol consumption, smoking, cohort, maternal self-esteem	Bayley MDI, all subjects Bayley MDI, high maternal self-esteem Bayley MDI, low maternal self-esteem assessed at ages 12-36 mo	-0.013 (-0.033, 0.0007) 0.027 (-0.037, 0.09) -0.02 (-0.04, -0.001)
Pilsner et al. (2010)	255 children age 24 mo born 1994-1995 Mexico City, Mexico	Prenatal (cord blood) mean (SD): 6.7 (3.6)	Linear regression model adjusted for maternal age, maternal IQ, marital status, parity, gestational age, inadequate folate intake, MTHFR genotype	Bayley MDI assessed at age 24 mo	-0.051 (-0.087, -0.016)
Hu et al. (2006)	146 children born 1997-1999 followed prenatally to age 24 mo Mexico City, Mexico	Prenatal (maternal blood Pb) in 1st trimester mean (range): 7.1 (1.5-43.6)	Log linear regression model adjusted for concurrent blood Pb, sex, maternal age, current weight, height-for-age Z score, maternal IQ	Bayley MDI assessed at age 24 mo	-0.36 (-0.70, -0.01)
Studies not included in figure due to lack of sufficient data to calculate z-scores					
Surkan et al. (2007)	534 children ages 6-10 yr Boston, MA and Farmington, ME	Concurrent mean (SD): 2.2 (1.6)	Analysis of covariance adjusted for child IQ, caregiver IQ, age, SES, race, birth weight	Reading score Math score Assessed using WIAT at age 6-10 yr	-5.20 (-9.45, -0.95) -4.02 (-7.6, -0.43), blood Pb level 5-10 µg/dL vs. 1-2 µg/dL[d]
Chandramouli et al. (2009)	488 children born 1991-1992 followed from birth to age 7-8 yr Avon, U.K.	Early childhood (age 30 mos.) mean (SD): 4.22 (3.12)	Log linear regression model adjusted for sex, child IQ, maternal education, home ownership, maternal smoking, HOME score, paternal SES, family adversity index, parenting attitudes at 6 mos.	Standardized Assessment Test assessed at age 7-8 yr	-0.61 (-0.82, -0.46), continuous blood Pb -1.08 (-1.71, -0.69), blood Pb 2-5 µg/dL vs. 0-2 µg/dL[d] -0.49 (-0.79, -0.31), blood Pb 5-10 µg/dL vs. 0-2 µg/dL[d] -0.44 (-0.93, -0.21), blood Pb >10 µg/dL vs. 0-2 µg/dL[d]

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Cognitive Index	Effect Estimate (95% CI)[a]
Solon et al. (2008)	502 children ages 6-35 mo 377 children 3-5 yr, 2003-2004 Visayas, Philippines	Concurrent mean (range): 7.1	Two-stage linear regression model to account for determinants of blood Pb (sex, roof materials, water source, breastfed for ≥ 4 months) and cognitive function (HOME score, maternal education, maternal smoking, born premature, region of residence)	BSID-II (ages 6-35 mo) WPPSI-III (ages 3-5 yr)	-3.32 (-5.02, -1.60) -2.47 (-4.58, -0.35)

MDI = Mental Development Index, MTHFR = methylenetetrahydrofolate reductase, WIAT = Weschler Individual Achievement Test
[a]Effect estimates are transformed to a z-score and standardized to a 1 µg/dL increase in blood Pb level.
[b]95% CI was constructed using a standard error that was estimated for a p-value of 0.01. Authors specified a p-value of <0.01. Effect estimate represents association of blood Pb with correct answers on test.
[c]Standard error was estimated from p-value.
[d]Effect estimates compare test performance of children in higher blood Pb groups to children in lowest blood Pb group.

Krieg et al. (2010) examined an older subset of children (ages 12-16 years) from NHANES III (1988-1994) that were previously examined in Lanphear et al. (2000). Similar to Lanphear et al. (2000), Krieg et al. (2010) found associations of increasing concurrent blood Pb level with decrements in block design, digit span, reading score, and arithmetic score in these older children (Figure 5-4 and Table 5-4). In another study of Korean children (ages 8-11 years) with similar blood Pb levels (mean: 1.9 µg/dL [SD: 0.67]), Cho and colleagues (2010) found concurrent blood Pb levels to be associated with decreases in in tests of attention (errors in responding to targets); however associations were not statistically significant in models that adjusted for urinary cotinine levels (Figure 5-4 and Table 5-4). Although associations of blood Pb levels with word and color naming were negative, effect estimates were associated with wide 95% CIs.

Krieg et al. (2010) additionally provided additional information on effect modification by vitamin D receptor (VDR) variants. Although there were not differences in blood Pb levels among the various haplotypes of VDR, various polymorphisms and haplotypes modified the association between blood Pb level and a range of neurocognitive tests. The VDR regulates calcium absorption and metabolism, and effect modification by VDR variants is consistent with the well-established mode of action of Pb in mimicking calcium in shared transport and metabolic pathways. However, several inconsistencies were observed by Krieg et al. (2010) in that a particular variant was associated with a lower Pb-associated decrement in performance for some tests and a greater Pb-associated decrement in performance for other tests. For example, among children ages 12-16 years, the VDR rs2239185 CC genotype was associated with the largest blood Pb-associated decrease in digit span score and reading score. The slopes for digit span score (95% CI) per 1 µg/dL increase in blood Pb level were -1.5 (-2.2, -0.71) for the CC genotype and -0.26 (-0.99, 0.46) for the TT genotype. Conversely, the TT genotype was associated with the greatest Pb-associated decrease in arithmetic score. The slopes (95% CIs) for math score per 1 µg/dL increase in blood Pb level were -8.4 (-11.5, -5.2) for the TT genotype and -2.7 (-10.1, 4.6) for the CC genotype.

Effect modification by VDR rs731236 was more consistent across cognitive tests, with larger decrements in blood Pb-associated cognitive performance among children with the CC genotype.

Multiple studies in different Mexico City mother-child dyads recently reported on associations between blood Pb levels (e.g., maternal, cord blood, or child postnatal Pb levels) and mental development in children at age 24 or 36 months (H. Hu et al., 2006; Pilsner et al., 2010; Surkan et al., 2008). Hu et al. (2006) assessed the impact of timing of exposure prenatally among 146 mother-child dyads meeting the following criteria: born at 37 weeks or greater gestational age, at least one valid Pb measurement during pregnancy, complete information on maternal age and IQ, and child's blood Pb level at 24 months when the 24-month Bayley Mental Development Index (MDI) was ascertained. Comparing whole blood and plasma Pb levels collected at each of 3 trimesters, this group found that 1st trimester blood Pb (or plasma Pb) was the best predictor of subsequent 24-month Bayley Scale MDI scores. Another study excluding the first trimester demonstrated an inverse association between IQ assessed at age 6-10 years and third trimester maternal blood Pb level, but not with maternal blood Pb levels measured at other times during pregnancy or within child Pb blood levels averaged over 6 to 10 years (Schnaas et al., 2006).

Surkan et al. (2008) found negative associations (statistically nonsignificant) of concurrent blood Pb levels with Bayley MDI and Psychomotor Development Index overall in a population of 379 Mexico City children between ages 12 and 36 months. However, when data were stratified by maternal self-esteem, negative associations were observed among children with mothers with in the lowest three quartiles of self-esteem but not among children with mothers in the highest quartile of self-esteem (Figure 5-4 and Table 5-4). These findings indicate that higher maternal psychosocial functioning (e.g., stress, anxiety, depression, self-esteem) may contribute to better caregiving, which in turn may improve neuropsychological functioning of the child. These limited data in humans are well-supported by findings from animal studies that have shown that environmental enrichment can reverse the effects of early stress experiences on reactions such as depressed behavior, HPA activation, and immunosuppression (Francis et al., 2002; Laviola et al., 2008; Laviola et al., 2004; Moreley-Fletcher et al., 2003). With specific regards to Pb exposure, Schneider et al. (2001) and Guilarte et al. (2003) demonstrated that the social isolation or enrichment can exacerbate or protect against, respectively, the neurological effects from Pb exposure. It is worth mentioning in this context that the potential programming effects of stress on childhood health outcomes may occur at an even more fundamental level, i.e., through epigenetic programming (Dolinoy & Jirtle, 2008). Pb exposure of animals and blood Pb levels in humans have been associated with altered DNA methylation patterns which in turn, may be associated with altered gene expression patterns (Section 5.3.6.11 and Section 5.10.4).

In a recent study in Mexico City, Mexico, investigators found increasing cord blood Pb levels to be associated with lower MDI scores among children at age 24 months (-0.051 points [95% CI: -0.087, -0.016] in standardized score per 1 µg/dL increase in blood Pb level) (Pilsner et al., 2010). Investigators additionally examined effect modification by variants in the methylenetetrahydrofolate reductase

(MTHFR) gene. The MTHFR enzyme is involved in folate metabolism, specifically, catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methylenehydrofolate, which, in turn, is involved in homocysteine methylation to the amino acid methionine. The transfer of methyl groups that results from folate metabolism is important for biological processes including Phase II detoxification reactions and epigenetic regulation of gene expression. The MTHFR gene has common functional variants, including the C677T SNP, which produces an enzyme with lower metabolic activity and is associated with lower serum folate levels (Kordas et al., 2009). Although Pilsner et al. (2010) found that both cord blood Pb levels and the MTHFR 677T allele were associated with lower child MDI score at age 24 months, they did not find a statistically significant interaction between blood Pb level and the MTHFR 677T allele. Results from stratified analyses were not reported, thus differences in the magnitude of association between genotypes could not be compared.

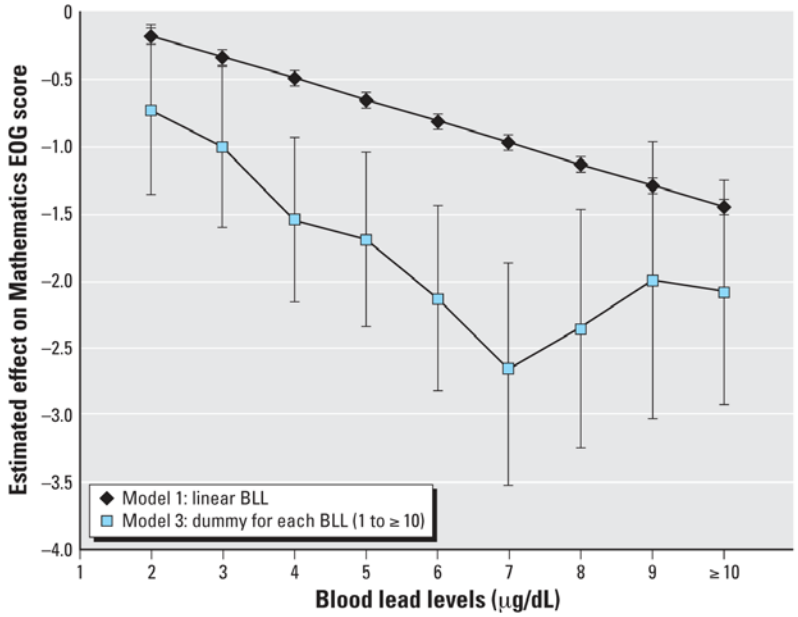
Instead of analyzing MTHFR genetic variants to represent folate metabolism, Solon et al. (2008) measured red blood cell folate levels among children in the Phillippines. Not only did investigators find an association between increasing blood Pb level and lower cognitive test performance (Table 5-4), but they found effect modification by red blood cell folate levels. Among children with folate levels less than or equal to 230 µg/mL, blood Pb level had a negative marginal effect on MDI (-0.80 to -2.44 points), whereas among children with higher folate levels, blood Pb level did not have a negative marginal impact. Thus, in contrast with those from Pilsner et al. (2010), findings from Solon et al. (2008) indicate that children with folate deficiencies may be at increased susceptibility to Pb effects on cognitive function.

Academic Performance in Children

Although the preponderance of evidence for Pb-associated neurodevelopmental deficits is for IQ and specific indices of cognitive function, academic achievement and school performance are corollaries to aptitude that may be more objective measure of one's abilities and skills and have important implications for success later in life. Aptitude tests are used to predict future performance of an individual on a task or test. Achievement tests and school performance, in comparison, assess an individual's actual knowledge in subject areas the individual has studied and measure the acquired knowledge of that subject. Studies reviewed in the 2006 Pb AQCD consistently demonstrated associations of Pb biomarkers with measures of academic achievement and performance including scores on math or vocabulary tests, class rank, teacher's assessment of academic functioning, and high school completion. Several studies found that blood or dentin Pb levels measured at an early age (ages 2-8 years) were associated with outcomes at older ages (ages 8-18 years), suggesting early exposure to Pb may have persistent effects (Bellinger et al., 1992; Leviton et al., 1993; Needleman et al., 1990). Results from the longitudinal study by Bellinger et al. (1992) was particularly noteworthy for examining associations of blood Pb at several ages with scores on the Kaufman Test of Educational Achievement at age 10 years. Only blood Pb level at age 2 years showed

a statistically significant association with lower predicted academic achievement. Additionally, the association was robust to adjustment for IQ, indicating that blood Pb levels may be associated with reduced performance on academic tasks not reflected in indices of IQ. Several studies also found associations between concurrent blood Pb levels and academic achievement (Al-Saleh et al., 2001; Kordas et al., 2006; Lanphear et al., 2000; C.-L. Wang et al., 2002). Among recent studies, academic performance was examined less frequently; however, findings are consistent with the extant body of evidence.

Miranda et al. (2007) linked blood Pb surveillance data collected between 0 and 5 years with end-of-grade (EOG) testing data at 4th grade for 7 of the largest counties in North Carolina. Approximately 22-30% of children in these counties were screened for Pb poisoning, and the total sample size in the analysis was approximately 8,600 children for both math and reading achievement tests. For both reading and math, achievement test scores were inversely associated with early childhood blood Pb screening data (Figures 5-4 and 5-5 and Table 5-4).

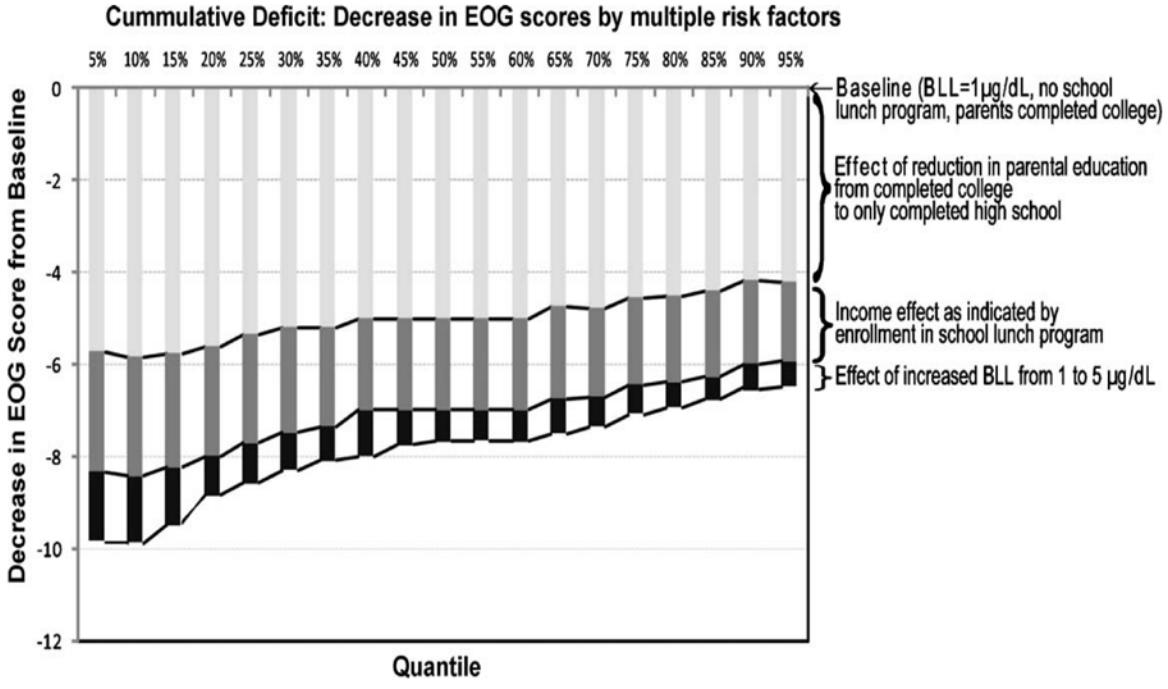


Source: Used with permission from Elsevier Science, Miranda et al. (2007).

Figure 5-5. Comparing model results for 4th-grade EOG mathematics scores.Based on a referent individual who was screened at 2 years of age and is a white female, living in Wake County, NC, parents with a high school education, not enrolled in the school lunch program, and who does not use a computer every day. Baseline score is 262.6.

Similar results were obtained in a subsequent study expanded to the entire state of North Carolina (Miranda et al., 2009) (Figure 5-4 and Table 5-4). Investigators additionally used quantile regression to

estimate effects for conditional percentiles of EOG (e.g., what is the 10th percentile of EOG test scores conditioned on early childhood blood Pb levels) rather than conditional means. Compared with linear regression, quantile regression is more robust in response to outliers and predicts outcomes at the top and bottom tails of the distribution of the outcome rather than at the mean. The distributions in EOG scores for children with blood Pb levels greater than or equal to 10 µg/dL were more spread out than those for children with lower blood Pb levels. With increasing blood Pb levels, the lower tail of the EOG distribution was stretched out more so than the middle or upper tail of the distribution. For example, in comparisons of children with blood Pb levels of 5 µg/dL versus children with blood Pb levels of 1 µg/dL, children in the 5th percentile of EOG have a greater decrease in EOG score compared with children in the 95th percentile of EOG (Figure 5-6). These findings indicate that children residing at the lowest performance regions of the EOG distribution may be differentially affected by blood Pb levels as compared with children in the middle or higher regions. Similarly, using quantile regression, Miranda et al. (2009) showed that, in addition to elevated blood Pb levels, cumulative social risk (lower parental education, being enrolled in a school lunch program) further enhanced the negative effects on academic achievement in these children.



Source: Used with permission from Elsevier Science, Miranda et al. (2009)

Figure 5-6. Reduction in EOG achievement test scores at each percentile of the test distribution.Note greater effect of Pb at low end of the distribution.

Similar to Miranda et al. (2009), Chandramouli et al. (2009) observed associations between early childhood blood Pb levels (age 30 months) and later academic performance (Standard Assessment Tests at age 7 years) among participants of the Avon Longitudinal Study of Parents and Children conducted in the U.K (Figure 5-4 and Table 5-4). While these aforementioned recent studies found negative associations for early childhood blood Pb levels, unlike the longitudinal assessment by Bellinger et al. (1992), they did not have available blood Pb measurements at other lifestages to compare associations with blood Pb levels at other lifestages. Therefore, results from these recent studies do not preclude associations with blood Pb levels at other lifestages. Among children (ages 6-10 years) participating in NECAT, increasing concurrent blood Pb level was associated with poorer performance on the Wechsler Individual Achievement Test, even when adjusted for IQ (Surkan et al., 2007). In analyses adjusted for child IQ, caregiver IQ, age, SES, race, and birth weight, children with concurrent blood Pb levels 5-10 µg/dL scored 5.2 (95% CI: 0.95, 9.45) points and 4.0 (95% CI: 0.43, 7.6) points lower on reading and math composite scores on the respectively, compared to children with levels of 1-2 µg/dL. Blood Pb levels were similarly associated with other tests of cognitive function (e.g., FSIQ, working memory, cognitive flexibility and a number of executive functioning domains [i.e., ability to formulate, test, and adapt hypotheses]). Children with blood Pb levels 3-4 µg/dL had lower scores compared with children with blood Pb levels 1-2 µg/dL; however, differences were not statistically significant.

Age-based Susceptibility to Lead-associated Neurodevelopmental Deficits

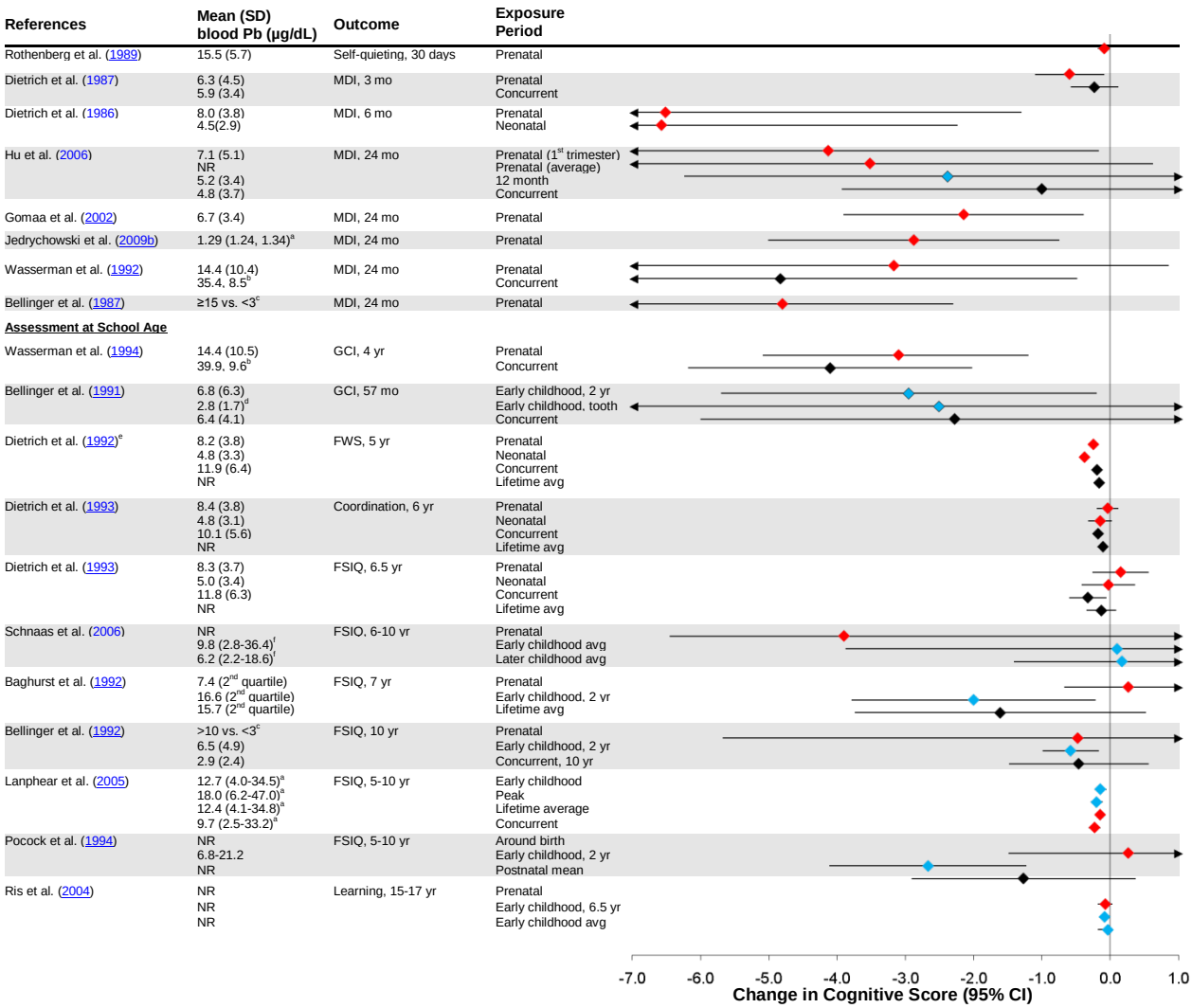
Plasticity is a consequence of environmental exposures during critical life periods affecting key physiological systems that orchestrate underlying developmental processes (Feinberg, 2007). Exposure to environmental toxins during prenatal and/or early postnatal development may alter the normal course of morphogenesis and maturation that occurs in utero and early in life, resulting in changes that affect structure or function of the central nervous system via altered neuronal growth and/or synaptogenesis/pruning structure (Landrigan et al., 1999; Rice & Barone, 2000). This hypothesis is well-supported by findings in animals that prenatal Pb exposure alters brain development via changes in synaptic architecture (Section 5.3.6.5) and neuronal outgrowth (Section 5.3.6.10) and leads to impairments in memory and learning (Section 5.3.2.2) and emotional and depressive changes postnatally (Section 5.3.3.4). Unlike other organ systems, the unidirectional nature of CNS development limits the capacity 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 may persist into adult life even in the absence of current exposure, magnifying the public health impact. Supporting evidence is provided by toxicological studies that find that Pb exposure during neonatal development but not in adulthood leads to neurodegenerative amyloid plaque formation in the brains of aged rodents and monkeys (Section 5.3.5.2).

With repeated assessment of children prenatally to later childhood and early adulthood, the prospective cohort studies have aimed to distinguish among the effects of blood Pb levels at different periods of development. In the collective body of evidence, neurocognitive decrements have been associated with prenatal, early childhood, childhood average, and concurrent blood Pb levels. In these studies, the identification of developmental periods when children are most sensitive to Pb-associated neurocognitive decrements has been complicated by the high degree of correlation in children's blood Pb levels over time and the confounding of age and peak blood Pb levels (Dietrich, Berger, & Succop, 1993; Lanphear et al., 2005; Needleman et al., 1990).

As described in detail in the 2006 Pb AQCD, several studies with varying lengths of follow-up demonstrated associations of prenatal blood Pb levels with neurodevelopmental deficits thoughout childhood and into early adulthood (U.S. EPA, 2006). The prenatal period may be susceptible lifestage of Pb exposure not only because of the nervous system developmental process occurring as described above but also because of factors that result in elevated Pb exposures. Substantial fetal Pb exposure may occur from mobilization of maternal skeletal Pb stores even related to remote exposures (Gulson et al., 2003; H. Hu & Hernandez-Avila, 2002). Pb can cross the placenta to affect the developing fetal nervous system (Rabinowitz, 1988). Maternal and umbilical cord blood Pb levels generally are highly correlated, indicating that a newborn infant's blood Pb levels reflects that of the mother (Schell et al., 2003).

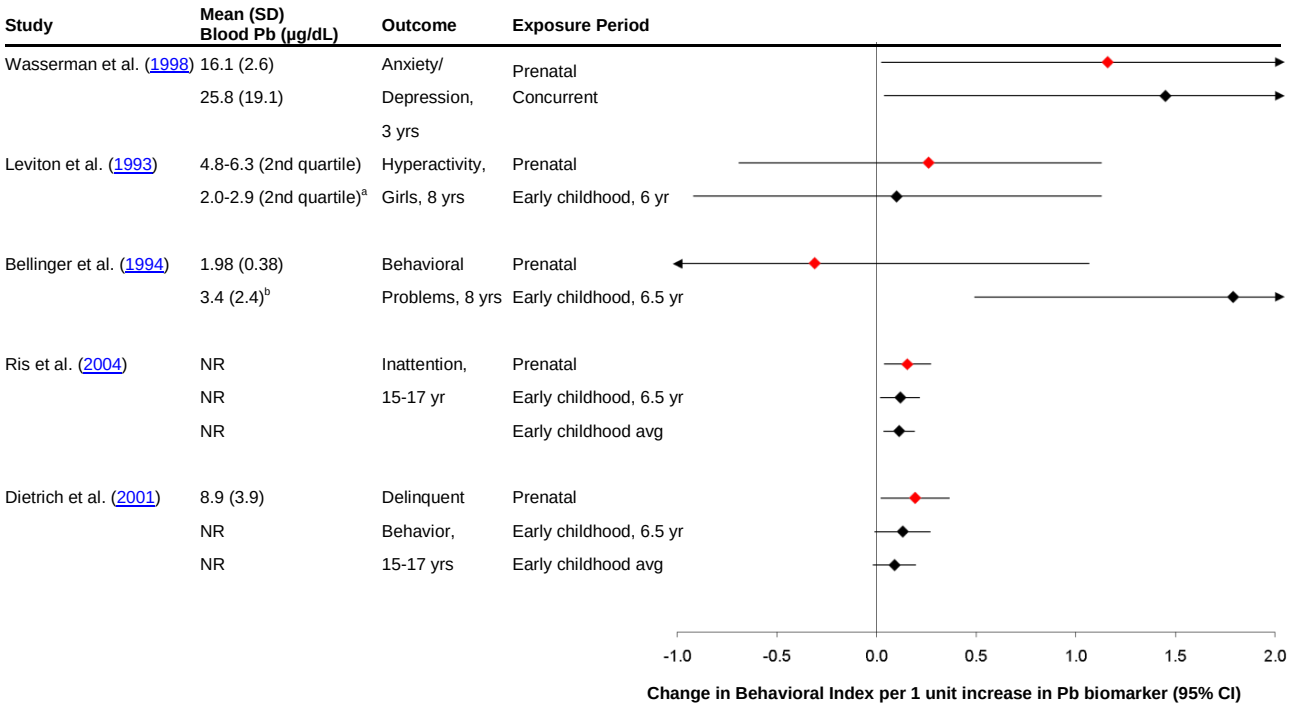
Associations with prenatal blood Pb levels were demonstrated most consistently for cognitive function and behavior assessed between infancy and age 3 years (Figures 5-7 and 5-8 and Table 5-5). Among studies examining associations of early-life blood Pb measures (maternal, cord, or neonatal blood), results were mixed as to whether prenatal (Bellinger et al., 1984; H. Hu et al., 2006) or concurrent blood Pb levels (G. Wasserman et al., 1992; G. A. Wasserman et al., 1998) were associated with a greater decrement in cognitive function. Several studies found that prenatal or neonatal blood Pb levels were associated with neurodevelopmental decrements assessed in neonates (within 30 days) or early in infancy (within 3 months), which indicated that relatively short-durations of Pb exposure may be associated with negative neurological effects (Dietrich et al., 1987; Ernhart et al., 1986; Rothenberg et al., 1989; Shen et al., 1998).

A recent analysis of 444 children participating in the Krakow Prospective Cohort Study corroborated previous findings for prenatal exposure and found associations at lower umbilical cord blood Pb levels than those in previous studies. Children in Jedrychowski et al. (2009b) had a median (range) of cord blood Pb levels of 1.23 (0.44-6.9 µg/dL), and increasing umbilical cord blood Pb levels was associated with lower 36-month Bayley MDI (Figure 5-4 and Table 5-4). Investigators also observed a larger magnitude of effect in males compared with females (Figure 5-9). These findings are consistent with the hypothesis that the developing male central nervous system may be more vulnerable than females' to environmental insults resulting in later behavioral problems (Moffitt et al., 2001).



Note: Effect estimates are standardized to a blood Pb levels of 1 µg/dL. Studies are arranged in order of ascending age of cognitive function assessment. Cognitive function test scores are not standardized to a similar scale because not all studies provided sufficient data.Red = prenatal or neonatal blood Pb levels, Blue = Early childhood levels, Black = concurrent or lifetime average levels. MDI = Mental Development Index, Bayley Scales; NR = Not reported; GCI = General Cognitive Index, McCarthy Scales; FWS = Filtered Word Test, Kaufman Assessment Battery for Children, FSIQ = Full-scale IQ. a = 95% CI for blood Pb levels. b = values represent mean blood Pb levels in the two towns studied. c = Effect estimate compares children in different categories of blood Pb levels. d = mean (SD) and effect estimate for tooth Pb levels (µg/g). e = Sufficient data were not provided in order to calculate 95% CIs. f = range of blood Pb levels.

Figure 5-7. Associations of blood Pb measures at various lifestages with cognitive function in children.



Note: Positive effect estimates represent an increase in behavioral index. Effect estimates are standardized to a blood Pb levels of 1 µg/dL. Studies are arranged in order of ascending age of behavioral assessment. Behavioral asssessment scores are not standardized to a similar scale because not all studies provided sufficient data. Red = prenatal blood Pb levels; Black = blood Pb levels at other lifestages. a = second quartile levels (µg/g) and effect estimate for tooth Pb levels b = mean (SD) in ppm and effect estimate for tooth Pb levels.

Figure 5-8. Associations of Pb biomarkers at various lifestages with behavioral indices in children.

Table 5-5. Additional characteristics and quantitative results for studies presented in Figures 5-7 and 5-8

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[a]
Rothenberg et al. (1989)	42 children followed prenatally to child age 30 days Mexico City, Mexico	Maternal week 36 gestation mean (SD): 15.0 (6.4) Maternal at birth mean (SD): 15.5 (5.7)	Regression model adjusted for smoking, single mother, problems in pregnancy, alcohol use in previous month, use of spinal block, gravidity, income	Self-quieting ability (regulation of state) at age 30 days Assessed using Newborn Brazelton Assessment System	Prenatal: -0.091 (-0.18, 0)
Dietrich et al. (1986)	305 children followed prenatally to age 6 mo. in Cincinnati, OH	Prenatal (maternal) mean (SD): 8.0 (3.8) Concurrent mean (SD): 5.9 (3.4)	Log linear regression model adjusted for birth weight, gestation, sex	Bayley MDI assessed at age 6 mo	Prenatal: -0.6 (-1.1, -0.09) Concurrent: -0.23 (-0.58, 0.12)
Hu et al. (2006)	146 children born 1997-1999 followed prenatally to age 24 mo Mexico City, Mexico	Prenatal (maternal blood Pb) in 1st trimester mean (range): 7.1 (1.5-43.6) Early childhood (12 mo) mean (SD): 5.2 (3.4) Concurrent mean (SD): 4.8 (3.7)	Log linear regression model adjusted for concurrent blood Pb, sex, maternal age, current weight, height-for-age Z score, maternal IQ	Bayley MDI assessed at age 24 mo	Prenatal 1st trimester: -4.1 (-8.1, -0.17) Prenatal (avg): -3.5 (-7.7, 0.63) 12 month: -2.4 (-6.2, 1.49) Concurrent: -1.0 (-3.9, 1.9)
Gomaa et al. (2002)	197 children followed prenatally to age 24 mo Mexico City, Mexico	Prenatal (cord blood) mean (SD): 6.7 (3.4)	Log linear regression model adjusted for maternal IQ, maternal age, sex, parental education, marital status, breastfeeding duration, child hospitalization status	Bayley MDI assessed at age 24 mo	Prenatal: -2.1 (-3.9, -0.39)
Jedrychowski et al. (2009b)	444 children born 2001-2004 followed prenatally to age 36 mo Krakow, Poland	Prenatal (cord blood) geometric mean (range): 1.29 (0.44-5)	Linear regression model adjusted for maternal education, birth order, prenatal ETS, sex	Bayley MDI assessed at age 36 mo	Prenatal: -2.9 (-5.0, -0.75)
Wasserman et al. (1992)	392 children followed prenatally to age 24 mo Kosovo, Yugoslavia (K. Mitrovica, Pristina)	Prenatal (cord blood) mean (SD): 14.4 (10.4) Concurrent means: K. Mistrovica: 35.4, Pristina: 8.5	Log linear regression model adjusted for sex, birth order, birth weight, ethnic group, HOME score, years of maternal education, maternal age, maternal intelligence	Bayley MDI assessed at age 24 mo	Prenatal: -3.2 (-7.2, 0.86) Concurrent: -4.1 (-6.2, -2.0)
Bellinger et al. (1987)	249 children followed from birth (1979-1981) to age 36 mo Boston area, MA	Prenatal (cord blood) mean (SD): 6.6 (3.2)	Regression and longitudinal analyses adjusted for the mother's age, race, IQ, education, number of years of cigarette smoking, number of alcoholic drinks per week in the third trimester, mean family social class over the period of the study, quality of the care-giving environment, infant's sex, birth weight, gestational age, birth order	Bayley MDI assessed at age 6, 12, 18, 24 mo	Prenatal: -4.8 (-7.3, -2.3), blood Pb levels ≥ 15 µg/dL vs. blood Pb levels <3[b]
Cognitive function assessments at school age					
Wasserman et al. (1994)	332 children followed prenatally to age 3-4 yr Kosovo, Yugoslavia (K. Mitrovica, Pristina)	Prenatal (cord blood) mean (SD): 14.4 (10.4) Concurrent means: K. Mistrovica: 39.9 Pristina: 9.6	Log linear regression model adjusted for HOME score, maternal age, maternal intelligence, maternal education, language, birth weight, sex	McCarthy GCI assessed at age 3-4 yr	Prenatal: -3.2 (-5.1, -1.2) Concurrent: -4.1 (-6.2, -2.0)

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[a]
Bellinger et al. (1991)	170 children followed from birth (1979-1981) to age 57 mo Boston area, MA	Early childhood (24 mo) mean (SD): 6.8 (6.3) Early childhood tooth mean (SD): 2.8 (1.7) µg/g Concurrent mean (SD): 6.4 (4.1)	Log linear regression model adjusted for family social class, maternal IQ, marital status, preschool attendance, HOME score, out of home care, number of residence changes, recent medication use, number of adults in household, sex, race, birth weight, birth order	McCarthy GCI assessed at age 57 mo	Early childhood blood: -3.0 (-5.7, -0.2) Early childhood tooth: -2.5 (-10.2, 5.2) Concurrent blood: -2.3 (-6.0, 1.4)
Dietrich et al. (1992)	259 followed from birth (1979-1984) to age 5 yr Cincinnati, OH	Prenatal (cord blood) mean (SD): 8.2 (3.8) Neonatal (10 days) mean (SD): 4.8 (3.3) Concurrent mean (SD): 11.9 (6.4)	Linear regression model adjusted for fetal distress and growth, perinatal complications, postnatal indices of health and nutritional status, sociodemographic characteristics, HOME score	Total FWS assessed using KABC at age 5 yr	Prenatal: -0.25, $p \leq 0.01$[c] Neonatal: -0.38, $p \leq 0.01$[c] Concurrent: -0.19, $p \leq 0.01$[c] Lifetime avg: -0.16, $p \leq 0.01$[c]
Dietrich et al. (1993)	245 children followed from birth (1979-1984) to age 6 yr Cincinnati, OH	Prenatal (cord blood) mean (SD): 8.4 (3.8) Neonatal (10 days) mean (SD): 4.8 (3.1) Concurrent mean (SD): 10.1 (5.6)	Linear regression model adjusted for obstretric complications, perinatal status, sex, social class, maternal intelligence, quality of rearing environment, earlier measures of neurobehavioral status	Bruininks-Oseretsky Test of Motor Proficiency assessed at age 6 yr	Prenatal: -0.04 (-0.20, 0.12) Neonatal: -0.15 (-0.33, 0.03) Concurrent: -0.18 (-0.26, -0.10) Lifetime avg: -0.11 (-0.19, -0.03)
Dietrich et al. (1993)	253 children followed from birth (1979-1985) to age 6.5 yr Cincinnati, OH	Prenatal (cord blood) mean (SD): 8.3 (3.7) Neonatal (10 days) mean (SD): 5.0 (3.4) Concurrent mean (SD): 11.8 (6.3)	Linear regression model adjusted for fetal distress and growth, perinatal complications, prenatal maternal substance abuse, postnatal indices of health and nutritional status, sociodemographic characteristics, maternal IQ, HOME score	FSIQ assessed using WISC-R at age 6.5 yr	Prenatal: 0.15 (-0.26, 0.56) Neonatal: -0.03 (-0.42, 0.36) Concurrent: -0.33 (-0.60, -0.06) Lifetime avg: -0.13 (-0.35, 0.09)
Schnaas et al. (2006)	150 children followed from prenatally (1987-1992) to age 6-10 yr Mexico City, Mexico	Prenatal (maternal 28-36 wk gestation): NR Early childhood avg (1-5 yr) mean (range): 9.8 (2.8-36.4) Later childhood avg (6-10 yr): 6.2 (2.2-18.6)	Log linear mixed effects model adjusted for blood Pb levels at other lifestages, sex, birth weight, SES, maternal IQ, First FSIQ measurement	FSIQ assessed using WISC-R at ages 6-10 yr	Prenatal (28-36 weeks gestation): -3.9 (-6.5, 1.4) Early childhood avg: 0.10 (-3.9, 4.1) Later childhood avg: 0.17 (-1.4, 1.8)
Baghurst et al. (1992)	494 children followed from birth (1979-1982) to age 11-13 yr Port Pirie, Australia	Prenatal mean of second quartile: 7.4 Early childhood (2 yr) mean of second quartile: 16.6 Lifetime avg mean of second quartile: 15.7	Log linear regression model adjusted for sex, birth weight, birth order, feeding method, breastfeeding duration, parental education, maternal age, parental smoking, SES, quality of home environment, maternal IQ, parents living together	FSIQ assessed using WISC-R at age 7-8 yr	Prenatal: 0.26 (-0.67, 1.5) Early childhood: -2.0 (-3.8, -0.21) Lifetime avg: -1.6 (-3.7, 0.52)
Bellinger et al. (1992)	148 children followed from birth (1979-1981) to age 15-17 yr Boston area, MA	Prenatal: NR Early childhood (2 yr) mean (SD): 6.5 (4.9) Concurrent mean (SD): 2.9 (2.4)	Linear regression model adjusted for HOME score (age 10 and 5), child stress, race, maternal IQ, SES, sex, birth order, marital status	FSIQ assessed using WISC-R at age 10 yr	Prenatal: -0.48 (-5.7, 4.7), blood Pb >10 µg/dL vs. <3 µg/dL[b] Early childhood: -0.58 (-0.99, -0.17) Concurrent: -0.46 (-1.5, 0.56)
Lanphear et al. (2005)	1333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts	Median (5th-95th) Early childhood: 12.7 (4.0-34.5) Peak: 18.0 (6.2-47.0) Lifetime avg: 12.4 (4.1-34.8) Concurrent: 9.7 (3.5-33.2)	Log linear regression model adjusted for HOME score, birth weight, maternal IQ, maternal education	FSIQ measured at ages 4.8-10 yr	Early childhood: -0.14 (-0.23, -0.06) Peak: -0.20 (-0.29, -0.11) Lifetime avg: -0.15 (-0.22, -0.09) Concurrent: -0.23 (-0.32, -0.14)

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[a]
Pocock et al. (1994)	Meta-analysis of 5 prospective (over 1100 children and 14 cross-sectional studies (3499 children)	Early childhood (2 yr) range in means: 6.8-21.2	Meta-analysis of combining effect estimates from individual studies	FSIQ assessed using various tests at ages 5-10 yr	Around birth: 0.26 (-1.5, 2.0) Early childhood: -2.7 (-4.1, -1.2) Postnatal mean: -1.3 (-2.9, 0.37)

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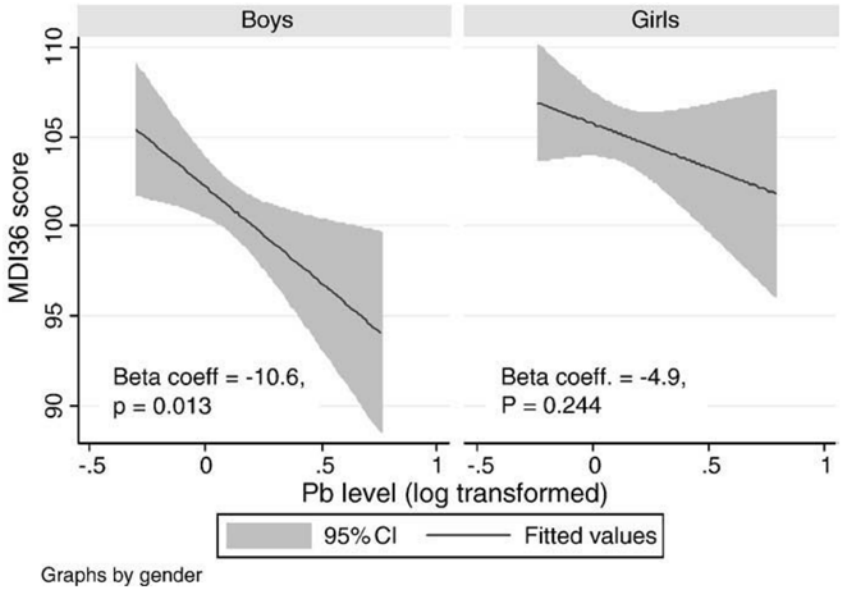
Ris et al. (2004)	195 children in followed from birth (1979-1985) to age 15-17 yr Cincinnati, OH	NR	Linear regression model adjusted for maternal IQ, sex, and average total HOME score	Learning/IQ composite assessed using WISC-III indices at age 15-17 yr	Prenatal: -0.08 (-0.18, 0.03) Early childhood, 6.5 yr: -0.08 (-0.17, 0.003) Early childhood avg: -0.03 (-0.18, 0.03)
Behavioral assessments					
Wasserman et al. (1998)	379 children followed prenatally to age 3 yr Kosovo, Yugoslavia (K. Mitrovica, Pristina)	Prenatal mean (SD): 16.1 (2.6) Concurrent mean (SD): 25.8 (19.1)	Hierarchical log linear regression analyses adjusted for town, sex, ethnicity, maternal education, HOME score	Anxiety/depression assessed using Child Behavior Checklist at 3 yr	Prenatal: 1.16 (0.02, 2.3) Concurrent: 1.45 (0.04, 2.86)
Leviton et al. (1993)	1923 children followed from birth (1979-1980) to age 8 yr Boston area, MA	Prenatal blood 2nd quartile: 4.8-6.3 Early childhood (tooth) second quartile: 2.0-2.9 µg/g	Log linear regression model adjusted for single-parent family, gestational age <37 wk, mother not a college graduate, self-identification as black, only child, daycare during first 3 yr	Hyperactivity assessed using Boston Teacher Questionnaire at age 8 yr	Prenatal, girls: 0.26 (-0.69, 1.13) Early childhood, girls: 0.10 (-0.92, 1.1)
Bellinger et al. (1994)	1,782 children followed from birth (1979-1980) to age 8 yr Boston area, MA	Prenatal (cord blood) mean (SD): 6.8 (3.1) Early childhood (tooth) mean (SD): 3.4 (2.4) ppm	Log linear regression analyses adjusted for prepregnant weight, race, delivery by cesarean section, marital status, paternal and maternal education, sex, birth weight, maternal smoking, prenatal care beginning after the first trimester, recipient of public assistance, number of children in family, child currently on medication	Problem behaviors (t-scores) assessed using Teacher Report Form of the Child Behavior Profile at age 8 yr	Prenatal: -0.31 (-1.7, 1.07) Early childhood: 1.8 (0.49, 3.1)
Ris et al. (2004)	195 children in followed from birth (1979-1985) to age 15-17 yr Cincinnati, OH	NR	Linear regression model adjusted for maternal IQ, sex, and average total HOME score	Inattention composite assessed using Continuous Performance Test	Prenatal: 0.16 (0.04, 0.27) Early childhood, 6.5 yr: 0.12 (0.02, 0.22) Early childhood avg: 0.11 (0.03, 0.19)
Dietrich et al. (2001)	195 children followed from birth (born1979-1985) to age 15-17 yr Cincinnati, OH	NR	Linear regression model adjusted for birth weight, HOME score, SES, parental IQ	Parental report of delinquent behavior	Prenatal: 0.19 (0.02, 0.37) Early childhood, 6.5 yr: 0.13 (-0.01, 0.27) Early childhood avg: 0.09 (-0.02, 0.20)

MDI = Mental Developmental Index, ETS = Environmental tobacco smoke, HOME = Home Observation for Measurement of the Environment, GCI = General Cognitive Index, FWS = Filtered Word Test, KABC = Kaufman Assessment Battery of Children, FSIQ = Full-scale IQ, WISC = Weschler Intelligence Scale for Children, NR = Not reported
[a]Effect estimates are standardized to a 1 µg/dL increase in blood Pb level in analyses of blood Pb as a continuous variable.
[b]Effect estimate represent comparisons between children in different categories of blood Pb level, with children in the lower blood Pb category serving as the reference group.
[c]Sufficent data were not provided in order to calculate 95% CI.



Source: Used with permission from Elsevier Science, Jedrychowski et al. (2009a).

Figure 5-9. Regression of fitted MDI score at 36 months on log-transformed concentration of cord blood Pb level by sex.

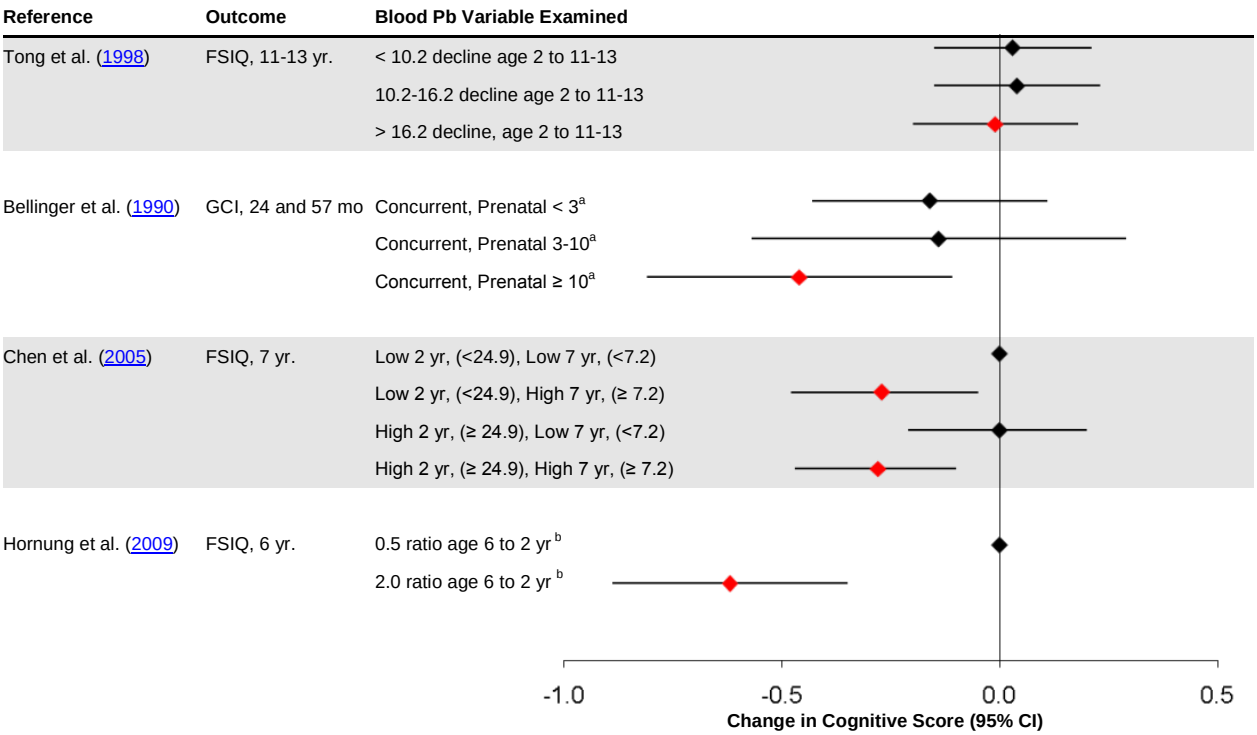
In studies that examined cognitive and behavioral indices measured in schoolaged children (ages 4-17 years), statistically significant, negative associations with prenatal or neonatal (10 days after birth) blood Pb measures were observed less frequently (Figures 5-7 and 5-8 and Table 5-5). Multiple studies conducted in the Cincinnati cohort found that blood Pb levels measured 10 days after birth but not maternal blood Pb during pregnancy were associated with impairments in cognitive function, auditory processing, and motor function as well as increased behavioral problems in children between ages 4 and 6 years (Dietrich, Berger, & Succop, 1993; Dietrich, Berger, Succop, et al., 1993; Dietrich et al., 1991; Dietrich et al., 1992). In these Cincinnati studies, concurrent blood Pb levels generally were estimated to have similar magnitudes of effect. In most of the studies examining associations of prenatal or neonatal blood Pb levels with neurodevelopmental outcomes in later childhood, early or cumulative childhood blood Pb levels were associated with the greater decrements in function. A larger effect estimate for peak blood Pb levels was corroborated in meta-analysis of results from five cohort studies (Pocock et al., 1994) (Figure 5-7 and Table 5-5). These findings may indicate the lack of persistence of early Pb exposures. Further, associations of cord blood Pb levels with neurodevelopmental measures in infancy may reflect associations with blood Pb levels in infancy, which are expected to be similar to those during the prenatal period. Cord blood Pb level may be a good surrogate of early postnatal blood Pb levels.

Early childhood blood Pb levels also were associated with diverse neurodevelopmental effects assessed later in childhood and into early adulthood in both recent (reviewed earlier in Section 5.3.2.1, Figure 5-4, and Table 5-4) and previous studies that did not compare various lifestages of Pb exposure

(Cecil et al., 2005; Tong et al., 2000; Yuan et al., 2006). This lag effect may be the result of a toxicological process in which some period of time is required for past Pb exposure to affect CNS function. Alternatively, Pb exposure may affect higher-order neurodevelopmental processes that are more reliably assessed at later ages when children's processes modalities are more highly differentiated. Early testing may lead to false-negative results and fail to identify a child who is at risk for later neurodevelopmental dysfunction. In some studies that have reported associations between early-childhood blood Pb levels and neurodevelopmental decrements later in life, children's blood Pb levels had not markedly changed over time Thus, the early-childhood blood Pb levels may have been serving as surrogates of concurrent or cumulative blood Pb levels.

As depicted in Figures 5-7 and 5-8 and Table 5-5, several studies estimated larger decreases in neurodevelopmental endpoints for concurrent or lifetime average blood Pb levels than blood Pb levels at other lifestages. These findings were substantiated in the analysis pooling data from seven prospective studies, in which concurrent, peak, average lifetime, and early childhood blood Pb levels were all negatively associated with IQ, with the largest magnitude of decrease associated with concurrent blood Pb levels (Lanphear et al., 2005). Childhood average blood Pb levels (Dietrich, Berger, & Succop, 1993; Dietrich, Berger, Succop, et al., 1993; Lanphear et al., 2005) and tooth Pb levels (Bellinger et al., 1994) have been associated with neurodevelopmental effects, indicating that biomarkers of cumulative childhood Pb exposure also may contribute to neurodevelopmental effects in children. Associations with concurrent blood Pb level were also demonstrated consistently in studies without comparisons to exposures at other lifestages (Figures 5-2 and 5-4 and Tables 5-3 and 5-4).

Some studies have aimed to improve assessment of age-based susceptibility by examining children with different degrees of changes in blood Pb levels over time (i.e., children whose blood Pb level ranking changed over time) (Bellinger et al., 1990; A. Chen et al., 2005; Hornung et al., 2009; Tong et al., 1998). Except for Tong et al. (1998), these studies have demonstrated stronger effects of concurrent blood Pb levels (Figure 5-10 and Table 5-6). Tong et al. (1998) found that early-life blood Pb level was associated with a larger deficit in IQ. As part of the Port Pirie, Australia cohort study, investigators separately examined intellectual attainment in 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. In comparisons of tertiles of change in blood Pb level between age 2 and 11-13 years, investigators found that intellectual attainment scores at ages 2, 4, 7, and 11-13 years did not significantly differ between children with the largest declines (>16 µg/dL) in blood Pb level and children with a lower decline (<10 µg/dL). These findings indicated a stronger effect of higher blood Pb levels early in life even among groups with lower concurrent levels.



Note: Effect estimates represent associations between concurrent blood Pb level and cognitive function (standardized to standard deviation) in children categorized by prenatal blood Pb level. bValues represent the ratio of blood Pb level at age 6 years to that at age 2 years. FSIQ = Full-scale IQ, GCI = General Cognitive Index. Cognitive function scores were standardized to their standard deviation. Effect estimates in red represent blood Pb level variables associated with the greater decrease in cognitive function.

Figure 5-10. Associations of cognitive function in children with different degrees of changes in blood Pb levels over time.

Table 5-6. Additional characteristics and quantitative results for studies presented in Figure 5-10

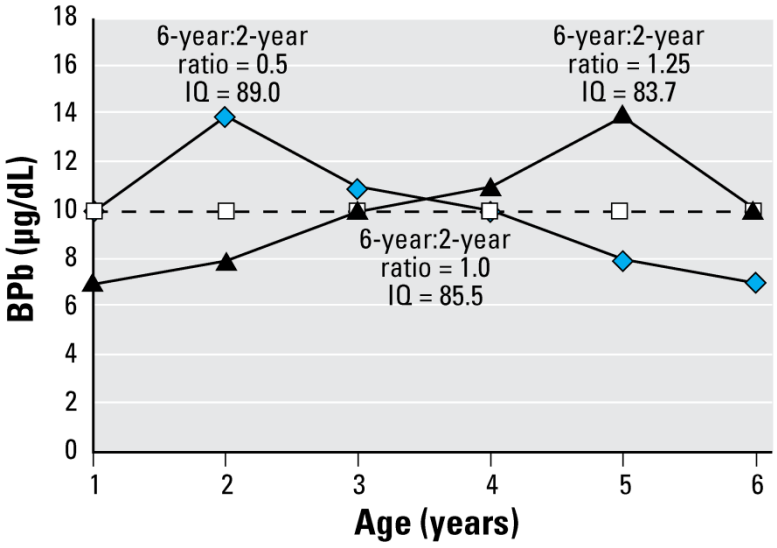
Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)a
Tong et al. (1998)	375 children followed from birth (1979-1982) to age 11-13 yr Port Pirie, Australia	Means: 21.2 (age 2 yr), 7.9 (age 11-13 yr)	Log linear regression model adjusted for sex, birth weight, birth rank, feeding style, breastfeeding duration, maternal IQ, maternal age, SES, HOME score, parental smoking, parents living together. ANOVA to assess association of change in IQ with change in blood Pb across time intervals	Change in cognitive function (z-scores) using Bayley MDI at age 2 yr, McCarthy GCI at age 4 yr, WISC-R at ages 7, and 11-13 yr	<10.2 µg/dL decline: 0.03 (-0.15, 0.21)[b] 10.2-16.2 µg/dL decline: 0.04 (-0.15, 0.23)[b] >16.2 µg/dL decline: -0.01 (-0.20, 0.18)[b]
Bellinger et al. (1990)	170 children followed prenatally to age 57 mo Boston area, MA	NR	Log linear regression adjusted for HOME score, social class, maternal IQ, maternal age, sex, ethnicity	Change in McCarthy GCI score (z-score) between age 57 and 24 mo	For concurrent blood Pb level[c] Prenatal <3 µg/dL: -0.16 (-0.43, 0.11) Prenatal 3-10 µg/dL: -0.14 (-0.57, 0.29) Prenatal ≥ 10 µg/dL: -0.46 (-0.81, -0.11)
Chen et al. (2005)	780 children participating in the TLC trial from age 12-33 mo to age 7 yr Baltimore, MD; Cincinnati, OH; Newark, NJ; Philadelphia, PA	Mean (SD): Age 2 yr: 26.2 (5.1) Age 5 yr: 12.0 (5.2) Age 7 yr: 8.0 (4.0)	Linear regression model adjusted for city, race, sex, language, parental education, parental employment, single parent, age at blood Pb measurement, caregiver IQ	WISC-III at age 7 yr	Low age 2 (<24.9 µg/dL, Low age 7 (<7.2 µg/dL): 0[d] Low age 2, High age 7: -0.27 (-0.48, -0.05) High age 2, Low age 7: 0 (-0.21, 0.20) High age 2, High age 7: -0.28 (-0.47, -0.10)
Hornung et al. (2009)	462 children followed from birth (1979-1984) to age 6 yr Rochester, NY and Cincinnati, OH	Geometric mean (5th-95th): Peak: 13.6 (4.6-34.4) Early childhood: 8.9 (3.0-23.8) Lifetime mean: 8.5 (3.0-22.1) Concurrent: 6.0 (1.9-17.9)	Linear regression model adjusted for city, HOME score, birth weight, maternal IQ, maternal education	FSIQ assessed using WISC-R at age 6 yr	0.5 ratio of blood Pb level at age 6 to age 2: 0 (reference) 2.0 ratio of blood Pb level at age 6 to age 2 yr: -0.70 (-1.0, -0.40)

[a]Effect estimates represent the cognitive function score or change in score over time standardized to its standard deviation.
[b]Investigators estimated changes in IQ in groups of children with different degrees of decline in blood Pb levels over the study period: children with <10.2 µg/dL decline, children with a 10.2-16.2 µg/dL decline, and children with >16.2 µg/dL.
çEffects are estimated for concurrent blood Pb level (continuous variable) in children in different categories of prenatal blood Pb level: <3 µg/dL, 3-10 µg/dL, and ≥ 10 µg/dL.
[d]Investigators compared IQs among children with different categories of blood Pb level early and later in childhood: low levels at age 2 (<11.4 µg/dL) and age 7 (<7.2 µg/dL), low levels at age 2 (<11.4 µg/dL) and high levels at age 7 (>7.2 µg/dL), high levels at age 2 (>11.4 µg/dL) and low levels at age 7 (<7.2 µg/dL), and high levels at age 2 (>11.4 µg/dL) and age 7 (>7.2 µg/dL). Cutoffs were based on the median blood Pb levels.

In several different U.S. cohorts of children, larger decrements on neurocognitive function were estimated for concurrent blood Pb levels (Bellinger et al., 1990; A. Chen et al., 2005; Hornung et al., 2009) (Figure 5-10 and Table 5-6). In the Boston cohort, Bellinger et al. (1990) found that at age 57 months, cognitive performance, as assessed by McCarthy GCI, was similar between children with higher (≥ 10 µg/dL) and lower (<3 µg/dL) prenatal blood Pb levels. Additionally, increasing concurrent blood Pb levels (age 57 months) were associated with the largest decline in GCI scores over time (score at age 57 months – score at age 24 months) among children with high prenatal blood Pb levels (≥ 10 µg/dL), which indicated an effect among children with both high early and concurrent blood Pb levels (Figure 5-10 and Table 5-6). These findings indicated that by age 5 years, children with higher prenatal blood Pb levels appear to recover the Pb-associated decrements in cognitive function unless concurrent blood Pb levels

remain high. The investigators also demonstrated that positive home and caregiving environment (e.g., HOME score >52, higher SES, higher maternal IQ) may also protect against decrements in cognitive function associated with higher postnatal Pb exposures.

As part of the multicenter Treatment of Lead-Exposed Children (TLC) trial, Chen et al. (2005) evaluated how change in blood Pb over time was related to IQ at later ages. The TLC was a clinical trial designed to examine the effect of chelation using succimer to prevent cognitive impairment in 780 urban children enrolled at 12 to 33 months of age with elevated blood Pb concentrations (20-44 µg/dL). Blood Pb and IQ were assessed at ages 24 and 36 months and 3, 5, and 7 years. Concurrent blood Pb level above the median level (>7.2 µg/dL) was associated with a larger decrease in IQ, regardless of whether prenatal blood Pb levels were low or high (less than or greater than the median of 11.4 µg/dL, respectively). Pooling the Cincinnati and Rochester cohorts (n = 397), Hornung et al. (2009) also created a new indicator of Pb exposure: the ratio of blood Pb level at 6 years of age to that at 2 years of age. The greatest decrease in cognitive and behavioral development was observed for children with blood Pb ratios greater than 1 (indicating an increase in blood Pb level from 2 to 6 years of age) (Figures 5-10 and 5-11 and Table 5-6). Presumably areas under the curve would be similar among children with blood Pb level ratios of 1, greater than 1, and less than 1, indicating that cumulative blood Pb levels would not be predictive. It is important to note that in these aforementioned studies, blood Pb levels were higher than those currently measured in among children in the U.S. Additionally, children in these study populations experienced larger decreases in blood Pb levels over time. It is unclear whether these findings would apply to children Blood Pb levels in the U.S. who currently are within the same age range and who would be expected to have smaller decreases in blood Pb levels over time.



Source: Hornung et al. (Hornung et al., 2009)

Note: All three patterns have an identical mean blood Pb level level of 10 µg/dL.

Figure 5-11. Estimated IQ in combined Cincinnati and Rochester cohorts for 3 patterns of blood Pb level levels from 1 through 6 years of age: peak at 2 years (blue diamonds), peak at 5 years (black triangles), and constant blood Pb level level (white squares).

In the collective body of epidemiologic evidence of children, it is difficult to ascertain which lifestage of Pb exposure is associated with the greatest susceptibility to Pb-associated neurodevelopmental effects. Associations have been observed with prenatal, early-childhood, lifetime average, and concurrent blood Pb levels as well as childhood tooth Pb levels. The assessment of age-based susceptibility is complicated further by the fact that blood Pb levels in children, although highly affected by recent dose, are also influenced by Pb stored in bone due to rapid growth-related bone turnover in children relative to adults. Thus, concurrent blood Pb level in children also may reflect cumulative dose (Section 4.3.5). Nonetheless, while the evidence indicates that prenatal and early-childhood blood Pb levels are associated with neurodevelopmental deficits, subsequent exposures that are reflected in concurrent, cumulative blood Pb levels or tooth Pb levels also are demonstrated to contribute to neurodevelopmental deficits throughout schoolage and into adolescence. Additional results from Hornung et al. (2009) and recent studies described earlier in the section support the conclusion from the 2006 AQCD that concurrent blood Pb level appears to be the best predictor of neurodevelopmental effects in children. These findings are consistent with the understanding that the nervous system continues to develop throughout childhood. Thus, the course of cognitive development may be modified in children, depending on concurrent blood Pb levels or positive caregiving environment.

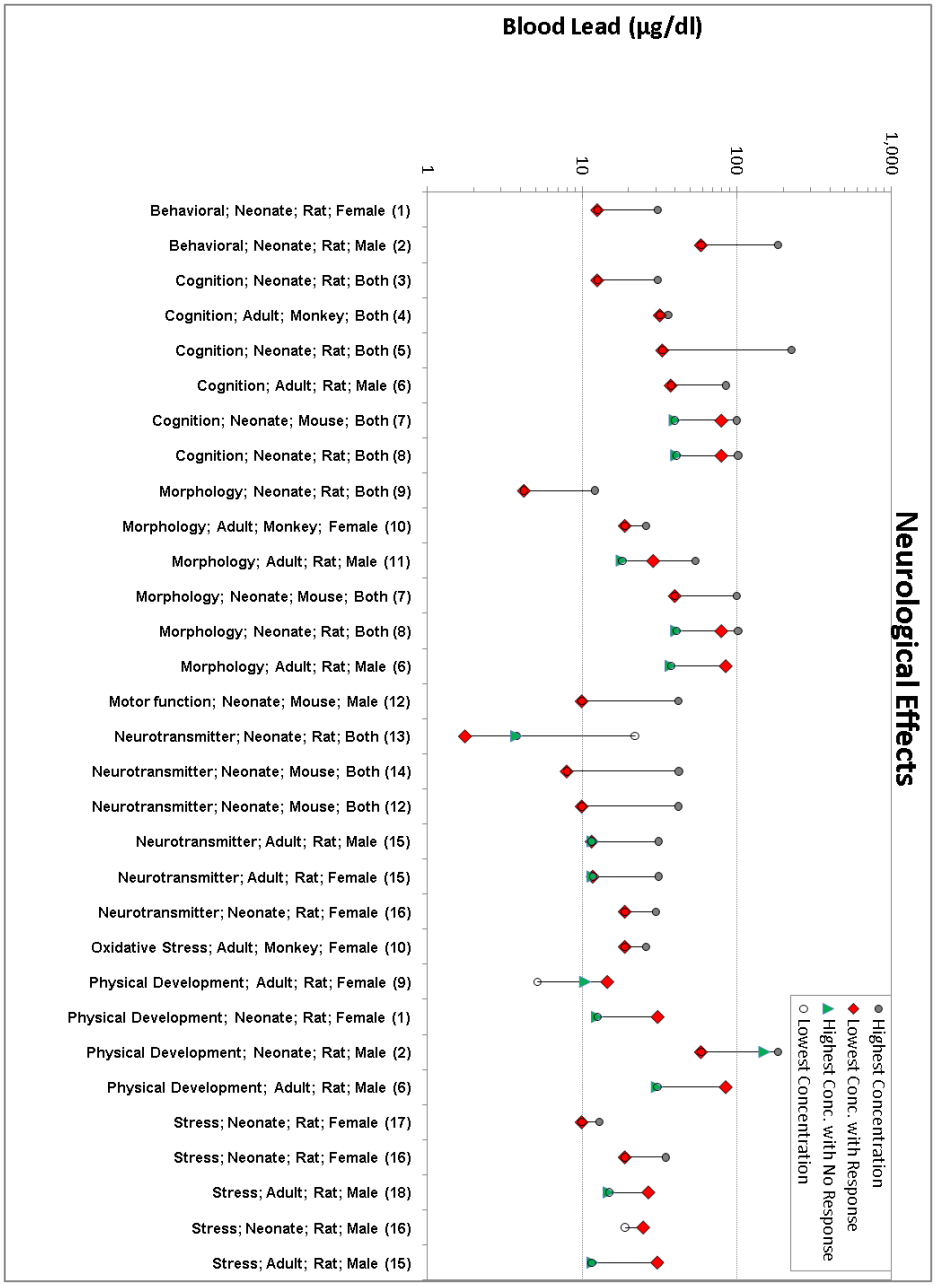


Figure 5-12. Neurological summary array of toxicological outcomes after Pb exposure. Dosimetric representation reported by blood Pb level. (ID corresponds to Table 5-7.)

Table 5-7. Summary of findings from neurotoxicological exposure-response array presented in Figure 5-12.

Study ID	Reference	Blood Pb Level (ug/dL)	Outcome
1	Beaudin et al. (2007)	13 & 31	Behavior, neonate: Lactational Pb exposure, offspring deficient in Reward Omission testing.
2	Kishi et al. (1983)	59 & 186	Behavior, neonate: Pb exposure (oral gavage of pups) during lactational period, Changed emotional behavior, males.
3	Stangle et al. (2007)	13 & 31	Cognition; Developmental Pb exposure (PND1-30): Impaired learning with visual discrimination task, heightened response to errors.
6	Gong & Evans (1997)	38 & 85	Cognition-Adult male 21 day Pb exposure: Hyperactivity with Habituation to new cage environment.
4	Rice (1990)	32 & 36	Cognition-Chronic Pb exposure from birth: Spatial discrimination reversal task impairment.
7	Li et al. (2009)	80 & 100	Cognition-Gestational & lactational Pb exposure: Morris water maze performance impaired.
8	Li et al. (2010)	80 & 102	Cognition- Gestational & lactational Pb exposure: Morris water maze performance impaired.
5	Overmann (1977)	33, 174 & 226	Cognition-Pb exposure (oral gavage of pups) during lactation: Response inhibition impaired.
17	Cory-Slechta et al. (2010)	10 & 13	Stress: Corticosterone-Lifetime Pb plus stress: Affects FI performance, dopamine and serotonin levels in female offspring.
16	Virgolini, Rossi-George, Weston et al. (2008)	25	Stress: Corticosterone-Maternal Pb plus stress: Affects FI performance.
16	Virgolini, Rossi-George, Weston et al. (2008)	19 & 35	Stress.
18	Virgolini et al. (2005)	15 & 27	Stress: Corticosterone-Chronic Pb plus stress: Affects neurotransmitters & FI performance.
15	Virgolini, Rossi-George, Lisek et al. (2008)	11 &/or 31	Stress: Corticosterone-Maternal Pb plus stress: Affects FI performance, dopamine, serotonin, and NE levels.
9	Hu et al. (2008)	4 & 12	Morphology; Gestational Pb exposure: Neurite outgrowth marker PSA-NCAM decreased in rat pups.
10	Wu et al. (2008)	19 & 26	Morphology: Elevated expression of Alzheimer's disease-related genes and Tc factors in aged brains of female monkeys (exposed to Pb as infants).
11	Tavakoli-Nezhad et al. (2001)	18, 29, & 54	Morphology; 3 to 6 weeks of Postnatal (starting at PND22) Pb exposure in males: Decreased number of spontaneously active midbrain dopamine neurons.
7	Li et al. (2009)	40 & 100	Morphology; Gestational & lactational Pb exposure: Increased levels of inflammatory cytokines & exocytosis related proteins in brains of pups at weaning.
8	Li et al. (2010)	80 & 102	Morphology: Increased levels of Alzheimer disease-associated proteins in mice with gestational and lactational Pb exposure.
6	Gong & Evans (1997)	85	Morphology; 21 day Pb exposure to adult males: Marker of neuronal injury-elevated hippocampal glial fibrillary acidic protein (GFAP).
12	Leasure et al. (2008)	10 & 42	Motor function; Mouse maternal (dam) Pb exposure: Induced decreased rotarod performance in offspring (1 year-old male offspring).
13	Bielarczyk et al. (1996)	1.8, 3.8, 22	Neurotransmitter; Perinatal (GD16-PND28) Pb exposure: Decreased hippocampal ChAT activity and increased hippocampal tyrosine hydroxylase activity.
14	Fortune & Lurie (2009)	8 & 43	Neurotransmitter; Mouse maternal (dam) Pb exposure: Affects offspring superior olivary complex (auditory) neurotransmitters.
12	Leasure et al. (2008)	10 & 42	Neurotransmitter; Mouse maternal (dam) Pb exposure: Affects 1 year old male offspring dopamine homeostasis.
15	Virgolini, Rossi-George, Lisek et al. (2008)	31	Neurotransmitter; Gestational and lactational Pb exposure: Induced NE aberrations in adult rat offspring (both sexes).
16	Virgolini, Rossi-George, Weston et al. (2008)	19 & 30	Neurotransmitter; Gestational and lactational Pb exposure: Induced DA and 5HT changes in rat offspring.
10	Wu et al. (2008)	19 & 26	Oxidative stress: Elevated oxidative DNA damage in aged brains of female monkeys (exposed to Pb as infants).
9	Hu et al. (2008)	15	Physical developmen; t-Gestational Pb exposure: Early brain synapse development impaired (hippocampal PSA-NCAM and sialytransferase).
1	Beaudin et al. (2007)	13 & 31	Physical development; Postnatal Pb exposure (birth to 4 weeks of age): Pb-dependent development of over-reactivity to reward omission and errors is reversible with chelation treatment.
2	Kishi et al. (1983)	59 & 186	Physical development; Pb exposure during lactation (oral gavage): Delayed development of righting reflex in male rats.
6	Gong & Evans (1997)	85	Physical development; Adult male rats (21 day Pb exposure): Neurotoxicity measured with brain glial fibrillary acidic protein (GFAP).
16	Virgolini, Rossi-George, Weston et al. (2008)	19 & 35	Stress: Corticosterone levels affected.
17	Cory-Slechta et al. (2010)	10 & 13	Stress: Corticosterone-neurotransmitter-Lifetime Pb exposure in female rats plus stress: Dopamine homeostasis affected.

5.3.2.2. Toxicological Studies of Neurocognition, Memory and Learning

The 2006 AQCD reported deficits in the Morris water maze with Pb exposure. The Morris water maze tests memory and learning by having a mouse swim and locate or remember the location of a

platform submerged in opaque water. New research since the 2006 AQCD continues to show Pb-induced impaired Morris water maze performance. Data on neurocognition and learning as well as other neurotoxicological endpoints with dose responsive data are shown in Figure 5-12 and accompanying Table 5-7. Dams received Pb acetate dissolved in drinking water (0.1%, 0.5%, and 1% with corresponding blood Pb level of 4, 8 and 10 µg/dL at postnatal day [PND] 21) throughout gestation and lactation. Beginning at weaning, Pb exposed pups were subjected to Morris water maze performance testing. Pb exposed pups had significant increases in escape latency and number of crossings of the platform area at 0.5% and 1% Pb acetate exposure (blood Pb levels of 8 and 10 µg/dL, respectively, indicating impaired memory and learning (Li et al., 2009). The pups in Li et al. (2009) were not separated by sex. Another study found that dietary supplementation with various supplements listed below or with methioninecholine concomitant with Pb exposure in weanling males shortened the escape latency of Pb-exposed pups to more closely resemble the escape latency of control pups (G. Fan et al., 2009; Fan et al., 2010). Zinc or methionine were effective dietary supplements in the G. Fan et al. 2009 study (2009); glycine, taurine, vitamin C, vitamin B1, tyrosine had no effect on the Morris water maze results. These data on the effect of Pb on learning and memory in the Morris water maze confirm findings by two other labs. Jett et al. (1997) showed increased escape latency in adult rats exposed to Pb via direct injections into the dorsal hippocampus. Kuhlmann et al. (1997) used maternal Pb diet exposure (gestation and lactation), continuous Pb exposure (gestation through adulthood) or post-weaning Pb exposure and only found only significant impairments in the maternal and continuous exposure groups. This new study confirms the findings of earlier studies that learning and memory are significantly impaired in rodents who are exposed to Pb early in life.

Working Memory

Working memory is the ability to temporarily keep information in mind while using the information to perform a related or unrelated task. The Morris water maze is able to measure working memory in addition to learning. Using this test, the 2006 AQCD found working memory was significantly affected in chronic developmentally exposed (Pb Acetate in feed 10 days prior to mating through PND 21) female offspring at PND 21 (Jett, Kuhlmann, Farmer, et al., 1997). Delayed spatial alternation (DSA) is another test used to measure working memory. With DSA, an animal receives rewards based on responses at two separate levers. Work from the 2006 AQCD showed that Pb-exposed animals had deficits under DSA testing. These deficits included increased response errors, decreased percent of correct responses, and perseverance at one lever (repeatedly pressing the same lever without moving between the two locations). These results have been consistently shown with non-human primate studies (continuous Pb exposure or juvenile to adult exposure) and less consistently shown with rats (juvenile only or juvenile to adult exposure). Working memory is a subcategory of executive function or goal-oriented problem

solving. Deficits in working memory are thought to underlie associations between blood Pb levels and ADHD in humans.

Response Inhibition

Response inhibition is another measure of executive function and is measured with multiple tests that measure premature responses, decreased pause time between two scheduled events and increased perseverance. These tests include Differential Reinforcement of Low Rates of Responding (DRL), DSA, Fixed Interval testing (FI), FI with Extinction (FI-Ext), Fixed Ratio-FI (FR-FI), and Signal Detection with Distraction. Multiple studies from the 2006 AQCD and earlier literature have shown that early life Pb exposure contribute to response inhibition across the spectrum of these aforementioned tests. Monkeys with moderate blood Pb levels (11-13 µg/dL) learned the DRL task more slowly but eventually acquired reinforcement rates equal to controls. Newer data from female rats exposed to Pb (Stangle et al., 2007) continued to show animals with premature responses after Pb exposure or response inhibition decrements.

Learning Ability, Schedule-Controlled Behavior

The 2006 AQCD discussed learning or cognition as measured with schedule controlled-behaviors including fixed interval (FI) and fixed ratio (FR) operant conditioning and found that FI response rate was affected differentially with low level and high level Pb exposures increasing and decreasing FI response rate in females, respectively. This curvilinear response has since been further explored in more recent work, much of which also includes the effect of psychological stress on Pb exposure.

Learning Ability with Stress

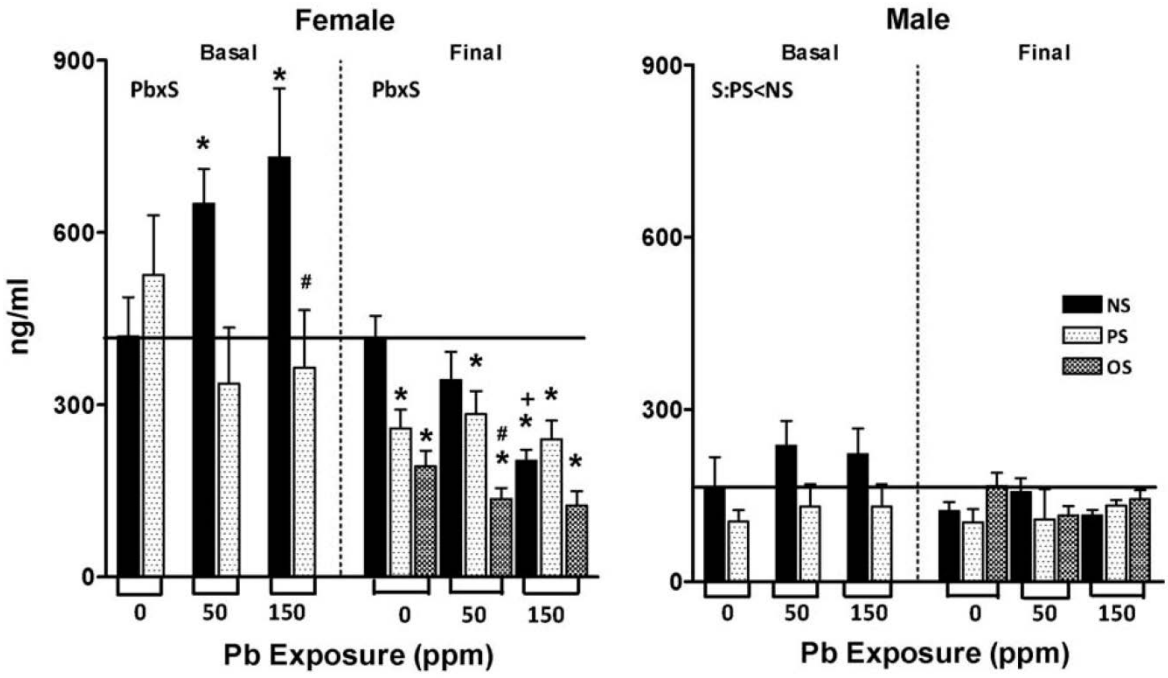
The combined paradigm of Pb exposure and stress experienced by a person or a laboratory animal is now being studied by multiple investigators who are focusing on the common pathway of HPA axis alteration and altered brain neurotransmitter levels. Data on stress and Pb with dose responsive data endpoints are shown in Figure 5-12 and accompanying Table 5-7. Cory-Slechta and colleagues have conducted multiple investigations in this area. Most recently, they have shown enhanced learning deficits in female rats (offspring) following lifetime Pb exposure combined with maternal restraint or prenatal stress (Cory-Slechta et al., 2010). This exposure paradigm used dams who were exposed to Pb for 2 weeks prior to mating through lactation and pups from a mixed sex litter received drinking water Pb (50 ppm) exposure through the remainder of their lifetime resulting in blood Pb levels of dams and pups ranging from 5-13 µg/dL.

Pb plus stress-related outcomes were followed in female offspring of dams who were exposed to Pb from 2 months prior to mating through lactation, i.e., developmental Pb exposure (2 exposure groups:

50 or 150 ppb Pb acetate drinking water solutions) (Virgolini, Rossi-George, Lisek, et al., 2008). Dams underwent restraint stress at gestational 16-17. Marked increases in response rates on FI performance in the Pb-stress female offspring versus control was found in animals whose mean blood Pb level was 11 µg/dL (50 ppb Pb acetate). Because these animals did not show effects with maternal stress or Pb exposure alone, this effect was potentiated in the animals exposed to Pb plus additional stress.

Similarly, lifetime Pb exposure (50 or 150 ppm, blood Pb level 11-16 and 25-33 µg/dL, respectively) plus stress (maternal or offspring) also induced FI aberrations at the post-reinforcement pause (PRP) period in female offspring, another potentiated effect (Rossi-George et al., 2011) (Table 5-8). Within the FI schedule, the PRP represents timing capacity or proper temporal discrimination. Namely, the PRP is the period during which the animal must wait or pause before depressing the lever for a reward. In this case, Pb plus stress exposed animals start responding too early due to a decreased pause or PRP interval. Abberant FI performance in infants and children has been used as a marker for impulsivity. Separately, overall FI response rate was significantly increased in Pb exposure alone and with maternal or offspring stress at the 50 ppm exposure dose. At 150 ppm, stress (maternal or offspring) increased FI response rate but Pb alone had no effect on FI. Biochemical analysis of possible mechanistic contributions to these aberrations revealed alterations in frontal cortex norepinephrine, reductions in dopamine homeostasis in the nucleus accumbens and enhancement of the striatial monoamine system. This study on the effect of lifetime Pb exposure with or without stress on FI testing itself or during the PRP component of FI testing further confirm learning deficits and provide possible mechanistic explanations.

Pb exposure over various exposure windows has been shown to affect corticosterone levels in rodents. Maternal Pb exposure (150 ppm drinking water from 2 months prior to mating through lactation with restraint stress as detailed above) induced increased basal corticosterone in female and male offspring at 9 months of age; no interactions of Pb and stress were seen in this model (Cory-Slechta et al., 2004). By 14 months of age, these offspring had reduced corticosterone concentrations versus control animals, indicating a possible acceleration of age-related decreases in basal corticosterone levels (Cory-Slechta et al., 2008) that were enhanced with maternal stress. Postnatal exposure of male rodents to Pb (PND 21-5 months of age) showed significant decrements in baseline corticosterone; this effect produced a U-shaped concentration-response curve with significant decrements in basal corticosterone levels in the 50 ppm exposure group versus control (Virgolini et al., 2005). In summary, developmental (gestational and lactational) and post-weaning exposure to Pb induced permanent changes in the HPA axis (corticosterone levels) in both sexes which are dynamic as the animal ages.



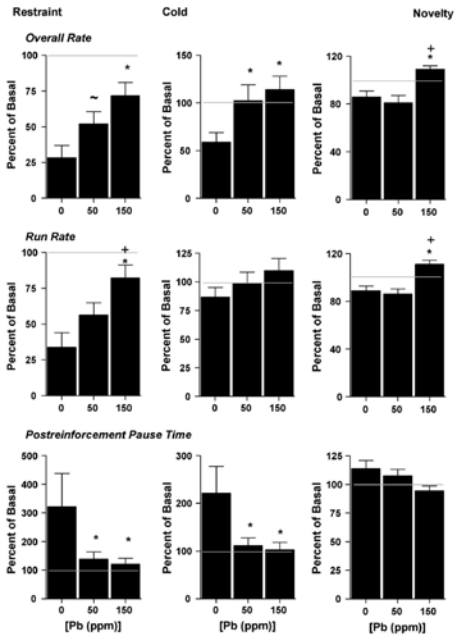
Source: Rossi-George et al. (2011)

Figure 5-13. Mean basal corticosterone levels of female and male offspring exposed to lifetime Pb (0, 50, 150 ppm) and/or stress (PS (dam stress) or OS (offspring stress)).*denotes significantly different from 0NS control; # denotes significantly different from corresponding Pb-NS value; + differs from 50-NS.

Mechanistic understanding of the cognitive deficits seen with Pb and/or stress exposure was explored in a recent study. HPA hypofunction following dam Pb exposure (pup gestational and lactational Pb exposure) with or without maternal stress was reported (Rossi-George et al., 2009). This study used the same model of developmental Pb exposure as is detailed in the preceding paragraph. Outcomes were followed in both male and female offspring. At 5-6 months of age, basal corticosterone in females was significantly increased at 150 ppm and unaffected at 50 ppm (Figure 5-13). In males, basal corticosterone of the 50 ppm group was significantly decreased and 150ppm was unaffected. These authors also explored the function of the glucocorticoid negative feedback loop and found that Pb and/or maternal stress significantly impacted this negative feedback. This negative feedback loop was more greatly impacted at the lower dose (50 ppm v 150 ppm Pb acetate, blood Pb level at PND 21 of 19 and 32 µg/dL, respectively). To test the effect of an outside stress on the HPA axis, mice in this study were subjected to vehicle injections. The corticosterone response to this vehicle injection stress was prolonged in a non-linear dose response manner in both sexes with the most profound effects seen at the lower 50 ppm dose. Maternal stress also prolonged the corticosterone stress response to vehicle injection and enhanced the Pb effect in males. To test the negative feedback of the HPA axis, exogenous dexamethasone (DEX) was administered to suppress endogenous corticosterone. The DEX test revealed HPA axis hypofunction.

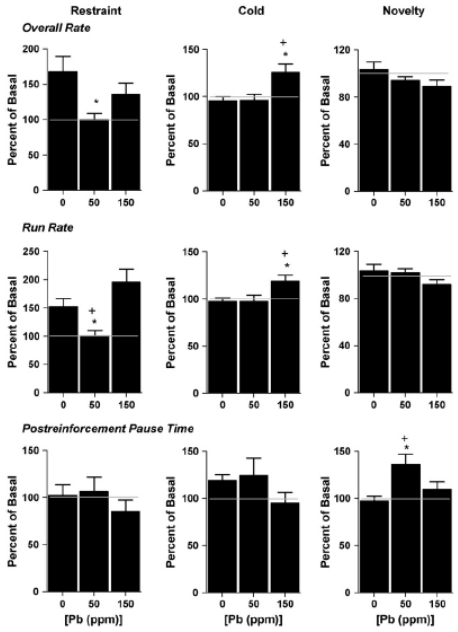
Specifically Pb and Pb plus maternal stress initially reduced the ability of DEX to suppress corticosterone. With time, the effect of this DEX test in males induced prolonged corticosterone suppression or failure to return to baseline as seen in control animals. In summary, dam Pb exposure induced negative feedback hypofunction in both sexes with an inverse U dose response function. A similar recent study explored the effect of lifetime Pb exposure on the HPA axis, looking at basal corticosterone levels in male and female offspring at two time periods (2 months old and 10 months old; before and after behavioral testing, respectively) in adulthood (Rossi-George et al., 2011). Pb and stress have no effect on basal corticosterone levels in males at either time period (Figure 5-13). At the first time period, Pb exposure elevated basal corticosterone levels in a dose-dependent fashion in females, and Pb plus stress attenuated the PB-dependent elevations in corticosterone to baseline levels (Figure 5-13). At the second time period, Pb and stress accelerated the age-dependent decrease in basal corticosterone levels in females (Figure 5-13).

These two studies of lifetime exposure (Rossi-George et al., 2009) reported different basal stress hormone levels with Pb exposure. Males with lifetime Pb exposure had no significant corticosterone response to Pb exposure; whereas males with dam Pb exposure had significant decreases in corticosterone at 5 months of age in the 50 ppm exposure group only (not seen in 150 ppm Pb exposure group). On the other hand, females had dose-dependent corticosterone responses to Pb exposure in both exposure models (lifetime Pb exposure and dam Pb exposure.



Source: Used with permission from Elsevier Science, Virgolini, Rossi-George, Weston, et al. (2008).

Figure 5-14. Changes in FI performance (FI overall performance, run rate, PRP) in female offspring with maternal Pb exposure with various stressors in adulthood (restraint, cold, novelty).



Source: Used with permission from Elsevier Science, Virgolini, Rossi-George, Weston, et al. (2008).

Figure 5-15. Changes in FI performance (FI overall performance, run rate, PRP) in male offspring with maternal Pb exposure with various stressors in adulthood (restraint, cold, novelty).

Maternal stress alone also led to HPA axis negative feedback hypofunction. Pb plus maternal stress enhanced negative feedback in males and attenuated this effect in females. Pb exposure with or without maternal stress prolonged the effect of DEX-dependent corticosterone supression in males. These data together show that HPA axis alterations could be an underlying mechanism linking commonalities between the contribution of Pb and stress to adverse health outcomes.

Schedule-control behavior is often measured using FI or FR testing. Because the FI animals are regularly handled by laboratory personnel and participate in 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 who have participated in behavior testing (FI) and those who have not (NFI) have been compared. Specifically, the corticosterone differences between FI and NFI animals after developmental Pb exposure (dam-only Pb exposure) have been measured. Virgolini et al. (2008) found that basal corticosterone levels were significantly different between FI and NFI animals. Also, the combination of dam Pb exposure with maternal stress was explored in FI and NFI animals. At the baseline age of 4-5 months, NFI animals who were not behaviorally trained displayed significant differences from FI animals. Pb exposure with or without stress did not induce differences in corticosterone levels in FI females. The corticosterone level of male FIs was affected by Pb and stress exposure (Figure 5-13). In the FI males, the 50 ppb Pb exposure group (50Pb) had decreased corticosterone versus control (no Pb exposure) and the 150 ppb Pb exposure group (150Pb) had elevated corticosterone versus control. Male NFI animals demonstrated a U shaped dose response corticosterone curve with 50Pb significantly less than control or 150Pb. In the NFI males, stress did not affect corticosterone levels or interact with the effect of Pb. NFI females exposed to 150Pb had significantly elevated corticosterone versus control (no Pb exposure). When drawing conclusions about the effects of Pb exposure, these data demonstrate that behaviorally trained animals have an altered HPA axis and response to Pb exposure versus animals who are housed under conditions without daily handling by caregivers.

Another study looked at female rats with lifetime Pb exposure combined with prenatal stress and found enhanced learning deficits(drinking water 50 ppm Pb acetate, offspring blood Pb 7-13 µg/dl) (Cory-Slechta et al., 2010). Learning was evaluated with multiple schedule of repeated learning (RL) and performance testing. Repeated learing was impaired but performance was not affected with Pb exposure. The impaired RL was further enhanced with prenatal stress. There were significant associations between Pb/stress and coticosterone concentration, dopamine from the frontal cortex, dopamine turnover in the nucleus accumbens, and total number of responses required to learn a sequence. Also Pb exposed offspring with and without maternal stress exposure had significant decreases in hippocampal nerve growth factor (NGF) versus control. Thus, this study demonstrates that lifetime Pb exposure with or without prenatal stress induced learning deficits in female mice. In a similar study, the authors proposed that associations of Pb and stress with learning deficits (F_1 testing in females) may be related to

aberrations in corticosterone and dopamine (Rossi-George et al., 2011). Earlier work has shown that dam or prenatal stress (PS) affects the HPA axis of the offspring. A newer study was conducted to determine the influence of low level dam Pb exposure and prenatal stress on offspring stress challenge responsivity (intermittent stress as an adult) (Rossi-George et al., 2011). In a similar study, the authors proposed that associations of Pb and stress with learning deficits (F1 testing in females) may be related to aberrations in corticosterone and dopamine (Rossi-George et al., 2011). Dam Pb exposure (50 or 150 ppm Pb acetate) followed by intermittent stressors (cold, novelty or restraint) to offspring as adults induced significant changes in FI response rate. Females were more sensitive to the adult intermittent stressors at the higher dose of Pb (150 ppm) with significant increases in FI response rate and decreased PRP, i.e. increased impulsivity (Figure 5-14). Males were more sensitive (decreased FI response rate due to decreased run rate) to the restraint stress at the lower Pb dose (50 ppm). At the higher dose of Pb, males were more sensitive to the cold stress (increased FI response rate and increased run rate) (Figure 5-15). Corticosterone levels were followed in this study and showed dose dependent correlations with FI outcomes in females but were independent of dose in males.

Table 5-8. Summary of effects of maternal and lifetime Pb exposure on FI performance water[a].

Pb (ppm)	Maternal Pb[b]		Lifetime Pb[c]	
	Overall rate	PRP	Overall rate	PRP
0 ppm				
0-PS	NO SIGNIFICANT EFFECT[d]	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

Note: *Dam blood Pb levels ranged from 5-13 µg/dL over gestation and lactation; offspring blood Pb ranged from 7-13 µg/dL from early life time points out to ten months of age. Thus, this study demonstrates that lifetime Pb exposure with or without prenatal stress induced learning deficits in female mice. Mechanistically, these authors propose that associations of Pb and stress with learning deficits may be related to aberrations in corticosterone and dopamine.

[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). *Denotes significant effect versus 0ppm control (p<0.05).
[c]Data from current study. *Denotes significant effect versus 0ppm control (p<0.05).

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

Cognitive Flexibility

Cognitive flexibility is the ability to reallocate mental resources when situations change (Monsell, 2003). Discrimination reversal learning is used to measure cognitive flexibility, which is a subclass of executive function. The 2006 AQCD reported reversal learning deficits in monkeys with blood Pb of 11-20 µg/dL. Rats also showed similar deficits but the authors attributed the changes to learning related problems instead of cognitive flexibility (Garavan et al., 2000; Hilson & Strupp, 1997). Interestingly, recent work has shown that NMDA receptors and D2-like receptors, two known targets of Pb, are involved in discrimination reversal learning (Herold, 2010). Another test of cognitive flexibility is called concurrent random interval (RI-RI) scheduling in which depression on two response levers is reinforced at different frequencies. The 2006 AQCD reported monkeys with cognitive flexibility impairment under RI-RI (Newland et al., 1994).

Selective Attention

Few animal toxicology studies measure selective attention. Those that do employ signal detection with distraction, a test looking for increased omissions after exposure to an external distraction. The newest publication in this area showed no effect with this test after juvenile through adolescence exposure in female rats (Stangle et al., 2007). The two dose groups yielded blood Pb levels of 13 µg/dl and 31ug/dl (Stangle et al., 2007).

5.3.2.3. Toxicological Studies on the Effects of Chelation

Earlier work in the animal toxicology literature has shown that succimer or chelation treatment of Pb exposed lab animals was able to normalize various aberrant Pb-induced behaviors including activity level, habituation (Gong & Evans, 1997) and forced-swim immobility (P. W. Stewart et al., 1996). A more recent study looked at the effect of succimer treatment on various neurobehaviroal and neurocognitive outcomes in control and neonatally Pb-exposed female animals (PND 1-30 Pb acetate exposure, 300 ppm dam through lactation and either 30 or 300 ppm pup water) by drinking water, generating a moderate Pb (m-Pb) exposure and a high Pb (h-Pb) exposure group. Pb blood levels at PND52 in the control, m-Pb, h-

Pb, m-Pb+succimer, and h-Pb+succimer are 1.5, 12.6, 31, 2.8, and 8.5 µg/dL, respectively; brain Pb levels at the same time for the same groups are 41, 1040, 3690, 196, and 1370 ng/g dry weight, respectively. Succimer treatment significantly attenuated the m-Pb induced impaired learning ability. Effects on arousal that were significantly affected in h-Pb rats were significantly attenuated with succimer treatment. Succimer treatment in the h-Pb animals only slightly improved learning ability and did not improve the impaired inhibitory control (Stangle et al., 2007). These are important findings because they provide evidence that certain adverse neurobehavioral or cognitive outcomes associated with Pb exposure appear to be reversible with chelation therapy.

A 3-week course of Pb acetate (PND 1-17, dam drinking water) plus or minus succimer/chelator (PND 31–52) treatment was performed to determine if succimer could alleviate behavioral deficits in rats exposed to Pb for the first 4 weeks of life. Pb-exposed animals had altered reactivity and increased reward omission and errors. Pb-exposed animals receiving chelation therapy had normalized reactivity to reward omission and errors (Beaudin et al., 2007). Adverse Pb-induced behavioral outcomes were attenuated with chelation therapy in Pb-exposed animals.

Another study (G. Fan et al., 2009) looked at methionine choline supplementation in Pb exposed animals to understand its effect on Pb disposition in various tissues (blood, bone, brain) and how this might contribute to neurocognitive or neurobehavioral changes. As a sulfur source, methionine is a chelator and a free-radical scavenger. Choline is important for cell membranes and neurotransmitter synthesis (Zeisel & Blusztajn, 1994). In this model, methionine choline attenuated Pb-dependent memory and learning defecits (Section 5.3.2.2). Exposure of weanling male rats to Pb acetate in drinking water (300 mg/L) out to PND60 produced a blood Pb level of 60 µg/dL, bone Pb of 165 µg/g and brain Pb of 0.63 µg/g. Methionine choline supplementation significantly attenuated blood Pb and bone Pb but produced a non-significant attenuation of brain Pb (0.51 µg/g) in mice that had significant improvements in learning and memory (Section 5.3.2.2).

Also, in another study the metal chelators DP-109 and DP-460 are neuroprotective in the ALS mouse neurodegenerative model or Tg(SOD1-G93A) (Petri et al., 2007).

In summary, succimer or chelation therapy appears to be able to restore Pb-dependent impairments of learning and arousal as well as being neuroprotective in a dose dependent fashion. In these studies succimer use was most efficacious at lower doses of Pb exposure. Chelation does not restore Pb-dependent impaired inhibitory control. Chelation with the supplement methionine choline affected the disposition of Pb in various tissues, significantly attenuating blood and bone Pb levels and non-significantly attenuating brain Pb.

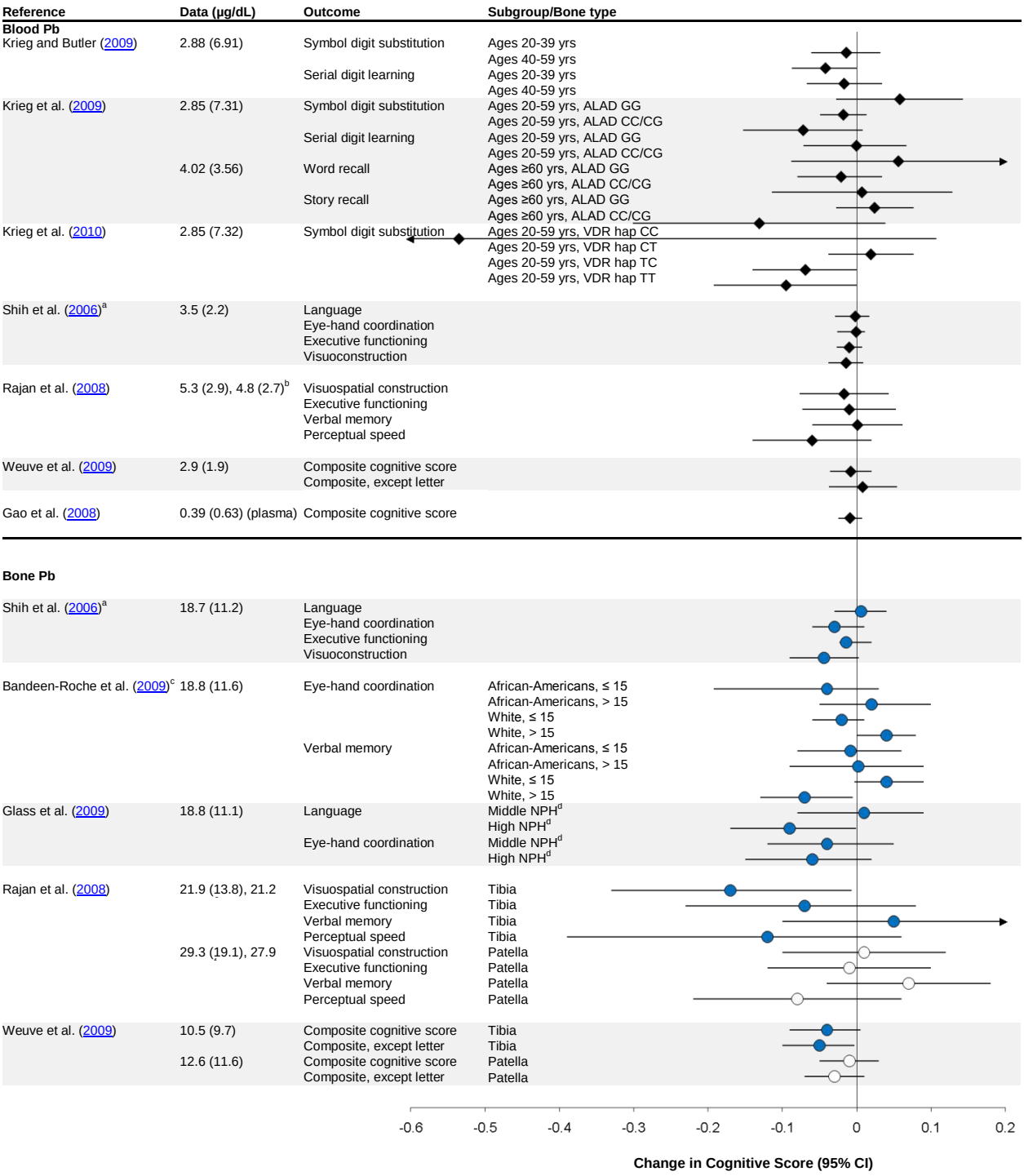
5.3.2.4. Epidemiologic Studies of Cognitive Function in Adults

Adults without Occupational Lead Exposures

The 2006 AQCD cited more consistent associations of bone Pb levels but not concurrent blood Pb levels with cognitive performance in environmentally-exposed adults (U.S. EPA, 2006). Studies published since the 2006 Pb AQCD continue to support the previous findings. Several studies analyzed data from NHANES III (1991-1994) and investigated effect modification by age and genetic variants. Krieg et al. (2009) overall did not find blood Pb level to be associated consistently with poorer performance on cognitive testing among 2,090 adults 20-59 years of age nor among 1,796 adults 60 years of age and older. There were also few statistically significant differences in the association with blood Pb between ALAD genotypes (E. F. Krieg, Jr. et al., 2009). Among the 20-59 year-old adults, a borderline significant difference was observed for mean reaction time, however, among subjects in the CC and CG ALAD genotype groups combined (ALAD2 carriers in the terminology above) (n=161), reaction time improved (i.e., faster reaction time) with increasing blood Pb level. In contrast, ALAD2 subjects had a greater increase in the number of errors on a symbol-digit substitution task ($p = 0.07$) in association with increasing blood Pb level. In the same study, contrasting observations were made in adolescent NHANES participants. Effect modification by ALAD2 is not entirely clear as it may increase susceptibility to Pb-associated health effects by increasing blood Pb levels or diminish Pb bioavailability by maintaining it in a sequestered state in the bloodstream. Krieg et al. (2010) also found differences in the association between blood Pb level and scores on a symbol-digit substitution test by the VDR variants, rs731236 and VDR rs2239185, as well as VDR haplotype. Similar to observations in children (Section 5.3.2.1), results were not consistent across the various tests. However, blood Pb level generally was associated with greater decrements in cognitive performance among adults with the CC genotypes of VDR variants.

Shih et al. (2006) studied participants in the Baltimore Memory Study (BMS), a longitudinal study of men and women 50-70 years of age residing in Baltimore, MD with a mean (SD) blood Pb level of 3.46 (2.23) µg/dL. A total 1,140 out of 2,351 (48.5%) subjects participated from neighborhoods that represented a diversity of race and SES. Of these, 991 had complete data for blood and tibia bone Pb, cognitive testing, and covariates. After excluding the three participants with the highest bone Pb values, the two participants with the two most negative bone Pb values, and one person with a blood Pb level 10 standard deviations greater than the mean, the analytic sample was 985. Scores on individual cognitive tests from a battery of 20 in-person administered tests were grouped into different cognitive domains (language, processing speed, eye-hand coordination, executive function, verbal memory and learning, visual memory, and visuoconstruction) by transforming individual test scores into z-scores and averaging. Negative associations were observed more consistently for tibia Pb levels than for blood Pb levels (Figure 5-16 and Table 5-9). Tibia bone Pb levels were associated with worse performance on tests in all domains

in models adjusted for age, sex, testing technician, and presence of the apolipoprotein (APO)E-ε4 allele. The magnitudes and statistical significance of associations were attenuated with additional adjustment for education, race, and wealth. In thesefully-adjusted models, all domains except language and processing speed were negatively associated with tibia bone Pb, but only the association with visuoconstruction was borderline statistically significant. Analysis of a quadratic term for tibia Pb indicated no evidence of nonlinearity, thus results for linear models were presented. In linear models, visuoconstruction scores decreased by 0.0044 SDs (95% CI: -0.0091, 0.0003) per 1 μg/g bone increase in tibia Pb level. The mean (SD) tibia Pb in this group was 18.7 (11.2) μg/g bone. Of particular note in this study is that it was the first such study with a large proportion of African-Americans (n=395). In a subsequent analysis, increasing tibia Pb levels were associated with a greater decrease in cognitive performance among subjects living in neighborhoods with a greater number psychosocial hazards (Glass et al., 2009) (Figure 5-16 and Table 5-9).



Note: Black diamonds = blood Pb, blue circles = tibia Pb, white circles = patella Pb. ALAD = aminolevulinate dehydratase, VDR = vitamin D receptor, NPH = neighborhood psychosocial hazard. [a]Effect estimates for Model B are presented. [b]Blood Pb levels represent levels in ALAD wildtype and ALAD2 carriers, respectively. [c]Effect estimates for the following strata: African-Americans with tibia Pb levels ≤ 15 µg/dL, African-Americans with tibia Pb levels >15 µg/dL, whites with tibia Pb levels ≤ 15 µg/dL, and whites with tibia Pb levels >15 µg/dL. [d]Effect estimates for the interaction between tibia Pb levels and NPH tertile, with the lowest NPH tertile serving as the reference group. [e]Tibia Pb levels represent levels in ALAD wildtypes and ALAD2 carriers, respectively. [f]Patella Pb levels represent levels in ALAD wildtypes and ALAD2 carriers, respectively.

Figure 5-16. Associations of blood and bone Pb levels with cognitive function among adults without occupational exposures to Pb.

Table 5-9. Additional characteristics and quantitative results for studies presented in Figure 5-16

Study	Population/ Location	Pb Biomarker Data	Statistical Analysis	Cognitive Test	Subgroup/ Model	Effect Estimate (95% CI)[a]
Blood Pb Studies						
Krieg and Butler (2009)	2823 adults, ages 20-59 yr, U.S. NHANES III (1991-1994)	Blood mean (SD): 2.88 (6.91) µg/dL	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	Symbol Digit Substitution (mean total latency, sec) Serial digit learning score Assessed using the Neurobehavioral Evaluation System 2	Ages 20-39 yr Ages 40-59 yr Ages 20-39 yr Ages 40-59 yr	-0.014 (-0.061, 0.032)b -0.042 (-0.087, 0.0009)b -0.017 (-0.067, 0.034)b 0.058 (-0.028, 0.143)b
Krieg et al. (2009)	2090 adults, ages 20-59 yr 1976 adults, ages ≥60 yr U.S. NHANES III (1991-1994)	Blood mean (SD): 20-59 yr: 2.85 (7.31) µg/dL ≥60 yr: 4.02 (3.56) µg/dL	Log linear regression model adjusted for sex, age, education, family income, race-ethnicity, computer or video game familiarity, alcohol use in the last 3 hrs, test language (20-59 yr) and sex, age, education, family income, race-ethnicity, test language (≥60 yr)	Symbol Digit Substitution (mean total latency, sec) Serial digit learning score Word recall Story recall Assessed using the Neurobehavioral Evaluation System 2	Ages 20-59 yr ALAD GG ALAD CC/CG Ages 20-59 yr ALAD GG ALAD CC/CG Ages ≥60 yr ALAD GG ALAD CC/CG Ages ≥60 yr ALAD GG ALAD CC/CG	 -0.018 (-0.049, 0.013)b -0.072 (-0.153, 0.008)b -0.003 (-0.072, 0.067)b 0.056 (-0.088, 0.201)b -0.021 (-0.08, 0.034) 0.007 (-0.114, 0.129) 0.024 (-0.028, 0.077) -0.131 (-0.301, 0.039)
Krieg et al. (2010)	2093 adults, ages 20-59 yr 1799 adults, ages ≥60 yr U.S. NHANES III (1991-1994)	Blood mean (SD): 20-59 yr: 2.85 (7.32) µg/dL ≥60 yr: 4.02 (3.39) µg/dL	Log linear regression model adjusted for sex, age, education, family income, race-ethnicity, computer or video game familiarity, alcohol use in the last three hours, test language (20-59 yr) and sex, age, education, family income, race-ethnicity, test language (≥60 yr)	Symbol Digit Substitution (mean total latency, sec) Serial digit learning score (# errors, pos is bad) Word recall Story recall Assessed using the Neurobehavioral Evaluation System 2	Ages 20-59 yr VDR haplotype CC VDR haplotype CT VDR haplotype TC VDR haplotype TT Ages 20-59 yr VDR haplotype CC VDR haplotype CT VDR haplotype TC VDR haplotype TT Ages ≥60 yr VDR haplotype CC VDR haplotype CT VDR haplotype TC VDR haplotype TT Ages ≥60 yr VDR haplotype CC VDR haplotype CT VDR haplotype TC VDR haplotype TT	 -0.535 (-1.18, 0.107)b 0.019 (-0.038, 0.077)b -0.069 (-0.140, 0.002)b -0.095 (-0.192, 0.001)b -0.346 (-0.665, 0.026)b -0.044 (-0.126, 0.039)b 0.061 (0.074, 0.135)b 0.006 (-0.107, 0.119)b -0.226 (-0.536, 0.087) -0.028 (-0.118, 0.067) -0.010 (-0.139, 0.115) -0.028 (-0.264, 0.209) 0.043 (-0.494, 0.581) 0.001 (-0.057, 0.060) 0.010 (-0.095, 0.116) -0.049 (-0.128, 0.064)
Shih et al. (2006)	985 adults, mean age: 59 yr Baltimore Memory Study, Baltimore, MD	Blood mean (SD): 3.5 (2.2) µg/dL	Linear regression adjusted for: Model A: age, sex, technician, presence of APOE-ε4 allele Model B: Model I, years of education, race/ethnicity, wealth	Language Eye-hand coordination Executive functioning Visuoconstruction Assessed using Raven's Colored Progressive Matrices	Model A Model B Model A Model B Model A Model B Model A Model B	-0.0060 (-0.029, 0.017) -0.0019 (-0.020, 0.016) -0.0110 (-0.032, 0.01) -0.0076 (-0.026, 0.011) -0.0143 (-0.034, 0.005) -0.0101 (-0.027, 0.007) -0.0191 (-0.046, 0.008) -0.0143 (-0.038, 0.009)
Rajan et al. (2008)	720 males, ages ≥45 yr Normative Aging Study, Boston, MA	Blood mean (SD): 5.3 (2.9) µg/dL (ALAD wildtype) 4.8 (2.7) µg/dL (ALAD2 carriers)	Linear regression adjusted for blood Pb main effect, ALAD genotype, age at cognitive test, education, alcohol consumption, cumulative smoking, English as first language	Visuospatial, constructional praxis Executive function verbal fluency Verbal memory, word recall Perceptual speed, mean latency Assessed using CERAD, Neurobehavioral Evaluation Sysem, WIAS-R		-0.017 (-0.077, 0.043)c -0.01 (-0.073, 0.053)c 0.001 (-0.06, 0.062)c -0.06 (-0.14, 0.02)c

Study	Population/ Location	Pb Biomarker Data	Statistical Analysis	Cognitive Test	Subgroup/ Model	Effect Estimate (95% CI)[a]
Weuve et al. (2009)	587 females, ages 47-74 yr Nurses' Health Study, Boston, MA	Blood mean (SD): 2.9 (1.9) µg/dL	Generalized estimating equations adjusted for age, age-squared at Pb assessment, age at cognitive assessment, education, husband's education, alcohol consumption, smoking status, physical activity, aspirin use, ibuprofen use, use of Vitamin E supplements, menopausal status and postmenopausal hormone use	Composite cognitive score Composite except letter fluency Assessed using Telephone Interview for Cognitive Status and East Boston Memory Test		-0.008 (-0.036, 0.020) 0.008 (-0.037, 0.054)
Gao et al. (2008)	188 adults, mean age 69.2 yr Sichuan and Shandong Provinces, China	Plasma mean (SD): 0.39 (0.63) µg/dL	ANCOVA adjusted for age, sex, education, BMI, APOE ε4	Composite cognitive score Assessed using CERAD, CSID, IU story recall, Animal fluency test, IU token test		-0.009 (-0.025, 0.007)
Excluded From Figure Due Insufficient Data To Standardize Test Scores						
Weuve et al. (2006)	720 males, ages ≥45 yr Normative Aging Study, Boston, MA	Blood mean (range): 5.2 (≤1-28) µg/dL	Linear mixed effects regression adjusted for smoking status, alcohol consumption, calorie adjusted calcium intake, regular energy expenditure on leisure time physical activity, diabetes	MMSE score	ALAD wildtypes ALAD2 carriers	-0.013 (-0.053, 0.027) -0.087 (-0.180, 0.007)
Bone Pb Studies						
Shih et al., (Shih et al.)	985 adults, mean age: 59 yr Baltimore Memory Study, Baltimore, MD	Tibia mean (SD): 18.7 (11.2) µg/g	Linear regression adjusted for: Model A: age, sex, technician, presence of APOE-ε4 allele Model B: Model I, years of education, race/ethnicity, wealth	Language Eye-hand coordination Executive functioning Visuoconstruction Assessed using Raven's Colored Progressive Matrices	Model A Model B Model A Model B Model A Model B Model A Model B	-0.08 (-0.13, -0.04) 0.006 (-0.03, 0.04) -0.08 (-0.12, -0.04) -0.03 (-0.06, 0.01) -0.08 (-0.11, -0.04) -0.014 (-0.05, 0.02) -0.022 (-0.17, -0.07) -0.044 (-0.09, 0.003)
Bandeen-Roche et al. (2009)	1140 adults, ages 50-70 yr Baltimore Memory Study cohort Baltimore, MD	Tibia mean (SD): 18.8 (11.6) µg/g	Marginal longitudinal linear regression models adjusted for age, household wealth, education, race/ethnicity Demographic characteristics, socioeconomic status, race/ethnicity	Eye hand coordination African-Americans White Verbal memory African-Americans White Visual memory African-Americans White	 Pb ≤ 15 µg/g Pb >15 µg/g Pb ≤ 15 µg/g Pb >15 µg/g Pb ≤ 15 µg/g Pb >15 µg/g Pb ≤ 15 µg/g Pb >15 µg/g Pb ≤ 15 µg/g Pb >15 µg/g Pb ≤ 15 µg/g Pb >15 µg/g	 -0.04 (-0.112, 0.03) 0.02 (-0.05, 0.10) -0.02 (-0.06, 0.01) 0.04 (-0.002, 0.08) -0.008 (-0.08, 0.06) 0.002 (-0.09, 0.09) 0.04 (-0.003, 0.09) -0.07 (-0.13, -0.005) 0.001 (-0.007, 0.09) -0.0009 (-0.011, 0.09) 0.003 (-0.003, 0.08) -0.004 (-0.011, 0.03)
Glass et al. (2009)	1001 adults, mean age 59 yr Baltimore Memory Study, Baltimore, MD	Tibia Pb mean (SD): 18.8 (11.1) µg/g	Multilevel hierarchical regression model adjusted for age, sex, race/ethnicity, education, testing technician, time of day	Language Eye-hand coordination Executive functioning Visuoconstruction Assessed using Raven's Colored Progressive Matrices	Middle NPH High NPH Middle NPH High NPH Middle NPH High NPH Middle NPH High NPH	0.01 (-0.08, 0.09)d -0.09 (-0.17, -0.001)d -0.04 (-0.12, 0.05)d -0.06 (-0.15, 0.02)d -0.02 (-0.10, 0.06)d -0.10 (-0.18, -0.02)d -0.003 (-0.14, 0.08)d -0.006 (-0.17, 0.05)d

Study	Population/ Location	Pb Biomarker Data	Statistical Analysis	Cognitive Test	Subgroup/ Model	Effect Estimate (95% CI)[a]
Rajan et al. (2008)	720 males, ages ≥45 yr Normative Aging Study, Boston, MA	Mean (SD): Tibia: 21.9 (13.8) µg/g (ALAD wildtype), 21.2 (11.6) µg/g (ALAD2 carriers) Patella: 29.3 (19.1) µg/g (ALAD wildtype), 27.9 (17.3) µg/g (ALAD2 carriers)	Linear regression adjusted for bone Pb main effect, ALAD genotype, age at cognitive test, education, alcohol consumption, cumulative smoking, English as first language	Visuospatial, constructional praxis Executive function verbal fluency Verbal memory, word recall Perceptual speed, mean latency Assessed using CERAD, Neurobehavioral Evaluation System, WIAS-R	Tibia Patella Tibia Patella Tibia Patella Tibia Patella	-0.17 (-0.33, -0.007) 0.01 (-0.10, 0.12) -0.07 (-0.23, 0.08) -0.01 (-0.12, 0.10) 0.05 (-0.10, 0.21) 0.07 (-0.40, 0.18) -0.12 (-0.39, 0.06) -0.08 (-0.22, 0.06)
Weuve et al. (2009)	Nurses' Health Study cohort 587 subjects Age range: 47-74 yr All females Boston, MA	Tibia mean (SD): 10.5 (9.7) µg/g Patella mean (SD): 12.6 (11.6) µg/g	Generalized estimating equations adjusted for age, age-squared at Pb assessment, age at cognitive assessment, education, husband's education, alcohol consumption, smoking status, physical activity, aspirin use, ibuprofen use, use of Vitamin E supplements, menopausal status and postmenopausal hormone use	Composite cognitive score Composite except letter fluency Assessed using Telephone Interview for Cognitive Status and East Boston Memory Test	Tibia Patella Tibia Patella	-0.04 (-0.09, 0.005) -0.01 (-0.05, 0.03) -0.05 (-0.10, -0.003) -0.03 (-0.07, 0.01)
Excluded From Figure Due Insufficient Data To Standardize Test Scores Or Calculate Effect Estimates						
Weisskopf et al. (2007)	1089 males, mean age 68.7 yr Normative Aging Study, Boston, MA	Mean (IQR): 20 (13-28) µg/g (Tibia) 25 (17-37) µg/g (Patella)	Linear repeated measures analysis adjusted for age, age squared, education, smoking, alcohol intake, yr between bone Pb measurement and first cognitive test, yr between cognitive tests	Visuospatial, pattern comparison (pos is bad, latency) Executive function verbal fluency Short-term memory, word list Assessed using CERAD, Neurobehavioral Evaluation System, WIAS-R, MMSE, VMI	Tibia Patella Tibia Patella Tibia Patella	0.79 (0.40, 1.2)e 0.73 (0.40, 1.2)e -0.40 (-1.6, 0.80)e -0.86 (-2.00, 0.30)e -0.28 (-1.2, 0.60)e -0.81 (-1.7, 0.05)e
Weuve et al. (2006)	720 males, ages ≥45 yr Normative Aging Study, Boston, MA	Median (1st-3rd quartile): Tibia: 19 (13-28) µg/g Patella: 27 (18-39) µg/g	Linear mixed effects regression adjusted for smoking status, alcohol consumption, calorie adjusted calcium intake, regular energy expenditure on leisure time physical activity, diabetes	MMSE score	Tibia ALAD wildtype ALAD2 carrier Patella ALAD wildtype ALAD2 carrier	 -0.03 (-0.14, 0.07) -0.11 (-0.30, 0.06) -0.03 (-0.11, 0.04) -0.12 (-0.30, 0.06)
Wang et al. (2007)	358 males, median ages: 67.2 yr (HFE wild-type) 67.7 yr (HFE variant) Normative Aging Study, Boston, MA	Median: 19 µg/g (Tibia), 23 µg/g (Patella)	Linear regression adjusted for age, years of education, nonsmoker, former smoker, pack-years, nondrinker, alcohol consumption, English as first language, computer experience, diabetes	MMSE	HFE wildtype One HFE variant Two HFE variants	-0.20 (-1.0, 0.70)e -1.40 (-3.3, 0.40)e -6.3 (-10.4, -2.1)e

Study	Population/ Location	Pb Biomarker Data	Statistical Analysis	Cognitive Test	Subgroup/ Model	Effect Estimate (95% CI)[a]
Van Wijngaarden et al. (2009)	47 adults, mean age 61.5 yr Rochester, NY	Mean (SD): 2.0 (5.2) µg/g (Tibia) 6.1 (8.5) µg/g (Calcaneus)	Linear regression adjusted for age, gender, educational level, history of hypertension	Delayed matching, % correct Lowest tertile Medium tertile Highest tertile Lowest tertile Medium tertile Highest tertile Total trials Lowest tertile Medium tertile Highest tertile Lowest tertile Medium tertile Highest tertile Assessed using CANTAB and Montreal Cognitive Assessment	Calcaneus Tibia Calcaneus Tibia 	87.56f 86.67 80.67, p = 0.027 85.42f 87.08 82.44, p = 0.25 2.54f 2.61 2.72, p = 0.21 2.62f 2.59 2.66

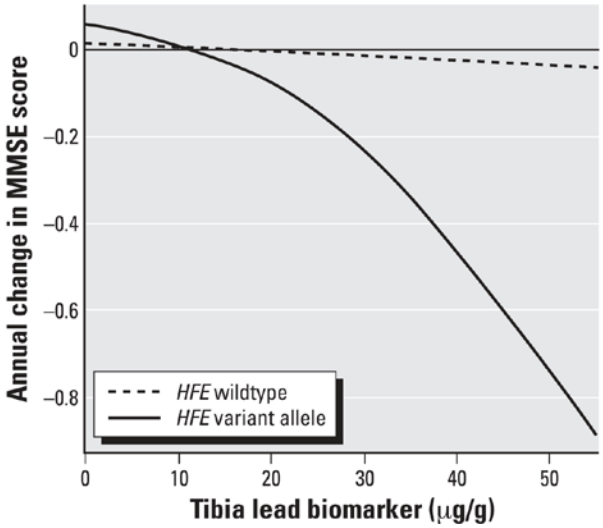
[a]Effect estimates have been standardized to the standard deviation of the cognitive test scores and standardized to a 1 µg/dL increase in blood Pb and 10 µg/g increase in bone Pb.
[b]The directions of effect estimates were changed to indicate a negative slope as a decrease in cognitive performance.
[c]Effect estimates indicate interactions between Pb and ALAD genotype.
[d]Effect estimates indicate interactions between Pb and category of neighborhood psychosocial hazard (NPH), with the lowest tertile of NPH serving as the reference group.
[e]Effect estimate refers to the change in cognitive function score over time.
[f]Results refer to mean cognitive function scores among tertiles of bone Pb.

Weuve et al. (2009) studied the association of blood and bone Pb levels with cognitive function among a subset of 587 women from the Nurses Health Study from whom blood and bone Pb measurements were taken between the ages of 47 and 74 years. The mean (SD) blood Pb level in this group was 2.9 (1.9) µg/dL. The women had had Pb measured as part of their participation in two separate sub-studies, one of osteoporosis and the other of hypertension. The inclusion criteria included residence in the Boston area and absent of major chronic diseases at the time of Pb measurement. Cognitive function was assessed via a battery of telephone administered tests (Telephone Interview of Cognitive Status, digit span backwards, alphabetizing span, animal naming [category fluency], "f" naming [letter fluency], and a composite verbal memory score) an average of 5 years after Pb measurement. As in the aforementioned studies of adults, negative associations were observed more consistently for tibia and patella Pb levels than for blood Pb levels (Figure 5-16 and Table 5-9). Contrary to expectation, scores on the "f" naming test were positively associated with patella and tibia bone Pb levels. In separate models, the "f" naming test was omitted from a composite index of all cognitive tests, and a one SD (10 µg/g bone) increase in tibia Pb level was associated with 0.051-point decrease (95% CI: -0.010, -0.003) in the standardized composite score. This effect size of tibia bone Pb level was approximately equivalent to the effect size for 3 years of age among these women. The magnitude of effect was smaller for patella Pb levels (-0.033 [95% CI: -0.080, 0.014) per 1 SD unit increase in patella Pb level)

Several analyses of Normative Aging Study (NAS) have contributed to greater understanding of the relationship between biomarkers of Pb dose and neurocognitive effects in adults. The NAS is conducted at the VA Outpatient Clinic in Boston, MA and is a multidisciplinary longitudinal investigation of the

aging process that was established in 1961 and originally involved 2,280 men residing in the Greater Boston area aged 21 to 80 years with no current or past chronic medical conditions. Participants are evaluated every 3-5 years with in-person clinical assessments, self-administered questionnaires. In-person administered cognitive tests included those in the general domains of attention/executive function, short-term memory, visuospatial, and verbal/language. About 60-70% of participants, depending on the specific cognitive test, took individual tests twice approximately 3.5 years apart. The average (SD) age of the men at baseline was 69 (± 7) years, and the median blood Pb level was 5 µg/dL. Beginning in 1991, measurement of bone Pb levels began, with 68% of active NAS subjects participating. All analyses were adjusted for age, age squared, education, smoking, and alcohol intake. In one study of Mini-Mental State Examination (MMSE) tests scores among 720 men 45 years of age and older, blood Pb levels were negatively associated with MMSE score among ALAD2 carriers (Weuve et al., 2006). A 3 µg/dL increment in blood Pb level (the interquartile range) was associated with a 0.26 point lower mean MMSE score (95% CI: -0.54, -0.01) among ALAD2 carriers and a 0.04 point lower score (95% CI: -0.16, -0.07) among noncarriers. A subsequent study in the same cohort considered performance on a battery of oher cognitive tests and did not find a consistent pattern of modification of the association between blood or bone Pb levels and cognitive function by ALAD genotype (Rajan et al., 2008). Nonetheless, Rajan et al. (2008) found tibia Pb levels to be associated more consistently with lower cognitive performance compared with blood or patella Pb levels (Figure 5-16 and Table 5-9).

The NAS also examined effect modification by hemochromatosis (HFE) gene variants. In models adjusted for age, years of education, nonsmoker, former smoker, pack-years, nondrinker, alcohol consumption, English as first language, computer experience, and diabetes, an interquartile range increase in tibia bone Pb level (15µg/g) was associated with a 0.22 point steeper annual decline (95% CI: -0.39, -0.05) in MMSE score among men with any variant HFE allele (either H63D or C282Y). The magnitude of association was less negative among those with only HFE wildtype alleles (F. T. Wang et al., 2007) (Figure 5-17). As indicated in Figure 5-17, a nonlinear association was observed between tibia Pb levels and change in MMSE score, with a steeper decline per unit increase in tibia Pb level at higher tibia Pb levels. Effect modification by nongenetic factors also were examined in the NAS cohort. Increasing bone Pb levels were associated with greater decreases in cognitive function among individuals with higher individual-level perceived stress (Peters et al., 2007).



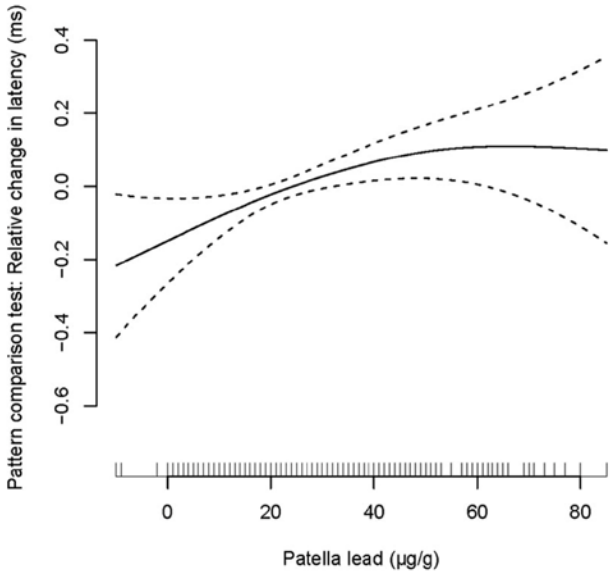
Source: Wang et al. (2007).

Note: The lines indicate curvilinear trends estimated from the penalized spline method. Among HFE wild-types, the optimal degree of smoothing was 1, meaning that the association between tibia Pb and annual cognitive decline was nearly linear, but among variant allele carriers, the association tended to deviate from linearity (p = 0.08), with an optimal 1.68 degree of smoothing. The model was adjusted for age, years of education, nonsmoker, former smoker, pack-years, nondrinker, alcohol consumption, English as first language, computer experience, and diabetes.

Figure 5-17. Exploration of nonlinear association of tibia Pb concentration with annual rate of cognitive decline, by class of HFE genotype.

Two large NAS studies examined the relationship of blood and bone Pb levels with the change in cognitive function over time. Weisskopf et al. (2007) expanded on an earlier study of Pb biomarkers and cognitive function (Payton et al., 1998) The average (SD) age of the men at baseline was 69 (±7) years, and the median blood Pb concentration was 5 μg/dL. Two measurements of cognitive function, collected approximately 3.5 years apart, were available 60-70% of participants. All analyses were adjusted for age, age squared, education, smoking, and alcohol intake. There was little association between blood Pb levels and cognitive test scores, except possibly for vocabulary scores, although this association was greatly weakened when the five men with the highest blood Pb levels (>15 μg/dL) were excluded. For bone Pb analyses, the authors used repeated measures analysis with an interaction term between bone Pb level and time in order to estimate the association between bone Pb level and decline in cognitive test score over time. There were no main effect associations with bone Pb levels; however, increases in patella and tibia bone Pb levels were associated with decreases in cognitive performance over time. The only test for which this reached statistical significance ($p<0.05$), however, was increased response latency on a pattern comparison test. Contrary to expectation, bone Pb levels were associated with fewer errors on the same pattern comparison test. The authors proposed that this may be related to slowing reaction time to improve accuracy. When the 9 men with the highest bone Pb levels were removed, the association was no longer statistically significant.

In an examination of the patella Pb concentration-reaction time relationship, Weisskopf et al. (2007) found a statistically significant nonlinear association, with latency times on the pattern comparison test becoming worse over time (i.e., larger values or slower response latencies) up to approximately 60 µg/g bone mineral, but the change over time leveling off at higher concentrations (Figure 5-18). Below 60 µg/g, a 20 µg/g difference in patella Pb level was associated with a decrease in pattern comparison test score of approximately 0.15 ms. Intriguingly, however, the cognitive tests where the association with bone Pb was significantly worse among ALAD-2 carriers (constructional praxis and pattern comparison) were the same tests that were significantly associated with bone Pb in an earlier study (Weisskopf, Proctor, et al., 2007).



Source: Used with permission from Williams & Wilkins, Weisskopf et al. (2007).

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 concentrations (>89 µg/g bone mineral) were removed. The estimate is indicated by the solid line and the 95% confidence interval by the dashed lines. Patella Pb concentrations of all individual subjects are indicated by short vertical lines on the abscissa.

Figure 5-18. Nonlinear association between patella bone Pb concentration and the relative change in response latency over time on the pattern comparison test (reference = 0 at mean of patella Pb concentration).

In a somewhat similar approach to that taken in the NAS, BMS investigators took advantage of repeat cognitive testing of study subjects (91% of the original cohort returned for a second round of testing and 83% for a third round each at approximately 14-month intervals) to analyze associations of blood and bone Pb levels with changes in cognitive performance over time cohort (Bandeen-Roche et al., 2009). An interquartile range increase in tibia Pb level (12.7 µg/g) was associated with a 0.019 units per

year decrease in eye-hand coordination z-score. The association was somewhat stronger among African-Americans than it was among whites (Figure 5-16 and Table 5-9). Of the other tests, only change in verbal memory and learning suggested some association with tibia Pb levels. In analyses of what they term persistent effects (analogous to the main effects analyses of longitudinal data in the NAS), a similar pattern was found as that reported by Shih et al. (2006) in the original BMS cross-sectional analyses. Increasing tibia Pb levels were associated with decreases in cognitive performance; however, the effect sizes decreased as more covariates were added to models. In models adjusted for age, sex, race, and education, performance on executive function, verbal memory and learning, and visual memory test decreased with increasing tibia Pb levels, with p-values ranging from 0.16 to 0.26. In contrast to the results of the longitudinal analyses described above, race-stratified analyses of persistent effects indicated that tibia Pb levels were associated with greater decreases in performance on tests of eye-hand coordination, executive functioning, and verbal memory and learning among whites compared with African-Americans.

Other studies generally confirm the same pattern as described in the larger studies above. One study of 188 rural Chinese men and women found a weakly negative association between plasma Pb levels and a composite cognitive score based on a battery of in-person administered tests (S. J. Gao et al., 2008). It should be noted, though, that Pb in plasma makes up a very small fraction of all Pb in blood and is a different, and much less used, biomarker than Pb in whole blood. The relevance of this Pb fraction is not entirely clear. Pb in plasma is not bound to erythrocytes, as is about 99% of blood Pb. Thus, it has been postulated that plasma Pb may be more toxicologically active (Chuang et al., 2001; Hernandez-Avila et al., 1998). In another study of 47 men and women in Rochester, NY (55-67 years of age), subjects in the higher two tertiles of calcaneal bone Pb level had lower scores on a delayed matching-to-sample task (van Wijngaarden et al., 2009) (Table 5-9). The pattern was similar for a paired associates learning task, although the results did not reach statistical significance. In analyses of tibia Pb levels, subjects in the highest tertile of tibia Pb level performed worse on cognitive tests (Table 5-9).

In summary, among adults without occupational exposures to Pb, there is weak evidence for an association between blood Pb levels and cognitive function. The strongest evidence of association between blood Pb levels and cognitive function in adults was provided by the various NHANES analyses of various age and genetic variant subgroups (E. F. Krieg, Jr. & Butler, 2009; E. F. Krieg, Jr. et al., 2009; E. F. Krieg, Jr. et al., 2010). These NHANES analyses did not have bone Pb measurements for comparison. Consistent with the conclusion of the 2006 Pb AQCD, recent studies continued to demonstrate associations between bone Pb levels and cognitive deficits in adults (Figure 5-16 and Table 5-9). Recent studies also demonstrated that bone Pb levels may be associated specifically with cognitive decline over time. Among recent studies that analyzed both blood and bone Pb levels, bone Pb levels, in particular tibia Pb levels, were associated with greater decreases in cognitive performance than were blood Pb levels across of the various cognitive tests that were performed. The discrepant findings for

blood and bone Pb levels indicate that biomarkers of cumulative Pb exposure, including higher levels in the past, may the best predictors of neurocognitive function in adults. The effects associated with cumulative Pb exposure are also demonstrated by observations that tibia Pb levels were associated with larger decreases in cognitive performance than were patella Pb levels. Tibia bone has a slower rate of turnover compared with patella bone and is an indicator of longer-term Pb exposure.

Adults with Occupational Lead Exposures

The 2006 Pb AQCD concluded that the evidence for an association between blood Pb levels and cognitive function in adults was most consistent among adults occupationally exposed to Pb. Results from a few recent studies of occupationally-exposed adults support the previous conclusions. Dorsey et al. (2006) followed-up on a cohort of Pb-exposed workers in Korea with a mean age of 43.4 years, on whom patella bone Pb measurements were made. This group represented a typically highly-exposed occupational group with an average blood Pb level of 30.9 μg/dL. In this cohort, both blood and tibia Pb levels previously were associated with poorer performance on a battery of neurocognitive tests (B. S. Schwartz et al., 2005; B. S. Schwartz et al., 2001). Dorsey et al. (2006) found patella Pb levels to be associated with poorer performance in the domains of manual dexterity, sensory PNS function, and depression symptoms. In this occupational cohort, however, the associations between patella Pb levels and cognitive function were not as strong as the associations with either blood or tibia Pb levels.

A follow-up study of the original 1982 Lead Occupational Study was conducted in 2001-2004 among 83 of the original 288 Pb-exposed workers and 51 of the original 181 controls (Khalil, Morrow, et al., 2009). Those originally in the exposed workers group had last worked in a job with Pb exposure from 0.02 to 16 years (median = 6) prior to follow-up testing. While the follow-up participation was somewhat low, participants did not appear to differ from nonparticipants on most baseline cognitive tests except for performing slightly better on aspects of the grooved pegboard test and recall on a paired associates learning task. This suggests that the follow-up participation was not biased to poor performers. At follow-up, the former Pb-exposed workers performed significantly ($p < 0.05$) worse than the controls in total cognitive score and in the spatial and general intelligence domains. They also performed worse in all other domains (e.g., motor, executive, and memory) although not significantly so. A similar pattern was observed in analyses using tibia Pb levels measured at the follow-up visit as the exposure variable. Associations also were seen with blood Pb levels (median among the exposed: 12 μg/dL), although these generally did not reach statistical significance. Among the former Pb workers, tibia Pb levels were associated with a greater decrease in total score and scores for spatial and executive domains between baseline and follow-up. Tibia Pb level were associated inversely with other domains as well, although they were not statistically significant.

Two additional studies aimed to characterize factors that either mediate or modify the association between Pb biomarkers and cognitive function. A study among 61 current Pb smelter workers with an average age of 40 years and blood Pb of 29.1 μg/dL found that both a time-weighted integrated blood Pb measure ($p = 0.09$) and tibia Pb level ($p = 0.08$) were associated with longer times to complete the grooved pegboard test (Bleecker, Ford, Vaughan, et al., 2007). In another study to examine the modifying effects of cognitive reserve (assessed by performance on the Wide Range Achievement Test-R for reading) on the Pb-cognitive function association among 112 Pb smelter workers, a time-weighted integrated blood Pb measure (an index of cumulative exposure) was associated with decrements in motor performance ($p<0.05$), and among those with low cognitive reserve, tests of attention tasks and a digit symbol task as well (Bleecker, Ford, Celio, et al., 2007).

Iwata et al. (2005) examined the association between blood Pb level and aspects of postural sway among 121 Pb-exposed workers in Japan with blood Pb levels between 6 and 89 μg/dL (mean: 40 μg/dL). In multiple regression analyses adjusted for age, height, and smoking and drinking status, increasing blood Pb level was associated with increases in sagittal sway with eyes open ($p <0.05$) and eyes closed ($p <0.01$) and transversal sway with eyes closed ($p <0.05$). The authors calculated a benchmark dose level (Budtz-Jorgensen et al., 2001; NRC, 2000) of 14.3 μg/dL from a linear dose-response model of their data. Although the data were slightly better fit with a supralinear dose-response function, the linear function was also statistically significant.

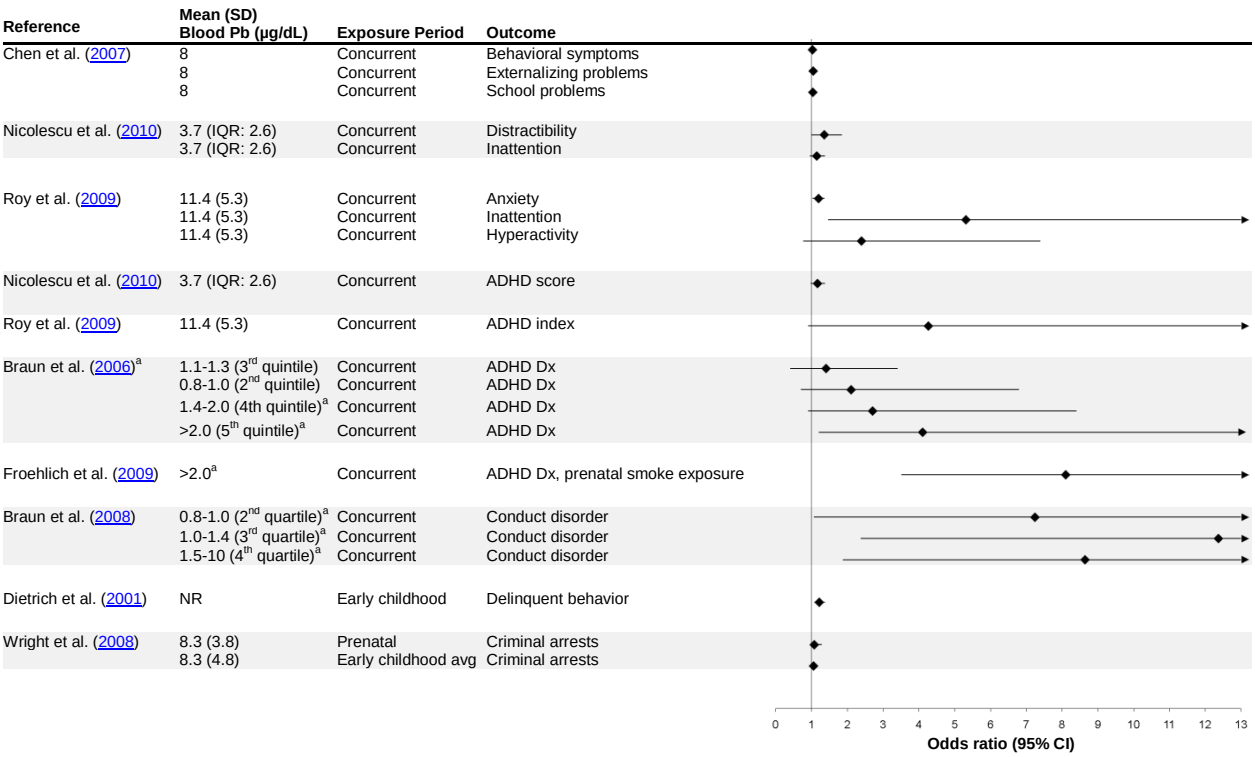
Apolipoprotein E is a transport protein for cholesterol and lipoproteins. The gene appears to regulate synapse formation (connections between neurons) and may be particularly critical in early childhood. A genetic variant, called the ApoE-ε4 allele is a haplotype between 2 exonic SNPs and is perhaps the most widely studied genetic variant with respect to increasing risk of neurologic disease. Carriers of the E4 variant are at twofold increased risk of developing Alzheimer's disease, although the majority of such individuals still do not develop the disease. A study of occupationally-exposed adults found that among individuals with at least one ApoE-ε4 allele, Pb was associated with greater decrements in tests such as digit symbol, pegboard assembly, and complex reaction time (W. F. Stewart et al., 2002).

5.3.3. Neurobehavioral Effects

5.3.3.1. Epidemiologic Studies of Behavioral Effects in Children

Several epidemiologic studies reviewed in the 2006 Pb AQCD reported associations between blood Pb levels and problems with behavior and social conduct that ranged from inattentiveness to self-reported delinquent behaviors to criminal activities (Bellinger et al., 1994; Bellinger & Rappaport, 2002; Burns et al., 1999; Dietrich et al., 2001; Needleman et al., 2002; Needleman et al., 1996; G. A. Wasserman et al., 1998). Recent studies continue to demonstrate similar associations and provide new evidence for

associations of blood Pb levels with ADHD diagnosis and diagnostic indices (Figure 5-19 and Table 5-10). Noncognitive effects of Pb are more complex to study relative to IQ tests. However, domain-specific neuropsychological assessments are advantageous as they may provide greater insight into the underlying CNS damage that may be associated with exposures (e.g., structural, neural system, neurotransmitter) (R. F. White et al., 2009). Most of these studies found that blood or dentin Pb levels measured at an early age (e.g., 2-6 years of age) were associated with behavioral problems later in childhood and early adulthood (7-22 years of age). Most of these studies examined associations with blood Pb levels assessed at one time point; however, even the prospective studies with serial measurements of blood Pb levels, found associations with both prenatal and early childhood blood Pb levels (Dietrich et al., 2001; J. P. Wright et al., 2008) and lifetime average blood Pb levels (Burns et al., 1999). Therefore, uncertainty remained over what was the critical time period of Pb exposure for increasing risk of behavioral problems and misconduct. In many of the studies noted above, blood Pb level also was associated with IQ and other endpoints of cognitive function, thus it was unclear whether blood Pb-associated neurocognitive deficits, poor school performance, and attention problems, in turn, progressed to antisocial and delinquent behavior later in life.



Note: Studies are presented in order of increasing severity of behavioral outcome. Odds ratios are standardized to a 1 µg/dL increase in blood Pb level in analyses of blood Pb level as a continuous variable. IQR = Interquartile range, ADHD = attention deficit hyperactivity disorder, NR = Not reported. [a]Effect estimate compares children in higher quantiles of of blood Pb level, with children in the lowest blood Pb quantile serving as the reference group.

Figure 5-19. Associations of blood Pb levels with behavioral indices in children.

Table 5-10. Additional characteristics and quantitative results for studies presented in Figure 5-19.

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Odds ratio (95% CI)a
Chen et al. (2007)	780 children participating in TLC trial followed between ages 2-7 yr Baltimore, MD; Cincinnati, OH; Newark, NJ; Philadelphia, PA	Concurrent mean (range): 8 (0-26)	Linear regression model adjusted for city, race, sex, language, parental eduction, parental employment, single parent, age at blood Pb measurement, caregiver IQ	Behavioral problems Externalizing problems School problems At age 5-7 yr assessed using CPRS-R, BASC-TRS, and BASC-PRS	1.02 (0.99, 1.06) 1.04 (1.00, 1.07) 1.03 (1.00, 1.06)
Nicolescu et al. (2010)	83 children ages 8-12 yr tested in 2007 Bucharest and Pantelimon, Romania	Concurrent mean (IQR): 3.7 (2.6)	Log-linear regression model adjusted for city, sex, age, computer experience, handedness, eye problems, number of siblings, parental education, prenatal smoking, family psychopathology	Distractibility Inattention At age 8-12 yr assessed using German version of Teacher's Conner's scales	1.35 (0.99, 1.85) 1.14 (0.95, 1.37)
Roy et al. (2009)	756 children ages 3-7 yr tested 2005-2006 Chennai, India	Concurrent mean (SD): 11.4 (5.3)	Linear regression model adjusted for age, sex, hemoglobin, average monthly income, parental education, number of other children, clustering in school and classroom	Anxiety Inattention Hyperactivity At age 3-7 yr assessed using Conners' ADHD/DSM-IV Scales and Behavior Rating Inventory of Executive Function	1.19 (1.03, 1.36) 5.31 (1.46, 19.3) 2.39 (0.77, 7.39)

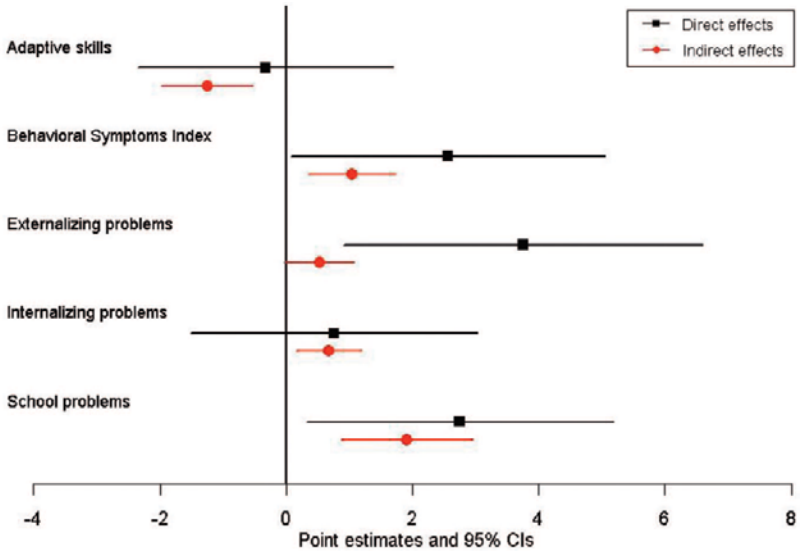
Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Odds ratio (95% CI)a
Nicolescu et al. (2010)	83 children ages 8-12 yr tested in 2007 Bucharest and Pantelimon, Romania	Concurrent mean (IQR): 3.7 (2.6)	Log-linear regression model adjusted for city, sex, age, computer experience, handedness, eye problems, number of siblings, parental education, prenatal smoking, family psychopathology	ADHD score At age 8-12 yr assessed using German version of Conner's scales	1.16 (0.98, 1.37)
Roy et al. (2009)	756 children ages 3-7 yr tested 2005-2006 Chennai, India	Concurrent mean (SD): 11.4 (5.3)	Linear regression model adjusted for age, sex, hemoglobin, average monthly income, parental education, number of other children, clustering in school and classroom	ADHD index At age 3-7 yr assessed using Conners' ADHD/DSM-IV Scales and Behavior Rating Inventory of Executive Function	4.26 (0.90, 20.08)
Braun et al. (2006)	4,704 children ages 4-15 yr U.S. NHANES 1999-2002	Concurrent 3rd quintile: 1.1-1.3	Logistic regression model adjusted for postnatal ETS, prenatal ETS, age, sex, race, childcare attendance, health insurance coverage, ferritin levels	ADHD Dx or medication use at age 4-15 yr	1.4 (0.4, 3.4), blood Pb 0.8-1.0 µg/dL vs. blood Pb <0.8 µg/dL[b] 2.1 (0.7, 6.8), blood Pb 1.1-1.3 µg/dL vs. blood Pb <0.8 µg/dL[b] 2.7 (0.9, 8.4), blood Pb 1.4-2.0 µg/dL vs. blood Pb <0.8 µg/dL[b] 4.1 (1.2, 14.0), blood Pb > 2.0 µg/dL vs. blood Pb <0.8 µg/dL[b]
Froehlich et al. (2007)	2,588 children, ages 8-15 yr U.S. NHANES 2001-2004	2nd quartile: 0.9-1.3	Logistic regression model adjusted for current household ETS exposure, sex, age, race/ethnicity, income, preschool attendance, maternal age, birth weight, and interaction terms for Pb and prenatal ETS interaction	ADHD Dx	8.1 (3.8, 18.7), blood Pb level > 2.0 µg/dL plus prenatal ETS exposure vs. blood Pb level <0.8 µg/dL and no prenatal ETS exposure[b]
Braun et al. (2008)	Children ages 8-15 yr U.S. NHANES 2001-2004	2nd quartile: 0.8-1.0	Logistic regression with sample weights applied to produce national estimates, adjusted for oversampling of minorities and young children and adjusted for age, poverty income ratio, maternal age, sex, race, prenatal ETS, cotinine, blood Pb levels	Conduct disorder at age 8-15 yr	7.24 (1.06, 49.47), blood Pb level 0.8-1.0 µg/dL vs. blood Pb <0.8 µg/dL[b] 12.37 (2.37, 64.56), blood Pb level 1.1-1.4 µg/dL vs. blood Pb <0.8 µg/dL[b] 8.64 (1.87, 40.04), blood Pb level 1.5-10 µg/dL vs. blood Pb <0.8 µg/dL[b]
Dietrich et al.(2001)	195 children followed from birth (1979-1985) to age 15-17 yr Cincinnati, OH	Early childhood (0-6 yr avg): NR	Linear regression model adjusted for HOME score, parental IQ, current SES	Delinquent behavior assessed at ages 15-17 yr using the Self-Report of Delinquent Behavior	1.21 (1.08, 1.37)
Wright et al. (2008)	250 adults followed from birth (1979-1985) to age 19-24 yr Cincinnati, OH	Early childhood mean (SD): 8.3 (4.8)	Negative binomial regression models adjusted for maternal IQ, sec, SES, maternal education	Criminal arrests at ages 20-23 yr	1.05 (1.00, 1.09)

[a]Effect estimates are standardized to a 1 µg/dL increase in blood Pb level.
[b]Odds in higher quantile of blood Pb level compared to that in lowest quantile of blood Pb level.

Burns et al. (1999) and Silva et al. (1988) aimed to characterize the direct association of blood Pb level with behavior by adjusting for child IQ in their models. They found positive associations, suggesting that blood Pb level may have an independent effect on behavior. However, because a decrement in IQ may on the causal pathway to behavioral problems, including both IQ and behavioral problems may result in an underestimate of the effect on behavior. Hence, to account for the relationship between IQ and behavior, Chen and colleagues (2007) used structural equation modeling (specifically path analysis) to estimate the direct effects of blood Pb level on behavioral problems as well as indirect effects mediated

through child's IQ (assessed using the WPPSI-R at age 5 years and the WISC-III) at age 7 years). Among 5- and 7-year-old participants from the TLC trial (described in Section 5.3.2.1), conduct problems were assessed using the Conners' Parent Rating Scale-Revised, and other behaviors were assessed with the Behavior Assessment System for Children-Teacher Rating Scale (BASC-TRS) and Behavior Assessment System for Children-Parent Ratings Scale (BASC-PRS). In models that adjusted for sex, race, clinical site, language (English or Spanish), parent's education, parent's employment, age at blood Pb testing, caregiver's IQ, and treatment group, peak blood Pb levels at age 2 years were not associated with conduct problems at age 5 years or any of the BASC scores at age 7 years. Concurrent blood Pb levels had statistically significant direct effects on behavioral symptoms index, externalizing problems and school problems as assessed by BASC-TRS and externalizing problems (outbursts of behavior) as assessed by BASC-PRS (Figures 5-20 and 5-21).

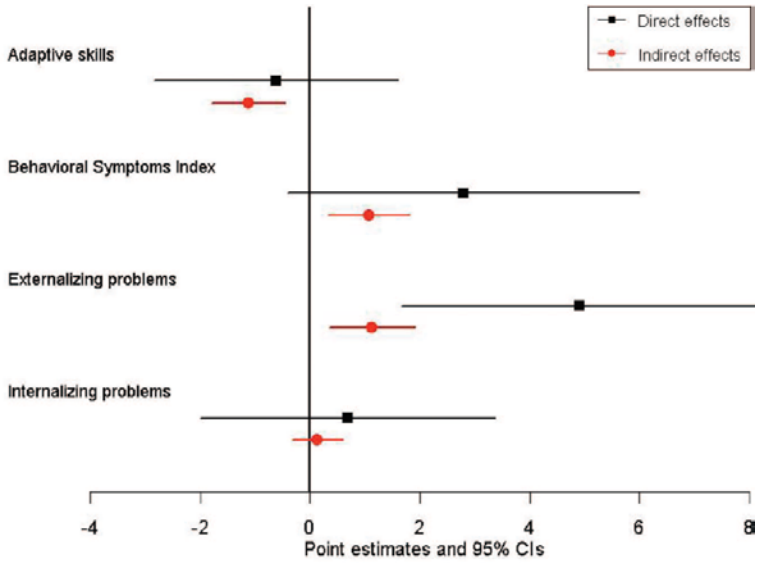
Chen et al. (2007) also found significant indirect effects (mediated through child's IQ) of blood Pb levels at age 7 years with all measurements except the BASC-TRS externalizing problems and BASC-PRS internalizing problems (repressing problems). However, because blood Pb level was estimated to have direct effects on several behavioral endpoints and, in for some endpoints, a larger magnitude of direct effect, the authors inferred IQ to only partially mediate associations between blood Pb levels and behavioral outcomes (Figures 5-20 and 5-21). The study also had limitations, including the lack of adjustment for HOME score and lack of information on other covarying family and neighborhood characteristics that may be relevant (i.e., social stressors). Also, these findings may not be generalizable to the general population given that the children in the study population had been referred for chelation therapy at enrollment because of high blood levels. In this study, only concurrent blood Pb levels were examined, and it is uncertain whether the observed associations were due to the residual effect of high blood Pb levels (20-44 µg/dL) four years earlier.



Source: Used with permission from the American Academy of Pediatrics, Chen et al. (2007)

Note: Values represent change in behavioral score per 10 µg/dL increase in blood Pb level.

Figure 5-20. Estimates and 95% CI of direct and indirect effects of concurrent blood Pb concentrations at age 7 on BASC-TRS.



Source: Used with permission from the American Academy of Pediatrics, Chen Chen et al. (2007)

Note: Values represent change in behavioral score per 10 µg/dL increase in blood Pb level.

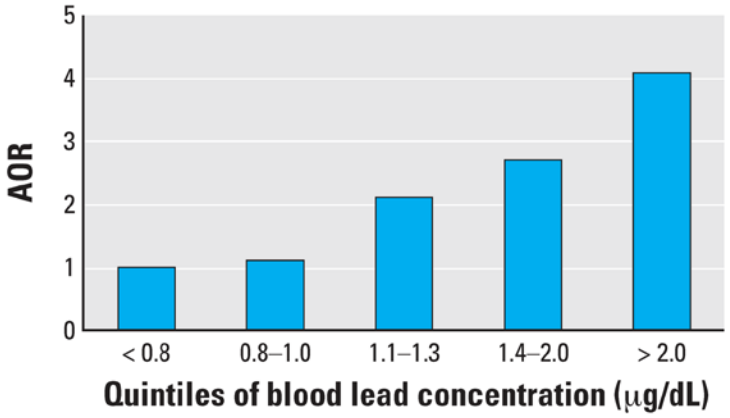
Figure 5-21. Estimates and 95% CI of direct and indirect effects of concurrent blood Pb concentrations at age 7 on BASC-PRS.

The 2006 Pb AQCD summarized indirect evidence for associations of blood Pb level with behavioral features of ADHD including distractibility, poor organization, lacking persistence in completing tasks, and daydreaming (Bellinger et al., 1994; Fergusson et al., 1993; Needleman et al., 1979;

G. A. Wasserman et al., 2001; G. A. Wasserman et al., 1998). Prior studies examining the association of blood Pb level with a diagnosis of ADHD were limited by small sample size, and results were inconclusive (David et al., 1972; Gittleman & Eskenazi, 1983). In addition, many earlier studies of inattention and impulsivity included children with higher blood Pb levels than those observed in contemporary children.

A recent analysis of data from NHANES (1999-2002) found a positive relationship between blood Pb level and ADHD (parent-report of a diagnosis of ADHD or use of stimulant medication) (Braun et al., 2006). This analysis included 4,704 children aged 4-15 years. These authors reported a monotonic increase in odds of ADHD from the lowest to highest quintile of blood Pb level (Figure 5-22). Children in the fifth quintile of blood Pb level (>2.0 µg/dL) had the highest odds of ADHD compared with those in the lowest quintile (<0.8 µg/dL) (OR: 4.1 [95% CI: 1.2, 14]). Children in the other three higher quintiles of blood Pb level also had increased odds of ADHD relative to the reference group; however, the associations were not statistically significant (Tables 5-19 and 5-10).

In the same NHANES dataset, however, restricted to children ages 8-15 years, Froehlich and colleagues (2009) demonstrated the joint effects of prenatal tobacco smoke (maternal report) and blood Pb levels. They found independent effects on ADHD for prenatal tobacco smoke exposure (OR: 2.4 [95% CI: 1.5, 3.7]) and concurrent blood Pb levels (OR: 2.3 [95% CI: 1.5, 3.8]) in children with blood Pb levels >1.3 µg/dL compared with children with blood Pb levels < 0.8 µg/dL. A statistically significant interaction also was found. Compared to children in the lowest tertile of blood Pb levels with no exposure to prenatal tobacco smoke, children in the highest tertile of blood Pb level with exposure to prenatal tobacco smoke had the greatest odds of ADHD (OR: 8.1 [95% CI: 3.5, 18.7]).



Source: Braun et al. (2006)

Note: Adjusted for child's age, gender, race/ethnicity, preschool attendance, serum ferritin, prental tobacco smoke exposure, smoker in the household, and insurance status (p for trend = 0.012).

Figure 5-22. Adjusted odds ratio for ADHD among U.S. children (ages 4-15 years) from NHANES 1999-2002 by quintile of blood Pb level.

Pb has long been known to impact dopaminergic neurons. Pb will inhibit depolarization-evoked neurotransmitter release and stimulate spontaneous neurotransmitter release, including effects specific to dopaminergic neurons (Section 5.3.6.9). This background would make dopamine related genes strong candidates as effect modifiers of Pb-associated neurodevelopmental effects. There are several known functional variants in dopamine related genes, including the dopamine 2 receptor, dopamine 4 receptor (DRD4), and the dopamine transporter. The DRD4 gene has a 48 base pair long repeat in exon 3. The longer repeat alleles (DRD4.7) appear to have less binding affinity for dopamine and have been associated with ADHD. However, DRD4.7 also has been associated with sustained attention, response inhibition, and quicker response time. Thus, it is not clear whether DRD4.7 would increase or decrease susceptibility to Pb-associated ADHD. Froehlich et al. (2007) studied 174 children from the Rochester prospective birth cohort and measured DRD4 genotype, blood Pb level, and several functional domains using the Cambridge Neuropsychological Testing Automated Battery. Increasing blood Pb levels were associated with poorer rule learning and reversal, spatial span, and planning. These negative associations were larger in magnitude among boys and those lacking DRD4.7.

In a population of children in a similar age range (8-11 years) and similar blood Pb levels (mean 1.9 µg/dL) as in the NHANES studies, Cho et al. (2010) found a statistically significant relationship of blood Pb levels with parent- and teacher-rated ADHD symptoms (i.e., inattentiveness, hyperactivity, and total score).

Nicolescu et al. (2010) examined the relationship between blood Pb level and ADHD-related behaviors among 83 children, ages 8-12 years, living near a metal-processing plant in Romania. These authors examined associations of Pb, mercury, and aluminum biomarkers with performance on four different attention tasks on the computerized German Kinder-KITAP as well as behavior ratings using the parent and teacher reports on the Connors scale. Only concurrent blood Pb levels, and not other metals, were consistently associated with increased odds for ADHD-related behaviors (OR: 1.16 [95% CI: 0.98, 1.37] per 1 µg/dL increase in blood Pb level. Notably, blood Pb levels ranged from 1.1 to 14.3 µg/dL, and when analyses were repeated taking out the 5 children with blood Pb levels at or above 10 µg/dL, associations with both questionnaire-based ADHD ratings and KITAP-performance remained statistically significant.

Roy and colleagues (2009) examined associations between blood Pb level and a range of behavioral problems in 756 children, ages 3-7 years, in Chennai, India. In this population, mean (SD) blood Pb level was 11.4 (5.3) µg/dL. Anxiety, social problems, inattention, hyperactivity, and ADHD as well as executive function were assessed based on the teacher's report of the Conners' Teacher Rating Scales-39, Conners' ADHD/Diagnostic and Statistical Manual for Mental Disorders, 4th Edition Scales, and the Behavior Rating Inventory of Executive Function questionnaire. In generalized estimating equations, increasing blood Pb level was associated with higher anxiety, social problems, and ADHD index scores (Figure 5-19 and Table 5-10).

Recent studies add to the collective body of evidence demonstrating associations between blood Pb level and a variety of conduct problems including conduct disorder (Braun et al., 2008), oppositional defiant disorder (Nigg et al., 2008) and more serious behaviors such as criminal behavior (J. P. Wright et al., 2008). These findings were corroborated in a recent meta-analysis on Pb and conduct problems (Marcus et al., 2010) that included 19 studies with a total of 8,561 children and adolescents (mean ages ranging from 3.5 years to 18.4 years). An overall medium effect size was estimated (r across studies was 0.19, $p<0.001$). Moreover, the meta-analysis demonstrated a consistent relationship between increasing blood Pb levels and conduct problems despite considerable heterogeneity across studies (e.g., ways in which conduct problems were defined and assessed, participant ages, participant blood Pb levels). In the meta-analysis, covariates such as SES, birth weight, parental IQ, and family environment did not attenuate the relationship between blood Pb level and conduct problems. Interestingly, a larger magnitude of effect was estimated for hair Pb levels compared with bone or blood Pb levels. Although the authors suggested that hair Pb may be a better indicator of cumulative Pb exposure compared to bone Pb levels, due to the high turnover of bone in throughout childhood and into adolescence, an empirical basis for interpreting hair Pb measurements in terms of body burden or exposure has not been firmly established (Section 4.3.4.2).

In addition to examining ADHD in the NHANES population, Braun and colleagues (2008) also examined conduct disorder defined using DSM-IV criteria. The prevalence of conduct disorder was higher among males, older children (13-15 years, relative to those less than 13 years), and children with higher blood Pb levels. In analyses adjusted for child's age, gender race/ethnicity, SES, mother's age at child's birth, prenatal tobacco smoke exposure (based on maternal report), and child's cotinine levels, children in the highest quartile of blood Pb level (>1.5 µg/dL) had increased odds of conduct disorder relative to children in the lowest quartile (0.2-0.7 µg/dL) (OR: 8.7 [95% CI: 1.9, 40]). Poisson regression models showed that compared with children in the lowest quartile of blood Pb level, children in the highest quartile had 1.73 (95% CI: 1.2, 2.4) times as many conduct disorder symptoms.

In their longitudinal study of children in the U.K., Chandramouli et al. (2009) found associations of blood Pb level at age 30 months not only with hyperreactivity but also with antisocial behavior at ages 7 and 8 years. Child behavior was assessed using three methods: parent- and teacher-report on the Strengths and Difficulties Questionnaire at age 7 years, the Development and Well-being Assessment at age 8 years, and the Anti-social Behaviour Interview at age 8 years. Attention was measured using the Test of Everyday Attention for Children at age 8 years. Blood Pb levels showed statistically significant positive association with antisocial behavior. Similar to Chen et al. (2007) and Burns et al. (1999), increasing blood Pb level was associated with antisocial behavior, independently of IQ.

Wright et al. (2008) recently examined the relationship between prenatal and postnatal blood Pb levels and arrests for criminal offenses at ages 19-24 years in the CLS. In this birth cohort, prenatal and postnatal blood Pb levels previously have been reported to be associated with self- and parent-reported

delinquent and social acts at ages 16-17 (Dietrich et al., 2001). Data on criminal arrests for participants and their mothers were obtained from a computer search of Hamilton County, Ohio criminal justice records. They also examined the absolute change in arrest rates between participants with higher levels of blood Pb compared to those with lower blood Pb levels defining attributable risk as the average difference in annual arrest rates between participants at the 95th percentile of blood Pb and those at the 5th percentile. Mean blood Pb concentrations were 8.3 µg/dL (range 1 to 26) for the prenatal period (maternal blood), 13.4 µg/dL (range 4 to 37) for the average between birth and age 6 years, and 8.3 µg/dL (range 2 to 33) at age 6 yr. In models that adjusted for maternal IQ, sex, SES score, and maternal education, 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 average childhood blood Pb level, and 1.05 (95% CI: 1.01, 1.09) for blood Pb level at age 6 years. Relative risks for violent criminal arrests were also larger for prenatal blood Pb levels and age 6-year blood Pb levels. The RRs for arrests involving violent crimes per 1 µg/dl increment in blood Pb level were 1.06 (95% CI: 0.97, 1.15) for prenatal blood Pb level, 1.05 (95% CI: 1.01, 1.10) for average childhood blood Pb level, and 1.08 (95% CI: 1.03, 1.14) for blood Pb level at age 6 years. Although interactions terms of blood Pb by sex were not statistically significant, the attributable risk for males was considerably higher for males (0.85 arrests/year [95% CI: 0.48, 1.47]) than for females (0.18 [95% CI: 0.09, 0.33]). Similar to findings from Dietrich et al. (2001), prenatal and early childhood blood Pb levels were associated positively with risk of criminial behavior in early adulthood. Results from the two studies combined suggest that in addition to the prenatal period, early childhood blood Pb levels may also predict criminal behavior in adulthood. However, it is important to note that in these CLS studies, concurrent blood Pb levels were not analyzed.

5.3.3.2. Epidemiologic Studies of Behavior, Mood, and Psychiatric Effects in Adults

Effects of blood Pb levels on emotional regulation in adults have received far less attention than that in children and cognitive function in adults. Nonetheless, evaluation of mood states is an integral part of the World Health Organization's (WHO) neurocognitive test battery and, indeed, it has been suggested that the assessment of mood with the Profile Of Mood States may be particularly sensitive to toxicant exposures (1987). With respect to Pb exposure, several early studies of Pb-exposed workers (mean blood Pb levels ranging from 23.5 to 64.5 µg/dL) found higher prevalence of symptoms of mood disorders and anxiety among Pb-exposed workers than unexposed controls (mean blood Pb levels ranging from 15-38 µg/dL) (Baker et al., 1984; Baker et al., 1985; Lilis et al., 1977; Maizlish et al., 1995; Parkinson et al., 1986; B. S. Schwartz et al., 2005). In one of the few previous studies of adults without occupational exposure to Pb and more relevant to blood and bone Pb levels measured currently in the U.S. among adults without occupational exposures, an association was observed between both blood (mean: 6.3 µg/dL

[SD: 4.16] and bone (tibia mean: 21.9 µg/g [SD: 13.5]) Pb levels and depression and anxiety symptoms (Brief Symptom Inventory, BSI) among men in the NAS (Rhodes et al., 2003).

More recently, subsequent assessments of the NAS men have provided understanding of effect modification of the associations of blood, patella, and tibia Pb levels with psychiatric symptom dimensions by ALAD genotype (Rajan et al., 2007). In addition to corroborating associations of Pb with BSI symptoms reorted by Rhodes et al. (2003), Rajan et al. (2007) found that of eight symptom dimensions considered, associations of tibia bone Pb levels with phobic anxiety, positive symptom total, and anxiety scale were modified by ALAD genotype, with interaction terms attaining statistical significance for phobic anxiety and positive symptom total. For all psychiatric symptoms, the association with tibia Pb was worse among ALAD 1-1 carriers, which was the opposite genotype observed to have larger Pb-associated decrements in cognitive performance (Section 5.3.2.4).

A study of 1,987 adults age 20-39 years participating in NHANES 1999-2004 was the largest study of psychiatric disorders and the study with the lowest blood Pb levels (mean 1.61 µg/dL [SD: 1.72]) (Bouchard et al., 2009). Investigators examined cases of major depressive disorder (MDD), panic disorder and generalized anxiety disorder (GAD) as determined using the WHO Composite International Diagnostic Interview, which follows criteria defined in DSM. Compared with those in the lowest quintile of blood Pb level (<0.7 µg/dL), adults in the highest quintile (≥ 2.11 µg/dL) had increased odds of MDD (OR: 2.32 [95% CI: 1.13, 4.75]) and panic disorder (OR: 4.94 [95% CI: 1.32, 18.48]). Odds ratios were even larger in analyses excluding current smokers. While those in higher quintiles of blood Pb level (>0.7 µg/dL) had increased odds to GAD, the associations were not statistically significant. Although studies in adults without occupation Pb exposure are sparse, consistent with studies of occupationally-exposed adults and experimental evidence, they demonstrate associations of blood (as low as 2.11 µg/dL) and bone Pb level (population mean tibia Pb level 22 µg/g) with psychiatric outcomes.

5.3.3.3. Toxicological Studies of Neurobehavioral Outcomes

Pb is a known risk factor for neurobehavioral changes with preferentially targeted sites including the prefrontal cerebral cortex, cerebellum, and hippocampus; affected functions include cognition, execution of motor skills, and memory/behavior, respectively. As discussed in earlier AQCDs, young animals are especially susceptible to the effects of Pb due to differences in structure of the nervous system and to the ongoing development with greater Pb absorption and retention. Pb exposure has been documented to induce neurobehavioral changes in exposed animals including effects on learning, social behavior, memory, attention, motor function, locomotor ability and vocalization. At the cellular level, Pb impairs axon and dendritic development and contributes to neurochemical changes in proteins, membranes, redox/antioxidant balance, and neurotransmission through a multitude of mechanisms, many of which involve the ability of Pb to mimic calcium. Very early research on neurobehavioral endpoints

failed to capture the disposition of Pb and its resulting body burden or blood Pb and is thus difficult to use in risk assessment.

Since then, the 1986 AQCD reported the literature on rodent and nonhuman primate Pb-induced aberrant operant conditioning tasks in rodents and non-human primates (blood Pb 11 µg/dL to 15 µg/dL) with other studies yielding hyperactive or inappropriate Pb-mediated responses (U.S. EPA, 1986) possibly of hippocampal origin with a curvilinear response, decreasing at higher doses possibly due to impairment of motor function at the high doses (Crofton et al., 1980; Ma et al., 1999). Pb exposure in lab animals contributed to distractibility, reduced adaption capacity to changes in behavior, impaired ability to inhibit inappropriate responses and perservation (U.S. EPA, 2006). Pb is known to impair learning (blood Pb 11 µg/dL) measured using Fixed Interval tasks (FI) defined as scheduled reinforcement delivered after a fixed period since the last reward, with premature responses not rewarded. The 2006 AQCD found FI response rates (blood Pb 58 to 94 µg/dL) were sensitive to Pb exposure, which was primarily accounted for by decreased interresponse times. Inter-response rates and overall run rate are the two subcomponents of FI response rate. Spatial and non-spatial discrimination reversal or reversal of a previously learned habit is significantly affected after developmental Pb exposure and is exacerbated with distracting stimuli; discrimination reversal has been shown to be especially sensitive to Pb exposure. Repeat-acquisition testing revealed that these deficits are likely not due to sensory or motor impairment at this dose. The results from different studies testing the effect of Pb on memory are mixed with impaired memory shown at blood Pb level of 10 µg/dL and improved memory in other studies. Low dose Pb does not appear to affect short-term memory. Memory tests may give incorrect results when opportunities exist for impaired attention to contribute to test results (U.S. EPA, 2006). Together, the data from the 2006 AQCD showed that social behavior and learning in rodents and nonhuman primates is significantly affected by Pb exposure (blood Pb 15-40 µg/dL).

In the new literature, gestationally-Pb exposed (GLE) male mice (low and high dose Pb, 10 µg/dL and 42 µg/dL blood Pb at PND10) were significantly less active than control mice and low dose GLE mice were significantly less active than high dose GLE mice, demonstrating a non-linearity of GLE dose responsiveness (Leasure et al., 2008). A similar Pb dose response non-linearity (baseline corticosterone) was seen in male mice exposed post-weaning to Pb (Virgolini et al., 2005). Activity level of GLE female mice versus control was unaffected (Leasure et al., 2008). Amphetamine induced motor activity was monitored in male and female GLE mice at 1 year of age. Amphetamine-induced activity of male low and high dose GLE offspring was significantly elevated versus control; GLE females had no change in sensitivity to amphetamine-induced motor activity (Leasure et al., 2008).

Rotarod performance measures endurance, balance and coordination in mice. GLE male mice had significantly shorter mean latencies to fall from the rotarod compared with controls; females were unaffected. Further, low dose GLE male mice had significantly poorer rotarod performance than high dose GLE male mice (fell off quicker, 10 µg/dL and 42 µg/dL blood Pb at PND10), showing non-linearity

of dose-responsiveness to GLE (Leasure et al., 2008). Other rotarod experiments at higher doses of Pb exposure and at various speeds of rotarod rotation yielded mixed results (Grant et al., 1980; Kishi et al., 1983; Overmann, 1977).

Herring gull chicks exposed to a single IP bolus dose (100 mg/kg Pb acetate) of Pb on post-natal day two at a dose created to be similar to that which wild herring gulls are exposed to in the wild were found to have neurobehavioral deficits, including learning deficits. Pb-exposed chicks displayed multiple deficits related to impaired survival skills including decreased time spent begging the parent for food, decreased accuracy at pecking for food in the parent bird's mouth, decreased time spent in the shade (behavioral thermoregulation), decreased learning in food location testing, decreased recognition of familiar individuals (caretaker or sibling), and slower development of motor skills (treadmill test) versus control birds (Burger & Gochfeld, 2005). The impaired thermoregulation with Pb exposure agrees with earlier work in rat pups who also showed impaired thermoregulatory behavior, i.e., impaired ultrasonic vocalization (Davis, 1982). These studies in herring gull chicks demonstrate that a single early life exposure to Pb can induce neurobehavioral deficits that affect survival skills.

5.3.3.4. Toxicological Studies of Mood Alterations

Neurotoxicological studies often focus on motor, sensory, behavioral or cognitive outcomes and often fail to evaluate psychological pathologies. Recent epidemiologic studies have reported that prenatal or early life blood Pb levels or ALAD changes, a biomarker of Pb exposure, may be a risk factor for development of mood disorders in adulthood (i.e. schizophrenia, major depression or panic disorders) (Bouchard et al., 2009; Opler et al., 2004). With this in mind, animal studies of Pb exposure during pregnancy and lactation and outcomes in offspring look to address the role of developmental Pb exposure on emotional state and mood disorder-like behavior in adult offspring. Wistar rats exposed to 10mg of Pb acetate daily by gavage during pregnancy (G) or pregnancy plus lactation (G+L) produced pups that were then tested in the open field test or the forced swimming test also known as Porsolt's test. Blood Pb levels in the pups at PND70 was 5-7 µg/dL. The open field test can measure emotion and exploratory behavior; Pb (G+L) treated male rats had increased emotionality with the open field test. Pb exposed (G+L) female offspring had a significant increased depressive phenotype in the forced swim test (de Souza Lisboa et al., 2005). It is interesting to note that this is one of many Pb-induced changes that seem to be sex-specific.

Depression may seem initially like an unexpected comorbidity for immune inflammatory dysfunction, but many forms of depression are linked with the same cytokine imbalances that occur with Pb-induced innate immune dysfunction (Maes; T. W. W. Pace & Miller, 2009). Some researcher use sickness behavior and its associated malaise as a model for depression. Examples from animal models include the study by Dyatlov and Lawrence (2002) with Pb exposure in mice. In this study, sickness behavior, which is due to an interaction of the immune system and the CNS in Pb-exposed mice, was

potentiated by Pb-exposure (blood Pb level 17 µg/dL) and this correlated with depletions in specific thymic T-cell populations. Pb-exposure also potentiated the infection-dependent elevation in IL-1β, a cytokine which has been shown to inhibit hippocampal glutamate release in young and not aged animals. Sickness behavior was induced with Listeria monocytogenes infection; Pb exposure was from birth through lactation and continued for a brief period after weaning until the experiment was terminated. Sickness behavior is characterized by overall malaise, decreased food intake, immobility and changes in core body temperature. Pb potentiated the sickness behavior in exposed animals.

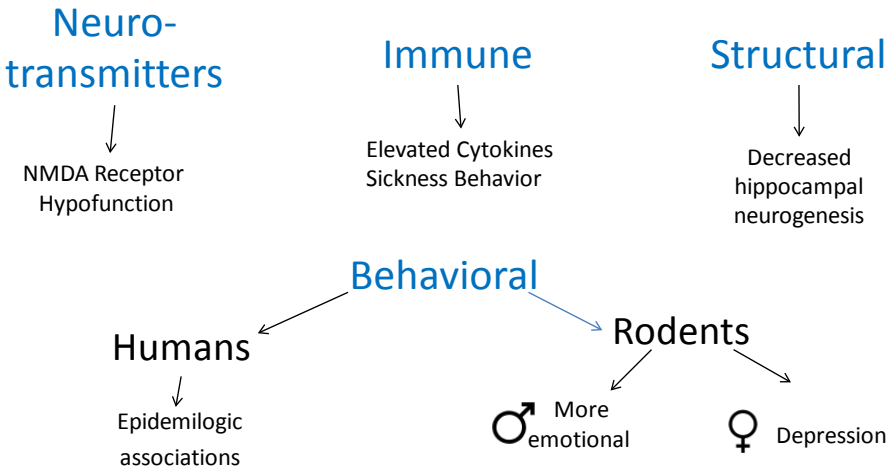


Figure 5-23. Animal toxicology evidence of possible Pb-dependent contributors to the development of mood disorders.

Schizophrenia is associated with a shortened lifespan in humans as reflected by increased standardized mortality ratio (McGrath et al., 2008). An environmental origin of schizophrenia was proposed years ago (Tsuang, 2000) but the specific link between prenatal Pb exposure, using ALAD as a biomarker, and schizophrenia is just beginning to appear in the literature and has been described in two publications (Opler et al., 2004; Opler et al., 2008). Because of this, the animal toxicology literature is beginning to explore the mechanisms that may contribute to schizophrenia development and has proposed two explanations. These are Pb-induced NMDA receptor (NMDAR) hypofunction and Pb-induced decreases in hippocampal neurogenesis (Figures 5-23, 5-24, and 5-25). Pb may bind a divalent cation site in the NMDAR and allosterically inhibit glycine binding (Hashemzadeh-Gargari & Guilarte, 1999); human studies of patients with schizophrenia have shown aberrations at this site (Coyle & Tsai, 2004). These findings are consistent with the glutamatergic hypothesis of schizophrenia which shows that NMDAR noncompetitive antagonist use in patients with schizophrenia exacerbates their psychotic symptoms and that

administration of antagonist to non-psychotic subjects can induce a schizophrenic phenotype. The second mechanistic hypothesis for Pb-associated schizophrenia induction is decreased hippocampal degenerate gyrus (DG) neurogenesis, which is seen in patients with schizophrenia (Kempermann et al., 2008; Reif et al., 2006), in animal models of schizophrenia {M, 2007, 670321} and in animal models with developmental Pb exposure (Jaako-Movits et al., 2005; Verina et al., 2007) (Figures 5-23, 5-24, and 5-25). Animal models of schizophrenia (i.e., phencyclidine administration) show decreased hippocampal DG neurogenesis that can be reversed by treatment with clozapine, which is often used in schizophrenia (Maeda et al., 2007). These DG pathways are also NMDAR-dependent. Studies cited in this section are further detailed in other sections of the ISA. Thus, a Pb-dependent contribution to mood disorders exists in the toxicology literature with support from behavioral, neurochemical and ultrastructural data.

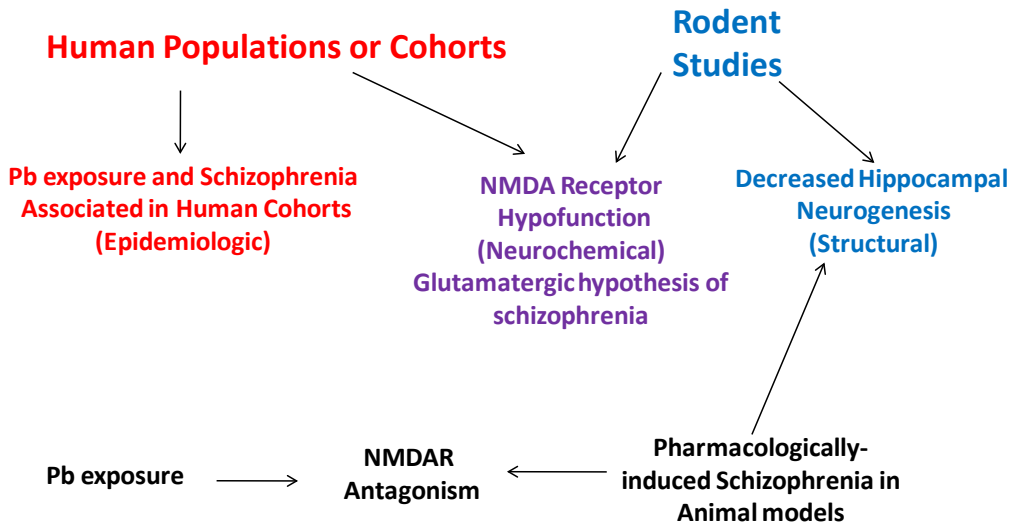
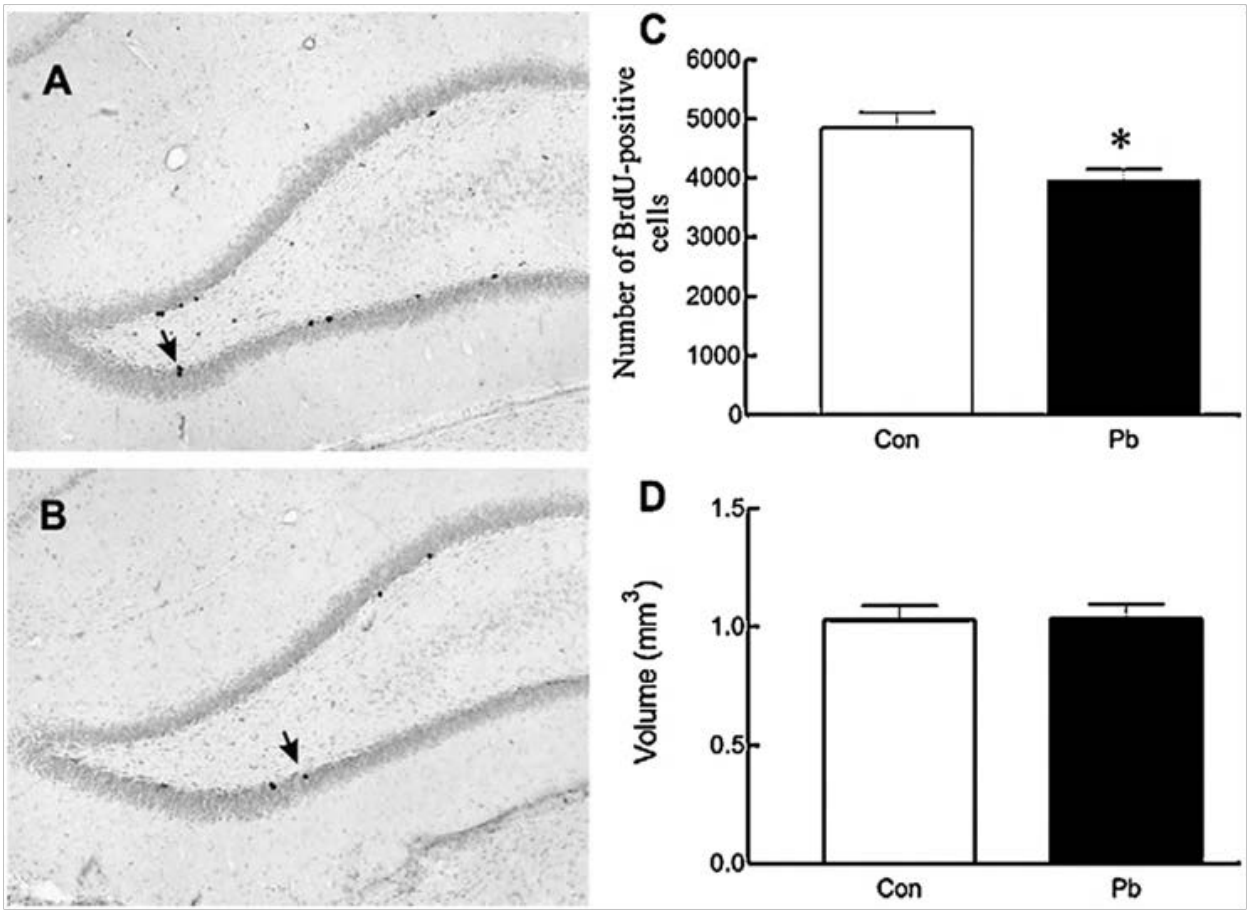


Figure 5-24. Schematic representation of the contribution of Pb exposure to the development of a phenotype consistent with schizophrenia.

As recently reviewed by Wright (2009), social stress and physical environmental toxins impact overlapping biological processes which determine adaptive plasticity in early neurodevelopment. Development of CNS organization into functional neuronal and synaptic networks can be determined by environmental signals which modify neuronalgenesis, synaptic formation and synaptic pruning (LeDoux, 2003). Environmental factors can promote or disrupt this process depending on whether they are positive (social supports, good nutrition, etc.) or negative (psychosocial stress, chemical toxicants, malnutrition, trauma, etc.). While plasticity allows recovery from short-term toxic exposures, the neural mechanisms

underlying the plasticity of the developing brain exposed to chronic toxic exposures could induce permanent structural or organizational changes via altered neuronal growth and/or synaptogenesis/pruning. While historically research has focused on how social and physical environmental factors independently affect children's health, evolving theory and methodologies underscore the importance of studying integrated effects (L. D. White et al., 2007; R. J. Wright, 2009). Recent studies highlight how social conditions influence susceptibility to future environmental exposures and, when contemporaneously exposed, how social-physical environmental interactions may account for more variance in explaining risk than main effects of either factor alone (R. J. Wright, 2009).



Source: Used with permission from Elsevier Science, Fox et al. (2010)

Figure 5-25. Neurogenesis (production of new cells) in the rat dentate gyrus after postnatal Pb exposure.(A) Control; (B) Pb exposed light micrograph pictures of Brd-U positive cells; (C) Counts of Brd-U positive cells (proliferating cells); and (D) volume of dentate gyrus. *p<0.05 v. control.

5.3.4. Sensory Acuity

5.3.4.1. Epidemiologic Studies of Children

Although not as widely examined as cognitive and behavioral outcomes, several studies demonstrated associations of blood Pb level with increased hearing thresholds and decrements in auditory processing in children (U.S. EPA, 2006). Such evidence has been limited largely to studies described in the 2006 Pb AQCD, including large U.S. studies, including NHANES II (J. Schwartz & Otto, 1987) and the Hispanic Health and Nutrition Examination Survey (HHANES) (J. Schwartz & Otto, 1991). In these studies, concurrent blood Pb level (median 8 µg/dL) from 6 to 18 µg/dL was associated with a 2-db loss in hearing and an increase in the percentage (15%) of children with a substandard hearing threshold (2,000 Hz). Blood Pb level also was associated with increases in hearing threshold across several frequencies in a population of children in Poland with similar blood Pb levels (median 7.2 µg/dL [range: 1.9 to 28]) (Osman et al., 1999). In the HHANES and Polish studies, associations persisted in analyses restricted to subjects with blood Pb levels below 10 µg/dL. In the CLS, blood Pb level was associated with impaired auditory processing, albeit at higher concentrations. In this cohort, the mean (SD) lifetime average blood Pb level was 17.4 µg/dL (8.8), and a 1 µg/dL increase in lifetime average blood Pb was associated with a 0.07-point ($p < 0.05$) lower score on the Filtered Word test, indicative of incorrect identification of filtered or muffled words (Dietrich et al., 1992). Despite the higher blood Pb levels in the CLS, the observed associations with auditory function were consistent with those with related endpoints, including cognitive deficits (Section 5.3.2.1) and behavioral problems (Section 5.3.3.1).

5.3.4.2. Epidemiologic Studies of Adults

Rather than evidence for effect on hearing thresholds, among adults, evidence of association between blood Pb levels and auditory function comprised changes in auditory evoked brainstem potentials (U.S. EPA, 2006). Two studies of hearing thresholds came to somewhat different conclusions. One study examined 183 Pb-workers with blood Pb levels from 1 to 18 µg/dL and among multiple frequencies examined, found correlations between increasing blood Pb level and increased hearing threshold at 4 kHz (Forst et al., 1997). A second study included 220 Pb-battery workers with higher blood Pb levels (mean: 56.9 µg/dL [SD: 25.3]) (Wu et al., 2000). Although hearing impairment was associated with a measure of cumulative Pb exposure based on years of work and ambient Pb measurements, no association was found with blood Pb levels at the time of hearing testing in analyses adjusted for age, gender, and duration of employment. These findings may indicate that in an occupational setting with high Pb exposure, any one blood Pb measure may not be the best biomarker for cumulative exposure over the duration of work.

Studies published since the 2006 Pb AQCD have produced findings consistent with previous studies. While studies of Pb-exposed workers continued to dominate, an NAS study provided new evidence for increasing bone Pb levels being associated with hearing loss in adult males without occupational Pb exposures (Park et al., 2010). A hospital-based case-control study recruited workers referred for hearing testing (average hearing thresholds above 25 dB) as cases and workers with normal hearing thresholds who were having occupational health examinations for other reasons as controls (Chuang et al., 2007). The 121 cases had a geometric mean blood Pb level of 10.7 μg/dL, and the 173 controls had a geometric mean blood Pb level of 3.9 μg/dL. In models that adjusted for age, smoking, alcohol consumption, years of noise exposure, as well as Mn, As, and Se levels in blood, blood Pb levels was associated with a statistically significant higher average hearing threshold (0.5-6 kHz).

Similar findings were reported in a study of 259 steel plant workers with no parental history of ear-related problems, no congenital abnormalities, no occupational organic solvent exposure, and hearing loss difference no more than 15 dB between both ears (Y.-H. Hwang et al., 2009). The participants had an average blood Pb level of 54.3 μg/dL (SD: 34.6). Average noise levels also were measured in work areas and dichotomized at 80dB. In analyses adjusted for age and work area noise (dichotomized at 80dB), workers with blood Pb ≥ 7 μg/dL had a statistically significant increased odds (range of ORs: 3.06 to 6.26) of hearing loss at frequencies of 3, 4, 6, and 8 kHz compared to workers with blood Pb levels ≤ 4 μg/dL.

Park et al. (2010) analyzed data from 448 men in the NAS with an audiometric hearing test within 5 years of bone Pb measurements (all but 5 of these men had audiometric testing before the bone Pb measurement) and who did not have unilateral hearing loss. In cross-sectional analyses adjusted for age, race, education, body mass index, pack-years of cigarettes, diabetes, hypertension and occupational noise (based on a job-exposure estimate from occupations), and presence of a noise notch (indicative of noise-induced hearing loss), higher patella bone Pb level was associated with a statistically significant higher hearing thresholds for frequencies greater than 1 kHz. In analyses of pure tone average hearing loss, a 21 μg/g increase in patella bone Pb level (interquartile range) was associated with an OR of 1.48 (95% CI: 1.14, 1.91) after controlling for all covariates. Similar, but slightly weaker associations were seen with tibia bone Pb levels. Audiometric data collected from the same men approximatly 20 years earlier (median observations/participant: 5; median follow-up: 23 years) were used to assess the association between tibia bone Pb levels and the change in hearing thresholds over that time. Increasing tibia Pb level was associated with faster rate of increase in hearing threshold for frequencies of 1, 2, and 8 kHz and a pure tone average. For the pure tone average, a 15 μg/g increase in tibia bone Pb level (interquartile range) was associated with an increased in hearing threshold of 0.05 dB per year. Blood Pb was not examined in this study. Together with those from studies of Pb-exposed workers, findings from the NAS study primarily indicate that biomarkers of cumulative Pb exposure are associated with increased hearing thresholds in adults. Because only bone Pb levels were examined in the NAS study, further investigation

is required to characterize potential differences between measures of cumulative and recent Pb exposures in the effects on hearing in adults without occupational exposures.

5.3.4.3. Toxicological Studies of Sensory Organ Function

Pb affects multiple aspects of the nervous system including the sensory organs. The 1986 and 2006 Pb AQCDs detailed the effects of Pb on the retina, CNS visual processing areas, and the auditory system and described possible or known mechanisms of action where available. The new research examines effects on these systems at even lower Pb exposures and blood Pb levels.

The Effect of Lead on Vision

The selective action of Pb on retinal rod cells and bipolar cells is well documented in earlier AQCDs and research in this area continues to date (Fox et al., 1997; Fox & Sillman, 1979). Pb exposure during perinatal development and adulthood has also been shown to affect the visual cortex (L. G. Costa & Fox, 1983) and subcortical neurons (Cline et al., 1996). The 1986 AQCD mentioned that neonatal rats exposed to Pb out to PND21, through gestational and lactational exposure, had significant alterations in visual evoked responses and impaired visual acuity; it was hypothesized that a decreased number of cholinergic receptors and alterations in the ratio of inhibitory to excitatory systems in the cerebrospinal axis may be the underlying mechanisms leading to these retinal changes (U.S. EPA, 1986). A 1996 publication detailing environmentally relevant doses of Pb administered to tadpoles showed that Pb inhibited the growth of developing neurons in the subcortical retinotectal pathway, the main efferent from the retina (Cline et al., 1996). The 2006 AQCD evolved to detail Pb-induced impairment of retinal function in non-human primates as well as focusing on mechanisms of action for specific physiological retinal changes using both in vitro and in vivo evidence, where available. With Pb-induced retinal effects, decreased maximal ERG amplitude or sensitivity and increased mean ERG latency was linked to increased retinal cGMP both in vitro and in vivo. Delayed dark adaptation and increased response thresholds at scotopic backgrounds were linked to in vivo apoptotic endpoints including rod bipolar cell death, increased Bax (apoptosis protein) translocation, increased cytochrome c release (apoptosis trigger), and decreased rhodopsin; in vitro evidence also included retinal apoptosis from the calcium/Pb signal localized to the mitochondrial permeability transition pore. Other endpoints seen in Pb-induced impaired retinal function included competitive inhibition of cGMP phosphodiesterase (PDE) in vitro and decreased stimulated cGMP PDE in vivo; also decreased retinal Na+/K+ ATPase activity has been reported both in vivo and in vitro. The effects of early life Pb exposure on the retina in monkeys was detailed in work by Reuhl et al. (1989) in the 2006 AQCD. Chronic Pb exposure from birth to age 6 produced cytoarchitectural changes in visual projection areas of the brain of rodents; maximum blood Pb level in the low and high dose group reached 20 µg/dL and 220µg/dL, respectively. Liliental et al. (1988) found

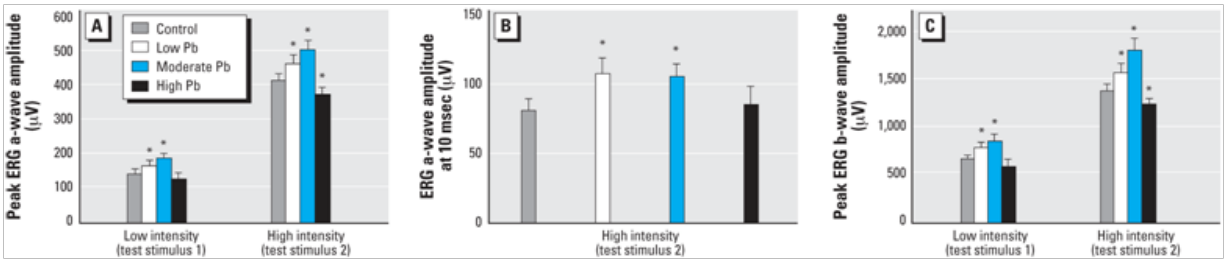
decreased amplitudes and increased latencies in visual evoked potentials from electroretinograms. Pb affected amplitude under dark conditions (dark adaptation, B waves affected) and latencies under bright conditions; blood Pb levels were 40 and 50 µg/dL in the 350 or 600 ppm Pb groups, respectively. Earlier work in rodents found that a moderate to high postnatal Pb exposure induced ERG subnormality (Fox & Chu, 1988; Fox & Farber, 1988; Otto & Fox, 1993). Thus, the historical animal toxicology literature shows multiple effects on vision from Pb exposure (Table 5-11, high dose).

Table 5-11. Summary of toxicological studies of Pb on the retina.

High Dose Pb	Low Dose Pb
Gestational (GLE) or Postnatal Pb exposure Persistent subnormal scotopic ERG Delayed Dark Adaptation Decreased thickness of ONL INL (GLE) Dose dependent decreased DA homeostasis (GLE) Increased retinal cell apoptosis (postnatal Pb) No increased rod neurogenesis (GLE) No increased progenitor cell proliferation (GLE)	Gestational Pb exposure Persistent scotopic ERG supeanormality Increased thickness of the ONL Dose dependent decreased DA homeostasis Males affected, females not affected No increase in retinal apoptosis Increased progenitor cell proliferation Increased neurogenesis of rods Larger size of retina

The current literature for this ISA has more work by the Fox lab showing retinal effects in rodents after human equivalent gestational Pb exposure (GLE, gestationally out to PND10); this is developmentally equivalent to the in utero retinal development period in humans. Pb exposure during various developmental windows and at specific doses has been shown to significantly affect electroretinographs (ERG) both in Pb-exposed humans and rodents (Fox et al., 2008; Rothenberg et al., 2002) (Table 5-11 and Figure 5-26). Consistent with low dose Pb exposure associated ERG supernormality in children (Rothenberg et al., 2002), Fox et al. (2008) found low and moderate ose gestational Pb-exposure (GLE) induced persistent supernormal scotopic ERGs in rodents. Low and moderate GLE also induced increased rod and rod bipolar cell neurogenesis (proliferation) and increased thickness and cell number of the outer and inner neuroblastic layers of the retina (ONL and INL) (Fox et al., 2008; Giddabasappa et al., 2011). Rodents with moderate dose GLE (blood Pb level 25 µg/dL) had 27-fold increased retinal progenitor cell proliferation (Giddabasappa et al., 2011). Extension of the in vivo studies to isolated cultured cells showed GLE increased and prolonged proliferation of retinal progenitor cells (Giddabasappa et al., 2011). Nitric oxide has been shown to regulate retinal progenitor cell proliferation in chick embryos (Magalhaes et al., 2006).

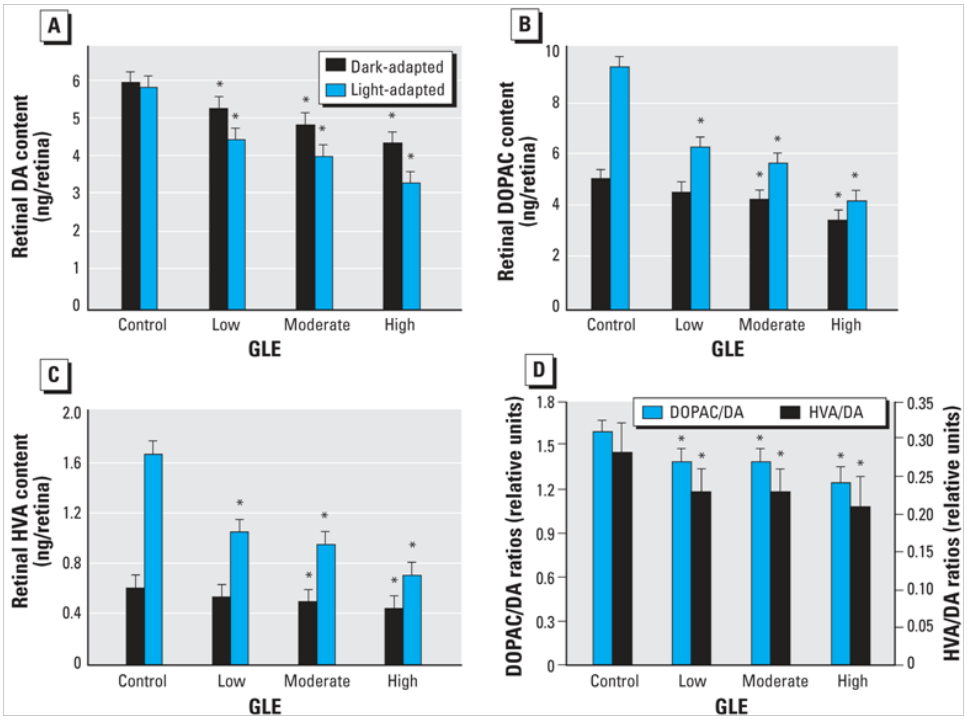
Because Pb exposure has been shown to impair NOS activity in other organs (Section 5.2.4.5), these authors postulate that impaired NO production may be linked to aberrant retinal cell proliferation (Giddabasappa et al., 2011). GLE did not significantly affect apoptosis during retinal development (Giddabasappa et al., 2011) but it did contribute to increased proliferation of retinal cells. GLE induced decreased DA synthesis and use in a dose-dependent fashion (Fox et al., 2008) (Figure 5-27).



Note: *p <0.05

Source: Fox et al. (2008)

Figure 5-26. Retinal a-wave and b-wave ERG amplitude in GLE adult males.



Note: *p <0.05

Source: Fox et al. (2008)

Figure 5-27. Retinal dopamine metabolism in adult control and GLE rats.

Many outcomes in this study showed an inverse U Pb dose-response curve as is shown with the high dose exposures having vastly different effects from low dose GLE. GLE exposure at high doses produced ERG subnormality, rod cells loss, and decreased rod neurogenesis (Fox et al., 2008) (Table 5-11). The high dose GLE rodents showed dose-dependent decreased DA synthesis and use (Figure 5-27). These new animal toxicology data confirm the epidemiologic data showing ERG supernormality at low dose GLE. They provide further insight into retinal changes showing increased proliferation of Pb-

exposed retinal progenitor cells without changes in apoptosis. GLE induced dose-dependent decrements in retinal DA use and synthesis.

Lead-Induced Auditory Effects

The 2006 Pb AQCD mentioned auditory effects on non-human primates who were exposed to Pb throughout gestation and out to age 8-9 (blood Pb levels 33-56 µg/dL during Pb exposure period). Auditory evoked potentials, which are used as a general test to assess neurological auditory function, revealed Pb related effects that persisted even after Pb exposure had ceased and blood Pb level had returned to baseline levels. In Pb-exposed animals, half of the pure tone detection thresholds were outside of the control range at certain frequencies (Rice, 1997), which is consistent with data from humans developmentally exposed to Pb. Thus, these authors found that early life Pb exposure impaired auditory function. The cochlear nerve in both developing and mature humans appears to be especially sensitive to the Pb insult. At low to moderate Pb exposures, elevated thresholds and increased latencies are seen in brainstem auditory evoked potentials. There is coherence between the animal and the human literature on the effects of chronic Pb exposure on auditory function.

5.3.5. Neurodegenerative Diseases

5.3.5.1. Epidemiologic Studies of Adults

The 2006 Pb AQCD described several studies examining associations of blood and bone Pb levels with neurodegenerative diseases such as Alzheimer's disease and dementia. Two NAS studies found associations between increasing bone Pb levels and decreasing MMSE scores (Weisskopf et al., 2004; R. O. Wright et al., 2003), which pointed to an association with dementia, given that the MMSE is widely used as a screening tool for dementia. Overall, studies had sufficient limitations, and findings were inconclusive (U.S. EPA, 2006). New studies on dementia are not available to assess further the association of Pb biomarkers with dementia. Similarly, new studies examining Alzheimer's disease are not available, and as in 2006, the evidence is inconclusive regarding the association between Pb biomarkers and Alzheimer's disease. In contrast, there has been additional investigation of ALS, Parkinson's disease (PD), and essential tremor, which is described below.

Amyotrophic Lateral Sclerosis

Most studies of the association between Pb and ALS have relied on indirect methods of assessing Pb exposure and overall, have produced inconsistent results. The 2006 Pb AQCD reviewed two case-control studies that measured blood Pb levels. One study found no difference in mean blood Pb levels

between the 16 ALS cases (mean: 12.7 µg/dL) and 39 controls (mean: 10.8 µg/dL) (Vinceti et al., 1997). Another study that examined blood and bone Pb levels in a New England-area population found increased odds of ALS among subjects with blood Pb levels ≥ 3 µg/dL (Kamel et al., 2002). In analyses of tibia or patella Pb tertiles, subjects in the highest two tertiles (≥ 10 µg/g patella Pb and ≥ 8 µg/g tibia Pb) had elevated odds of ALS; however, associations were not statistically significant. In analyses of Pb biomarkers as continuous variables, odds ratios for all three biomarkers were similar; however, only associations for blood Pb level were statistically significant. Kamel et al. (2002) also found that an estimate of cumulative Pb exposure based on occupational history to be significantly associated with ALS (Kamel et al., 2002). Thus, the stronger findings for blood Pb level were surprising given that bone Pb level is a better biomarker of cumulative Pb exposure than is blood Pb level. One explanation for these findings is that the association could be the result of reverse causality since the half-life of blood Pb is only about 30 days, and blood was collected from people who already had ALS. If, for example, reduced physical activity among those with ALS led to more bone turnover, then more Pb would be released from bones into circulation leading to elevations in blood Pb levels among cases as a result of effects of the disease.

Since the 2006 Pb AQCD, additional studies have been conducted in the New England-area case-control study. One study indicated that the association between blood Pb level and ALS was not modified by the ALAD genotype (Kamel et al., 2005). Another study examined survival with ALS among 100 of the original 110 ALS cases (Kamel et al., 2008). Higher tibia Pb levels were associated with longer survival time. Findings were similar for patella and blood Pb levels, however, they were associated with smaller increases in survival time. These paradoxical findings raise the concern that in a case-control study of ALS, the association between bone Pb levels and ALS may be biased because the case group may comprise more individuals with longer survival time. Consequently, their bone Pb levels may be higher because they reflect a longer period of cumulative exposure. However, because the strongest findings for survival were found for tibia Pb, it might be expected a bias would be most apparent in a study examining associations of tibia Pb levels and ALS incidence. However, this was not observed in the one study that had bone and blood Pb biomarkers (Kamel et al., 2002).

Another case-control study examined blood Pb levels and odds of 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 frequency matched by age, gender, race, and past use of the Veterans Administration system for health care. A doubling of blood Pb levels was associated with an OR (95% CI) of 2.6 (1.9, 3.7). Associations did not differ substantially by indicators of bone turnover but were 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 suggests that reverse causation is not likely driving the association between blood Pb level and ALS. Whether other types of reverse causality are occurring, however, cannot be ruled out. This study did not have measures of bone Pb and therefore could not assess the association with biomarkers of cumulative Pb exposure.

In summary, several studies have found associations of blood and bone Pb levels with ALS; however, the issues of reverse causality and bias due to survival time make it difficult to draw firm conclusions.

Parkinson's Disease

A few studies, some ecological (Aquilonius & Hartvig, 1986; Rybicki et al., 1993) and some case-control relying on questionnaire data or occupational history (Gorell et al., 1997; Gulson et al., 1999; Tanner et al., 1989) have indicated associations between exposure to heavy metals, particularly Pb, and risk of PD, although the evidence is limited and far from conclusive. Coon et al. (2006) expanded on earlier studies that had found more than a twofold increased risk of PD among adults occupationally-exposed to Pb for more than 20 years (Gorell et al., 1997; Gulson et al., 1999) by examining associations with bone and whole-body Pb levels. Coon et al. (2006) included 121 PD patients and 414 age-, sex-, and race-, frequency-matched controls all receiving health care services from the Henry Ford Health System. Subjects in the highest quartile of both tibia (OR: 1.62 [95% CI: 0.83, 3.17] for levels $\geq$ 15 µg/g) and calcaneus (OR: 1.50 [95% CI: 0.75, 3.00] for levels $\geq$ 25.29 µg/g) bone Pb levels were at elevated odds of PD compared to those in the lowest quartile. The highest OR for PD was estimated for subjects in the highest quartile of whole-body lifetime exposure to Pb, compared to the lowest quartile of exposure (OR: 2.27 [95% CI:1.13, 4.55] for levels $\geq$ 80.81 µg/g).

The second study to explore the association between biomarkers of Pb and PD was published recently (Weisskopf et al., 2010). This study was based in the Boston, MA area and had more than twice as many cases as Coon et al. (2006): 330 cases and 308 controls. Subjects in the highest quartile of tibia Pb level (>16.0 µg/g) had elevated odds of PD compared to those in the lowest quartile (OR: 1.91 [95% CI: 1.01, 3.60]). In this study, cases and controls were recruited from several different sources including movement disorder clinics and community-based cohorts, which could have introduced some biases. However, when analyses were restricted to cases recruited from movement disorder clinics and to their spouse, in-law, or friend as controls, the results were even stronger (OR: 3.21 [95% CI: 1.17, 8.83]). Although the use of spouse, in-law, and friend controls can introduce bias, this is expected to be towards the null as these groups are likely to share many exposures.

Essential Tremor

The 2006 Pb AQCD described two studies that found associations between blood Pb levels and odds of PD in New York City-based populations (Louis et al., 2005; Louis et al., 2003). Since then, Dogu et al. (2007) reported on a case-control study of 105 essential tremor cases from a movement disorder clinic in Turkey and 105 controls (69 spouses and 36 other relatives living in the same district). Blood Pb levels in this study were comparable to those found in the earlier New York based studies: the median blood Pb was 2.7 μg/dL among cases and 1.5 μg/dL among controls. After adjusting for age, sex, education, cigarette smoking (yes versus no), cigarette pack-years, and ethanol use (yes vs. no), a 1 μg/dL increase in blood Pb level was associated with more than a fourfold increase in odds of essential tremor (OR: 4.19 [95% CI: 2.59, 6.78]). This OR was much larger than that obtained in the New York 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, but past occupation that could have contributed to Pb exposure (possibly stored in bone from where it could have contributed to blood Pb levels at the time of the study) was not assessed. One of the earlier New York studies also found a very high OR for essential tremor among ALAD2 carriers (per 1 μg/dL increase in blood Pb level, OR: 71.8 [95% CI: 1.08, 4789.68); however, the very wide 95% CI indicated lack of precision in the effect estimate.

Several studies of essential tremor have reported a very strong association between blood Pb levels and essential tremor, although studies employing biomarkers of Pb have had relatively small sample sizes and have produced imprecise effect estimates.

5.3.5.2. Toxicological Studies

In epidemiological studies, Pb level (bone and blood) is associated with increased odds of ALS (Kamel et al., 2005) and paradoxically with longer survival time in patients diagnosed with ALS (Kamel et al., 2008). Chronic Pb exposure (Pb acetate in drinking water at 200 ppm from weaning onward, blood Pb level 27 μg/dL) reduced astrocyte reactivity and induced increased survival time in the superoxide dismutase transgenic (SOD1 Tg) mouse model of severe ALS (Barbeito et al., 2010). In this model, Pb exposure does not significantly increase the onset of the ALS disease in SOD1 Tg mice, but Pb exposure was associated with longer survival time in SOD1 Tg mice (Barbeito et al., 2010). Baseline levels of VEGF are elevated in astrocytes from the ventral spinal cord of untreated SOD1 Tg control mice versus control, non-transgenic animals. VEGF was not induced in Pb treated non-transgenic astrocytes. Further, Pb-exposed SOD1 Tg mice had significant elevations of astrocyte VEGF versus vehicle treated SOD1 animals (Barbeito et al., 2010). Control animals exposed to Pb showed no elevation in VEGF expression above control vehicle-treated animals (Barbeito et al., 2010). Other research has suggested that ALS

initiation is dependent on motor neuron function and ALS progression is dependent on astrocyte and microglia function (Boillee et al., 2006; Yamanaka et al., 2008).

Consistent these findings, other have 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, Barbeito et al (2010) found that an up-regulation of VEGF production by astrocytes in the Pb-exposed SOD1 Tg mice is protective against motor neuron death in the SOD1 Tg cells (Barbeito et al., 2010). Chronic Pb exposure in a mouse model of ALS was associated with increased survival time and correlated with higher spinal cord VEGF levels, making astrocytes less cytotoxic to surrounding motor neurons (Barbeito et al., 2010). Also, in another study the metal chelators DP-109 and DP-460 are neuroprotective in the ALS mouse model or Tg(SOD1-G93A) (Petri et al., 2007).

Improper activation of microglia and release of inflammatory cytokines and metabolites can contribute to neurodegeneration (L. Qian & Flood, 2008; D. Zhang et al., 2010). These two cell types are known to accumulate or sequester Pb in the nervous system. Researchers have implicated dysfunctional astrocytes as playing an important role in the chain of misregulated inflammation leading to neurodegenerative conditions (Barbeito et al., 2010; De Keyser et al., 2008).

Cell Death Pathways

Earlier work has documented that Pb exposure could induce cell death or apoptosis in various models including rat brain (Tavakoli-Nezhad et al., 2001), retinal rod cells (L. H. He et al., 2000; 2003), cerebellar neurons (Oberto et al., 1996), and PC12 cells (Sharifi & Mousavi, 2008). This study addresses chronic (40 days) Pb exposure-induced hippocampal apoptosis in young (exposure starting at 2-4 weeks of age) and old (exposure starting at 12-14 weeks of age) male rats exposed to 500 ppm Pb by drinking water (blood Pb, 98 µg/dL); apoptosis was verified by light and electron microscopy, and increased pro-apoptotic Bax protein levels (Sharifi et al., 2010). Another study followed the developmental profile of changes in various apoptotic factors in specific brain regions of animals exposed to Pb acetate (0.2% dam drinking water) during lactation. Male offspring blood Pb at the end of lactation or PND20 was 80 µg/dL. These data showed hippocampal mRNA for various apoptotic factors including caspase-3, Bcl-x and Brain-derived neurotrophic factor (BDNF) was significantly upregulated on PND12, PND15 and PND20. The cortex of these male pups also showed upregulation of Bcl-x and BDNF on PND 15 and PND20 (Chao et al., 2007). The cerebellum did not have elevated apoptotic mRNA levels in this model. This study shows temporal and regional changes in activation of death protein message levels in male offspring. Thus, the new data continue to confirm that Pb exposure induced apoptosis in brains of exposed animals.

Lead-Induced Neuronal Plaque Formation

Studies from the 2006 Pb AQCD highlighted the importance of Pb exposure in early life in promoting Alzheimer's like pathologies in the adult brain. Pb has been recognized for decades as having a more profound effect on children than adults who receive the same exposure. In the last decade, the developmental origins of adult disease (DoAD) paradigm and the similar Barker hypothesis have reported that early life exposures can result in aberrant adult outcomes. Epidemiology data show that blood Pb level effects on neurological outcomes fit this paradigm. Recent evidence in the toxicological literature points to latent effects in rodent and non-human primate models of gestational and/or early life exposure to Pb including neurodegeneration similar to Alzheimer's like pathologies, obesity (in males only), retinal aberrations (male only), and neurobehavioral aberrations. Immune outcomes fitting the DoAD paradigm involve tissue inflammation and loss of organ function. Bolin et al. (2006) demonstrated the connection between developmental exposure to Pb in the rat with early life programming and the resulting inflammation-associated DNA damage with neurodegenerative loss in the adult brain. Wu and colleagues (2008) had similar findings in a study using infantile exposure to Pb in monkeys. The investigations reinforce the need to directly examine the long-term effects of developmental exposure to xenobiotics rather than relying on adult exposure information to predict probable health risks from prenatal, neonatal or juvenile exposure (Dietert & Piepenbrink, 2006). Mechanistically, some of these pathologies have been associated with changes in the epigenome. Multiple Pb studies point to sensitive windows of exposure; early life or developmental exposures are far more sensitive than adult exposures. Alzheimer's disease is characterized by amyloid-beta peptide (Ab) accumulation, hyper-phosphorlyation of the tau protein, neuronal death and synaptic loss. The toxicological evidence for Pb in contributing to the AD pathology follows.

The fetal basis of amyloidogenesis has been explored extensively by Zawia's laboratory in both rodents and non-human primates. Amyloid deposits in the brain are seen in patients with Alzheimer's disease and in the aged brain of individuals with Down Syndrome who display an Alzheimer's-like pathology. Mechanistically, amyloid plaques originate from the cleavage of the amyloid precursor protein (APP) to Ab, which comprises the plaque. By exposing rodents to Pb as neonates or as aged animals, it was determined that neonatal Pb exposure is a sensitive window for induction of the amyloidogenesis in the aged animal brains and that exposure to Pb in old animals did not contribute to plaque formation. Following cortical APP gene expression over the lifetime of male rodents exposed neonataly via lactation to Pb (PND1-PND20 exposure, dam drinking water Pb acetate 200 ppm, pup PND20 blood Pb level 46 µg/dL and cortex 0.41 µg/g wet weight of tissue), one sees a biphasic significant increase above control animals in APP expression with the first increased phase manifesting neonatally and the second phase manifesting in old age (82 weeks of age) (M.R. Basha et al., 2005). A concomitant biphasic response is seen in specificity protein 1 (Sp1), a transcription factor known to be related to APP expression. Ab, the

amyolid plaque constituent, was also significantly elevated in these aged animals developmentally exposed to Pb. A separate subset of rodents exposed to Pb only as aged adults (18-20 weeks of age) were unresponsive in APP or Sp1 expression or Ab production after Pb exposure, indicating the developmental window and not adult exposure as the susceptible period for Pb-dependent amyloidogenesis. The Zawia lab (J. Wu et al., 2008) has confirmed similar amyloid findings in brains of monkeys that were exposed to Pb as infants (PND1-PND400), i.e., significantly higher message levels 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 (Rice, 1990, 1992). After weaning and when still being dosed with Pb, the monkeys had blood Pb level of 19-26 µg/dL. As a caveat, by the time neonatally Pb-exposed animals become aged and manifest with amyloid plaques, blood Pb level and brain cortex Pb levels have returned to control or baseline levels. Thus, the rodent and non-human primate toxicology studies concur and show that developmental Pb exposure induced elevations in neuronal plaque proteins in the aged animals.

Transcription factors are essential in the regulation of the developing brain. Pb exposure is known to perturb DNA binding of transcription factors including SP1 at essential sites like zinc finger proteins. In Long-Evans hooded rat pups exposed during lactation, these Pb-induced developmental perturbations of SP1 DNA binding can be ameliorated by exogenous zinc supplementation (M. R. Basha et al., 2003).

The mechanism by which Ab peptide formation is affected by Pb exposure has been explored by Behl et al. It has been shown that the choroid plexus is able to remove beta-amyloid peptides from the brain extracellular matrix and that Pb impairs this function and may be mediated by the metalloendopeptidase, insulin-degrading enzyme (IDE), which metabolizes Ab (Behl et al., 2009).

Further studies with developmental Pb exposure (gestational plus lactational, dam drinking water solutions of 0.1%, 0.5% or 1%, blood Pb level 400, 800 and 1,000 µg/L) showed that the hippocampus contained neurofibriallary changes as early as PND21. These changes manifested with Tau hyper-phosphorylation, and increased tau and beta amyloid hippocampal protein levels in Pb-exposed offspring (Li et al., 2010). The multiple new studies on Pb-dependent changes in the neurofibrillary proteins show that developmental Pb exposure induced significant increases in neuronal plaque associated proteins, indicating that early life Pb exposures may contribute to dementia in adulthood.

Data from the animal toxicology literature point to an early life window in which Pb exposure can contribute to pathological brain changes consistent with those seen in Alzheimer's disease including Ab peptide accumulation and activation of Ab supporting transcription factors as well as tau hyperphosphorylation.

5.3.6. Studies of Mechanisms of the Neurological Effects of Lead

5.3.6.1. Effects on Brain Physiology and Activity

A growing body of epidemiologic evidence demonstrates associations of Pb biomarkers with electrophysiologic changes in the brain. By providing insight into the underlying mechanisms by which Pb exposure may disrupt brain function, findings from these studies have provided biologically plausible evidence for the effects of Pb exposure on cognitive, psychological and behavioral consequences observed in children and adults. Much of the early work was conducted by Otto and colleagues (Otto et al., 1985; Otto & Fox, 1993), which found associations of blood Pb level with auditory and visual evoked potentials. Rothenberg et al. (1994) and Rothenberg et al. (2000) reported similar findings; however, the direction of association differed between prenatal (maternal) and postnatal (ages 1-4 years) blood Pb level. Postnatal blood Pb level was associated with a decrease in interpeak intervals in auditory evoked potentials at age 5-7 years. Prenatal blood Pb level showed a biphasic relationship, with a negative association at blood Pb levels of 1-8 µg/dL and a positive association at blood Pb levels of 8-30 µg/dL. These findings provide mechanistic support for observations of Pb-associated changes in sensory acuities (Section 5.3.4.1).

Studies using magnetic resonance imaging (MRI) or spectroscopy (MRS) as clinical outcome measures have been limited in number and sample size, but have shown associations of blood Pb level with alterations in brain physiology such as reduced levels of N-acetylaspartate, creatine, or choline in young adults (Cecil et al., 2005; Meng et al., 2005; Trope et al., 2001; Trope et al., 1998; Yuan et al., 2006). These changes have been linked to decreased neuronal density or loss and alteration in myeline. Notably, Trope et al. (2001; 1998) and Meng et al. (2005) reported that all subjects had normal MRIs with no evidence of structural abnormalities. Thus, the clinical significance of the observed physiological changes is unclear. Additionally, these studies compared subjects with relatively high childhood blood levels (23-65 µg/dL) to those with childhood blood Pb levels <10 µg/dL. Therefore, it is unclear whether physiological changes would be observed in association with lower blood Pb levels. Cecil et al. (2005) and Yuan et al. (2006) conducted functional MRI in 42 adult (ages 20-23 years) participants from the CLS cohort during a verb generation language task and found that mean childhood blood Pb level was associated with decreased activation in the left frontal gyrus and left middle temporal gyrus, regions traditionally associated with semantic language function. Although these findings were in adults, they were consistent with findings in the same cohort of associations of blood Pb level with other indices of language in childhood.

Since the 2006 Pb AQCD, studies examining MRI data have been limited to CLS cohort participants as adults (ages 19-24), and recent results continue to support associations of childhood blood

Pb levels with physiological changes in the brain in adulthood. These recent studies expanded on previous studies by including larger sample sizes and aiming to characterize Pb effects more precisely by examining blood Pb levels at different periods in childhood and aiming to link changes in brain activity to neurodevelopmental deficits. Brubaker et al. (2009) used diffusion tensor imaging to examine associations between mean childhood blood Pb and white matter integrity in 91 young adults, hypothesizing that childhood Pb exposure may alter adult white matter architecture via deficits in axonal integrity and myelin organization. Fractional anisotropy (FrA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were measured on an exploratory voxel-wise basis. In adjusted analyses, mean childhood blood Pb levels were associated with decreased FrA throughout white matter. Regions of the corona radiata demonstrated highly significant Pb-associated decreases in FrA and AD and increases in MD and RD. The genu, body, and splenium of the corpus callosum demonstrated highly significant Pb-associated decreases in RD, smaller and less significant decreases in MD, and small areas with increases in AD. The results of this analysis suggest multiple insults appear as distinct patterns of white matter diffusion abnormalities in the adult brain which may be indicative of altered myelination and axonal integrity. Additionally, childhood blood Pb levels appear to differentially affect neural elements, which may be related to the stage of myelination development at various periods of exposure.

Another study of 157 CLS participants provided evidence of region-specific reductions in adult gray matter volume in association with childhood blood Pb levels and by examining associations between MRI-assessed brain volume and historic neuropsychological assessments, provided insight into the potential clinical significance of changes in brain physiology associated with blood Pb levels (Cecil et al., 2008). Using conservative, minimum contiguous cluster size and statistical criteria (700 voxels, unadjusted $p < 0.001$), approximately 1.2% of the total gray matter was significantly and inversely associated with mean childhood blood Pb level. The most affected regions included frontal gray matter, specifically the anterior cingulate cortex and the ventrolateral prefrontal cortex (i.e., areas traditionally related to executive functions, mood regulation, and decision-making). Comparing neuropsychological factor scores with gray matter volume, investigators found that fine motor factor scores 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 are notable in light of other studies linking brain volume changes with altered function and suggest that MRI changes association with blood Pb levels may be indicative of decrements in neurocognitive and neurobehavioral function. Schwartz and colleagues (2007) showed that larger RO1 volumes were associated with better cognitive function in 5 or 6 cognitive domains (visuoconstruction, processing, speed, visual memory, executive functioning, and eye-hand coordination). More recent studies by Raine and colleagues suggest that deficits in cortical volume or activity found in select brain regions, including the prefrontal gray matter, may predispose individuals to impulsive, aggressive, or violent behavior.

In a subsequent analysis, Brubaker et al. (2010) investigated the developmental trajectory of childhood blood Pb levels on adult gray matter. Adjusted voxel-wise regression analyses were performed for associations between adult gray matter volume loss and yearly mean blood Pb levels from 1 to 6 years of age in the entire cohort and by sex. Investigators observed significant inverse associations between gray matter volume loss and yearly mean blood Pb levels from 3 to 6 years of age. The extent of prefrontal gray matter loss associated with yearly mean blood Pb levels increased with advancing age of the subjects. These results indicate that blood Pb levels later in childhood are associated with greater losses in gray matter volume than are childhood mean or maximum blood Pb levels. This study demonstrates that maximum blood Pb levels do not fully account for gray matter changes, particularly in the frontal lobes of young men. Notably, although they did not consider Pb, Yang et al. (2005) reported volume reduction in gray matter in psychopaths, adding additional evidence that these physiological changes may be related to overt deleterious outcomes. Consistent with Wright et al. (2008), Cecil et al. (2008) found that gray matter volume loss associated with childhood blood Pb levels was much larger in CLS male adults than female adults. In an expanded analysis of the developmental trajectory of childhood blood Pb levels on adult gray matter, Brubaker et al. (2010) found that the inverse associations between gray matter volume loss and yearly mean blood Pb levels were more pronounced in the frontal lobes of men than women for blood Pb levels measured at all ages.

Whereas the aforementioned CLS studies examined associations of childhood blood Pb levels, a recent analysis of the NAS participants indicated that biomarkers of cumulative, long-term Pb exposure also may be associated with changes in brain structure and function in older adults. Weisskopf et al. (2007) found increasing bone Pb level to be associated with increased myoinositol (mI)/Cr ratio with increasing bone Pb concentration among 31 elderly men from the NAS. A higher mI/Cr ratio may be indicative of glial activation and is a signal reportedly seen in the early stages of HIV-related dementia and Alzheimer's disease.

Studies of Pb-workers also found associations of blood and bone Pb levels with changes in brain structure and physiology. Stewart et al. (2006) studied 532 former organolead workers with a mean age of 56 years and found that an estimate of past peak tibia bone Pb (mean: 23.9 µg/g) was significantly associated with more white matter lesions (WML). Higher estimated peak tibia Pb also was associated with smaller total brain volume and volumes of frontal and total gray matter, parietal white matter, the cingulate gyrus, and insula. In this same group, Caffo et al. (2008) found evidence that the association between tibia Pb level and cognitive function, in particular, visuo-construction domain tasks, and to a lesser degree, executive function and eye-hand coordination, were mediated by the association between tibia Pb levels and brain region volume changes. In a similar study of 61 current Pb smelter workers with an average age of 40 years, higher estimates of cumulative Pb exposure were also associated with WML, and there was evidence that this association accounted for, in part, the association between higher cumulative Pb exposure and worse performance on the grooved pegboard test (Bleecker, Ford, Vaughan,

et al., 2007). Another small study of 15 current occupationally Pb-exposed workers (mean blood Pb level: 63.5 µg/dL) and 19 non-Pb exposed controls (mean blood Pb level: 8.74 µg/dl) found smaller hippocampal volumes in MRI scans among the Pb workers (Jiang et al., 2008). A lower ratio of the brain metabolites N-acetyl-aspartate (NAA) and creatine (Cr), indicative of neuronal density, was also found among the Pb workers, as well as an increased lipid to creatine ratio, indicative of cell membrane or white matter damage. Similarly, a study of 22 Pb paint factory workers and 18 controls found lower NAA/Cr ratios among the Pb exposed workers as well as lower choline/Cr, possibly indicative of reduced cell membrane turnover (Hsieh et al., 2009).

5.3.6.2. 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 manifested with Pb-induced cholesterogenesis and phospholipidosis in brain tissue (Ademuyiwa et al., 2009). Pb-dependent changes in brain cholesterol produced an inverse U dose response curve with the highest brain cholesterol at 200 ppm followed by 300 ppm Pb. Animals exposed to 400 ppm Pb did not have significant changes in brain cholesterol. Mechanistically, Pb exposure has been shown to depress the activity of cholesterol-7-a-hydroxylase, an enzyme involved in bile acid biosynthesis (Kojima et al., 2005); bile acids are the route by which cholesterol is eliminated from the body. Pb exposure produced significant increases in brain triglycerides with an 83% increase at 300 ppm and a 108% increase at 400 ppm. At 200 ppm, Pb exposure induced a non-significant decrease in brain triglycerides. Pb exposure across all three dose groups induced significantly increased brain phospholipids. Interestingly, plasma free fatty acids were significantly elevated in a dose-dependent fashion; plasma triglycerides and cholesterol were unaffected by Pb exposure. The molar ratio of brain cholesterol to phospholipids, an indicator of membrane fluidity (Abe et al., 2007), 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 at 0, 200, 300, and 400 ppm were 7, 41, 61, and 39 µg/dL, respectively. In summary, Pb exposure significantly increased brain cholesterol, triglycerides, and phospholipids as well as significantly increased plasma free fatty acids. These effects were sometimes more prominent at lower doses of Pb. Future characterization of molecular and cellular pathways affected by Pb exposure may bring insight to this Pb-dependent phospholipidosis and cholesterogenesis.

5.3.6.3. Oxidative Stress

Pb has been shown to induce oxidative stress in multiple animal models and this oxidative stress can contribute to DNA damage, which can be measured with the biomarker 8-hydroxy-2'-deoxyguanosine (8-oxo-dG). The contribution of reactive oxygen or nitrogen species to these Pb induced

changes was assayed by looking at the ratio of 8-oxo-dG to 2-deoxyguanosine (2-dG). 2-dG is a DNA nucleoside enzyme that can generate 8-oxo-dG from a parent compound forming a DNA adduct or biomarker during conditions of nitrosative or oxidative stress. The 8-oxo-dG to 2-dG ratio data from rodent male offspring recapitulated the amyloid data with significant biphasic elevations in developmentally Pb-exposed animals (0.2% Pb acetate in dam drinking water from PND1-20) versus control, non-Pb exposed animals at early (PND5) and late life time points (80 weeks of age) (Bolin et al., 2006). Activity of the base-excision DNA repair enzyme oxoguanine glycosylase or Ogg1 was unaffected by Pb exposure (Bolin et al., 2006). Interestingly, the monkey data were the same as the rodent data. The ratio of 8-oxo-dG to 2-dG in the brains of aged monkeys (23 years) after being exposed as infants, was significantly elevated above controls (J. Wu et al., 2008). Similar to the amyloid data, the oxidative stress markers showed no significant changes above baseline when animals were exposed to Pb as aged adults (Bolin et al., 2006; J. Wu et al., 2008). Thus, the data for biomarkers of oxidative stress concur with the amyloidogenesis data with both demonstrating kinetically similar biphasic significant elevations in markers of oxidative stress and amyloidogenesis with early life Pb exposure.

Because the brain has the highest energy demand and metabolism of any organ, energy homeostasis is of utmost importance. Pb has been shown to inhibit various enzymes involved in energy production or glucose metabolism including glyceraldehydes-3 phosphate dehydrogenase, hexokinase, pyruvate kinase, and succinate dehydrogenase (Regunathan & Sundaresan, 1984; Sterling et al., 1982; Verma et al., 2005; Yun & Hoyer, 2000). 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 neuotransmission. The mitochondrial Na/K ATPase is important in maintaining the inner mitochondrial membrane potential (delta omega sub m) and the health of the mitochondria. To address the effect of Pb exposure on these mitochondrial parameters, mice were mated, produced offspring and nursed the offspring until PND8 at which time the brains were collected from the pups (Baranowska-Bosiacka et al., 2011). Cerebellar granular cells were harvested from these PND 8 control and Pb-exposed animals (0.1% Pb acetate in dam drinking water, blood Pb level 4 µg/dL and cerebella Pb 7.2 µg/g dry weight). These neuronal cells were cultured for 5 days in vitro, at which point various mitochondrial parameters were measured. With Pb exposure, reactive oxygen species 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 control. Neuronal Na/K ATPase activity was significantly lower in cortical granule cells from Pb-exposed mice versus controls. Mitochondrial mass was unaffected with Pb treatment, but mitochondrial membrane potential was significantly decreased with Pb exposure. Pb-exposed crayfish who are placed under hypoxic conditions adapt to the situation by decreasing their metabolism (Morris et al., 2005), manifesting with whole organism findings consistent with these cell data. These data show impaired mitochondrial function and energy production in neuronal cells from mice

with gestational and lactational Pb exposure with concomitant increases in mitochondrial and cellular ROS production.

5.3.6.4. Nitrosative Signaling and Nitrosative Stress

The nitric oxide system is increasingly being recognized as a signaling system in addition to its more classical role as a marker of cellular stress. In studies of learning and memory using the Morris water maze, hippocampal changes in NO were noted after completion of the test. Pb exposure has been repeatedly shown to increase the escape latency in Pb-exposed animals (Section 5.3.2.2). Chetty (2001) initially reported decreased hippocampal nNOS with perinatal Pb exposure. Namely, with repeated swim tests, control animals more quickly find a submerged platform, i.e. escape, than do Pb-exposed animals. After either 4 or 8 weeks of Pb exposure to weanling male rats (blood Pb level 0.3 umol/L), hippocampal NOS and NO are significantly decreased. Dietary supplementation concomitant with 8 weeks of Pb exposure induced significant increases in hippocampal NOS (taurine or glycine) and decreases in hippocampal NOS (vitamin C, methionine, tyrosine, or vitamin B1). These animals also had significant changes in hippocampal NO with supplementation. NO increased with taurine and decreased with vitamin C, tyrosine or glycine co-exposure with Pb. Dietary supplementation after 4 weeks of Pb exposure in weanling males (4 week blood Pb level 2.3 umol/L & 8 week Blood Pb level 0.39 umol/L), induced significant increases in NO with the supplements tyrosine, methionine, or ascorbic acid. Zinc supplementation in this model had no effect on the NO system. The conclusions of this study are that various combinations of nutrients significantly attenuate Pb-dependent decreases in NO/NOS. Specifically, nutrients prevented (8 weeks Pb plus concomitant exposure to methionine, zinc, ascorbic acid, and glycine) or restored (4 weeks Pb exposure followed by 4 weeks nutrient exposure, taurine and thiamine) Pb-dependent decrements in NO/NOS concentrations (G. Fan et al., 2009).

5.3.6.5. Synaptic Changes

Work in earlier criteria documents as well as earlier publications in the scientific literature point to an effect of developmental Pb exposure on synapse development, which mechanistically may contribute to multiple Pb related aberrant outcomes, including changes in long-term potentiation (LTP) and facilitation. Earlier work has shown that developmental Pb exposure is responsible for altered density of dendritic hippocampal spines (Király & Jones, 1982; Petit & LeBoutillier, 1979), aberrant synapse elimination (Lohmann & Bonhoeffer, 2008) and abnormal long-term and short-term plasticity (MacDonald et al., 2006). Newer research using the Drosophila larval neuromuscular junction model has shown that stimulation with multiple action potentials (also called AP trains) induced significant increases in intracellular calcium and significant delays in calcium decays back to baseline levels at the pre-synaptic neuronal bouton in developmentally Pb exposed larvae versus control. Pb-exposed larvae had

reduced activity of the plasma membrane calcium ATPase, which is responsible for extravasations of calcium from the synaptic terminal (T. He et al., 2009). Intracellular calcium in Pb exposed larvae was no different from 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 low dose body burden (100 µM) of Pb calculated to be 13-48 µM per larvae. Facilitation of a neuronal terminal is defined as the increased ability to transmit an impulse down a nerve due to prior excitation of the nerve. 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 (T. He et al., 2009). The data from this synapse study demonstrate that developmental Pb exposure affected the plasma membrane calcium 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.

A study by Li et al. (2009) focused on inflammatory endpoints and synaptic changes after gestational plus lactational dam drinking water Pb exposure (solutions of 0.1%, 0.5% or 1%, offspring blood Pb level 40, 80 and 100 µg/dL, respectively at PND 21). Hippocampal TNF-alpha 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 (Li et al., 2009). Thus, Li et al. (2009) found significant difference in hippocampal synaptic protein composition and increased pro-inflammatory cytokine levels in the brains of Pb-exposed offspring.

Neurotransmission is an energy-dependent process with calcium-dependent ATP releases found at the synaptic cleft. At the synapse, ATP is metabolized by ecto-nucleotidases. In heme synthesis, Pb is known to substitute for the cation zinc in another nucleotidase, pyrimidine 5′-nucleotidase, and is thus used as a biomarker of Pb exposure. Acute exposure (96h) of male and female zebrafish to Pb acetate (20 µg/L) in their water induced significant decreases in ATP hydrolysis in brain tissue. This dose is deemed to be an environmentaly relevant dose. With a chronic exposure (30 days), Pb acetate promoted the inhibition of ATP, ADP and AMP hydrolysis; these data are consistent with findings in rodents (Baranowska-Bosiacka et al., 2011). The authors hypothesize that at 30 days, this change in nucelotide hydrolysis was likely due to post-translational modification because message level of enzymes responsible for the hydrolysis, NTPDase1 and 5′-nucleotidase, were unchanged (Senger et al., 2006). Thus, Pb is shown to affect nucleotidase activity in the central nervous system of zebrafish, possibly contributing to aberrant neurotransmission.

Another enzyme important in synaptic transmission at cholinergic junctions in the CNS and at neuromuscular junctions peripherally is acetylcholinesterase (AChE). After 24 hours of exposure to environmentally relevant concentrations of PbAcetate (20 ug/L water), AChE activity was significantly inhibited in zebrafish brain tissue. In Pb-exposed fish, AChE activity returned to baseline by 96 hours and

maintained baseline activity after chronic exposure of 30 days. Thus, Pb is also able to affect synaptic homeostasis of AChE in the brain of exposed zebrafish (Richetti et al., 2010).

Pb is known to act as an antagonist of the NMDA receptor. The NMDAR is essential for proper pre-synaptic neuronal activity and function. Primary cultures of mouse hippocampal cells were exposed to Pb (10 or 100 µM solutions in media) during the period of synaptogenesis (Neal et al., 2010). This exposure induced the loss of two proteins necessary for presynaptic vesicular release, synaptophysin (Syn) and synaptobrevin (Syb), without affecting a similar protein synaptotagmin (Syt). This deficit is seen in both GABAergic and glutamatergic neurons. Pb also induced an increase in number of presynaptic contact sites. But, these sites may be non-functional as they lack the protein receptor complexes necessary for proper vesicular exocytosis. Another factor involved in growth and signaling of pre-synaptic neurons is BDNF, which is synthesized and released by post-synaptic neurons. BDNF is regulated by the NMDAR and acts in a retrograde fashion, participating in pre-synaptic maturation. In this model, both pro-BDNF and BDNF release were significantly attenuated with Pb exposure. Further, exogenous BDNF administration rescued the aforementioned Pb-dependent pre-synaptic effects. Thus, this cell culture model shows that Pb-dependent pre-synaptic aberrations are controlled by NMDAR-dependent BDNF effects on synaptic transmission.

5.3.6.6. Blood Brain Barrier

Two barrier systems exist in the body to separate the brain or the central nervous system from the blood. These two barriers are the blood brain barrier (BBB) and the blood cerebrospinal fluid barrier (BCB). The blood brain barrier, 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 this barrier. Pb exposure during various developmental windows is known to affect the blood brain barrier even at low Pb concentrations resulting in increased permeability (Dyatlov et al., 1998; Moorhouse et al., 1988; Struzynska, Walski, et al., 1997; Sundstrom et al., 1985). Because the BBB is under-developed early in life, prenatal and perinatal Pb exposure results in higher brain Pb accumulation than does similar exposures later in life (Moorhouse et al., 1988). Earlier AQCDs have shown that the chemical form of Pb, and its ability to interact with proteins and other blood components affects its ability to penetrate the BBB (U.S. EPA, 2006). Pb compromises the function of the BCB, and decreased the CSF level of transthyretin, a thryoid binding protein made in the choroid plexus. The choroid plexus and cerebral endothelial cells that form these BBB and BCS tight junctions are known to accumulate Pb more than other cell types and regions of the CNS. Hypothyroid status can contribute to impaired learning and IQ deficits. More recent research with weanling rats fed Pb through Pb acetate drinking water exposure manifested histologically with leaky cerebral vasculature as detected with lanthum nitrate staining of the brain parenchyma, an

indication of BBB impairment, that was ameliorated or that resembled controls after iron supplementation. These weanlings also had significant Pb-induced decreases in the BBB tight junction protein occludin in the hippocampus, brain cortex, and cerebellum that were rescued to control levels with iron supplementation (Q. Wang et al., 2007). These data demonstrate that Pb induced a leaky BBB in weanling rats with associated decreases in the junctional protein occludin; dietary supplementation with iron was able to ameliorate these Pb-induced impairments of the BBB in male rats.

5.3.6.7. Cell Adhesion Molecules

Classic cell adhesion molecules including NCAM and the cadherins are junctional or cell surface proteins that are critical for cell recognition and adhesion. Cell adhesion molecules, particularly the cadherins, are calcium-dependent and thus interaction from competing cations like Pb can contribute to nervous system barrier function disruption, tissue development dysregulation, immune dysfunction, and affect learning and memory (Prozialeck et al., 2002).

5.3.6.8. Glial Effects

Astroglia and oligodendroglia are supporting cells in the nervous system that maintain the extracellular space in the brain and provide support and nutrition to neurons via nutrient transport, strucutral support to neurons, and myelination, among other effects. Glial cells are known to serve as Pb sinks in the developing and mature brain (Tiffany-Castiglioni et al., 1989) by sequestering Pb. This glial sequestration of Pb has been shown to decrease brain glutamine concentrations at a dose of 0.25 ± 1.0 μM Pb acetate via Pb-dependent reduction in glutamine synthestase activity in the astroglia; astroglia take up glutamate after its release and convert it to glutamine. Pb causes hypomyelination and demyelination (F. Coria et al., 1984) mediated through the oligodendrocytes with younger animals being more susceptible to the effects of (Tiffany-Castiglioni et al., 1989). Unfortunately, Pb accumulation in young glial cells may contribute to a lifelong exposure to this Pb sink in the brain as it is released over time where it can damage surrounding neurons (Holtzman et al., 1987).

Glial transmitters

To determine the contribution of the gliotransmitter serine to Pb mediated changes in long-term potentiation (LTP), Sun et al. (2007) performed in vitro patch clamp monitoring of rat hippocampal CA1 section LTPs collected from pups exposed to Pb Acetate in utero, lactationally and through drinking water out to PND28. D-serine supplementation relieved the chronic Pb exposure dependent impaired magnitude of hippocampal LTP (H. Sun et al., 2007), which is known to be regulated by the NMDAR (Bear & Malenka, 1994). The use of 7-chlorokynurenic acid, an antagonist of the glycine binding site of the NMDAR-the binding site of D-serine, effectively abolished D-serine's rescue of the LTP. NMDAR-

independent LTP hippocampal neurotransmission with slices from Pb-exposed mossy-CA3 synapses was not rescued by exogenous D-serine supplementation. These data indicate that glial transmission may provide promising therapeutic targets for intervention after Pb exposure or with other affective or cognitive disorders known to manifest with aberrant NMDAR-dependent neurotransmission.

5.3.6.9. 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 signals for its homeostasis. Pb-dependent alterations in neurotransmission are discussed in further detail below.

Monoamine Neurotransmitters and Stress

Combined exposures of maternal stress and Pb exposure can synergistically enhance behavioral and neurotoxic outcomes in exposed offspring, sometimes even potentiating an effect that would otherwise be sub-threshold. Virgolini et al. (2008) found that effects on the central nervous system by developmental Pb exposure (2 months prior to mating through lactation, 50 or 150 ppm Pb acetate drinking water exposure, blood Pb level 11 µg/dL and 35 µg/dL, respectively) are enhanced by combined maternal and offspring stress. Offspring neurotransmitter concentrations were significantly affected with Pb exposure, but the most interesting findings were those of potentiated effects or effects that were not seen with Pb exposure alone or stress alone. These potentiated effects were only seen when Pb was combined with stress (maternal [MS] and/or offspring stress [OS]). Potentiation of serotonin (5HT) levels in females was significant in the frontal cortex in females and in the nucleus accumbens (NAC) in the male offspring (50 and 150 ppm Pb drinking water exposure) (Cory-Slechta et al., 2009). Regional 5HT levels were unaffected in offspring with no stressors and Pb exposure. Thus, Pb alone did not significantly affect 5HT levels. 5HIAA concentration was significantly increased with Pb exposure alone in the striatum of male offspring at 150 ppb Pb exposure; with the remaining exposures, Pb plus stress potentiated striatal and frontal cortex 5HIAA in males. Potentiated 5HIAA levels in females were significant in the NAC at both Pb doses; stress alone also significantly increased 5HIAA levels in 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

The monoamine neurotransmitters include DA, 5HT, and NE. Earlier work has shown that perinatal rat Pb exposure induced increased tyrosine hydroxylase, increased DA and increased cerebral cortex catecholamine neurotransmission (Bielarczyk et al., 1996; C. B. Devi et al., 2005; Leret et al., 2002). Earlier publications detailing the window of exposure, duration of exposure and dose of Pb used have varying effects on monoamine transmitters. In more recent work, these neurotransmitters, among others, have been implicated in auditory function in the brainstem in various integration centers there including the lateral superior olive (LSO), and the superior olivary complex (SOC). The SOC is vital for sound detection in noisy settings among other functions. Low level Pb exposure has been associated with altered processing of auditory temporal signals in animal studies (Finkelstein et al., 1998; Lurie et al., 2006). Because Pb alters auditory processing, the monoamine system is a potential target for Pb-mediated interactions. Blood Pb levels for control, very low Pb (VLPb) and low Pb (LPb) exposure groups are 1.4, 8.0, and 42.2 µg/dL, respectively. Developmental Pb exposure from the formation of breeding pairs to PND21, which is at the end of auditory development in the mouse, led to significant decreases in immunostaining of LSO and SOC brainstem sections for monoamine vesicular transporter VMAT2, and for 5HT and dopamine beta-hyrdoxylase (DbH), a marker for NE. This immunochemistry was significant for both VLPb and LPb exposure for VMAT2 and DbH but 5HT only had significant decrements with VLPb. Immunostaining for TH 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 relating to auditory integration with interaction from the monoamine neurotransmitter system are affected by developmental Pb exposure (Fortune & Lurie, 2009).

Dopamine

The 2006 AQCD detailed low dose Pb-dependent decreased dopaminergic cell activity in the substantia nigra and ventral segmental areas. Earlier studies with moderate to high dose postnatal or adult Pb exposure have reported changes in dopamine (DA) metabolism, DA and DOPAC, a DA metabolite. Thus, these were measured in various brain regions of year old males to determine if GLE affected DA metabolism. Low and high dose GLE in male rodents induced significant elevations in the DOPAC to DA ratio, and DOPAC concentration in the forebrain. In the forebrain, DA was significantly decreased in low dose GLE males and significantly elevated in GLE high dose males compared to controls. In the striatum, DOPAC was significantly elevated in both low and high dose GLE exposed males, but DA concentration was only significantly elevated in high dose GLE males. The striatum ratio of DOPAC to DA was not significantly different from control. These new data expand upon the monoamine literature base which reports perinatal rat low concentration Pb exposure induced increased sensitivity of the dopamine receptors (D2 and D3) (Cory-Slechta et al., 1992; Gedeon et al., 2001), produced higher DA levels (C. B.

Devi et al., 2005; Leret et al., 2002), and enhanced catecholamine neurotransmission in the cerebral cortex, cerebellum, and hippocampus (C. B. Devi et al., 2005).

The interaction of dopamine and the nitric oxide system in the striatum was studied after prenatal Pb exposure. Blood Pb was not reported in this study, but similarly treated Wistar rat pups in other studies report 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 dopamine release in the rat striatum (Nowak et al., 2008). Prenatal Pb exposure attenuated 7-NI's facilitatory effect on dopamine release in the striatum. This interaction is ROS-independent; using spin trap measurements, there were no significant concentration changes in hydroxyl radical with Pb exposure (Nowak et al., 2008). Thus, the neuronal NO system appears to be involved in specific aspects of Pb-dependent dopaminergic changes.

Dopamine and Vision

In various experimental animal models, the loss of retinal dopamine or zinc is associated with supernormal rod-mediated scotopic electroretinograms (ERGs), pointing to the retina as a sensitive target of low dose Pb exposure (19 µg/dL). In the human and non-human primate literature, GLE is associated with increased amplitude (supernormality) of ERGs (Lilienthal et al., 1994; Lilienthal et al., 1988; Rothenberg et al., 2002) and in the animal toxicology literature postnatal Pb exposure induces subnormality of the ERGs. New research in the animal toxicology literature recapitulated the low dose human literature, showing that low-and moderate (LPb or MPb) level gestational Pb exposure in the rat produced supernormal ERGs with associated significant increases in retinal neurogenesis and significant decreases in retinal dopamine use and dopamine turnover, DA and DOPAC:DA ratio, respectively. High gestational Pb exposure (HPb) produced significant subnormal ERGs, similar to the findings with postnatal human Pb exposures. Rats (dams) were exposed to Pb acetate in drinking water starting 2 weeks prior to mating and throughout gestation and lactation until PND 10, a period of developmental exposure that is equivalent to gestational exposure in humans with peak blood Pb PND1-10 of 12, 24, and 46 µg/dL in LPb, MPb, and HPb, respectively. LPb and MPb gestational exposure induced increased cellularity or retinal thickness in the outer nuclear layer, inner nuclear layer and total retina (Leasure et al., 2008). In conclusion, the retina is a sensitive site to low-dose Pb exposure and gestational Pb exposure produced dose-dependent decreases in DA use and turnover; inverted U shaped Pb dose response curves were reported for retinal endpoints including ERG and retinal thickness.

NMDA

NMDA receptors (NMDAR) have been shown to contribute to synaptic plasticity and Pb-exposure at different developmental stages is known to contribute to aberrations in LTP or LTD in the hippocampus via reduced NMDA current, among other mechanisms (L. Liu et al., 2004). The 2006 AQCD detailed that

Pb induced decreases in stimulated glutamate release that affected LTP. Further, it detailed that the Pb-dependent decreased magnitude and increased threshold of the LTP in the hippocampus is biphasic or non-linear. NMDAR subtypes have been shown to be significantly decreased with developmental Pb exposure (Guilarte & McGlothan, 1998). Recent work looking at supplement use, found Pb-dependent decreases in message and protein level of NMDAR subunit NR1 was rescued with methioninecholine coexposure in these weanling male rats (Fan et al., 2010). Fan et al. (Fan et al., 2010) found that Pb-dependent suppression of the NMDAR subunits NR2A and NR2B were not rescued with methioninecholine treatment. Other recent mechanistic studies have found that pretreatment of primary fetal brain neuronal rat cultures with glutamic acid, a NMDAR agonist, reversed Pb-dependent reductions in NMDAR subunits (S.-Z. Xu & Rajanna, 2006) whereas pretreatment with the NMDA antagonist MK-801 exacerbated Pb-induced NMDAR defecits (S.-Z. Xu & Rajanna, 2006). Thus, glutamic acid or methioninecholine may offer therapeutic possibilities for Pb-induced neuronal NMDAR decrements.

The Guilarte lab has made extensive contributions to the Pb animal toxicology literature and a recent publication details the effect of low dose developmental/lifetime Pb exposure on changes in hippocampal neurogenesis in adulthood (Verina et al., 2007), an emerging area of research affecting long-term potentiation, spatial learning, neuronal outgrowth, and possibly mood disorders like schizophrenia. NMDAR mediates the integration of new neurons into existing neuronal pathways in the adult hippocampal DG, which is important to learning and memory. Lifetime Pb chow exposure (dam Pb acetate exposure 10 days prior to mating through pregnancy out to PND50 or PND78) induced significant decrements in hippocampal granule cell neurogenesis or proliferation of new cells in adult rats. Also, Pb exposed animals had significant decreases in brain volume in the stratum oriens (SO) region of the hippocampus, specifically significant decreases in the mossy fiber terminals of the SO. A marker for immature or newly formed neurons showed a significant decrease in the length-density of these cells in the outer portion of the DG in Pb-exposed animals. These findings show that exposure to environmentally relevant doses of Pb induced significant aberrations in adult hippocampus granule cell neurogenesis and morphology, providing mechanistic explanations for Pb-induced neuronal aberrations. Guilarte et al. (2003) demonstrated that Pb exposure of rats from an enriched environment was associated with reversal of learning impairment, increased expression of hippocampal NMDA receptor subunit 1, and increased induction of brain derived neurotrophic factor mRNA (Guilarte et al., 2003).

5.3.6.10. Neurite Outgrowth

The 2006 AQCD reported Pb decreased neurite outgrowth at 20µg/dL noting that Pb interfered with neurite outgrowth via protein kinase mediated pathways (MAPK/ERK); earlier work has documented decreased primary DA neuron outgrowth with 0.001 µM Pb exposure (Lidsky & Schneider, 2004). More recent studies have shown that dam exposure to low dose Pb (blood Pb level 4 µg/dL) of

dams significantly decreased pup hippocampal neurite outgrowth (pup blood Pb level 12 µg/dL) and reduced the expression of hippocampal polysialylated neural cell adhesion molecule (PSA-NCAM), NCAM, and sialytransferase; PSA-NCAM is transiently expressed in newly formed neurons (Q. S. Hu et al., 2008) during the period of neurite outgrowth from embyrogenesis until the early postnatal period and is down-regulated in the adults except in areas known to exhibit synaptic plasticity (Seki & Arai, 1993). NCAM is important for memory formation, plasticity and synapse formation and early life Pb exposure in laboratory rodents affects its expression.

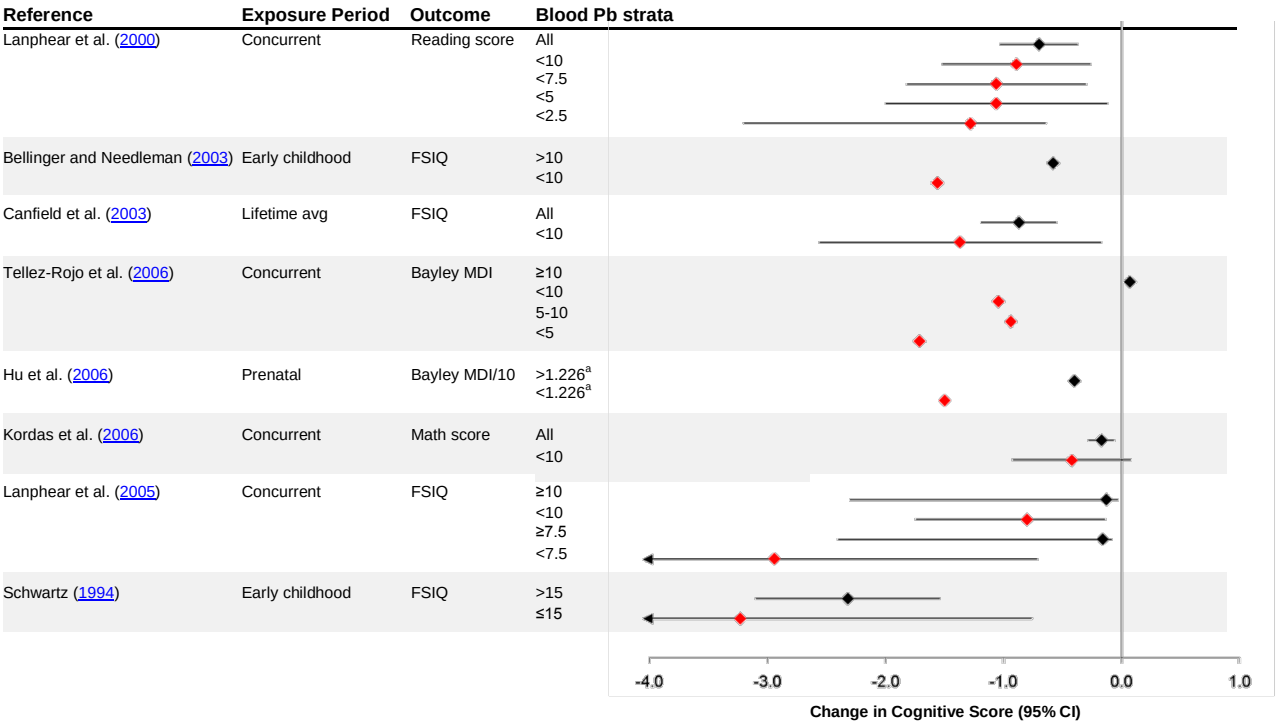
5.3.6.11. Epigenetics

DNA methyltransferase activity was significantly decreased in cortical neurons from both monkey (aged animals) and mouse brains (fetal cells exposed to Pb in culture, 0.1 µM Pb) after Pb exposure (J. F. Wu et al., 2008). 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.

5.3.7. Examination of the Lead Concentration-Response Relationship

With each successive Pb AQCD and supplement, epidemiologic and toxicological studies find that progressively lower blood Pb levels are associated with cognitive deficits and behavioral impairments. For example, among children, such decrements were observed in association with blood Pb levels in the range of 10-15 µg/dL in the 1986 Addendum and 1990 Supplement and 10 µg/dL and lower in the 2006 AQCD (U.S. EPA, 2006). Furthermore, in the 2006 AQCD, several individual studies, pooled analyses, and meta-analyses estimated a supralinear blood Pb concentration-response relationship in children, i.e., greater decrements in cognitive function per incremental increase in blood Pb level among children in lower strata of blood Pb levels compared with children in higher strata of blood Pb levels (Figure 5-28 and Table 5-12). While the majority of epidemiologic evidence indicated differences in effect estimates above and below 10 µg/dL, several studies of children with mean blood Pb levels less than 5 µg/dL estimated larger effects for children with <5 µg/dL (compared with children with blood Pb levels 5-10 µg/dL, and >0 µg/dL) (Tellez-Rojo et al., 2006), <2.5 µg/dL (compared with children with blood Pb levels <5 µg/dL, <.5 µg/dL, <10 µg/dL, and all subjects) (Lanphear et al., 2000), and <1.2 µg/dL (maternal plasma Pb, compared with plasma Pb levels >1.2 µg/dL) (H. Hu et al., 2006). Using data from NHANES 1999-1994, Lanphear et al. (2000) examined differences in effect estimates among multiple strata of blood Pb levels and found the largest deficit in reading score per 1 µg/dL increment in blood Pb among children with blood Pb levels less than 1 µg/dL. As lower concentrations of Pb exposure are being used experimentally, the toxicological literature reports nonlinear concentration-response relationships for

some endpoints and similar to the epidemiologic literature, shows larger effects in lower Pb exposure groups.



Note: a = Pb levels measured in plasma of maternal blood during 1st trimester of pregnancy. FSIQ = full-scale IQ, MDI = mental development index. Effect estimates are standardized to a 1 µg/dL increase in blood Pb level. Black symbols represent effect estimates among all subjects or in highest blood Pb stratum. Red symbols represent effect estimates in lower blood Pb strata. Effect estimates without error bars are from studies that did not provide sufficient information in order to calculate 95% CIs.

Figure 5-28. Comparison of associations between blood Pb and cognitive function among various blood Pb strata.

Table 5-12. Additional characteristics and quantitative results for studies presented in Figure 5-28

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Blood Pb stratum (µg/dL)	Effect Estimate (95% CI)[a]
Lanphear et al. (2000)	4853 children ages 6-16 yr NHANES 1988-1994	Concurrent mean (SE): 1.9 (0.1)	Linear regression model adjusted for sex, race/ethnicity, poverty index ratio, reference adult education level, serum ferritin level, serum cotinine level	WRAT reading subtest at ages 6-16 yr	All subjects <10 <7.5 <5 <2.5	-0.70 (-1.03, -0.37) -0.89 (-1.52, -0.26) -1.06 (-1.82, -0.30) -1.06 (-2.00, -0.12) -1.28 (-3.20, -0.64)
Bellinger et al. (1992) Bellinger and Needleman (2003)	148 children followed from birth (1979-1981) to age 10 yr Boston area, MA	Early childhood (age 2 yr) mean (SD): 6.5 (4.9)	Linear regression model adjusted for HOME score (age 10 and 5), child stress, race, maternal IQ, SES, sex, birth order, marital status	WISC-R at age 10 yr	>10 <10	-0.58[b] -1.56[b]

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Blood Pb stratum (µg/dL)	Effect Estimate (95% CI)[a]
Canfield et al. (2003)	172 children born 1994-1995 followed from infancy to age 3-5 yr Rochester, NY	Lifetime avg (3 or 5 yr) mean (SD): 7.4 (4.3)	Mixed effects models adjusted for sex, maternal race, parental smoking, child iron status, maternal income, maternal IQ, HOME score	Stanford-Binet at age 3 or 5 yr	All <10	-0.87 (-1.19, -0.55) -1.37 (-2.56, -0.17)
Tellez-Rojo et al. (2006)	294 children followed from birth (1994-1995, 1997-1999) to age 2 yr Mexico City, Mexico	Concurrent (age 2 yr) mean (SD): 4.28 (2.25)	Linear regression model adjusted for sex, birth weight, maternal IQ	Bayley MDI at age 2 yr	≥10 <10 5-10 <5	0.07 (p = 0.84)[b] -1.04 (p <0.01)[b] -0.94 (p = 0.12)[b] -1.71 (p = 0.01)[b]
Hu et al. (2006)	146 children followed prenatally (1997-1999) to age 24 mo Mexico City, Mexico	Maternal 1st trimester plasma Pb median: 1.226	Linear regression model adjusted for plasma Pb in 2nd trimester, plasma Pb in 3rd trimester, child 24 mo blood Pb, sex, maternal age, height-for-age Z score, maternal IQ	Bayley MDI at age 24 mo	>1.226 <1.226	-4.0[b] -15.0[b]
Kordas et al. (2006)	602 children in 1st grade Torreon, Mexico	Concurrent mean (SD): 11.4 (6.1)	Linear regression model adjusted for sex, age, hemoglobin, family possessions, forgetting homework, house ownership, crowding, maternal education, birth order, family structure, arsenic exposure, tester, school	Math achievement test in 1st grade	all <10	-0.17 (-0.28, -0.06) -0.42 (-0.92, 0.08)
Lanphear et al. (2005)	1333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts	Median (5th-95th) Peak: 18.0 (6.2-47.0)	Linear regression model adjusted for HOME score, birth weight, maternal IQ, maternal education	FSIQ measured at ages 4.8-10 yr	≥10 <10 ≥7.5 <7.5	-0.13 (-2.3, -0.03) -0.80 (-1.74, -0.14) -0.16 (-2.4, -0.08) -2.94 (-5.16, -0.71)
Schwartz (1994)	Meta-analysis of 7 studies with sample sizes 75-579 children	Early childhood (2-3 yr) range in study means: 6.5-23	Meta-analysis of combining effect estimates from individual studies	FSIQ measured at schoolage	Studies with mean >15 Studies with mean ≤15	-2.32 (-3.10, -1.54) -3.23 (-5.70, -0.76)

[a]Effect estimates are standardized to a 1 µg/dL increase in blood Pb level.
[b]Investigators did not provide sufficient information in order to calculate 95% CIs.

Using data pooled from seven prospective studies, Lanphear et al. (2005) fit various types of models to the data and observed that a cubic spline, log-linear model, and piece-wise linear model all supported a more negative concentration-response relationship at lower blood Pb levels. A linear model was found to be inadequate as the polynomial terms for concurrent blood Pb were statistically significant. These findings were corroborated by a separate analysis by Rothenberg and Rothenberg (2005) which found that the log-linear model fit the relationship between blood Pb level and IQ better than did a linear model.

Studies of adults have not widely examined the shape of the relationship between blood or bone Pb level and cognitive performance. In the various NHANES analyses, only log-linear models were used to fit the data (E. F. Krieg, Jr. & Butler, 2009; E. F. Krieg, Jr. et al., 2009; E. F. Krieg, Jr. et al., 2010). Other studies examined nonlinearity with the use of quadratic terms, penalized splines, or visual inspection of bivariate plots (Bandeen-Roche et al., 2009; Shih et al., 2006; Weisskopf, Proctor, et al., 2007). While there was some evidence for nonlinearity for some cognitive tests (Figures 5-17 and 5-18), the majority of results suggested linear associations. Shih et al. (2006) found that a quadratic term for tibia Pb was not

statistically significant and found a linear model fit adequately the relationship between tibia Pb level and various tests of cognitive performance. In contrast to most studies, Wang et al. (2007) found that among HFE variant carriers, there was a steeper decline in MMSE score at higher tibia Pb levels (20-25 μg/g, Figure 5-17).

Attenuation of the concentration-response relationships at higher exposure or dose levels has been reported in the occupational literature, and explanations have included greater exposure measurement error, competing risks, saturation of biological mechanisms at higher levels, larger proportion of susceptible populations at lower exposure levels, and variations in other risk factors among exposure levels (Stayner et al., 2003). Other explanations for nonlinearity include different mechanisms operating at different exposure levels, confounding by omitted or misspecified variables, and the lower incremental effect of Pb due to covarying risk factors such as low SES, poor caregiving environment, and higher exposure to other environmental factors.

The contribution of these factors to the supralinear relationship between blood Pb levels and neurocognitive function has not been examined widely in epidemiologic studies to-date. However, in several populations, higher blood Pb levels have been measured in susceptible groups such as those with higher poverty, greater exposure to tobacco smoke, lower parental education, and lower birth weight, which argues against a larger proportion of susceptible populations at lower blood Pb levels (Lanphear et al., 2000; Lanphear et al., 2005). It has been suggested that in populations of low SES, poorer caregiving environment, and greater social stress, the incremental effect of Pb exposure may be attenuated due to the overwhelming effects of these other risk factors (J. Schwartz, 1994). Several studies have found significant associations of these sociodemographic risk factors with neurocognitive deficits, and Miranda et al. (Miranda et al., 2009) found that indicators of SES (i.e., parental education and enrollment in a free/reduced fee lunch program) accounted for larger decrements in EOG scores than did blood Pb level (Figure 5-6). Few studies have compared Pb effect estimates among groups in different sociodemographic strata, and the limited data are mixed. Greater Pb-associated neurocognitive deficits in low-SES groups were reported by Bellinger et al. (1990). In a meta-analysis of eight studies, Schwartz (1994) found a smaller decrement in IQ per 1 μg/dL increase in blood Pb level for studies in disadvantaged populations (-2.7 points [95% CI: -5.3, -0.07]) than for studies in advantaged populations (-4.5 points [95% CI: -5.6, -2.8]). It is important to note that blood Pb is associated with deficits in neurocognitive function in both higher and lower SES groups; however, it is unclear what differences there are between groups in the decrement per unit increase in blood Pb and whether these differences can explain the nonlinear dose-response relationship.

Rothenberg and Rothenberg (2005) formally assesed the influence of residual confounding on the nonlinear blood Pb concentration-response relationship by comparing model fit between linear and spline transformations (df = 2) of covariates such as maternal IQ, HOME score, and maternal education. Inclusion of covariates as spline functions did not significantly improve model fit either with a linear

blood Pb term or log blood Pb term, which indicated that their inclusion as linear functions was adequate. These findings demonstrate that the improved model fit with log-specification of blood Pb level was not due to residual confounding by covariates.

Consistent with the epidemiologic literaure, toxicological studies also find nonlinear relationships between Pb exposure and neurological effects in animals. In particular, multiple studies have shown U- or inverse U-shaped curves with lower exposures of Pb having different or often the opposite effect from higher doses. U-shaped Pb exposure-responses include rotoarod performance, adult forebrain dopamine levels, amphetamine-induced motor activity, and latency to fall from rotarod (Leasure et al., 2008). Inverted U-shaped Pb dose-responses include histological findings such as the numbers of rod photoreceptors and bipolar cells, forebrain dopamine use, activity level, and adult body weight (Leasure et al., 2008) as well as ERG wave amplitudes (Fox et al., 2008) and hippocampal neurogenesis (Fox et al., 2008; Gilbert et al., 2005).

Because toxicological studies typically do not have confounding, exposure measurement error or other epidemiologic influences, i.e., susceptibility, they have permitted assessment of a mechanistic basis for nonlinear Pb exposure- or dose-response relationships. Several lines of evidence support the possibility of low-dose and high dose-Pb acting through differential activation of mechanisms. For example, in mice, lower Pb exposure (50 ppm) is associated with differential responses of the neurotransmitters dopamine and norepinephrine compared with control treatment and higher doses (150 ppm) (Leasure et al., 2008; Virgolini et al., 2005). These differential responses of neurotransmitter systems to lower versus higher Pb exposures may provide mechanistic understanding of the nonlinearity of Pb-induced behavioral changes in animals and may also explain the nonlinear blood Pb-neurocognitive and neurobehavioral associations reported widely among children. Additional evidence points to differences in hormonal homeostasis by Pb exposure level. In male mice with chronic Pb exposure (PND21-9 months of age), basal corticosterone levels are significantly lower in the 50 ppm exposure group than in the control or 150 ppm Pb exposure group.

Additional mechanistic understanding comes from differences in histological changes found in Pb-exposed animals. Compared with higher Pb exposure, lower Pb exposure stimulates greater induction of c-fos, a marker of neuronal activation and action potential firing (Lewis & Pitts, 2004). These findings may underlie the nonlinear association between Pb exposure and learning and the U-shaped behavioral dose-responsiveness seen with amphetamine-induced motor activity in males after GLE (Leasure et al., 2008).

Sensory organ findings in animals also show vastly different outcomes with low versus higher Pb exposure. Higher Pb exposure produces subnormal retinal ERGs and lower Pb exposure produces supernormal ERGs in both children (Rothenberg et al., 2002) and rodents (Fox & Chu, 1988; Fox & Farber, 1988; Fox et al., 1991). Inverted U-shaped dose-response curves have been seen for rod photoreceptor numbers or neurogenesis (Giddabasappa et al., 2011) and retinal thickness (Fox et al.,

2010). Thus, these dichotomous histological findings are coherent with the functional retinal test or the ERG where higher Pb exposure produces subnormal ERGs and lower exposure Pb produces supernormal ERGs.

Hierarchical enzyme activity also may explain nonlinear Pb concentrations-response relationships. The phosphatase enzyme calcineurin has been shown to be inhibited by higher Pb exposure and stimulated by lower Pb exposure (Kern & Audesirk, 2000). At lower Pb exposure, Pb displaces calcium at its binding sites on calmodulin and by acting as a calmodulin agonist at calcineurin's catalytic A subunit, stimulates calcineurin activity. At higher Pb exposure, Pb can bind directly to a separate calcium-binding B subunit, overriding the calmodulin-dependent effect and turning off the activity of calcineurin. Interestingly, mice with modulated calcineurin expression exhibit aberrant behavior related to schizophrenia or impaired synaptic plasticity and memory (Zeng et al., 2001). This example of the stimulatory effects of Pb at lower exposure and inhibitory effects at higher exposure gives another example of biological plausibility for the nonlinear concentration-response relationship reported for Pb in multiple studies.

The supralinear concentration-response relationship widely documented for Pb is consistent with the lack of a threshold for Pb-associated neurological effects as a smaller effect estimate would be expected at lower blood Pb levels if a threshold existed. Schwartz (1994) explicitly assessed evidence for a threshold in the Boston prospective cohort data by regressing IQ and blood Pb level on potential confounders including age, race, maternal IQ, SES, and HOME score and fitting a nonparametric smoothed curve to the residuals of both regression models (variation in IQ or blood Pb level not explained by covariates). A 7-point decrease in IQ was observed over the range of blood Pb residuals below 0, which corresponds to the mean blood Pb level in the study (6.5 µg/dL). Thus, in the Boston study, the association between blood Pb level and IQ was clearly demonstrated at blood Pb levels below 5 µg/dL.

An important limitation of previous studies in terms of characterizing the concentration-response relationship, in particular, identifying whether a threshold exists, has been the limited examination of effects in populations or blood Pb strata with blood Pb levels more comparable to the current U.S. population mean. While Schwartz (1994) did not find evidence for a threshold in the Boston study data, the mean blood Pb in that population was 6.5 µg/dL, and 56% of subjects had a blood Pb level >5 µg/dL. Recent studies indicate a downward shift in the distribution of blood Pb levels (i.e., 50% of subjects in the 2001-2004 NHANES population had a blood Pb <1 µg/dL (Braun et al., 2008). Additionally, more sensitive quantification methods have improved the detection limits, for example, from 0.6 µg/dL to 0.025 µg/dL in NHANES. This has allowed categorization of children in mulitple blood Pb quantiles below 1 µg/dL (Braun et al., 2008). Consequently, the examination of populations with large proportions of subjects at very low blood Pb levels has improved the ability to discern a threshold for Pb-associated neurological effects. Several recent studies reported associations between blood Pb levels and deficits in neurocognitive and neurobehavioral endpoints in populations with mean blood Pb levels <2 µg/dL (Braun

et al., 2008; Braun et al., 2006; Cho et al., 2010; E. F. Krieg, Jr. et al., 2010). In comparisons of various quantiles of blood Pb, Chandramouli et al. (2009) observed a lower SAT score among children in the U.K. with blood Pb levels 2-5 µg/dL compared with children with blood Pb levels 0-2 µg/dL. Likewise, Miranda et al. (2009) reported lower EOG scores in children in North Carolina with blood Pb levels of 2 µg/dL compared with children with blood Pb levels of 1 µg/dL. In the 2001-2004 NHANES population, Braun et al. (2008) found higher odds ratios for conduct disorder and ADHD among children with blood Pb levels 0.8-1.0 µg/dL (2nd quartile) compared with children with blood Pb levels 0.2-0.7 µg/dL (1st quartile). Collectively, these new findings in children, as summarized in this document, do not provide evidence for a threshold for the neurological effects of Pb in the ranges of blood Pb levels examined to-date.

It is important to note, however, that the lack of a reference population with blood Pb levels reflecting pre-industrial Pb exposures limits the ability to identify a threshold. Estimates of "background" blood Pb levels have been garnered from the analysis of ancient bones in pre-industrialized societies. These studies suggest that the level of Pb in blood in preindustrial humans was approximately 0.016 µg/dL (Flegal & Smith, 1992), approximately 65-fold lower than that currently measured in U.S. populations and lower than the levels at which neurological effects have been observed (1 µg/dL). Thus, the current evidence does not preclude the possibility of a threshold existing in the large range of blood levels between 1 µg/dL and preindustrial "background" levels.

5.3.8. Summary and Causal Determination

The 2006 Pb AQCD concluded that the collective body of epidemiologic studies provides clear and consistent evidence for the effects of Pb exposure on neurocognitive function in children. This conclusion was substantiated by the coherence of findings across studies of diverse design and populations (varying distributions of blood Pb levels, SES, parental intelligence, and quality of caregiving) that blood Pb levelswere associated with a broad spectrum of neurocognitive and neurobehavioral indices, including cognitive function (IQ), higher-order processes such as language and memory, academic achievement, behavior and conduct, delinquent and criminal activity, sensory acuities, and changes in brain structure and activity as assessed by MRI or MRS (Figure 5-29). Toxicological studies not only provided coherence with similarly consistent findings for Pb-induced impairments in parallel tests of learning, behavior and attention, and sensory acuities, but also provided biological plausibility by characterizing mechanisms for Pb-induced neurological effects (Figure 5-29). These mechanisms included Pb-induced inhibition of neurotransmitter release, decline in synaptic plasticity, decreases in neuronal differentiation, and decreases in the integrity of the blood-brain-barrier. Both epidemiologic studies in children and toxicological studies reviewed in the 2006 Pb AQCD demonstrated neurocognitive deficits in association with blood Pb levels at or below 10 µg/dL, and evidence from both disciplines also supported a nonlinear

concentration-response relationship, with greater cognitive or behavioral decrements per unit increase in blood Pb level estimated for lower blood Pb levels or estimated for lower Pb exposures. Among adults, although associations of blood Pb level with the spectrum of neurological effects (e.g., impairments in memory, attention, mood, balance, and motor function) were most consistently observed in occupationally-exposed adults with blood Pb levels ≥ 14 μg/dL, studies of adults without occupational Pb exposures indicated associations between biomarkers of cumulative Pb exposure, serial blood Pb or bone Pb measurements, and decrements in cognitive function.

Building on the strong body of evidence presented in the 2006 AQCD, recent studies continue to support associations of Pb biomarkers or exposures with neurological effects (Figure 5-29). Although fewer in number, recent studies in children corroborate findings from the several previous longitudinal and cross-sectional studies in demonstrating associations between blood Pb levels and FSIQ. In the cumulative body of evidence, negative associations between blood Pb level and IQ are best substantiated at mean blood Pb levels in the range of 5-10 μg/dL; however, an association was observed in a recent study with a mean blood Pb level of 1.73 μg/dL. A majority of recent epidemiologic studies in children has focused on examining specific indices of neurocognitive function such as reading and verbal skills, memory, learning, and visuospatial processing and has demonstrated associations with blood Pb levels as low as 2 μg/dL (population mean or quantiles). The consistently positive associations observed between blood Pb levels and this diverse set of neurocognitive indices provides coherence with findings for IQ, a global measure of cognitive function that reflects the integration of these individual domains. Additional coherence for findings in children is derived from evidence in animals that blood Pb levels of 11.6 μg/dL and higher are associated with changes in learning and memory (Figure 5-29). Recent toxicological studies continue to demonstrate that in utero and early postnatal exposure to Pb is the most sensitive window for Pb-dependent neurological effects. In the 2006 Pb AQCD, uncertainty was noted among studies in children regarding the relative importance of prenatal, early life, concurrent, and lifetime measures of Pb exposures. It also was noted that distinguishing among the effects of Pb exposures at different lifestages is difficult in epidemiologic studies due to the high correlations among children's blood Pb levels over time. Although blood Pb levels at all of these lifestages are associated with neurocognitive deficits in children, stronger effects generally are estimated for concurrent blood Pb levels, and recent evidence indicates that among both children with relatively lower or higher early childhood blood Pb levels, concurrent blood Pb levels are more strongly associated with neurocognitive deficits. In addition to performance on various neurocognitive tests, recent epidemiologic studies in children link blood Pb levels (in quantiles as low as 2 μg/dL) with factors that may be indicators of children's life success, including the level of educational attainment and end-of-grade score. In particular, observations of lower 4th grade end-of-grade score among children with blood Pb levels of 2 μg/dL compared with children with blood Pb levels of 1 μg/dL indicate that a threshold may not exist for the neurodevelopment effects of Pb in children.

Recent studies in children continue to support associations of blood Pb levels (population means or quantiles of 3-11 µg/dL) with a range of behavioral problems from anxiety and distractability to conduct disorder and delinquent behavior (Figure 5-29). Whereas previous evidence was not compelling, new evidence indicates associations between blood Pb levels and ADHD diagnosis and contributing diagnostic indices. In particular, a recent NHANES analysis demonstrated associations at blood Pb levels between 1 and 2 µg/dL). These findings for ADHD are well-supported by observations in animals of Pb-induced increased response rates and impulsivity. Additional coherence is provided by evidence in aquatic and terrestrial species for Pb affecting behaviors that decrease the ability of organisms to escape predators or capture prey (Chapter 7.1, 7.2). Both epidemiologic studies in children and adults as well as toxicological studies demonstrate associations of Pb biomarkers or exposure with deficits in visual acuity and hearing and auditory processing. New evidence from toxicological studies demonstrates the effects of lower blood Pb levels (<15 µg/dL) with retinal changes in male offspring. Combined evidence for Pb-associated neurocognitive deficits, inattention, conduct disorder, and effects on sensory function provides plausible mechanisms by which Pb exposure may contribute to academic underachievement and to more serious problems of delinquent behavior.

Studies in adults without occupational exposure to Pb have not provided consistent evidence for associations or blood or bone Pb levels with the range of neurological effects. Levels of Pb in bone, particularly in tibia, which is an indicator of cumulative Pb exposure, including higher exposures in the past, are better predictors of cognitive performance rather than a single blood Pb measurement. One explanation for the overall weaker body of evidence may be that cognitive reserve may compensate for the effects of Pb exposure on learning new information. Compensatory mechanisms may be overwhelmed with age, which may provide an explanation for more consistent associations between biomarkers of cumulative Pb exposure (serial blood measurements, tibia Pb levels) and neurocognitive deficits. Among recent studies of adults, blood Pb levels and bone Pb levels have been associated with essential tremor and Parkinson's Disease, respectively. These findings are well-supported by toxicological evidence for Pb-induced decreased dopaminergic cell activity in the substantia nigra, which contributes to the primary symptoms of Parkinson's disease. Biological plausibility also is provided by observations of developmental Pb exposures of monkeys and rats resulting in neurodegeneration in aged brains. Recent evidence also indicates associations between early-life ALAD activity, a biomarker of Pb exposure, and schizophrenia later in adulthood. Consistent with these findings, toxicological studies have observed Pb-induced emotional changes in males and depression changes in females. It is not surprising that Pb exposure may increase the risk of different neurological endpoints in children and adults given the predominance of different neurological processes operating at different ages, in particular, neurogenesis and brain development in children and neurodegeneration in adults.

Several host and environmental factors may modify the association between Pb exposure and neurological effects in children. Interactions of blood Pb levels with race/ethnicity and SES continue to be

poorly characterized. Although the 2006 Pb AQCD cited mixed epidemiologic evidence for effect modification by sex, recent epidemiologic evidence points to males having increased susceptibility for Pb-associated neurological effects. Toxicological studies continue to demonstrate increased susceptibility of males for endpoints such as sensory function, balance, stress hormone homeostasis, and brain membrane composition. Although limited, evidence suggests that risk of Pb-associated neurocognitive deficits in children also may be modified by variants in genes for apolipoprotein E and dopamine receptors. In addition to host factors, recent studies suggested that associations between blood Pb levels and neurological effects in children are greater with coexposures to environmental tobacco smoke and manganese. Historical animal toxicology findings demonstrate interactions between Pb exposure and stress. Namely, Pb-exposed animals reared in cages with enriched environments (toys) perform better in the Morris water maze than their Pb-exposed littermates who were reared in isolation. New findings indicate a potentiating effect of stress on behavior and memory with lower Pb exposures. In comparison, epidemiologic evidence for such interactions has been sparse. However, consistent with historical animal studies, a recent study indicated that positive social environment of children as characterized by maternal self-esteem, attenuates the negative association between blood Pb level and cognitive function. While effect modification by these host and environmental factors has not been examined widely in epidemiologic studies, new studies provide information on potentially susceptible populations that may benefit from early intervention to reduce the risk of neurological effects. Furthermore, the robust evidence for varying susceptibilities to Pb-induced neurological effects provides a basis to integrate mechanistically the findings of toxicology and epidemiology.

Extensive evidence from toxicological studies clearly substantiates the biological plausibility for epidemiologic findings by characterizing mechanisms underlying neurological effects. Pb exposure of animals induces dopamine changes in animals (Figure 5-29). Dopamine plays a key role in cognitive functions mediated by the prefrontal cortex and also motor functions mediated by the substantia nigra, and animal toxicological findings provide mechanistic support for associations in humans between blood Pb levels and neurocognitive deficits and in adults for associations with Parkinson's Disease. Current toxicological research has been expanded to document that early-life Pb exposure can contribute to neurodegeneration and neurofibrillary tangle formation in the aged brain. Pb induces complex neurochemical changes in the brain that differ by region of the brain, neurotransmitter type, age and sex of the organism. These changes remain aberrant over time but are dynamic in nature. The effect of Pb on NMDA receptors and the contribution of this paradigm to mood disorders is detailed. Synapse formation, adhesion molecules, and nitrosive stress continue to be areas of research with known Pb-associated adverse outcomes. Finally, the new area of epigenetics details that Pb exposure affects methylation patterns in rodent brains. These toxicological data complement the expanding epidemiologic data and often provide coherence between the two fields.

In summary, recent evidence substantiates and expands upon the established epidemiologic and toxicological literature of neurologic effects associated with Pb exposure. In epidemiologic studies of children, consistently positive associations of blood Pb levels with deficits in neurocognitive function, attention, and sensory acuities support observed associations with school performance as assessed by end-of-grade scores and level of educational attainment, which in turn, may explain associations with delinquent and criminal behavior. In particular, observations of lower academic achievement and ADHD among children in quantiles of blood Pb levels in the range of 1 to 2 μg/dL have not indicated that a threshold exists for the neurodevelopment effects of Pb in children. Epidemiologic findings are strengthened by the coherence and biological plausibility provided by toxicological findings for similar or parallel endpoints and for the mechanisms underlying the neurological effects (Figure 5-29). The collective body of evidence integrated across epidemiologic and toxicological studies and across the spectrum of neurological endpoints is sufficient to conclude that there is a **causal relationship between Pb exposures and neurological effects.**

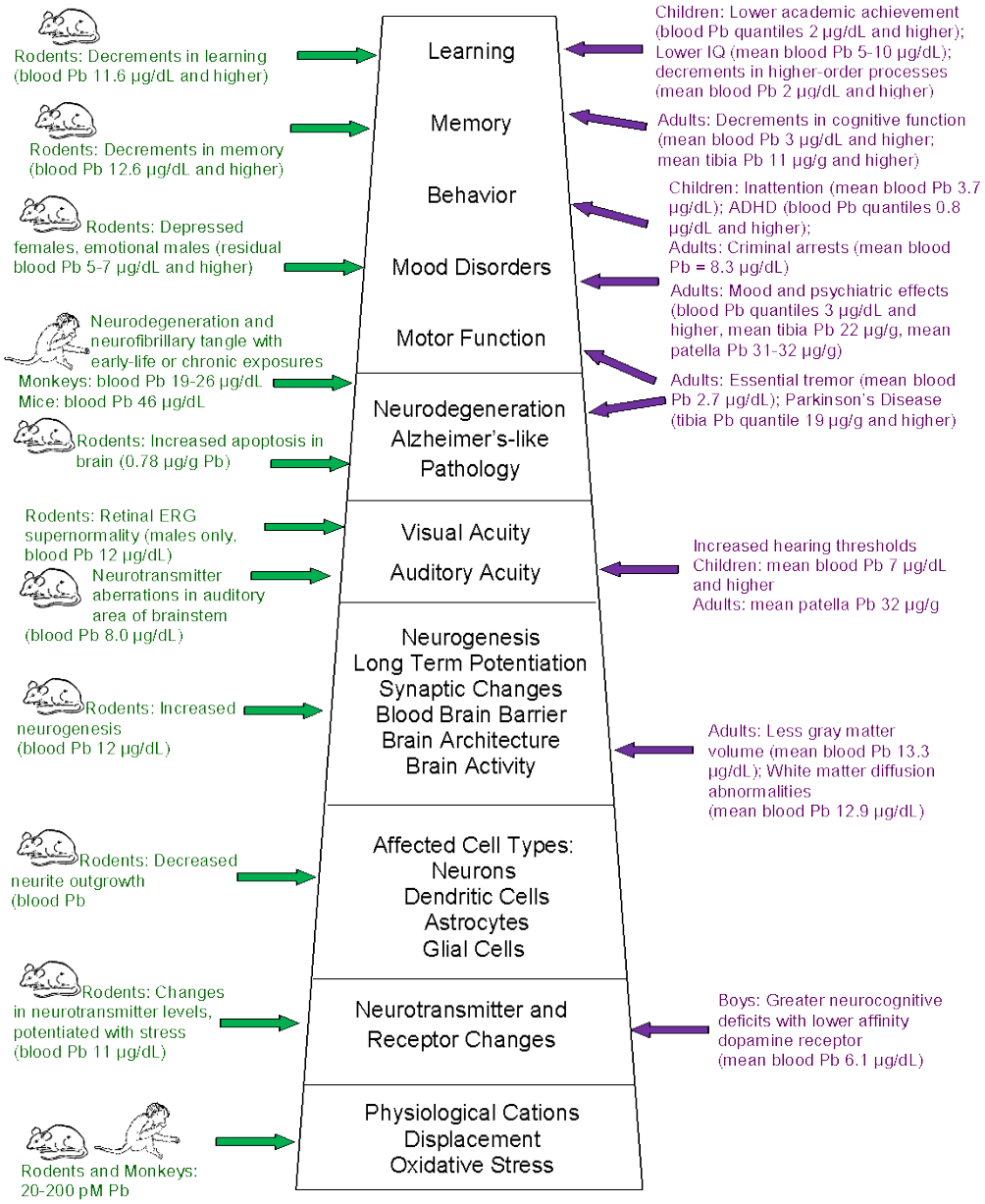


Figure 5-29. Snapshot of evidence for the spectrum of effects to the nervous system associated with Pb exposure.Green=animal toxicological studies (left side); purple=epidemiological studies (right side).

5.4. Cardiovascular Effects

5.4.1. Introduction

Both human and animal studies provide consistent evidence for an association of increased BP and arterial hypertension with chronic exposure to Pb resulting in adult blood Pb levels below 5 µg/dL. In addition, studies have suggested a connection between measures of Pb exposure and other cardiovascular diseases in adults such as ischemic heart disease, cerebrovascular disease, peripheral vascular disease, and cardiovascular disease related mortality. Toxicological studies explore the underlying mechanisms by which Pb exposure can lead to human cardiovascular health outcomes. Such studies have demonstrated that the Pb content in heart tissue reflects the increases in blood Pb levels (Lal et al., 1991). In general, associations between blood Pb and bone Pb (particularly in the tibia) with health outcomes in adults indicate acute effects of recent dose and chronic effects of cumulative dose, respectively. In some physiological circumstances of increased bone remodeling or loss (e.g., osteoporosis and pregnancy), Pb from bone of adults may also contribute substantially to blood Pb concentrations. Additional details on the interpretation of Pb in blood and bone are provided in Section 4.3.5. Additionally, as the cardiovascular and renal systems are intimately linked, cardiovascular effects can arise secondarily to Pb-induced renal injury (Section 5.5).

The previous Pb AQCD (U.S. EPA, 2006) concluded that both epidemiologic and animal toxicological studies support the relationship between increased Pb exposure and increased cardiovascular outcome, including increased BP, increased incidence of hypertension, and cardiovascular morbidity and mortality. Meta-analysis of these human studies found that each doubling of 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 (Nawrot et al., 2002). On a population-wide basis, the measured effect size translates into a large number of events for a moderate population size and thus has important health consequences for the occurrence of stroke, myocardial infarction, and sudden death. It was also noted that most of the reviewed studies using cumulative Pb exposure measured by bone Pb also showed increased BP (Y. Cheng et al., 2001; H. Hu et al., 1996) or increased hypertension with increasing bone Pb (B.-K. Lee et al., 2001). 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 ratio of hypertension between 1.28 and 1.86, depending upon the study. Two studies observed averaged increased 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. Since bone Pb measures Pb accumulation over time, duration of past exposure to Pb plays a role in increased BP.

The previous Pb AQCD also provided compelling evidence for a number of mechanisms leading to increased BP, and the development of hypertension and other cardiovascular diseases observed after Pb exposure. The strongest evidence supported the role of oxidative stress in the pathogenesis of Pb-induced

hypertension. Additionally, several studies focused on other pathways or cellular, molecular, and tissue events promoting the Pb-induced increase in BP. These mechanisms include inflammation, adrenergic and sympathetic activation, renin-angiotensin-aldosterone system (RAAS) activation, vasomodulator imbalance, and vascular cell dysfunction. Studies continue to support the observed increase in BP and hypertension development following Pb exposure, as well as build on the evidence for the biological pathways of these effects. This section reviews the published studies pertaining to the cardiovascular effects of Pb exposure in experimental animals, isolated vascular tissues, cultured vascular cells, and humans. Emphasis has been placed on studies published since the 2006 AQCD (U.S. EPA, 2006), however the large body of evidence that existed prior to that review has been summarized and incorporated into the current review.

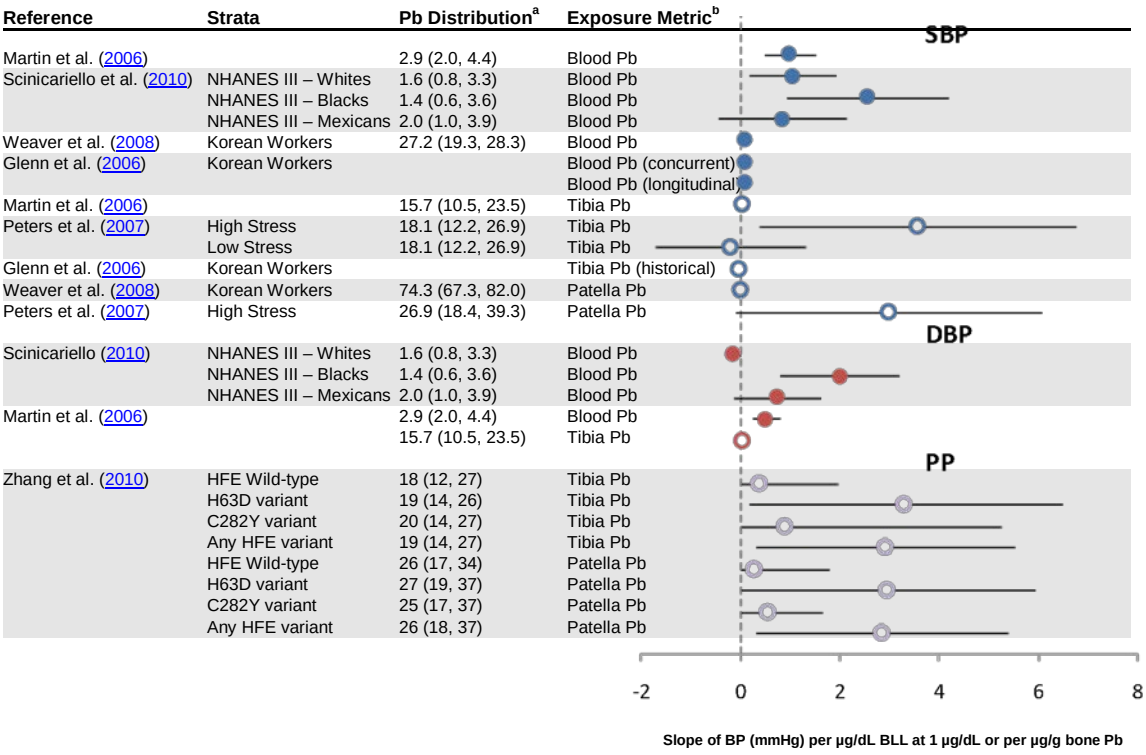
5.4.2. Blood Pressure and Hypertension

5.4.2.1. Epidemiology

The most commonly used indicator of cardiovascular morbidity is increased BP and its derived index, hypertension. Hypertension diagnoses in these studies require that the patient or subject have diastolic and/or systolic BP above certain cut-points or be taking anti-hypertensive medicines. These BP cut-points have historically been established by reference to informed medical opinion and as medical knowledge improves BP cut-points defining hypertension have been lowered over time. Consequently, different studies using "hypertension" as a cardiovascular outcome may assign different cut-points, depending on the year and location of the study and the individual investigator. All of the new studies in the current review used the same criteria for hypertension (e.g., systolic BP at or above 140, diastolic at or above 90 or taking anti-hypertensive medications). Studies in the medical literature show that increasing BP is associated with increased rates of cardiovascular disease including coronary disease, stroke, peripheral artery disease, and cardiac failure. Coronary disease (i.e. myocardial infarction, angina pectoris, sudden death) is the most lethal sequela of hypertension (Chobanian et al., 2003; Ingelsson et al., 2008; Kannel, 2000a, 2000b; Neaton et al., 1995; Pastor-Barriuso et al., 2003; Prospective Studies Collaboration, 2002).

Several recent general population and occupational cohort studies examined the associations of blood Pb and/or bone Pb with BP (Figure 5-30 and Table 5-13) as well as the associations of these Pb exposure metrics with hypertension (Figure 5-31 and Table 5-14). In a cross-sectional analysis, Martin et al. (2006) examined the association of blood and tibia Pb with BP and hypertension in a community-based cohort study of older adults (n = 964). Four models evaluated associations for BP and hypertension considering SES and race/ethnicity. Blood Pb but not tibia Pb was a strong and significant predictor of BP in all models with an approximately 1 mmHg increase in systolic BP with each 1 µg/dL increase in blood

Pb level and an approximately 0.5 mmHg increase in diastolic BP per 1 µg/dL increase in blood Pb level. 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 risk factors 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 independent of race/ethnicity and socioeconomic status). No evidence for effect modification by race/ethnicity was found. Overall, the results suggest that Pb has an acute effect on BP as a function of recent dose and a chronic effect on hypertension risk as a function of cumulative exposure.



Note: [a]Pb distribution is the median (IQR) estimated to make comparable. [b]Effect estimates were standardized to 1 µg/dL blood Pb or 10 µg/g bone Pb.

Figure 5-30. Slope of BP (mmHg) per µg/dL blood Pb level at 1 µg/dL or per 10 µg/g bone Pb (95% CI) for associations of blood Pb (closed circles) and bone Pb (open circles) with systolic BP (SBP; blue), diastolic BP (DBP; red), and pulse pressure (PP; purple).

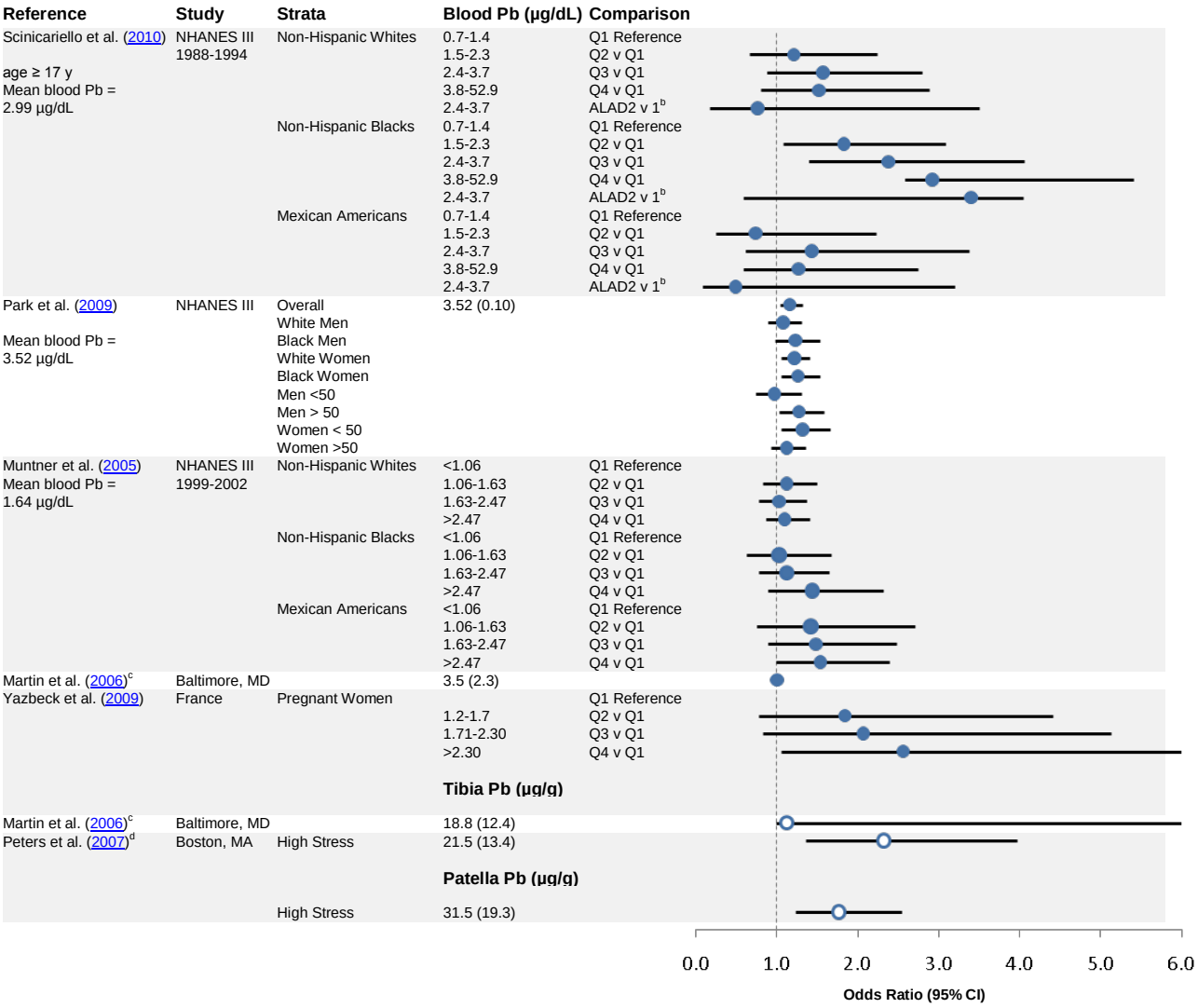
Table 5-13. Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for results presented in Figure 5-30

Study	Population /Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Martin et al. (2006)	964 men and women, 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)	Multiple linear regression base model 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 select models that are presented in Figure 5-30 and tabulated here.)	Blood Pb SBP: β=1.05 (0.53,1.58) DBP; β=0.53 (0.25, 0.81) Tibia Pb: SBP: β=0.07 (-0.05, 0.14) DBP: β=0.05 (-0.02, 0.08) mmHg per µg Pb /dL blood mmHg per µg Pb/g bone
Glenn et al. (2006)	575 Pb exposed workers, age 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 Tibia Pb, mean (SD): Visit 1: 28.2 (19.7), Women Visit 2: 22.8 (20.9), Women Visit 1: 41.7 (47.6), Men Visit 2: 37.1 (48.1), Men Patella Pb, mean (SD): Visit 3 49.5 (38.5) Women Visit 3 87.7 (117.0)	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 body mass index, sex, baseline BP lowering medication use, alcohol consumption, body mass index, sex, BP lowering medication use.	Model 1 (short-term) Blood Pb concurrent β=0.08 (-0.01, 0.16) Blood Pb (longitudinal) β=0.09 (0.01, 0.16) Model 4: short and longer-term) Blood Pb concurrent β=0.10 (0.01, 0.19) Blood Pb longitudinal: β=0.09 (0.01, 0.16) Per 10 µg/dL blood Pb
Weaver et al. (2008)	652 current and former Pb workers in South Korea (12/1999-6/2001)	BP	Blood Pb: Mean (SD): 30.9 (16.7) µg/dL Patella Pb: Mean (SD): 75.1 (101.1) µg/g	Linear regression model adjusted for age, gender, BMI, diabetes, antihypertensive and analgesic medication use, Pb job duration, work status, tobacco and alcohol use	SBP Patella Pb β=0.0059 (p=0.41) Blood Pb β=0.1007 (p=0.01) Interaction between blood Pb/patella Pb with ALAD and vitamin D receptor polymorphisms not significant.
Peters et al. (2007)	513 elderly men (mean 67 y) from Normative Aging Study 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 calcium intake, family history of hypertension, BMI, educational level, pack-years of smoking, alcohol consumption, and physical activity	SBP Tibia Pb/ High Stress: β=3.57 (0.39, 6.75) Low Stress: β=-0.21 (-1.70, 1.29) per SD increase in tibia Pb Patella Pb/ High Stress: β=2.98 (-0.12, 6.08) per SD increase in tibia Pb Patella Pb/ Low Stress: NR

Study	Population /Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Scinicariello et al. (2010)	6,016 NHANES III participants ≥ 17 yr	BP	Blood Pb: Overall Mean (SE): 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 calcium, glycosylated hemoglobin, and hematocrit	Ln blood Pb SBP Non-Hispanic whites: β±SE=1.05±0.37 (p=0.01) Non-Hispanic blacks: β±SE=2.55±0.49 (p=0.001) Mexican Americans: β±SE=0.84±0.46 (p=0.08) DBP Non-Hispanic whites: β±SE= -0.14±0.49 (p=0.77) Non-Hispanic blacks: β±SE=1.99±0.44 (p=0.0002) Mexican Americans: β±SE=0.74±0.38 (p=0.06) Significant interactions with blood Pb and ALAD observed in relation to SBP for non-Hispanic whites and non-Hispanic blacks
Zhang et al. (2010)	619 older adult males (mean 67 yr) enrolled in the VA-NAS in Greater Boston, MA area	PP	Wild type Tibia Pb: Med (IQR):8(12-27) µg/g Patella Pb: Med(IQR):26(17-37) µg/g C282Y Tibia Pb: Med(IQR):20 (14-27) µg/g Patella Pb: Med(IQR):25(17-37) µg/g H63D Tibia Pb: Med(IQR):19(14-26) µg/g Patella Pb: Med(IQR):27(19-37)µg/g	Linear mixed effects regression models with repeated measurements adjusted for age; education; alcohol intake; smoking; daily intakes of calcium, 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 Tibia Pb per 13 µg/g: Wild Types: β=0.38 (0,1.96) H63D: β=3.30 (0.16, 6.46) C282Y: β=0.89 (0, 5.24) Any HFE: β=2.90 (0.31, 5.51) Patella Pb per 19 µg/g: Wild Type: β=0.26 (0, 1.78) H63D: β=2.95 (0, 5.92) C282Y: β=0.55 (0, 1.66) Any HFE: β=2.83 (0.32,5.37)
Perlstein et al. (2007)	593 predominantly white men from VA-NAS in Greater Boston, MA area (1991-1997)	PP	Blood Pb: Overall mean (SD): 6.12 (4.03) µg/dL Mean (SD) quintiles: 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: Median: 19 µg/g Mean (SD) quintiles: 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	Tibia Pb: SBP r=0.06 (p=0.15) DBP r=-0.02 (p=0.63) Blood Pb: SBP r=0.05 (p=0.28) DBP r=0.12 (p=0.01) Pulse Pressure Tibia Pb: >Median: 4.2 (1.9, 6.5) mmHg (mean higher than men below the median) <Median: Referent 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 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

Study	Population /Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Navas-Acien et al. (2008)	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	Pooled Estimates per 10 µg/g increase in Tibia Pb Prospective/SBP β=0.33 (-0.44, 1.11) X-sectional SBP β=0.26 (0.02, 0.50) X-sectional DBP β=0.02 (-0.15, 0.19 x-Sectional hypertension OR=1.04 (1.01, 1.07) Pooled Estimates per 10 µg/g increase in patella Pb hypertension OR=1.04 (0.96, 1.12)
Gump et al. (2007)	122 children age 9.5 yr in Oswego, NY	BP, TPR (total peripheral vascular resistance)	Blood Pb: Mean (SD): 4.6 (2.5) µg/dL		Pb is a mediator/modifier and moderator in the analysis, no effects presented
Yazbeck et al. (2009)	971 pregnant women, age 18-45 yr, in France	BP	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; cadmium, manganese, and selenium 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.
Elmarsafawy et al. (2006)	471 elderly men (mean 67 yr) from Normative Aging Study 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	Tibia Pb High calcium group (>800 mg/d): SBP: β=0.40.(0.11, 0.70) Low calcium group (<800 mg/d): SBP: β=0.19 (0.01, 0.37) mmHg per µg/g tibia Pb

References not included in Figure 5-30 are included in this table.



Note: [a]The outcomes plotted are hypertension with the exception of Yazbeck et al. (2009) which measured pregnancy induced hypertension and Peters et al. (2007) which measured hypertension incidence. [b]ALAD2 v 1 indicates comparison between ALAD 2 carriers (e.g. ALAD1-2 and ALAD2-2) and ALAD 1 homozygotes (e.g., ALAD1-1). [c]Effect estimates were standardized to 1 µg/dL blood Pb. [d]Effect estimates were standardized to 1 µg/g bone Pb.

Figure 5-31. Odds ratio (95% CI) for associations of blood and bone Pb with hypertension measures.

Table 5-14. Additional characteristics and quantitative data for associations of blood and bone Pb with hypertension measures for results presented in Figure 5-31

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Scinicariello et al. (2010)	6,016 NHANES III participants ≥ 17 y	Hypertension (current use of antihypertensive medication, SBP ≥ 140 mmHg, or DBP ≥ 90 mmHg)	Blood Pb: Mean (SE): 2.99 (0.09) µg/dL Q1 0.7-1.4 µg/dL, Q2 1.5-2.3 µg/dL, Q3 2.4-3.7 µg/dL, Q4 3.8-52.9 µg/dL 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 calcium, glycosylated hemoglobin, and hematocrit	Non-Hispanic whites: Q1 Reference 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 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 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 POR for hypertension with *ALAD2* carriers across quartiles of blood Pb level also reported. *ALAD2* carriers associated with hypertension in non-Hispanic whites.
Park et al. (2009)	12,500 NHANES III participants	Hypertension	NHANES III Blood Pb 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's 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 (1.94, 1.26).

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Muntner et al. (2005)	9,961 NHANES (1999-2002) participants	Hypertension (current use of antihypertensive medication, SBP ≥ 140 mmHg, or DBP ≥ 90 mmHg)	Blood Pb: Overall Mean (CI): 1.64 (1.59-1.68) µg/dL quartile 1: <1.06 µg/dL, quartile 2: 1.06-1.63 µg/dL, quartile 3: 1.63-2.47 µg/dL, and quartile 4: ≥2.47 µg/dL	Multivariable logistic regression models adjusted for age, sex, diabetes mellitus, BMI, cigarette smoking, alcohol consumption, high school education, and health insurance status	Adjusted OR of having a blood Pb level of 10 µg/dL Non-Hispanic white: Q1 reference 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 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 Q2 OR=1.42 (0.75, 2.71) Q2 OR=1.48 (0.89, 2.48) Q3 OR=1.54 (0.99, 2.39) Significant trend (p=0.04)
Martin et al. (2006)	964 men and women, 50-70 y, 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	Blood Pb level OR=1.02 (0.87, 1.19) Tibia Pb OR=1.24 (1.05, 1.47) mmHg per µg Pb /dL blood mmHg per µg Pb/g bone
Peters et al. (2007)	513 elderly men (mean 67 y) from Normative Aging Study 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 calcium intake, family history of hypertension, BMI, educational level, smoking, alcohol consumption, baseline SBP and DBP, and physical activity	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
Yazbeck et al. (2009)	971 pregnant women, age 18-45 y, in France	PIH (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg after the 22nd wk of gestation)	Blood Pb: PIH group mean (SD): 2.2 (1.4) µg/dL No PIH group mean (SD): 1.9 (1.2) µg/dL Q1: <1.20 µg/dL Q2: 1.20-1.70 µg/dL Q3: 1.71-2.30 µg/dL Q4: >2.30 µg/dL	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 Log Blood Pb OR=3.29 (1.11, 9.74) per 1 µg/dL maternal blood Pb level Q1: reference Q2: OR 1.84 (0.77, 4.41) Q3: OR=2.07 (0.83, 5.13) Q4: OR=2.56 (1.05, 6.22)
Weaver et al. (2008)	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, gender, BMI, diabetes, antihypertensive and analgesic medication use, Pb job duration, work status, tobacco and alcohol use	None of the Pb exposure metrics examined were (blood, patella, and ln patella) were significantly associated with hypertension (results not reported)
Chen et al. (2006)	2,994,072 pregnant women in United States (1998)	PIH (gestational hypertension as increased SBP of ≥ 30 mmHg or DBP of ≥ 15 mmHg after 20th wk of gestation)	Pb in TSP Seasonal mean at conception: 0.0940 µg/m^3 Seasonal mean at birth: 0.0950 µg/m^3	Generalized estimating equations (GEEs) adjusted for maternal age, race, education, marital status, parity, adequacy of care, and tobacco use	OR at conception Q1 Referent Q2 1.07 (1.05, 1.08) Q3 1.22 (1.20, 1.25) Q4 1.16 (1.15, 1.18) 0.05 µg/m^3 increase:1.04 (1.03, 1.04) OR at birth Q1 Referent Q2 1.07 (1.05, 1.09) Q3 1.21 (1.19, 1.23) Q4 1.15 (1.13, 1.17) 0.05 µg/m^3 increase:1.04 (1.04, 1.05)

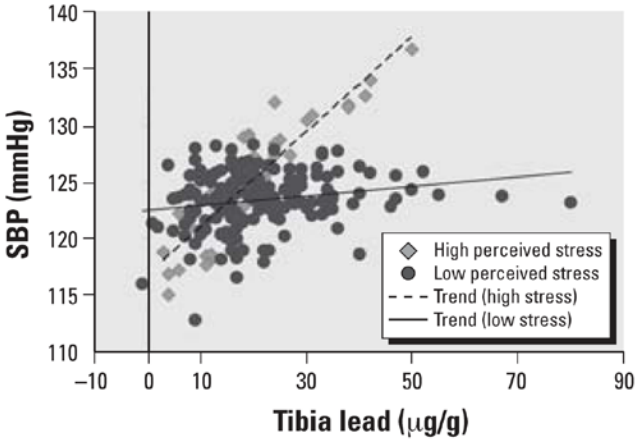
Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Elmarsafawy et al. (2006)	471 elderly men (mean 67 y) from Normative Aging Study in Greater Boston, MA area	Hypertension (mean SBP ≥ 160 mmHg, DBP ≥ 95 mmHg; and/or physician diagnosis with current use of antihypertensive 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 calcium group (<800 mg/d): Blood Pb: 1.07 (1.00, 1.15) Tibia Pb: 1.02 (1.00, 1.04) Patella Pb: 1.01 (1.00, 1.03) High calcium group (>800 mg/d): Blood Pb: 1.03 (0.97, 1.11) Tibia Pb: 1.01 (0.97, 1.04) Patella Pb: 1.01 (0.99, 1.03)

References not included in Figure 5-31 are included in this table.

In another cross-sectional analysis, Perlstein et al. (2007) examined the association of BP and pulse pressure (PP) among predominantly white older adults in the greater Boston area. 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. A statistically significant association between blood Pb and DBP was observed in adjusted models but the correlations of BP with tibia Pb were not significant. Men with tibia Pb above the median had a significantly higher mean PP compared to men with tibia Pb below the median (4.2 mmHg [95%CI: 1.9, 6.5]). The trend toward increasing PP with increasing tibia Pb was significant although none of the confidence intervals for PP referenced to the lowest quintile of tibia Pb excluded the null value.

Peters et al. (2007) examined the modification by self-reported stress of the associations of Pb exposure (tibia and patella Pb) with BP and hypertension in a cohort of subjects enrolled in the VA-NAS. Cross-sectional analyses of the effect of bone Pb and stress on BP and hypertension were conducted. Increased but nonsignificant associations between hypertension status and SBP with bone Pb were observed. Interaction of stress with tibia Pb (tibia Pb β=3.77 [CI: 0.46, 7.09]) and stress with patella Pb was significant (patella Pb β=2.60 [CI: -0.95, 6.15]) in systolic BP models (neither bone, self-reported stress, nor the interaction predicted DBP). Figure 5-32 shows the association between SBP and tibia Pb, comparing those with high and low self-reported stress. Peters et al. (2007) also used Cox proportional hazards models to assess the interaction of stress and bone Pb level on the association of bone Pb with the development of hypertension among those free of hypertension at baseline. The results of this analysis showed interactions between both tibia and patella Pb and the incidence of hypertension (RR of developing hypertension among those with high stress: 2.66 [CI: 1.43, 4.95] per SD increase in tibia Pb and 2.64 [CI: 1.42, 4.92] per SD increase in patella Pb). These provide information regarding factors that moderate or modify Pb effects on cardiovascular health. Gump et al. (2005) described significantly greater total peripheral vascular resistance (TPR) associated with increased blood Pb among 122 children (mean age 9.5 years) under acute stress, assessed by minor tracing or reaction time tasks which are consistent with α and β adrenergic activation. In a new analysis (Gump et al., 2007) significant effects of

SES on stress-induced reactivity of BP and TPR are reported. A significant SES by blood Pb level interaction is also reported suggesting possibly heightened effects of blood Pb level on stress changes in TPR and BP in low SES groups.



Source: Peters et al. (2007)

Figure 5-32. The relationship between tibia Pb and estimated SBP for those with high self-reported stress versus those with low self-reported stress.

Elmarsafawy et al. (2006) examined the modification of Pb effect by dietary calcium, with 467 subjects from the VA-NAS. Responses on a semi-quantitative dietary frequency questionnaire with one-year recall were converted to estimated calcium intake. Hypertension was modeled using logistic regression and included interaction terms between Pb (tibia, patella and blood Pb) and a dichotomized calcium intake variable (split at 800 mg/day). They also constructed alternative models stratified on the calcium variable. Nonsignificant increases in hypertension associated with elevated blood, tibia, and patella Pb were observed in both low and high calcium groups. The only significant interaction reported was between BMI and calcium in a tibia Pb model. The authors report that in linear regression models of BP stratified by calcium status, SBP increased 0.40 (95% CI: 0.11, 0.70) mmHg for every 1 μg/g increase in tibia Pb concentration in the high calcium group, and 0.19 (95% CI: 0.01, 0.37) mmHg in the low calcium group.

Glenn et al. (2006) simultaneously modeled the multiple Pb dose measures of individuals over repeated time periods, assessing cross-sectional as well as longitudinal relationships. The initial blood Pb level was used as a baseline covariate and the difference in blood Pb level between visits were computed for subsequent visits. The bone Pb measures were used to indicate historical exposure and cumulative exposure. Four models were specified: Model 1 was conceptualized to reflect short-term exposure; Model 2 to reflect longer-term exposure controlling for recent dose; Model 3 to reflect longer-term exposure controlling for cross-sectional influence of cumulative dose; and Model 4 to reflect both short-term

change with recent dose and longer-term change with cumulative dose. Concurrent and longitudinal measures of blood Pb were associated with SBP in Model 1 (short-term exposure) and Model 4 (short- and longer-term exposures). No associations with tibia Pb at baseline were observed while historical tibia Pb metric was negatively associated with SBP in each of the models. This study suggests that Pb exposure may act continuously on systolic BP and reduction in exposure may contribute to reductions in BP, while cumulative Pb burden may contribute to hypertension incidence by other mechanisms over longer time periods. Elevated BP may reflect an immediate response to Pb at a biochemical site of action as a consequence of recent dose or a persistent effect of cumulative doses over a lifetime.

In a separate analysis of the third year cross-sectional results of the same occupationally-exposed group, Weaver et al. (2008) examined associations between patella Pb and blood Pb level and SBP, DBP, and hypertension to determine interactions of the patella Pb effects with ALAD and vitamin D receptor (VDR) polymorphisms. None of the Pb exposure metrics were associated with DBP. Patella Pb alone was not significantly associated with SBP, while blood Pb, either alone or with patella Pb was positively and significantly associated with SBP. The patella Pb-age and blood Pb-age interactions were not significant. There were no significant effects of blood Pb or patella Pb on hypertension status, or effect modification by age or sex. Further, interactions between polymorphisms of the VDR and of ALAD with blood Pb and patella Pb on SBP were not 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 the Korean worker cohort (V. M. Weaver et al., 2008), with a focus on determining functional form of the concentration-response relationships. The coefficient indicates that every doubling of blood Pb level is 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 superior fit to data in non-nested models (Davidson & 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 systolic BP increase in this cohort is better described as a logarithmic function of blood Pb level within the blood Pb level range of the study than by a linear function.

Yazbeck et al. (2009) conducted a cross-sectional study examining a community-based group of pregnant women to determine the association of Pregnancy Induced Hypertension (PIH) with blood Pb level and unlike most other studies adjusted their model for metal blood concentrations of cadmium, manganese, and selenium. 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. An association between blood Pb and PIH was observed (OR 3.29 [95% CI: 1.11, 9.74]) between PIH cases (2.2 ± 1.4 µg/dL blood Pb) and normotensive patients (1.9 ± 1.2 µg/dL blood Pb). Cadmium and selenium 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. They observed no significant interactions among blood Pb level, any of the other elements, and maternal characteristics in predicting the risk of PIH. Interaction between selenium and Pb concentrations was not significant, and the putative protection effects of selenium through antioxidative properties were not confirmed in this study.

Chen et al. (2006) reported an ecological study of air Pb concentration and PIH, aggregated by all 50 U.S. states and the District of Columbia. The PIH data were taken from the live births and infant deaths up to 1 year of age compiled by the National Center for Health Statistics (CDC, 2000) for the year 1998. Associations between state level air Pb and state level PIH were reported (OR 1.04 [95% CI: 1.03, 1.04] per 0.05 μg/m^3 Pb). No individual level data were used in the analysis. Wells et al. (2011) measured the influence of cord blood Pb on BP in 285 women at admission to the Johns Hopkins Hospital in Baltimore, MD, during labor and delivery. Women in the fourth quartile of blood Pb elevations (>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 authors used Benchmark Dose Software V2.1, developed by the EPA, to estimate benchmark dose (BMD) and the associated lower confidence limit for benchmark dose (BMDL) for one standard deviation (SD) increase in BP, which is approximately equivalent to a 10% increase above the mean for the first quartile blood Pb "controls". The BMD approach is used here only as a means of characterizing the exposure level where effects might be found. These BMDL results indicate that the 95% lower bound confidence limit on the venous blood Pb level that is associated with a 1 SD increase is about 1.85 μg/dL for all BP outcomes. While these reported results are similar to those in the 2006 Pb AQCD as well as those found 25 years ago but with blood Pb levels an order of magnitude lower, the authors did not provide enough information to allow for verification of the BMD analysis.

Zhang et al. (2010) examined the effect of polymorphisms of the hemochromatosis gene (HFE) on the bone Pb effect on PP among older adult men participating in the VA-NAS. 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 the cross-sectional PP study of many of the same subjects of the VA-NAS group, without genotyping, reviewed above (Perlstein et al., 2007). The effect of bone Pb (tibia and patella) among those with the H63D variant was greater compared to those with the wild-type or the C282Y variant. In another gene-environment interaction analysis, Scinicariello et al. (2010) used NHANES III (1988-1994) data to examine the interaction between ALAD genotype and blood Pb in relation to BP in a cross-sectional analysis. A significant interaction between log blood Pb level and ALAD1-2/2-2b among non-Hispanic whites and non-Hispanic blacks was observed. In addition, associations of blood Pb with SBP and DBP across race/ethnicity strata were presented. The strongest associations were observed among non-Hispanic blacks. Scinicariello et al. (2010) also examined the association of blood Pb level with hypertension. Significant associations between 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 Q2 association for Mexican Americans.) In addition, non-Hispanic white ALAD2 carriers in the highest blood Pb level quartile had a significantly higher association with hypertension compared with ALAD1 homozygous individuals.

Muntner et al. (2005) also used the NHANES data (1999-2002) to examine the cross-sectional effect of blood Pb on hypertension, peripheral artery disease (PAD), and chronic kidney disease. The PAD results are discussed later in Section 5.4.3.4 and chronic kidney disease results are discussed in Section 5.5.2.2). Blood Pb increased regularly with age among those with blood Pb measurements (1.28 µg/dL [95% CI: 1.23, 1.33] in the 18-39 age group to 2.32 µg/dL [95% CI: 2.20, 2.44] in the 75 and older age group.) Associations were observed between blood Pb level and hypertension across race/ethnicity groups with significant trends observed for non-Hispanic blacks and Mexican Americans. Park et al. (2009) examined the association of blood Pb as well as bone Pb, which was predicted from blood Pb, with hypertension. The predicted bone Pb metrics were derived from models using VA-NAS data and applied to the NHANES (1988-1994) population. Blood Pb was associated with hypertension overall in the NHANES part of this study, with larger associations among black men and women as well as older adults. Associations with estimated bone Pb were also observed.

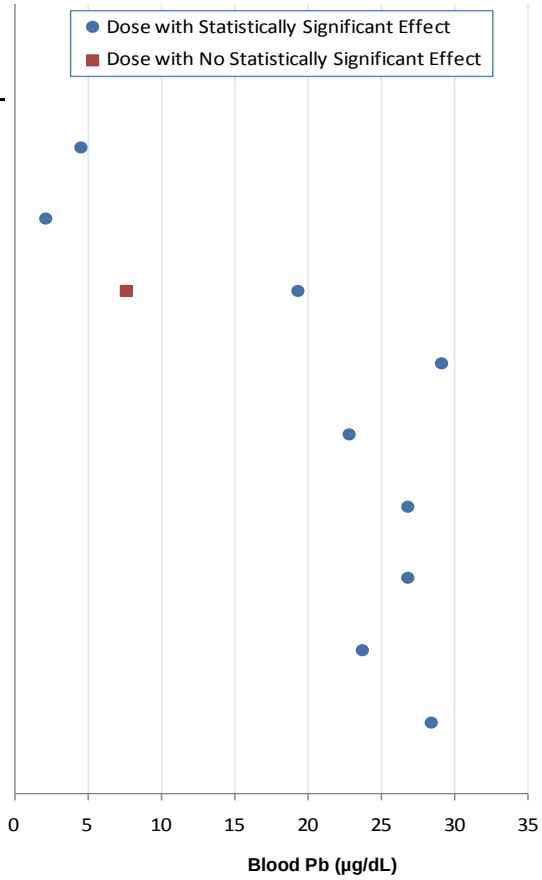
5.4.2.2. Toxicology

An array of studies have provided evidence that extended exposure to low levels of Pb (<5 µg/dL) can result in delayed onset of hypertension in experimental animals that persists long after the cessation of Pb exposure (U.S. EPA, 2006). Tsao et al. (2000) found significantly increased systolic and diastolic BP in rats with blood Pb levels relevant to human exposure (2.15 µg/dL). As this was the lowest Pb level tested, no evidence of a threshold was evident. After Pb exposure is removed, blood, heart, aorta, and kidney Pb levels decreased quickly within the first three months (H.-R. 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. Between 4 and 7 months after Pb exposure, the still-elevated BP did not decrease further, thus never returning to control BP levels. Decreases in BP were closely correlated with decreases in blood Pb level after exposure cessation. Prenatal Pb exposure in rats given a low calcium diet also resulted in increased arterial pressure (Bogden et al., 1995).

Experimental animal studies continue to provide evidence to conclude that Pb exposure results in delayed, yet sustained arterial hypertension. Increased systolic BP developed in rats after exposure to 90 - 10,000 ppm Pb (as Pb acetate in drinking water) for various time periods resulting in blood Pb level between 19.3-240 µg/dL (Badavi et al., 2008; Bagchi & Preuss, 2005; Bravo et al., 2007; Grizzo & Cordellini, 2008; Heydari et al., 2006; Reza et al., 2008; Vargas-Robles et al., 2007; L.-F. Zhang et al., 2009). However, past studies have shown statistically significant elevations in BP in rats with lower blood Pb level (Figure 5-33). Consistent with measurements of systolic BP, Pb exposure (100 ppm for 14

weeks; blood Pb level 24 µg/dL) also caused an increase in intra-aortic mean arterial pressure (Bravo et al., 2007). One study tested low levels of Pb exposure (30 ppm; blood Pb level 7.6 µg/dL) and did not find a statistically significant increase in systolic BP despite elevated blood Pb level after 8 weeks of treatment, however the data do represent a trend of increasing BP (Rizzi et al., 2009). Additionally, pups of Pb exposed dams (1,000 ppm through pregnancy and lactation) exhibited increased blood Pb level (58.7 µg/dL) and increased arterial systolic BP after weaning (Grizzo & Cordellini, 2008) suggesting a role for childhood Pb exposure leading to adult disease.

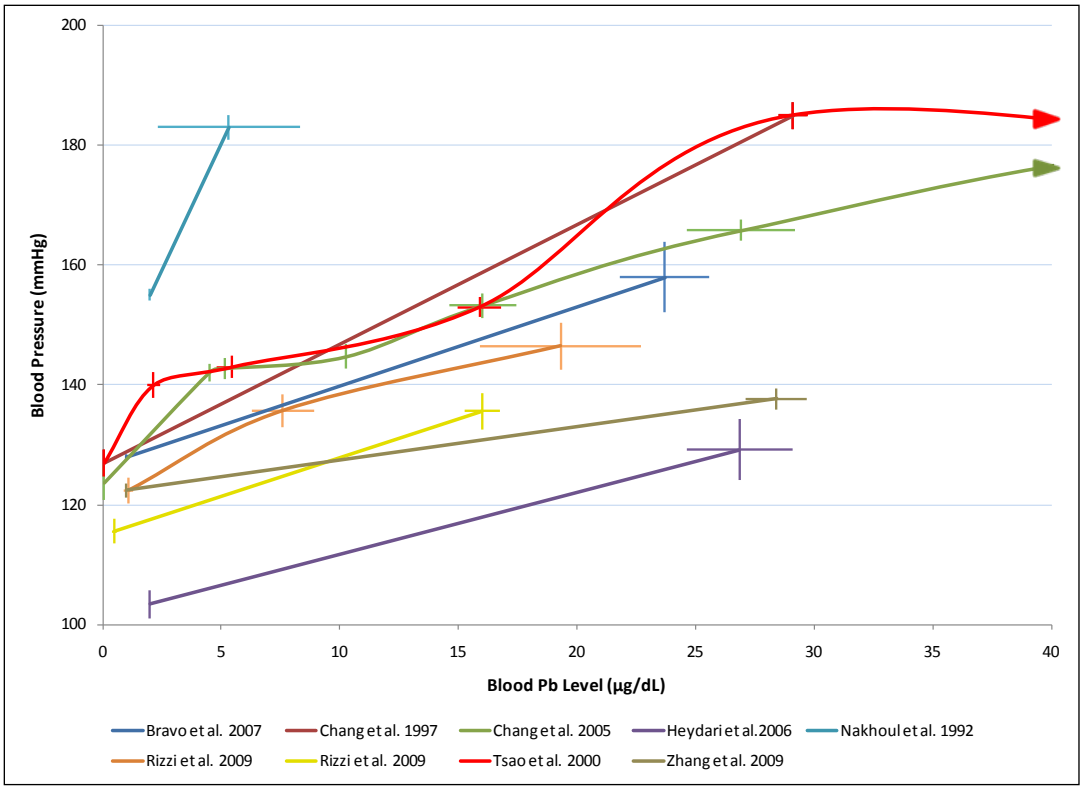
Reference	Duration	n	ΔSBP
Chang et al. (2005)		5	13.81
Tsao et al. (2000)	8 weeks	10	13
Rizzi et al. (2009)	8 weeks	11	13.3
Chang et al. (1997)	8 weeks	10	58
Carmignani et al. (2000)	12 weeks	12	17
Heydari et al. (2006)	12 weeks	6	25.8
Reza et al. (2008)	12 weeks	6	28.5
Bravo et al. (2007)	14 weeks	12	30
Zhang et al. (2009)	40 weeks	8-10	15.3



Note: Red square = blood Pb level where no statistically significant change in BP was observed; Blue circles = lowest blood Pb level reported with statistically significant changes in BP; Arrow line = higher blood Pb level reported in the same study with significant changes in SBP; Δ SBP = the change in SBP from control to first statistically significant blood Pb level in mmHg; n = number of animals in treatment group.

Figure 5-33. Rat blood Pb levels reported to be associated with changes in SBP from the current ISA and 2006 Pb AQCD.

Pb induced hypertension persists long after cessation of Pb exposure. Bagchi and Preuss (2005) found that elevated systolic BP was maintained for 210 days after Pb exposure cessation. 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. However, chelation therapy using $Na_2CaEDTA$ was able to return systolic BP to levels comparable to untreated rats (Bagchi & Preuss, 2005). Studies reporting the effect of Pb (as blood Pb level) on systolic BP in unanesthetized adult rats since 1992 report a positive increase in BP with increasing blood Pb level (Figure 5-34).



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 5-34. Changes in BP after Pb exposure (as blood Pb level) in unanesthetized adult rats across studies.

5.4.2.3. Hypertension Mechanisms

The previous Pb AQCD examined a number of mechanisms leading to Pb-induced hypertension, including oxidative stress, hormonal and blood pressure regulatory system dysfunction, vasomodulation, and cellular alterations. Further examination of these possible mechanisms of hypertension from Pb exposure in experimental animals and cells has been conducted and are provided below.

Oxidative Stress Response

Reactive Oxygen Species and Nitric Oxide

Studies discussed in the previous Pb AQCD suggest 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) (Attri et al., 2003; Dursun et al., 2005; Gonick et al., 1997; Khalil-Manesh et al., 1994; Khalil-Manesh, Gonick, Weiler, et al., 1993; Vaziri et al., 1997). Pb-induced reduction of biologically active ˙NO is not due to a reduction in ˙NO-production capacity (Vaziri & Ding, 2001; Vaziri, Ding, et al., 1999); instead it is a result of inactivation and sequestration of ˙NO by ROS

(Malvezzi et al., 2001; Vaziri, Liang, et al., 1999). 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., 2000; Ding et al., 2001), 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 plays a role in Pb-induced downregulation of sGC (Courtois et al., 2003; Farmand et al., 2005; M. Marques et al., 2001). The reduction of the vasodilator ˙NO leads to increased vasoconstriction and BP.

Pb-induced oxidative stress also induces 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 NFκB (Ramesh et al., 2001). Pb-induced hypertension, inflammation, and NFκB 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 continue to provide evidence for the role of ROS and ˙NO metabolism in Pb-induced hypertension and vascular disease. Increased SBP after Pb exposure has been accompanied by increased superoxide ($O_2^{\cdot-}$) and $O_2^{\cdot-}$ positive cells (Bravo et al., 2007; Vargas-Robles et al., 2007) , elevated urinary malondialdehyde (MDA) (Bravo et al., 2007), and increased 3-nitrotyrosine (Vargas-Robles et al., 2007). Inhibition of NAD(P)H oxidase, an enzyme that generates $O_2^{\cdot-}$ and hydrogen peroxide, was able to block Pb-induced (1 ppm) aortic contraction to 5-HT (L. F. Zhang et al., 2005). Increased SBP, intra-aortic mean arterial pressure, and MDA after Pb exposure (100 ppm; blood Pb level 23.7-27 µg/dL) were also prevented by treatment with the immunosuppressive, mycophenolate mofetil (MMF) (Bravo et al., 2007). MMF has been shown to inhibit endothelial NAD(P)H oxidase, which could explain the decrease in oxidative stress and BP. Red grape seed extract was also able to protect rats from Pb-induced (100 ppm) increased BP and heart rate, perhaps through the antioxidant properties of the extract (Badavi et al., 2008).

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 enzyme found in heart tissue (Alghazal, Lenártová, et al., 2008; Sobekova et al., 2009). Pb exposure (>100ppm) in female rats increased the activity of cardiac SOD, GST, GR, and GPx and increased cardiac TBARS (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 exposure (♂ 100 ppm: 205% of control, 1,000 ppm: 379%; ♀ 100 ppm: 246%, 1,000 ppm: 775%), which could explain the sex differences observed.

Oxidative stress can trigger a cascade of events that promote cellular stress, renal inflammation, and hypertension. As was shown previously (Rodriguez-Iturbe et al., 2004), Pb exposure can increase renal NFκB, 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 is also able to induce inflammation in human endothelial cells as a model for vessel intima hyperplasia (Zeller et al., 2010). The proinflammatory cytokine, interleukin-8 (IL-8) protein and mRNA were increased, dose 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 NFκB, 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).

Oxidative stress affects vascular reactivity and tone through inactivation and sequestration of ˙NO, causing a reduction in biologically active ˙NO. Recent studies confirm these past conclusions on the interplay of ROS and ˙NO metabolism in the cardiovascular effects of Pb. Elevated SBP and altered vasorelaxation after Pb exposure is accompanied by a decrease in total nitrates and nitrites (NOx) (Heydari et al., 2006; L. F. Zhang et al., 2007). Serum NOx levels in Pb-treated rats remained depressed for 8 weeks and then reversed after 12 weeks, despite continued elevation in SBP (Heydari et al., 2006). This return of serum NOx levels could be a result of compensatory increases in endothelial NOS (eNOS) attempting to replenish an over-sequestered ˙NO supply. With this in mind, studies have shown increased eNOS protein expression after chronic Pb exposure in kidney (L. F. Zhang et al., 2007) 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 (L. F. Zhang et al., 2007).

˙NO, also known as endothelium-derived relaxing factor, is a potent endogenous vasodilator. Studies continue to investigate the effects of Pb on ˙NO dependent vascular reactivity. Perinatal Pb exposure (1000 ppm through pregnancy and lactation, blood Pb level 58.7 µg/dL) resulted in a greater increase in maximal contraction to L-NAME, which decreases ˙NO production, with a greater effect in the endothelium than the smooth muscle cells (Grizzo & Cordellini, 2008). Additionally, blocking NOS with L-NAME abolished the relaxant response evoked by ACh, which triggers the release of ˙NO from the endothelial cell, in aortic rings of perinatally exposed rats. Cyclooxygenase (COX) inhibition decreased the EC50 of the ACh response in Pb treated animals. This study suggests that pups exposed to Pb through pregnancy and lactation have an altered vascular reactivity that is endothelium dependent and occurs due to the altered release of ˙NO and a COX-derived vasoconstrictor (Grizzo & Cordellini, 2008).

Similarly, a recent study provides evidence that acute Pb exposure increases rat tail artery reactivity in an endothelium dependent manner due to a COX-derived vasoconstrictor and in part free radicals and ˙NO (Silveira et al., 2010). Acute exposure of rat tail artery to Pb (100 µM, 1 h) increased reactivity to phenylephrine. Pb exposure decreased ACh induced relaxation, suggesting damage to the endothelium. Pb

did not affect smooth muscle integrity since sodium nitroprusside (SNP)-induced vasorelaxation was unchanged. Inhibition of NOS increased the Pb pressor response whereas COX inhibition eliminated the response to PHE. Treatment with the SOD mimetic Tempol decreased, but did not eliminate, the Pb pressor response (Silveira et al., 2010). A second study showed that Pb (90 ppm) exposure did not change the rat thoracic aortic ring relaxation response curves to the ˙NO donor, SNP (Rizzi et al., 2009).

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. In rat renal interlobar arteries, Pb exposure blunted the increase in AngII mediated contraction from NOS inhibition by L-NAME (Vargas-Robles et al., 2007).

Vascular Reactivity

Alteration of the adrenergic system from Pb exposure, which can increase peripheral vascular resistance, and thereby arterial pressure, may be one cause of Pb-induced hypertension. Pb exposure in animals can increase stimulation of the sympathetic nervous system (SNS), as shown by increased plasma norepinephrine and plasma catecholamines (Carmignani et al., 2000; H.-R. Chang et al., 1997), and decreased β adrenergic receptor density and β agonist-stimulated cAMP production in the aorta and heart (H.-R. Chang et al., 1997; Tsao et al., 2000). These stimulatory effects on the SNS paralleled effects on BP, cardiac contractility, and carotid blood flow. Increased Pb induced arterial pressure and heart rate were abrogated by ganglionic blockade (C.-C. Lai et al., 2002) and gradually decreased 7 months after Pb exposure cessation along with Pb-induced SNS alterations (H.-R. Chang et al., 2005).

Increased BP can be caused by vascular narrowing leading to increased total peripheral resistance, resulting from activation of the SNS. 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, norepinephrine (NE), and dopamine), hormones of the SNS. Indeed, the isolated mesenteric vessel bed from Pb treated rats (50 ppm blood Pb level: 11.2 µg/dL, but not 100 ppm blood Pb level: 17.3 µg/dL) exhibited increased reactivity to NE (Skoczynska & Stojek, 2005). Similarly, 100 ppm Pb did not affect the NE induced contractile response after 10 months of exposure (L.-F. Zhang et al., 2009), suggesting a small range of doses affecting 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 abolish Pb-induced aortic contraction (Fazli-Tabaei et al., 2006; Heydari et al., 2006). Phenylephrine-induced aortic contractions were enhanced by treatment with Pb (100 ppm; blood Pb level: 26.8 µg/dL), indicating a specific role for the α1-adrenergic receptor. Additionally, Pb blunted the isoproterenol-induced relaxation, supporting a role for the β-adrenoceptors (Heydari et al., 2006; Vassallo et al., 2008).

Recently, there has been mixed evidence for Pb disrupting vascular reactivity to other pressor agents. One study found that Pb (50 ppm; 12 weeks; blood Pb level: 11.2 µg/dL) increased acetylcholine (ACh) induced relaxation in rats (Skoczynska & Stojek, 2005). Additionally, studies have shown no change in ACh induced vasorelaxation after Pb exposure (Grizzo & Cordellini, 2008; Rizzi et al., 2009). However, Zhang et al. (2007) and Silveira et al. (2010) found that Pb (1 ppm and 100 µM, 1 h) blunted ACh induced relaxation in isolated rat thoracic aorta and tail artery, respectively. Another study investigated the influence of Pb on vasoconstriction from 5-hydroxytryptamine (5-HT). 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 (L. F. 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 (L.-F. Zhang et al., 2009).

Studies continue to investigate the role of ˙NO, also known as endothelium-derived relaxing factor, in Pb induced changes in vascular reactivity. A recent study provides evidence that acute Pb exposure increases rat tail artery reactivity in an endothelium dependent manner due in part to free radicals and ˙NO (Silveira et al., 2010). Acute exposure of rat tail artery to Pb (100 µM, 1 hour) increased reactivity to phenylephrine. Pb exposure decreased ACh induced relaxation, suggesting damage to the endothelium. However, Pb did not affect smooth muscle integrity since SNP-induced vasorelaxation was unchanged. Similarly, Pb (90 ppm) exposure did not change the rat thoracic aortic ring relaxation response curves to the ˙NO donor, SNP (Rizzi et al., 2009). Inhibition of NOS increased the Pb pressor response to PHE (Silveira et al., 2010). Another study showed that blocking NOS with L-NAME, abolished the relaxant response evoked by ACh, which triggers the release of ˙NO from the endothelial cell, in aortic rings of perinatally exposed rats (1,000 ppm through pregnancy and lactation, blood Pb level 58.7 µg/dL) (Grizzo & Cordellini, 2008). Additionally, perinatal Pb exposure resulted in a greater increase in maximal contraction to L-NAME, which decreases ˙NO production, with a greater effect in the endothelium than the smooth muscle cells. This study suggests that pups exposed to Pb through pregnancy and lactation have an altered vascular reactivity that is endothelium dependent and occurs due in part to the altered release of ˙NO (Grizzo & Cordellini, 2008). 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). NOS inhibition by L-NAME increased AngII-induced vasoconstriction in control but not Pb-exposed arteries, suggesting impaired ˙NO availability (Vargas-Robles et al., 2007). 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.

Renin-Angiotensin-Aldosterone and Kininergic Systems

The adrenergic system also affects the renin-angiotensin-aldosterone system (RAAS), which is responsible for fluid homeostasis and blood pressure regulation, and has been shown to be affected by Pb exposure. Meta-analysis found that Pb exposure (30-40 µg/dL) increases plasma renin activity and renal tissue renin in young rats, but not old (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 killikrein activities; and renal angiotensin II (AngII) positive cells (Carmignani et al., 1999; Rodriguez-Iturbe et al., 2005; Sharifi et al., 2004). 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 continue to implicate the RAAS in the development of Pb-induced hypertension, especially during early exposure in young animals. Low level Pb (100 ppm, 14 weeks; blood Pb level 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 SBP and was ablated by the anti-inflammatory antioxidant, MMF. Similarly, early high level Pb (1% Pb, 40 days; blood Pb level >240 µg/dL after exposure, 12-13 µg/dL after chelation after 1 year) accumulation resulted in sustained hypertension (Bagchi & Preuss, 2005). Treatment with the AngII receptor blocker, Losartan, resulted in a greater decrease in SBP in Pb exposed rats than control rats that continued into later periods of follow-up (day 283). Increased SBP 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. 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). These studies point to the activation of the RAAS in the course of Pb-induced hypertension.

Vasomodulators

The balance between production of vasodilators and vasoconstrictors is important in the regulation of blood pressure and cardiovascular function. The previous AQCD reported that the effects of Pb on vasomodulators are contradictory. Urinary excretion of the vasoconstrictor, thromboxane (TXB_2), and the vasodilatory prostaglandin, 6-keto-PGF1α, were unchanged in rats with Pb-induced hypertension (Gonick et al., 1998). However, in vitro Pb exposure promoted the release of the prostaglandin substrate, arachidonic acid, in vascular smooth muscle cells (VSMC) via activation of phospholipase A_2 (Dorman & Freeman, 2002). Plasma concentration and urinary excretion of the vasoconstrictive peptide, endothelin (ET) 3 was increased after low (100 ppm), but not high level (5000 ppm) Pb exposure in rats (Gonick et

al., 1997; Khalil-Manesh et al., 1994; Khalil-Manesh, Gonick, Weiler, et al., 1993). 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 dose dependent decline in the vasodilator, atrial natriuretic factor (ANF) (Giridhar & Isom, 1990). These studies suggest that Pb may interfere with the balance between vasodilators and vasoconstrictors forming the hormonal regulation of vascular contraction and blood pressure.

The imbalance in vasomodulators is one explanation for the concentration-dependent vasoconstriction observed after Pb exposure (Piccinini et al., 1977; Valencia et al., 2001; Watts et al., 1995). Vasoconstriction after Pb exposure was not reported in all studies (Shelkovnikov & 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 attenuation of acetylcholine- and NO-mediated vasodilation (M. 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 (Piccinini et al., 1977; Valencia et al., 2001; Watts et al., 1995).

One 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 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. This study suggests 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.

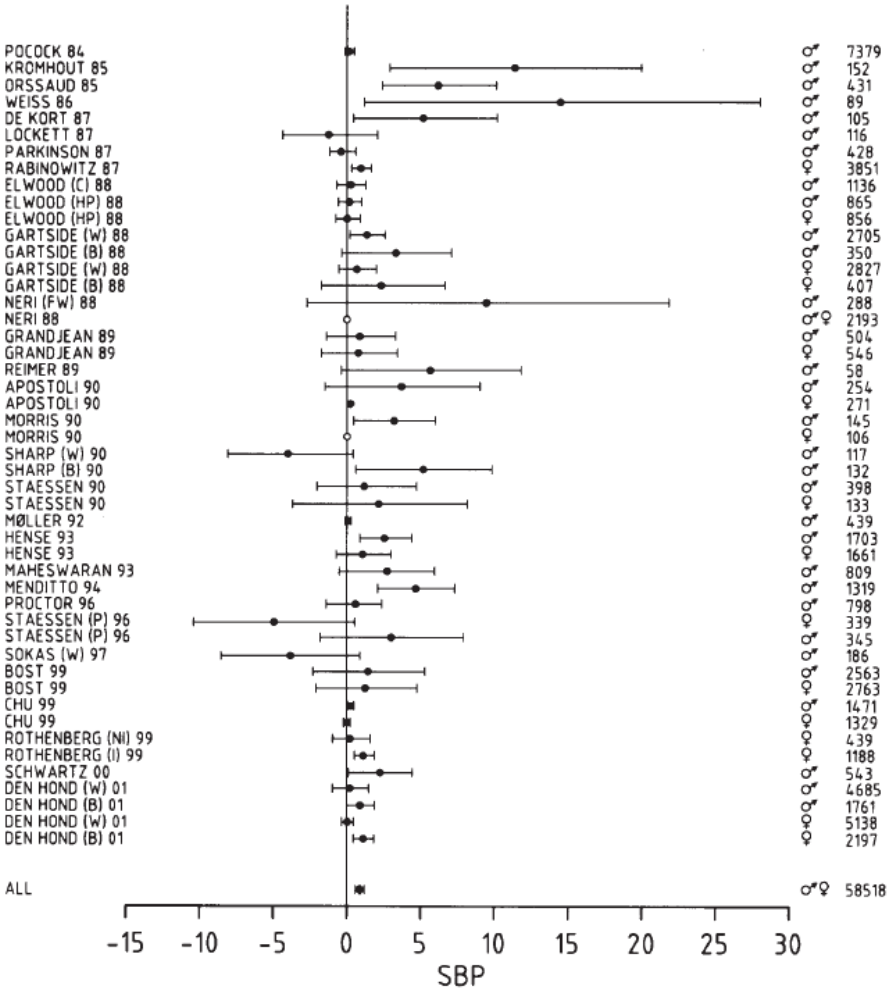
COX-2 blockade has been shown to prevent Pb-induced downregulation of sGC expression (Courtois et al., 2003). Inhibition of COX-2 also decreases the Pb induced pressor response to ACh (Grizzo & Cordellini, 2008) and PHE (Silveira et al., 2010) in experimental animals. These studies suggest that Pb induced vascular reactivity may depend on the participation of a COX-derived vasoconstrictor, such as prostaglandins, prostacyclins, or thromboxanes.

5.4.2.4. Summary

The 2006 Pb AQCD reported a clear positive association between blood Pb level and BP. The effect was modest, but highly significant, as determined by a meta-analysis (Nawrot et al., 2002) of over 30

studies comprising over 40,000 subjects (Figure 5-35), reporting that each doubling of blood Pb was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP. Recent studies support this conclusion at lower blood Pb levels (<2 µg/dL) and add to the evidence base on susceptibility factors and bone Pb associations with BP and hypertension at levels <20 µg/g. Associations of bone and/or blood Pb with systolic BP and hypertension were higher among non-whites, those reporting high stress, and those with the HFE H63D and ALAD genotypes.

A recent study in an ethnically diverse community-based cohort of women and men aged 50-70 years of age suggests that Pb has an acute effect on BP as a function of recent dose measured by blood Pb and a chronic effect on hypertension risk as a function of cumulative exposure measured by tibia Pb (Martin et al., 2006). This study verified other studies by demonstrating that with each increase of 1 µg/dL blood Pb level, systolic BP would increase 1 mmHg and diastolic BP would increase 0.5 mmHg. Additionally, recent epidemiologic studies provided evidence for associations between blood Pb and BP and hypertension at relatively low blood Pb level; a positive relationship was found in the NHANES data (1999-2002) at a geometric mean blood Pb level of 1.64 µg/dL (Muntner et al., 2005). Animal toxicological studies also provide support for effects of low blood Pb level on increased BP with statistically significant increases shown as low as 2 µg/dL (Tsao et al., 2000). Collectively, all animal toxicological studies providing blood Pb level and BP measurements report positive increases in BP with increasing blood Pb level (Figure 5-34). New studies also demonstrate reversibility of Pb-induced increased BP following Pb exposure cessation or chelation.



Source: Used with permission from MacMillan Press, Nawrot et al. (2002)

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.

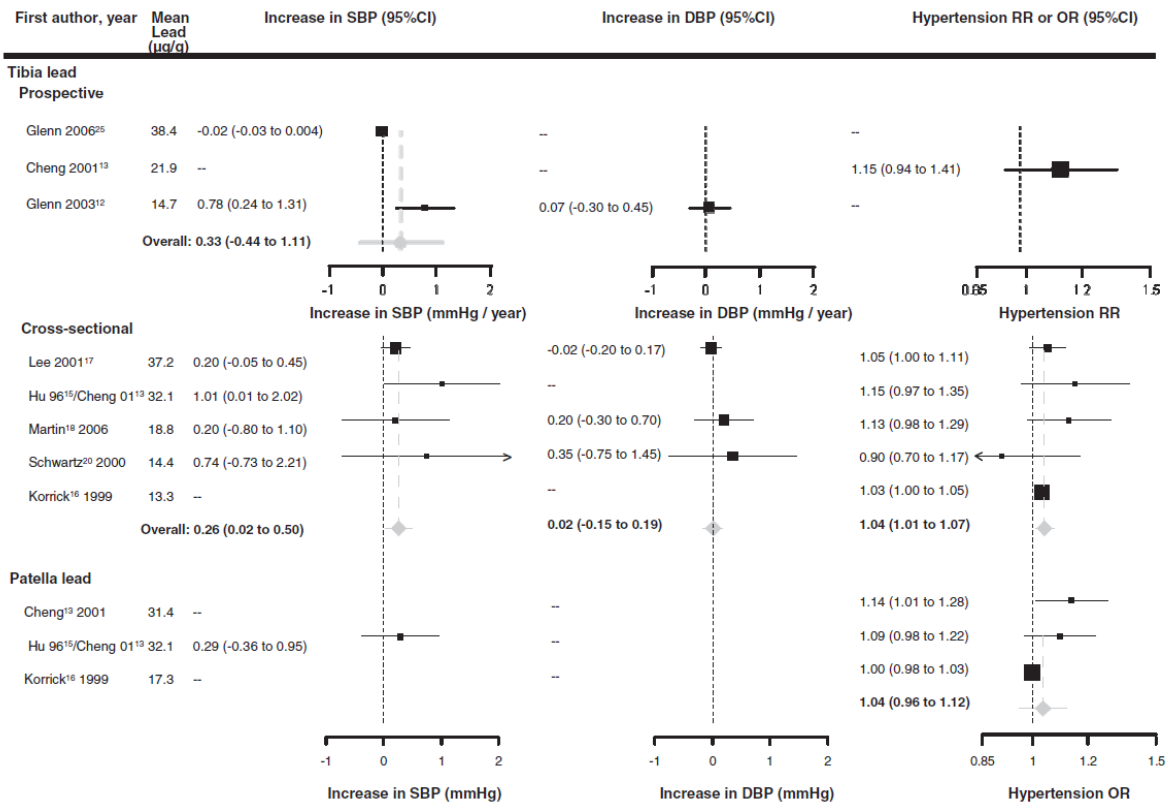
Note: Circles represent individual groups and squares represent the combined association sizes. Open circles denote a nonsignificant association size that was assumed to be zero.

Figure 5-35. Change in SBP, association size in mmHg with 95% CI, associated with a doubling in the blood Pb concentration.

Epidemiologic studies continue to investigate the relationship between bone Pb and increased BP. A recently published meta-analysis of epidemiological studies used bone Pb as an exposure index and BP or hypertension as the outcome (Figure 5-36) (Navas-Acien et al., 2008). The eight studies (three prospective, five cross-sectional) showed positive relationships between tibia Pb and systolic BP but not

diastolic BP. A pooled estimate for an increase in systolic BP of 0.26 mmHg (95% CI: 0.02, 0.50) for the cross-sectional studies was reported. The estimate for the longitudinal studies was 0.33 mmHg (95% CI: -0.44, 1.11). With the exception of one study, positive associations of bone Pb with hypertension were also reported. Pooled estimates 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.

Recent epidemiological studies have also emphasized the interaction between long-term Pb exposure and factors that moderate or modify the Pb effect, like chronic stress and metabolic syndrome, on BP and hypertension. Bone Pb coupled with high stress was associated with a strong and reliable increased risk of developing hypertension in an originally nonhypertensive group (Peters et al., 2007). Also, long duration Pb exposure interacted with components of the metabolic syndrome to drive HRV in directions associated with increased cardiovascular events (Park et al., 2006).



Source: Used with permission from Elsevier Publishers, Navas-Acien et al. (2008)

In the Normative Aging Study, Hu et al (1996) 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 SBP 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.

Figure 5-36. Prospective and cross-sectional increase in SBP and DBP and relative risk of hypertension per 10 µg/g increase in bone Pb levels.

Recent epidemiologic studies investigated the interaction of genotypes with effects of Pb on the cardiovascular system. Significant evidence was presented for modification of the effect of blood Pb level on BP by ALAD genotype (Scinicariello et al., 2010). Additionally, polymorphisms in the hemochromatosis gene modified the pulse pressure response to bone Pb exposure, where pulse pressure represents a good predictor of cardiovascular morbidity and mortality and an indicator of arterial stiffness (A. Zhang et al., 2010). Park et al. (2009) provided further evidence of gene variants, specifically those related to iron metabolism, impacting the effect of long-term Pb exposure on the cardiovascular system, evaluated by QT interval changes.

Not only has Pb exposure been shown to increase BP and hypertension, but Pb exposure can contribute to the development of other cardiovascular diseases. Recent epidemiologic and toxicological studies provide evidence for increased atherosclerosis, thrombosis, ischemic heart disease, peripheral artery disease, arrhythmia, and cardiac contractility.

Animal toxicological evidence continues to build on the evidence supporting the mechanisms leading to these cardiovascular alterations. Enhanced understanding of Pb-induced oxidative stress including ˙NO inactivation, endothelial dysfunction leading to altered vascular reactivity, activation of the RAAS, and vasomodulator imbalance provides biological plausibility for the consistently positive associations observed between blood and bone Pb and cardiovascular effects.

5.4.3. Vascular Effects and Cardiotoxicity

Not only has Pb exposure 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 & Kopp, 1985; Revis et al., 1981). Epidemiologic studies discussed in the previous AQCD provided limited evidence to support the association of ischemic heart disease (IHD) and peripheral artery disease (PAD) with increased blood Pb.

5.4.3.1. Effects on Vascular Cell Types

The endothelium layer is an important constituent of the blood vessel wall, which regulates macromolecular permeability, vascular SMC tone, tissue perfusion, and blood fluidity. Damage to the endothelium is an initiating step in development of atherosclerosis, thrombosis, and tissue injury. Given that chronic Pb exposure promotes a number of these diseases, numerous studies have investigated the role of Pb on endothelial dysfunction. The endothelial layer makes up only a small part of the vascular anatomy; the majority of the vessel wall is composed of vascular SMC, which work in concert with the 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, studies continue to investigate the effects of Pb on SMC.

Pb exposure (50 µM, 2 weeks) stimulated SMC invasiveness in isolated human arteries leading to the invasion of medial SMC into the vessel intima and development of intimal hyperplasia, a key step in atherosclerotic progression (Zeller et al., 2010). In addition, Pb exposure (50 µM, 12 hours) promoted SMC elastin expression and increased arterial extracellular matrix in isolated human arteries. SMC invasiveness was also increased in culture by treatment with supernatant of Pb-treated human EC (50 µM), suggesting that Pb-exposed EC secrete an activating compound. This compound was confirmed to

be interleukin-8 (IL-8). Pb exposure (5-50 µM) was able to dose-dependently 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 SMC invasion and arterial intima thickening (Zeller et al., 2010). This study provides evidence that Pb exposure stimulates EC to secrete IL-8 in an Nrf2-dependent manner that stimulates SMC invasion from the vessel media to intima leading to a vascular thickening and possibly atherogenesis.

A number of cardiovascular diseases, including atherosclerosis, are characterized by increased inflammatory processes. Numerous studies have shown that Pb exposure is able to induce an inflammatory environment in humans and animals by increasing inflammatory mediators like prostaglandin E_2 (PGE_2). Human aortic vascular SMC treated with Pb (1 µM, 1-12 hours) exhibited increased secretion of PGE_2 time-dependently through enhanced gene transcription (W. C. Chang et al.). This was preceded by a Pb-induced increase in gene expression of the rate limiting enzymes in the regulation of prostaglandins, cytosolic phospholipase A_2 ($cPLA_2$) and COX-2. The induction of these enzymes was mediated by activation of ERK1/2, MEK1, and MEK2. Further investigation into 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 $cPLA_2$ and COX activation and PGE_2 secretion. Overall this study suggests that Pb can induce proinflammatory events in vascular SMC in the form of increased PGE_2 secretion and $cPLA_2$ and COX-2 expression through activation of EGFR via ERK1/2 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; Kaji et al., 1995; Kishimoto et al., 1995; Ueda et al., 1997). However, Pb exposure does not 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 results in specific cytotoxicity (caspase3/7 activation) through endoplasmic reticulum (ER) stress that can be 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 Pb to EC induces *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 study suggests that the functional damage caused by Pb exposure to EC may be partly attributed to induction of ER stress.

5.4.3.2. Cholesterol

As blood cholesterol rises so does the risk of coronary heart disease. Early occupational studies (Ademuyiwa, Ugbaja, Idumebor, et al., 2005; Bener et al., 2001; Kristal-Boneh et al., 1999) at higher than current blood Pb levels reported higher total cholesterol levels related to Pb exposure, and mixed results for HDL, LDL, and triglycerides. More recently, Poreba et al. (2010), in an occupational study, reports no significant differences in parameters of lipid metabolism between Pb exposed and unexposed hypertensive patients. Other Pb studies (Menke et al., 2006) adjust models for total cholesterol to control for this coronary heart disease risk factor and note that mean total cholesterol was higher at higher blood Pb levels. In developing models to predict bone Pb levels, Park et al. (2009) noted that total and high-density lipoprotein cholesterol were selected as 2 of 18 predictors for the bone Pb level model. Their findings suggested that total and HDL cholesterols, as part of a larger groups of predictors, may be critical contributors to such prediction models. The major risk factor that lipids represent for heart disease make relating lipid levels to lead exposures an interesting but challenging hypothesis to test.

5.4.3.3. Heart Rate Variability

Pb has been shown to not only affect vascular contractility but also cardiac contractility. Park et al. (2006) investigated the interaction of key markers of the metabolic syndrome and bone Pb effect on heart rate variability (HRV) in a group of 413 older adults with patella Pb measurements from the VA-NAS. 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 blood pressure >130/85 mmHg, and high fasting glucose (>110 mg/dL). Those using antihypertensive medication or diabetes medications were counted as high BP or high fasting glucose, respectively. The strongest relationships with bone Pb were among those with three or more metabolic abnormalities. Tests for trend by number of metabolic abnormalities was significant at $p<0.05$ for patella Pb. These results suggest multiplicative effects of long duration Pb exposure 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 (nonsignificant) bone Pb main effects on HRV measures were linear. However, the relationship with LF/HF was linear in log(LF/HF).

Park et al. (2009) followed up a previous report (Y. Cheng et al., 1998), which found prolongation of corrected QT interval (QTc) with increasing bone Pb in men <65 years, but not in men ≥ 65. In the recent work, authors stratified multiple regression models on polymorphisms in three genes known to alter iron metabolism, hemochromatosis (HFE), transferrin (TF), and heme oxygenase-1 (HMOX-1), and related QTc intervals to blood, tibia and patella Pb level. They also used interaction models with cross-

product terms between genotype and the Pb biomarker. The distributions of all genotypes but the HFE mutant, H63D, were in Hardy-Weinberg equilibrium. Subjects homozygous for the other HFE mutant, C282Y, had higher bone Pb concentrations and those homozygous for H63D and heterozygous for both C282Y and H63D had lower bone Pb. The HMOX-1 variant alone, compared to the wild type, showed a significant interaction with tibia Pb (increased QTc [11.35 msec] for each 13 µg/g increase in bone Pb in L-allele variants). No other gene variant alone showed significant QTc differences from wild types, either for tibia and patella Pb or for (linear) blood Pb. Lengthening of QTc with increased tibia and blood Pb was more pronounced with an increase in the total number of gene variants, driven by a joint effect between HFE variant and HMOX-1 L allele. Tests for linear trend in QTc by increasing number of gene variants from 0 to 3 were significant for both tibia and blood Pb. This study provided further suggestive evidence of gene variants impact on long-term Pb exposure on the cardiovascular system.

Increased incidence of arrhythmia and atrioventricular conduction block increased in rats after 12 weeks of Pb exposure (100 ppm; blood Pb level 26.8 µg/dL) (Reza et al., 2008). Also, Pb exposure after 8 weeks increased heart rate and systolic BP. These corresponded with increased cardiac contractile force and prolonged ST interval, without alteration in QRS duration or coronary flow. In contrast, another study found that Pb (100 µM) exposure dose-dependently reduced myocardial contraction using rat right ventricular strips by reducing sarcolemmal Ca^{2+} influx and myosin ATPase activity (Vassallo et al., 2008). This study also found that Pb exposure could change 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 (Favalli et al., 1977; Lal et al., 1991; Piccinini et al., 1977), which could result in increased cardiac output and hypertension.

5.4.3.4. 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 were discussed previously, also examined the association of blood Pb with PAD. They observed an increasing trend in the prevalence of PAD with increasing 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). These results are consistent with a previous NHANES analysis of the association of blood Pb with PAD conducted by Navas-Acien et al. (2004).

Navas-Acien et al. (2004) reported a significant trend of increasing OR with increasing quartile of blood Pb or Cd. However, the authors reported a significant association of blood Cd with PAD and a non-significant association with blood Pb. 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 ($p < 0.001$). When blood Pb and blood Cd were in the same model together, the ORs were diminished slightly but both had significant trends of increasing OR with increasing quartile of the metal. Their interaction was not significant. In a later analysis, Navas-Acien et al. (2005) used the same 1999-2000 NHANES data set that they used in their 2004 paper, 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. Urine Cd, but not urine Pb, was reliably elevated in all models in PAD subjects, while there were indications of elevations in antimony and tungsten. Spot urine Pb measurements are less reliable compared to blood Pb measurements. In another NHANES analysis Navas-Acien et al. (2005) reported associations between PAD and urinary Pb level that were sensitive to adjustment for urinary creatinine.

5.4.3.5. Ischemic Heart Disease

A few studies discussed in the previous Pb AQCD reported association between Pb exposure and increased risk of cardiovascular outcomes associated with IHD, including left ventricular hypertrophy (J. Schwartz, 1991) and myocardial infarction (Gustavsson et al., 2001). Recently, Jain et al. (2007) reported the incidence of IHD (physician confirmed MI, angina pectoris) among older adult males enrolled in the VA-NAS during the period of September, 1991 to December, 2001. All subjects had blood Pb and bone Pb measurements with no IHD at enrollment. Fatal and nonfatal cases were combined for analysis. Blood, tibia, and patella Pb levels were log-transformed. Significant hazard ratios were reported for the association of IHD with blood Pb level and patella Pb level. When blood Pb and patella Pb were included simultaneously in the model, HR were 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 the 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). This suggests that both contribute independently to IHD. In an ecological study, Marchwinska-Wyrwal et al. (2010) report simple associations between 15-year air Pb averages in 15 Silesian cities in Poland with similar health and socioeconomic conditions but widely varying air contamination, including air Pb and air Cd. They report associations between air Pb and CVD by city when each city was weighted equally.

IHD, characterized by reduced blood supply to the heart, may result from increased thrombosis. A recent study suggests that Pb exposure promotes a procoagulant state that would contribute to thrombus formation (Shin et al., 2007). Pb exposure (i.v. 25 mg/kg, 1 hour) in a rat model of venous thrombosis resulted in increased thrombus formation. Additionally, increased blood coagulation (5 µM) and thrombin generation (2-5µM) was observed in a dose-dependent manner after Pb exposure to human erythrocytes.

This enhanced procoagulant activity in Pb-treated erthyrocytes was the result of increased outer cell membrane phosphatidylserine (PS) exposure (human RBC: 2-5 µM; rat RBC: 5 µM). Similar to these in vitro results, PS externalization on erythrocytes was increased in Pb exposed rats (50-100 mg/kg). Increased PS exposure was likely the result of increased intracellular calcium (5 µM), enhanced scramblase activity (5-10 µM), inhibited flippase activity (5-10 µM), and ATP depletion (1-5 µM) after Pb exposure (Shin et al., 2007).

5.4.3.6. Atherosclerosis

Studies provide evidence for increased atherosclerosis and intimal medial thickening (IMT) after Pb exposure. The association between stroke subtypes and severity of cerebral atherosclerosis was examined in relation to a single blood Pb level and total 72-hour urine Pb amount (body Pb store – EDTA mobilization test) in a cross-sectional study of 153 patients (mean age 63.7) receiving digital subtraction angiography in Chang Gung Memorial Hospital in Taiwan from 2002 to 2005 (T.-H. Lee et al., 2009). Body Pb stores were positively associated with the severity of artery stenosis in the intracranial carotid system but not the extracranial and vertebrobasilar systems. As development of atherosclerosis is a lifelong process, cumulative body Pb stores may be more sensitive than single blood Pb in the prediction of atherosclerosis.

Zeller et al. (2010) examined human radial and internal mammary arteries exposed to Pb in culture and reported a dose-dependent increase in arterial intimal thickness (nonsignificant at 5 µM, significant at 50 µM, 2 weeks) and intimal extracellular matrix accumulation (50 µM). Also, Pb exposure promoted endothelial cell (EC) proliferation (5 and 50 µM, 72 hours) and SMC elastin expression (50 µM, 12 hours), as discussed above (Section 5.4.3.1) (Zeller et al., 2010). A second study showed that Pb exposure (100 ppm, 10 months; blood Pb level 28.4 µg/dL) to rats also increased the aortic media thickness, media-lumen ratio, and medial collagen content (L.-F. Zhang et al., 2009). These morphological changes to the vessel do not only imply initiation of arteriosclerosis. These vascular changes could be to blame for decreased contractile response of the vessel due to altered visco-dynamic vessel properties or could be an effect of Pb-induced hypertension.

Table 5-15. Characteristics and quantitative data for associations of blood and bone Pb with other CVD measures.

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)[a]
Ischemic Heart Disease					
Jain et al. (2007)	837 men from VA-NAS in Greater Boston, MA area (1991-2001)	IHD (MI or angina pectoris)	Blood Pb: Non-cases 6.2 (4.3) µg/dL; Cases 7.0 (3.8) µg/dL Patella Pb: Non-cases 30.6 (19.7) µg/dL; Cases 36.8 (20.8) µg/dL Tibia Pb: 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's 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 OR= 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) Per 1 SD increase in Pb metric
Marchwinska-Wyrwal et al. (2010)	Residents from 13 Silesian cities, Poland	CVD	Air Pb (avg yearly rate): 127.8 - 359.9 ng/m^3	Linear regression models	β= 1.52 (0.76)
Peripheral artery disease					
Muntner et al. (2005)	9,961 NHANES (1999-2002) participants	PAD	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 (95% CI): 1.00 (Reference), 1.00 (0.45, 2.22), 1.21 (0.66, 2.23), 1.92 (1.02, 3.61)
Navas-Acien et al. (2005)	790 participants, age ≥ 40 y, from NHANES (1999-2000)	PAD	Urine Pb: Mean (10th-90th %): 0.79 µg/L (0.2-2.3)	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	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) Per IQR increase of urinary Pb Array of metals in urine also evaluated.
Heart rate variability					
Park et al. (2006)	413 men from Normative Aging Study in Greater Boston, MA area (11/14/2000 - 12/22/2004)	HRV (SDNN, HF, HF_{norm}, LF, LF_{norm}, LF/HF)	Patella Pb: Median (IQR): 23.0 (15-34) µg/g Estimated[a]: Median (IQR): 16.3 (10.4-25.8) µg/g Tibia Pb: Median (IQR): 19.0 (11-28) µg/g	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, calcium channel blockers, and/or ACE inhibitors	Tibia Pb: Model 2 Change (95%CI) HF: –0.9 (–3.8, 2.1) LF: 0.9 (–2.0, 3.9) Log LF/HF: 3.3 (–10.7, 19.5) Per 17 µg/g tibia Pb Patella Pb: Model 2 Change (95%CI) HF: –0.6 (–3.1, 1.9) LF: 0.6 (–1.9, 3.1) Log LF/HF: 3.0 (–8.7, 16.2) Per 15.4 µg/g patella Pb Among those with metabolic syndrome the strongest effects were observed.

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)[a]
Park et al. (2009)	613 men from Normative Aging Study 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 calcium, and diabetes.	Blood Pb: β: 1.3 (-0.76, 3.36) IQR: 3 µg/dL Patella Pb: β: 2.64 (0.13, 5.15) IQR: 19 µg/g Tibia Pb: β: 2.85 (0.29, 5.40) IQR: 13 µg/g

[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

5.4.3.7. Summary

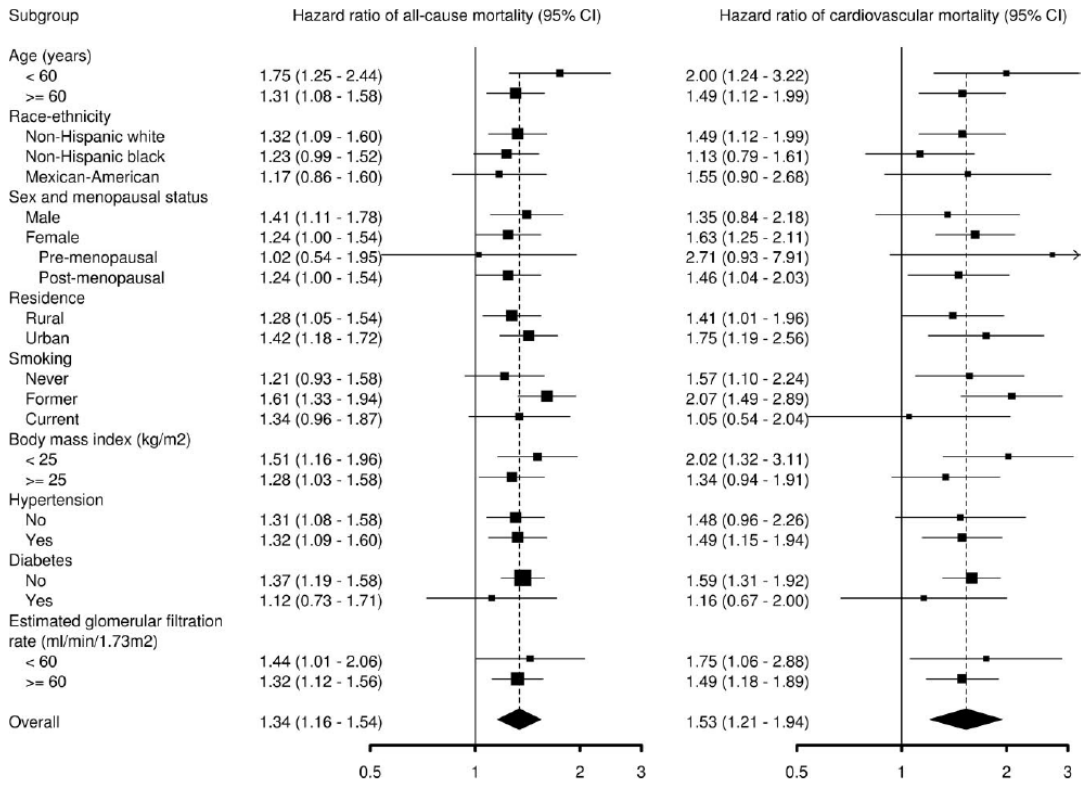
There are a limited number of studies that investigate the association between Pb exposure and cardiovascular effects other than hypertension (Table 5-15). These studies have presented associations between various measures of Pb, representing distinct exposure time periods, and atherosclerosis, IHD, PAD, and HRV. Also, limited, mixed evidence of occupational exposure to Pb and altered cholesterol have been reported. Additionally, studies in isolated vascular tissues and cells provided mechanistic evidence to support the biological plausibility of these other vascular effects and cardiotoxicity. A recent study provided evidence for the interaction between Pb exposure and gene variants for iron metabolism on the prolonged QT interval (Park, Hu, et al., 2009). Blood Pb (>2.5 µg/dL) was associated with greater risk for PAD in two NHANES analyses (Muntner et al., 2005; Navas-Acien et al., 2004). In addition, in the VA-NAS cohort of older adult men, blood Pb (≥ 5 µg/dL) and patella Pb levels were associated with increased morbidity from IHD (Jain et al., 2007). A recent study involving both human and toxicological studies elucidated the mechanisms for observed Pb-mediated arterial IMT, an early event in Pb-induced atherogenesis (Zeller et al., 2010). Studies in isolated tissues and cells found that Pb stimulated the synthesis and secretion of IL-8 in EC, which was responsible for stimulating SMC invasion into the vessel intimal layer. Pb exposure also increased extracellular matrix and elastin, primary sites for lipid deposition in the vessel wall. Overall, the relatively few studies that investigate associations between biomarkers of Pb exposure and other cardiovascular events provide supportive evidence for the role of Pb in the development of these diseases, yet further research is needed to understand these relationships.

5.4.4. Mortality Associated with Long-Term Lead Exposure

The previous Pb AQCD (U.S. EPA, 2006) stated that collectively the then available analyses of NHANES II and III data suggest a significant 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. This involved two NHANES analyses that examined the association of blood Pb with all cause and cause-specific mortality

(Lustberg & Silbergeld, 2002; Schober et al., 2006). As blood Pb levels in adults are comprised of both recent Pb exposure and Pb mobilization from bone, it is unclear whether the mortality associated with blood Pb levels are due to current or cumulative Pb exposures. Given the decline in ambient air Pb concentrations and population blood Pb levels, it is likely that study subjects had a much higher Pb exposure in their past than what they are experiencing currently. Using NHANES II data, Lustberg and Silbergeld (2002) found significant increases in all-cause, circulatory and cancer mortality, comparing those with blood Pb levels from 20-29 µg/dL to those with blood Pb levels less than 10 µg/dL. Using NHANES III data, Schober et al. (2006) found significant associations for all cause mortality, cardiovascular, and cancer mortality comparing those 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.

Three new studies make substantial additions to the evidence base. A further analysis of the NHANES III database by a different research group using different methods provides further information addressing uncertainties from other earlier analyses. Two longitudinal prospective studies in different cohorts conducted by different researchers with different methods in different parts of the U.S. provide coherence to the evidence base. Menke et al. (2006) examined the associations of all-cause and cause-specific mortality using NHANES III data. Subjects at least 18 years of age were followed up to 12 years after they were surveyed and 1,661 deaths were identified. Those with blood Pb levels from 3.63 to 10 µg/dL had significantly higher risks of all-cause (1.25 [95% CI: 1.04, 1.51]), cardiovascular (1.55 [95% CI: 1.08, 2.24]), MI (1.89 [95% CI: 1.04, 3.43]), and stroke (2.51 [95% CI: 1.20, 2.26]) mortality compared to those with blood Pb levels less than 1.93 µg/dL and non-significantly increased risk of cancer mortality. Hazard ratios were not higher comparing those with blood Pb levels from 1.94 to 3.62 µg/dL to those with blood Pb levels <1.93 µg/dL. However, trends of increasing hazard with increasing blood Pb tertile were significant ($p < 0.017$) for all models of all CVD presented. Menke et al. (2006) evaluated several of the model covariates (e.g. diabetes, hypertension, and GFR) in a subgroup analysis (Figure 5-37). The authors reported that there were no interactions between blood Pb and other adjusted variables and found a high consistency of HRs across models with a varying number of control variables (indicating little residual confounding). In the previous NHANES III analysis of the association of blood Pb with mortality, Schober et al. (2006) 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) reported significant associations comparing those with blood Pb levels ≥ 10 µg/dL to those with blood Pb levels <5 µg/dL for all-cause (1.59 [95% CI: 1.28, 1.98]), CVD (1.55 [95% CI: 1.16, 2.07]), and cancer (1.69 [95% CI: 1.14, 2.52]) mortality and generally non-significant increases comparing those with blood Pb levels from 5-9 µg/dL to those with blood Pb levels <5 µg/dL.

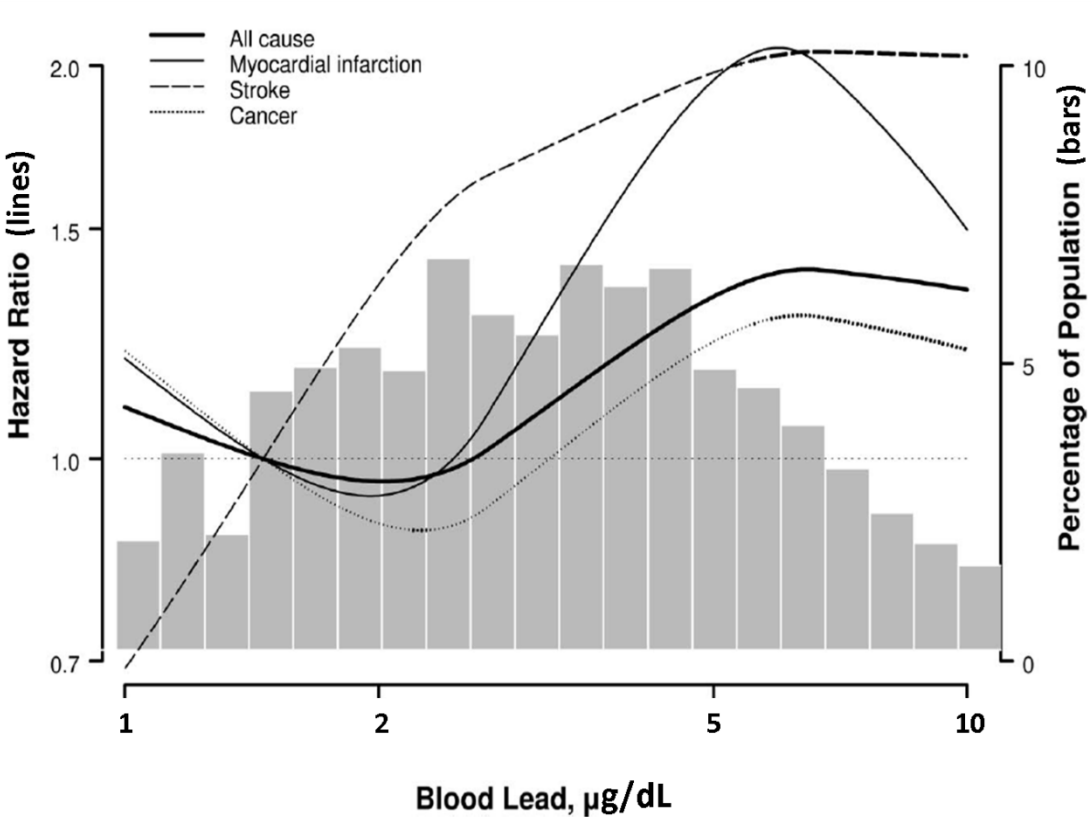


Source: Used with permission from Lippincott Wiliams & Wilkins, Menke et al. (2006)

Note: Hazard ratios were calculated for a 3.4-fold 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).

Figure 5-37. Multivariate adjusted relative hazards of all-cause and cardiovascular mortality.

Both Menke et al. (2006) and Schober et al. (2006) present mortality curves that plot the hazard ratios against blood Pb level. Figure 5-38 shows the mortality curves for both stroke and MI reported by Menke et al. (2006), which reach a peak around 6-7 µg/dL. The curves were fitted by restricted quadratic splines with knots at the 10th percentile (1.00 µg/dL; 0.048 µM/L), the 50th percentile (2.67 µg/dL; 0.287 µM/L), and the 80th percentile (5.98 µg/dL) blood Pb levels.

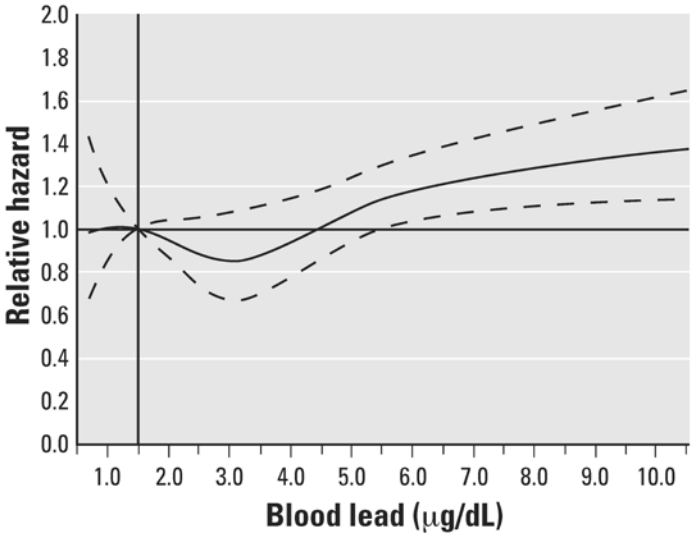


Source: Used with permission from Lippincott Wiliams & Wilkins, Menke et al. (2006)

Note: A histogram of blood Pb levels is superimposed in the background and displayed on the right axis.

Figure 5-38. Multivariate adjusted relative hazard (left axis) of mortality associated with blood Pb level between 1 µg/dL and 10 µg/dL.

Schober et al. (2006) examined proportional hazard assumptions, tested for linear trend across blood Pb tertiles, and evaluated log-transformed continuous blood Pb level as a 5-knot cubic spline (position of knots not stated). The test for linear trend by blood Pb tertile was significant at the $p<0.01$ level. 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) is shown in Figure 5-39. In contrast to the curve presented by Menke et al. (2006) the relative hazard axis and the blood Pb axis are both linear. Dashed lines are 95% CIs and the referent blood Pb level was 1.5 µg/dL. Despite differences in the age groups included, follow-up time, and categorization of blood Pb levels, results reported by Menke et al. (2006) and Schober et al. (2006) consistently conclude that higher blood Pb is associated with increased CVD mortality.



Source: Schober et al. (2006)

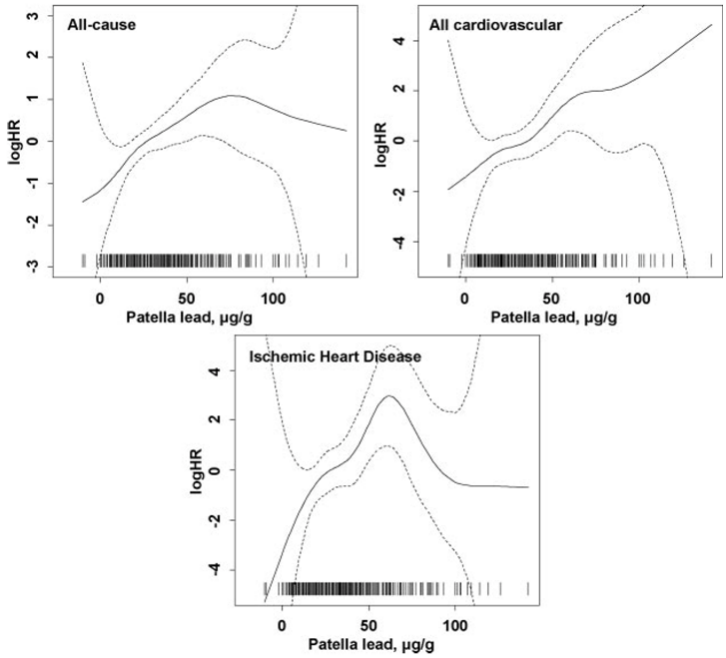
Note: The solid line shows the fitted five-knot spline relationship; the dashed lines are the point-wise upper and lower 95% CIs.

Figure 5-39. 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, two studies of older adult males (Weisskopf et al., 2009) and older adult females (Khalil, 2010; Khalil, Wilson, et al., 2009) were conducted. Weisskopf et al. (2009) used data from the VA-NAS to determine the association 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) in study subjects. The strongest associations were observed between mortality and patella Pb concentration. Non-significant increases in CVD mortality with tibia Pb and no association between blood Pb and mortality were observed. Tibia bone Pb concentration is thought to reflect a longer cumulative exposure period than patella bone Pb because the residence time of Pb in trabecular bone is shorter than in cortical bone. Ischemic heart disease contributed most to the relationship between patella Pb and all CVD death with HR of 2.69 (95% CI: 1.42, 5.08). Although there was high correlation between tibia and patella Pb (Pearson r = 0.77), trabecular bone Pb may have more influence on circulating blood Pb level, and thus organ concentration of Pb, than cortical bone Pb because of its shorter residence time in bone. In contrast to the NHANES analyses, blood Pb was not significantly related to cardiovascular mortality in this study. 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 youngest subjects at baseline in the Weisskopf et al. (2009) study were approximately 50-55 years old, compared to the youngest in the Menke et al. (2006) and Schober et al. (2006) studies, which were 17 and 40 years, respectively. Further the blood Pb tertile analysis of the Weisskopf study

could have been affected if the majority of a hypothesized non-linear effect was contained largely in the lowest (reference) blood Pb tertile.

Weisskopf et al. (2009) also conducted an exposure-response analysis. The test for linear trend of HR by bone Pb tertile was significant in both tibia and patella Pb models. The linear relationship using tertile patella Pb was confirmed in other models using continuous patella Pb and non-linear penalized spline terms, where higher order components were non-significant. The number of knots and their placement within the Pb variable, which can influence these results though the number and placement of knots of the penalized spline, were determined by an iterative best fit procedure to the data. Concentration-response relationships shown in Figure 5-40 are approximately linear for patella Pb on the log hazard ratio scale for all CVD, but appear non-linear for ischemic heart disease ($p < 0.10$). Peak HR is shown around 60 µg/g, beyond which HR tends to decrease, though the confidence limits are wide.

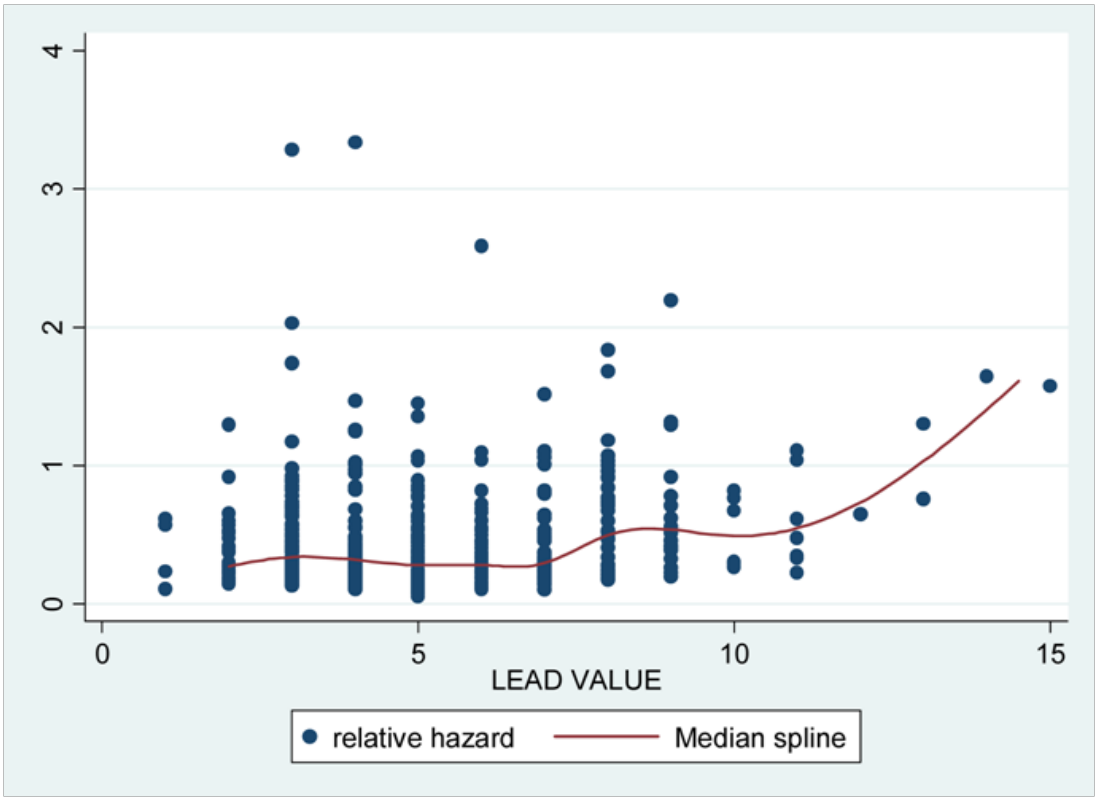


Source: Used with permission from Lippincott Wiliams & Wilkins, Weisskopf et al. (2009)

Note: The reference logHR = 0 at the mean of patella Pb concentration. The estimates are indicated by the solid line and the 95% pointwise 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 absicissa.

Figure 5-40. Nonlinear association between patella bone Pb concentration and the log of HR (logHR) for all-cause, cardiovascular, and ischemic heart disease adjusted for age, education, smoking status, and pack-years of smoking among participants without ischemic heart disease at baseline.

The association of Pb with mortality has also been examined among women enrolled in the Study of Osteoporotic Fractures (SOF) (Khalil, Wilson, et al., 2009). This prospective cohort (N = 533) enrolled female volunteers from two locations across the U.S. (Baltimore, MD and Monongahela Valley, PA). All-cause mortality comparing those with blood Pb levels >8 µg/dL to those with blood Pb levels <8 µg/dL was significantly increased (1.59 [95% CI: 1.02, 2.49]). Combined cardiovascular disease mortality (1.78 [95% CI: 0.92, 3.45]), coronary heart disease mortality (3.08 [95% CI: 1.23, 7.70]), but not stroke mortality (1.13 [95% CI: 0.34, 3.81]) HR was increased among the women enrolled in this study. In addition, Khalil et al. (2010) provided both tertile and quintiles analyses, as well as exposure-response results shown in Figure 5-41.



Source: Khalil et al. (2010)

Figure 5-41. Multivariate adjusted relative hazard (left axis) of mortality associated with blood Pb levels between 1 µg/dL and 15 µg/dL.

5.4.4.1. Summary

The mortality results in this review support and expand upon findings from the previous Pb AQCD (U.S. EPA, 2006), which included two NHANES mortality studies (Lustberg & Silbergeld, 2002; Schober et al., 2006). The recent NHANES mortality study discussed above (Menke et al., 2006) addressed many

of the limitations of the these earlier studies, including control for a wider range of potential confounders, testing for interactions with Pb, consideration of exposure-response relationships, extensive model evaluations, and including sub-categories of CVD. Further, an association with increased mortality was observed at lower population blood Pb concentrations. The mean blood Pb level of the NHANES III population was 2.58 µg/dL. The Pb attributable risk of increased cardiovascular mortality in the NHANES III analysis of Menke et al. (2006) reached its maximum at blood Pb levels between of 6 and 7 µg/dL. In addition, the first evidence that bone Pb, a metric of cumulative Pb exposure, is associated with increased mortality was reported. Several studies report associations between the accumulated Pb in bone and higher CVD morbidity, which are consistent with the mortality findings.

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 exposure measure, assumptions of linearity of the Pb exposure term, and choice of exposure metric. Quantitative differences in Pb-associated mortality across NHANES II and NHANES III studies or between different NHANES III may be explained by the use of continuous or ordered blood Pb exposure variables 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 national effects of Pb on cardiovascular disease-related mortality. The study uses the nationally representative NHANES III (1988 – 1994) sample allowing results to be generalized to the segment of the US population included in the sample. The results provide confirmation of earlier published studies and address some of the key weaknesses noted in those studies. 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 with trabecular bone Pb. The Khalil et al. (2010; 2009) Study of Osteoporotic Fractures provides supporting results in a different study cohort consisting of white females aged 65-87 years. Further, a number of prior studies have already found association between accumulated Pb reflected in bone Pb measurements and higher CVD morbidity (see CVD morbidity section), to which is added new findings of increased mortality due to CVD from long-term Pb exposure (bone Pb). Despite the differences in design and methods across studies, effects on mortality were consistently observed (Figure 5-42 and Table 5-16). In studies that broke out CVD-related mortality into sub-categories, MI, stroke, and IHD mortality, death-causes related to higher BP and hypertension were all significantly elevated as Pb exposure increased.

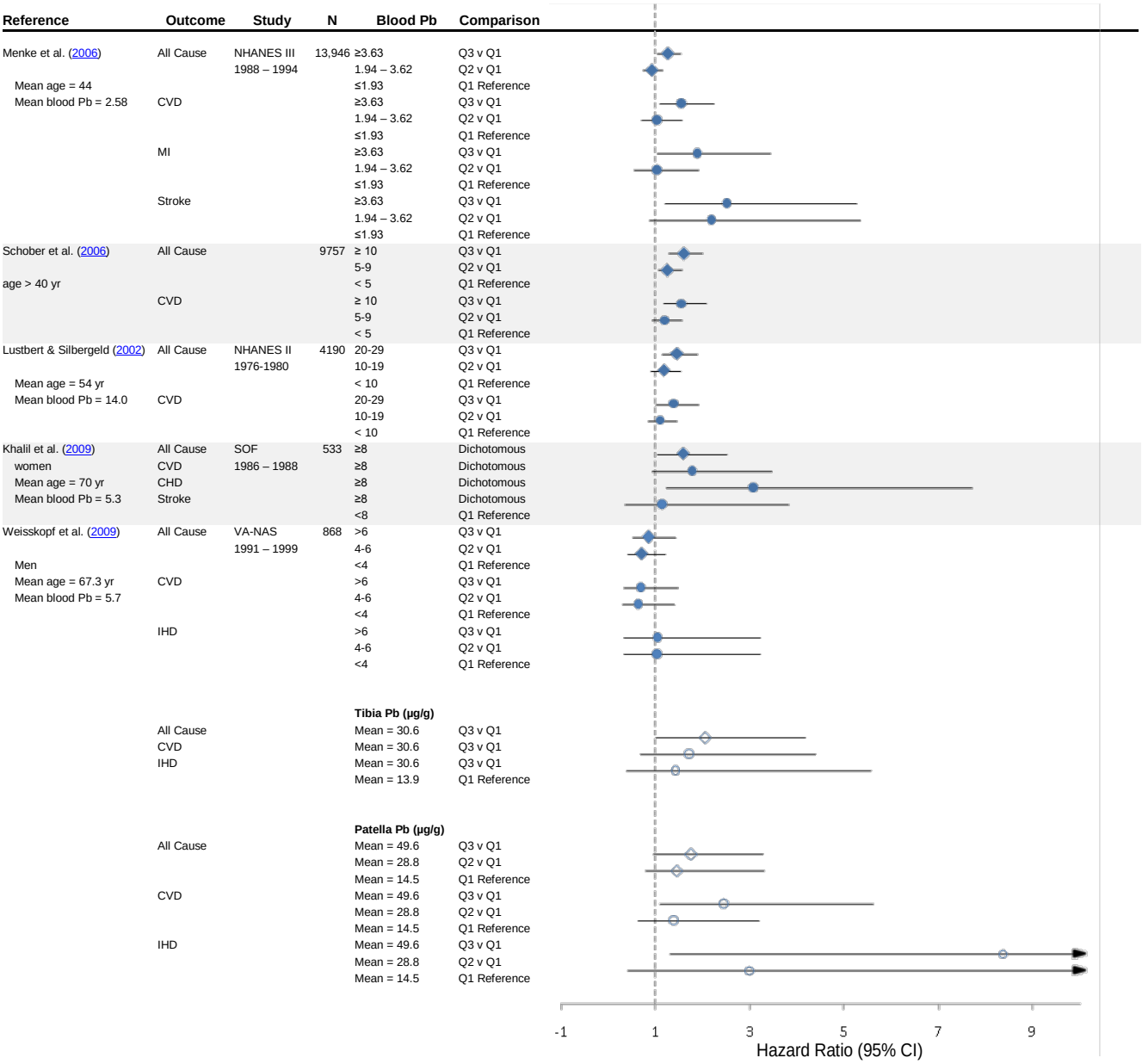


Figure 5-42. Hazard ratios between blood Pb (closed markers), bone Pb (open markers), all-cause mortality (diamonds), and cardiovascular mortality (circles).

Table 5-16. Additional characteristics and quantitative data for associations of blood and bone Pb with CVD mortality for results presented in Figure 5-42.

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Menke et al. (2006)	13,946 adult participants of NHANES III , ≥ 17 yr (1988-1994)	All cause and cause-specific mortality (through 2000) 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).	Blood Pb: 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: urban residence, cigarette smoking, alcohol consumption, education, physical activity, household income, menopausal status, BMI, CRP, total cholesterol, diabetes mellitus, Model 3: 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)
Schober et al. 2006 (2006)	9686 adult participant of NHANES III, ≥ 40 yr	All cause and cause-specific mortality	Ordered categorical blood Pb level: <5 µg/dL 5-9 µg/dL ≥10 µg/dL Also ln(blood Pb level)	Survey-design adjusted Cox proportional hazard adjusted for sex, age, race/ethnicity, smoking, education level, median follow-up time = 8.55 y	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)
Weisskopf et al. (2009)	868 men, >55 yr, 95% white, from Normative Aging Study in Greater Boston area, MA	All cause and cause-specific mortality	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)
Khalil et al. (2009)	533 women, 65-87 y, from Study of Osteoporotic Fractures cohort in Baltimore, MD and Monongahela Valley, PA	All cause and cause-specific mortality	Blood Pb: 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 BMD, walking for exercise, and diabetes, , mean follow-up time = 12 ± 3 y.	All cause (≥ 8 µg/dL vs. <8 µg/dL): 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)

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Neuberger et al. (2009)	Residents at or near Tar Creek Superfund site, Ottawa County, OK (exposed pop. 5,852, unexposed pop. 16,210)	Cause-specific mortality	Not reported	Standardized mortality ratio based on 2000 US 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)
Cocco et al. (2007)	933 male Pb smelter workers from Sardinia, Italy (1973-2003)	All cause and cause-specific mortality	Not reported	Standardized mortality ratio	All cause: 56 (46, 68) CVD: 37 (25, 55)

References not included in Figure 5-42 are included in this table.

5.4.5. Air Lead-PM Studies

5.4.5.1. Hospital Admissions

In addition to blood Pb, some recent epidemiologic studies have used Pb measured in PM_{10} and $PM_{2.5}$ air samples to represent Pb exposures. Some studies have analyzed Pb individually, whereas others have applied source apportionment techniques to analyze Pb as part of a group of correlated components. A common limitation of air-Pb studies is the variable size distribution of Pb-bearing PM (Section 3.5.3) and its relationship with blood Pb levels. Relative to studies of Pb biomarkers, time-series studies provide weak evidence for association between $PM_{2.5}$-Pb concentrations and cardiovascular hospital admissions and mortality in older adults. In a time-series study of 106 U.S. counties, Bell et al. (2009) found that an increase in lag 0 $PM_{2.5}$-Pb was associated with an increased risk of cardiovascular hospital admissions. Quantitative results were not presented; however, the 95% CI was wide and included the null value. Ostro et al. (2007) found that a 5 ng/m^3 (interquartile range) increase in lag 3 $PM_{2.5}$-Pb was associated with a 1.89% increase (95% CI: -0.57, 4.40%) in cardiovascular mortality in six California counties in the cool season. In both of these studies, statistically significant positive associations were observed for other PM components such as nickel, vanadium, and zinc also. In the absence of detailed data on correlations among components or results adjusted for copollutants, it is difficult to exclude confounding by these other components.

To address correlations among PM chemical components, some studies have applied source apportionment techniques to group components into common source categories. In these source-factor studies, it is difficult to attribute findings to a particular component in a group. In a study of 20 counties near Atlanta, GA, Sarnat et al. (2008) found that $PM_{2.5}$-Pb mass was explained by a "woodsmoke" factor, which was associated with a 2.4% (95% CI: 1.5, 3.3%) increased risk of cardiovascular hospital

admission (lag 0, per interquartile range increase). Less than 10% of variation in $PM_{2.5}$-Pb mass was explained by "woodsmoke," thus the association may not be attributable specifically to Pb. Andersen et al. (2007) found that in Copenhagen, Denmark, variation in PM_{10}-Pb was explained by a "vehicle" factor that also included copper and iron. A 0.6 μg/m^3 increase in the 3-day lagged sum of "vehicle" factor pollutants was not associated with increased risk of cardiovascular hospital admissions among adults age 65 years and older (RR: 0.999, [95% CI: 0.993, 1.004]).

5.4.5.2. Mortality

Time-series studies of $PM_{2.5}$-Pb have reported positive associations with all-cause mortality. 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-9th percentile) increase in $PM_{2.5}$-Pb. In six California counties, Ostro et al. (2007) found that a 5 ng/m^3 (interquartile range) increase in $PM_{2.5}$-Pb was associated with a 1.74% (95% CI: 0.24, 3.26%) increased risk in all-cause mortality during the cool season. The limitations of air-Pb studies have been described previously (Section 5.5.5.1), and are relevant to the interpretation of these findings for all-cause mortality.

5.4.6. Summary and Causal Determination

The 2006 Pb AQCD concluded that there was a relationship between increased blood Pb and bone Pb and increased adverse cardiovascular outcomes in adults, including increased BP and increased incidence of hypertension (U.S. EPA, 2006). This was substantiated by the coherence of effects observed across epidemiologic and toxicological studies. The large evidence base of epidemiologic studies conducted by many researchers in many locations using different designs found a clear positive association between blood Pb level and BP. Meta-analysis of these studies found that each doubling of 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 (Nawrot et al., 2002). In addition, most of the reviewed studies using cumulative Pb exposure measured by bone Pb levels also showed increased BP. Similarly, toxicological studies provided evidence for exposure to low levels of Pb resulting in increased BP in experimental animals that persists long after the cessation of Pb exposure. Also, animal toxicological studies provided mechanistic evidence to support the biological plausibility of Pb-induced hypertension, including oxidative stress, altered sympathetic activity, and vasomodulator imbalance. Studies in the 2006 Pb AQCD also suggested a connection between Pb exposure and other cardiovascular diseases such as ischemic heart disease, cerebrovascular disease, peripheral vascular disease, and cardiovascular disease related mortality, however this evidence was limited.

Building on the strong body of evidence presented in the 2006 Pb AQCD, recent studies continue to support associations between long-term Pb exposure and cardiovascular effects with recent

epidemiologic studies informing past uncertainties (e.g. confounding, low Pb exposures). A recent study in an ethnically diverse community-based cohort of women and men aged 50-70 years of age suggests that Pb has an acute effect on BP as a function of recent dose measured by blood Pb and a chronic effect on hypertension risk as a function of cumulative exposure measured by tibia Pb (Martin et al., 2006). This study verified other studies by demonstrating that with each increase of 1 µg/dL blood Pb level, systolic BP would increase 1 mmHg and diastolic BP would increase 0.5 mmHg. Additionally, recent epidemiologic studies provided evidence for associations between blood Pb and hypertension at relatively low blood Pb levels; a positive relationship was found in the NHANES data set at a geometric mean blood Pb level of 1.64 µg/dL (Muntner et al., 2005). Animal toxicological studies also provide support for effects of low blood Pb level on increased BP with statistically significant increases shown as low as 2 µg/dL (Tsao et al., 2000). Collectively, all animal toxicological studies providing blood Pb level and BP measurements report positive increases in BP with increasing blood Pb level (Figure 5-34). New studies also demonstrate reversibility of Pb-induced increased BP following Pb exposure cessation or chelation.

Epidemiologic studies continue to investigate the relationship between bone Pb, representing cumulative Pb exposure, and increased BP. Navas-Acien et al. (2008) published a meta-analysis of epidemiological studies examining this association. Studies passing the detailed inclusion criteria all showed positive relationships between bone Pb measures and BP and all but one that characterized hypertension showed positive risk or odds ratios associated with bone Pb. Recent epidemiological studies have also emphasized the interaction between long-term Pb exposure and factors that moderate or modify the Pb effect, like chronic stress and metabolic syndrome, on BP and hypertension. Bone Pb coupled with high stress was associated with a strong and reliable increased risk of developing hypertension in an originally nonhypertensive group (Peters et al., 2007). Also, long duration Pb exposure interacted with components of the metabolic syndrome to drive HRV in directions associated with increased cardiovascular events (Park et al., 2006).

Recent epidemiologic studies investigated the interaction of genotypes with effects of Pb on the cardiovascular system. Significant evidence was presented for modification of the effect of blood Pb level on BP by ALAD genotype (Scinicariello et al., 2010). Additionally, polymorphisms in the hemochromatosis gene modified the pulse pressure response to bone Pb exposure, where pulse pressure represents as a good predictor of cardiovascular morbidity and mortality and an indicator of arterial stiffness (A. Zhang et al., 2010). Park et al. (2009) provided further evidence of gene variants, specifically those related to iron metabolism, impacting the effect of long-term Pb exposure on the cardiovascular system, evaluated by QT interval changes.

Not only has Pb exposure been shown to increase BP and hypertension, but Pb exposure can contribute to the development of other cardiovascular diseases. Recent epidemiologic and toxicological studies provide evidence for increased atherosclerosis, thrombosis, ischemic heart disease, peripheral artery disease, arrhythmia, and cardiac contractility (blood Pb levels >2.5 µg/dL).

Animal toxicological evidence continues to build on the evidence supporting the mechanisms leading to these cardiovascular alterations. Enhanced understanding of Pb-induced oxidative stress including ˙NO inactivation, endothelial dysfunction leading to altered vascular reactivity, activation of the RAAS, and vasomodulator imbalance provides biological plausibility for the consistently positive associations observed between blood and bone Pb and cardiovascular effects.

New evidence extends the potential continuum of Pb-related cardiovascular effects by demonstrating associations between Pb exposure and both cardiovascular and all-cause mortality. All-cause mortality was positively associated with increased blood Pb level. The recent analysis of the nationally representative NHANES III (1988-1994) sample reported positive associations with cardiovascular mortality, with stronger associations with myocardial infarction and stroke mortality (Menke et al., 2006). These findings were supported by a community-based cohort of women age 65-87 years, in which effect estimates were increased for mortality from cardiovascular disease and coronary heart disease (Khalil, Wilson, et al., 2009). Weisskopf et al. (2009) published the first mortality study using bone Pb as an exposure index. This prospective study found that trabecular bone Pb levels were associated with increased mortality from cardiovascular disease and ischemic heart disease with hazard ratios of 5.6 and 8.4, respectively.

In summary, new studies evaluated in the current review support or expand upon the strong body of evidence presented in the 2006 AQCD that Pb exposure is causally associated with cardiovascular health effects. Both epidemiologic and toxicological studies continue to demonstrate a consistently positive relationship between Pb exposure and increased BP or hypertension development in adults and this relationship is observed at adult blood Pb levels (mean 2 μg/dL) lower than that reported in the 2006 AQCD. While some studies evaluate exposure-response relationships, the information is inconclusive. Recent studies investigate cumulative Pb exposure measures and suggest that bone Pb related strongly to hypertension risk. Evidence of Pb increasing the risk of developing other cardiovascular diseases has also been shown. By demonstrating Pb-induced oxidative stress including ˙NO inactivation, endothelial dysfunction leading to altered vascular reactivity, activation of the RAAS, and vasomodulator imbalance, toxicological studies have characterized the mode of action of Pb and provided biological plausibility for the consistently positive associations observed in epidemiologic studies between blood and bone Pb and cardiovascular effects. These observed associations between Pb exposure and cardiovascular morbidity are supported by recent reports of increased cardiovascular mortality. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of cardiovascular health endpoints is sufficient to conclude that there is a **causal relationship between Pb exposures and cardiovascular health effects**.

5.5. Renal Effects

5.5.1. Introduction

This section summarizes key findings with regard to effects of Pb on the kidney in animal toxicology and epidemiologic studies. After chronic Pb exposure, pathological changes in the renal system include proximal tubule (PT) cytomegaly, renal cell apoptosis, mitochondrial dysfunction, aminoaciduria, increased electrolyte excretion, ATPase dysfunction, oxidant redox imbalance, altered glomerular filtration rate (GFR), chronic kidney disease (CKD) development, and altered ˙NO homeostasis with ensuing elevated BP.

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 damages the kidneys and its vasculature and systemic hypertension ensues with effects on the cardiovascular and renal systems (Section 5.4). 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 cause 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 examining 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 interrelated nature of 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 blood Pb and bone Pb (particularly in the tibia) with health outcomes in adults indicate acute effects of recent dose and chronic effects of cumulative dose, respectively. In some physiological circumstances of increased bone remodeling or loss (e.g., osteoporosis and pregnancy), Pb from bone of adults may also contribute substantially to blood Pb concentrations. Blood Pb in children, although highly affected by recent dose, 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 4.3.5.

5.5.1.1. 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 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 epidemiology studies and clinical settings. Creatinine is the most commonly used endogenous compound; blood urea nitrogen (BUN) has also been used. 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 metabolized 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, which 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 & 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., 1999; Levey et al., 2000), which estimates GFR, has become the standard in the kidney epidemiology and clinical communities. With widespread use of the MDRD equation, it became clear that it 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 and the inability to adjust for muscle mass has led to evaluation of alternative serum biomarkers 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 with serum creatinine (Fried, 2009). Despite this, recent research indicates that serum cystatin C varies by age, sex, and race (Kottgen et al., 2008) and a cystatin C-based eGFR equation that includes age, sex, and race was recently developed (Stevens et al., 2008).

Most of the kidney outcome measures discussed above were developed for use in the clinical setting. Unfortunately, they are insensitive for detection of early kidney damage, as 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 & 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 (Devarajan, 2007; M. A. Ferguson et al., 2008).

5.5.2. Nephrotoxicity and Renal Pathology Related to Lead Effects

5.5.2.1. Toxicology

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. After an initial 3 months of Pb exposure (in a longitudinal 12-month exposure study to either 0.01% [low dose] or 0.5% [high dose] Pb acetate in drinking water, male rats), elevated GFR, consistent with hyperfiltration, and renal hypertrophy were observed; high dose animals also had increased NAG and GST (Khalil-Manesh, Gonick, Cohen, Bergamaschi, et al., 1992; Khalil-Manesh, Gonick, & Cohen, 1993; Khalil-Manesh, Gonick, Cohen, Alinovi, et al., 1992). At 6 months of exposure, GFR decreased, albuminuria was present, and pathology ensued with focal tubular

atrophy and interstitial fibrosis formation. This pathology remained consistent out to 12 months, and at 12 months glomeruli developed focal and segmental sclerosis. Similarly, GFR remained decreased after 12 months of exposure. The evidence provided by toxicological studies that showed a difference in GFR with acute Pb exposure (hyperfiltration) versus chronic exposure (decreased GFR) provided biological plausibility to epidemiological studies that observed a similar phenomenon by age in humans with Pb exposure. These dichotomous changes in GFR (acute versus chronic Pb exposure) are consistent between the toxicological and epidemiology literature.

Biomarkers of Pb-induced renal toxicity have been developed including the enzymes lysosomal NAG, GST, brush border antigens (BB50, BBA, HF5), and Tamm-Horsfall protein. GST functions as a biomarker since renal ALAD is protected by the kidney antioxidant GSH. Urinary NAG and GST levels increase after 3 months of high dose Pb exposure (blood Pb level 125 µg/dL) (Dehpour et al., 1999; Khalil-Manesh, Gonick, Cohen, Alinovi, et al., 1992), whereas only urinary NAG was increased following low dose Pb exposure (blood Pb level 29 µg/dL) (Khalil-Manesh, Gonick, & Cohen, 1993). Occupational studies found that urinary NAG correlated best with recent blood Pb changes.

The adverse effects of chronic Pb exposure as detailed above are partially rescued with chelation therapy such as DMSA (Khalil-Manesh, Gonick, Cohen, Bergamaschi, et al., 1992). Improvements include increased GFR, decreased albuminuria, and decreased inclusion body numbers but little change in tubulointerstitial scarring. Administration of an Indian herb to Pb exposed mice, as is discussed in further detail in the antioxidant section (Section 5.5.5), produced similar findings. There was a function rescue however Pb-induced pathology remained (Jayakumar et al., 2009). Thus, administration of various compounds (chelators, antioxidants) to Pb-exposed animals produced hemodynamic rescue.

Recent studies have confirmed the previously observed increase in serum creatinine following chronic Pb exposure in rats. Annabi Berrahal et al. (2011) reported on the effects of age-dependent exposure to Pb on nephrotoxicity in male rats. 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-dependent renal dysfunction are listed in Table 5-17 below. Other investigators have also shown that chronic Pb exposure has adverse effects on renal function. Pb exposed male rats (500 ppm Pb acetate in drinking water for 7 months) had elevated urinary pH, proteinuria, as well as glucose and blood in the urine (Navarro-Moreno et al., 2009).

Qiao et al. (2006) measured the effect of Pb on the expression of the renal fibrosis-related nuclear factor-kappa B (NFκB), transforming growth factor (TGF-β) and fibronectin in Sprague-Dawley rat kidney. Pb was administered at a dose of 0.5% Pb acetate, continuously for either one, two or three months. All growth factors increased by the end of three months of treatment but only NFκB 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.

Roncal et al. (2007) found that Pb accelerated arteriolopathy and tubulointerstitial injury in non-Pb-related CKD. Sprague-Dawley rats were administered Pb acetate at 150 ppm for 4 weeks, followed by remnant kidney surgery (left kidney mass reduced by 2/3 and right kidney removed) and then continuation of Pb exposure for an additional 12 weeks. Pb-treated rats had higher systolic BP, lower creatinine clearance, and higher proteinuria than 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. Low level 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 et al. (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 low blood Pb level may exacerbate pre-existing underlying kidney disease.

Table 5-17. Indicators of renal damage in male rats exposed to 50 ppm Pb for 40 and 65 days, starting at parturition

Biomarker	PND40 Control	PND40 Pb	PND65 Control	PND65 Pb
Blood Pb level (µg/dL)	1.8	12.7	2.1	7.5
Plasma Creatinine (mg/L)	4.5+0.21	5.35+0.25[a]	4.55+0.27	6.04+0.29[a]
Plasma Urea (mg/L)	0.37+0.019	0.47+0.021[a]	0.29+0.009	0.29+0.009
Plasma Uric Acid (mg/L)	7.51+0.44	7.65+0.32	9.39+0.82	5.91+0.53[a]

[a]p <0.001

Source: Used with permission from John Wiley & Sons, Berrahal et al. (2011)

Histological Changes

Historical studies discussed in previous Pb AQCDs have identified Pb-dependent renal damage by the presence of dense intranuclear inclusion bodies, which are capable of sequestering Pb (Goyer, May, et al., 1970). Chelators like $CaNa_2EDTA$ removed these inclusion bodies from affected nuclei (Goyer et al., 1978). Multiple endpoints indicate dysfunction in the PT after Pb exposure. Pb-induced formation of intranuclear inclusion bodies in the PT is protective; Pb is sequestered such that it is not in its bioavailable, free, toxicologically active form. Intranuclear inclusion bodies are seen in the kidney with acute Pb exposures but present to a lesser degree with chronic exposures (See Section 5.2.3 for further discussion). Other PT ultrastructural changes in Pb-induced nephropathy include changes to the PT epithelium, endoplasmic reticulum dilation, nuclear membrane blebbing, and autophagosome enlargement (Fowler et al., 1980; Goyer, Leonard, et al., 1970). Symptoms similar to the PT transport-associated Fanconi syndrome appear with Pb exposure, albeit often at high doses of Pb. These symptoms, which include increased urinary electrolyte excretion (zinc), decreased Na-K-ATPase activity, mitochondrial aberrations, and aminoaciduria, have also been reported in Pb exposed children.

New studies since the 2006 Pb AQCD are consistent with the historical findings and build upon the literature base by including the role of antioxidants in histological preservation. 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. (1992; 1992); in particular, the enlarged glomeruli are consistent with the early hyperfiltration caused by Pb.

A recent study has also reported inclusion body formation in the nuclei, cytoplasm, and mitochondria of PT cells of Pb exposed rats (50 mg Pb/kg bw i.p., every 48 h 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 in the BrdU-positive cells suggesting Pb was able to accelerate reentry into the cell cycle and cause proliferation in the PT. Pb-dependent proliferation has also been reported in the retina with gestational 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 of Pb-mediating nephrotoxic manifestations. 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 histopathological changes to the kidney. Ozsoy et al. (In Press) 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 (0.2% 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-Nekeety et al. (2009) found an extract of the folk medicine plant *Aquilegia vulgaris* to be protective against Pb acetate-induced kidney injury in Sprague-Dawley rats. Rats were treated with Pb (20 ppm; 2 weeks) and extract (administered before, during, or after Pb). Pb treatment resulted in tubular dilatation, vacuolar and cloudy epithelial cell lining, interstitial inflammatory cell infiltration, hemorrhage, cellular debris, and glomerulus hypercellularity. Concomitant exposure of Pb and extract produced histology indiscernible from control. Post treatment with extract partially rescued the Pb induced histopathology. El-Neweshy and El-Sayed studied the influence of vitamin C supplementation (20 mg/kg pretreatment every other day) on Pb-induced histopathological alterations in 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 seen. 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 are presented in more detail in Section 5.5.5 but they consistently show that some antioxidants are capable of preventing or rescuing Pb-dependent histopathological changes.

Alteration of Renal Vasculature and Reactivity

As discussed in Section 5.5.1, changes in renal vasculature function or induction of hypertension can contribute to further renal dysfunction. Pb will increase BP through the promotion of oxidative stress and altered vascular reactivity. 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 affected by Pb exposure. Antioxidants attenuated Pb-dependent oxidative/nitrosative stress in the kidney and abrogated the Pb-induced increased BP (Vaziri, Ding, et al., 1999). Administration of the vasoconstrictor endothelin-1 (ET-1) affected mean arterial pressure (MAP) and decreased GFR (Novak & Banks, 1995). Acute high dose Pb exposure completely blocked the ET-1-dependent 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 historic data detail the interaction of known modulators of vascular tone with Pb.

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 L-NAME in both Pb-treated and control animals. Pb exposure significantly increased BP (134 ± 3 versus 100 ± 6 mm Hg), 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 conclude that Pb exposure increases vascular tone through nitric oxide-dependent and independent mechanisms, increasing renal vascular sensitivity to vasoconstrictors.

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 adverse outcomes. All of these outcomes, in conjunction with Pb-dependent depletion of antioxidants (e.g. GSH) and elevation of lipid peroxidation point to possible susceptibility of the kidney to apoptosis or necrosis. Literature in this area is emerging.

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, NFκB activation and overexpression of tubulointerstitial angiotensin II.

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 microvillae, 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 speculate that Pb may compete with calcium in the establishment and maintenance of intercellular junctions.

Tubular necrosis was also observed in rats exposed to 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 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 conclude 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 & El-Sayed).

Wang et al. (2009) 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, were seen 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 were seen during Pb exposure. Pb-dependent 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.

Table 5-18 presents the acute and chronic renal effects of Pb exposure observed in recent and past animal toxicology studies.

Table 5-18. Acute and chronic effects of Pb on the kidney/renal system – evidence from animal toxicology studies.

Acute	Chronic
Mitochondrial dysfunction Renal cell apoptosis Nuclear Inclusion Body Formation Proximal Tubule Cytomegaly Glomerular Hypertrophy Increased GFR	Mitochondrial dysfunction Renal cell apoptosis Oxidant redox imbalance Altered NO homeostasis ATPase dysfunction Aminoaciduria Increased electrolyte excretion Elevated blood pressure Decreased GFR

5.5.2.2. Epidemiology in Adults

A number of significant advances in research on the impact of Pb on the kidney in the 20 years following the 1986 Pb AQCD (U.S. EPA, 1986) were noted in the 2006 Pb AQCD (U.S. EPA, 2006). These included research in general and CKD patient populations at much lower levels (5-10 µg/dL) of Pb exposure than previously studied. Furthermore, chelation therapy in CKD patients was evaluated, also at levels of exposure not previously thought to be amenable to chelation. Through these lines of research, it became clear that chronic Pb nephropathy, characterized by tubulointerstitial nephritis due to chronic, high-level Pb exposure (Bonucchi et al., 2007), is a small portion of the contribution that Pb makes to

kidney dysfunction overall in the population. Pb, at much lower doses than those causing chronic Pb nephropathy, acts as a cofactor with other more established kidney risks to increase the risk for CKD and disease progression in susceptible patients. The populations at risk for kidney dysfunction (diabetics and hypertensives) are increasing worldwide, particularly in countries where obesity is epidemic. Pb exposure continues to decline in many industrialized countries, although less so among minority populations, which, notably, are also higher risk groups for CKD. Thus, the extent of the public health impact of Pb on the kidney depends on the balance of these two factors.

In the 2006 Pb AQCD (U.S. EPA, 2006), several key issues could not be completely resolved based on the Pb-kidney literature published to date. These included the lowest Pb dose at which adverse kidney effects occur, whether associations at current Pb levels are due to higher past exposures, 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 cadmium. In the intervening five years, relevant data for several of these challenges have been published.

General Population Studies

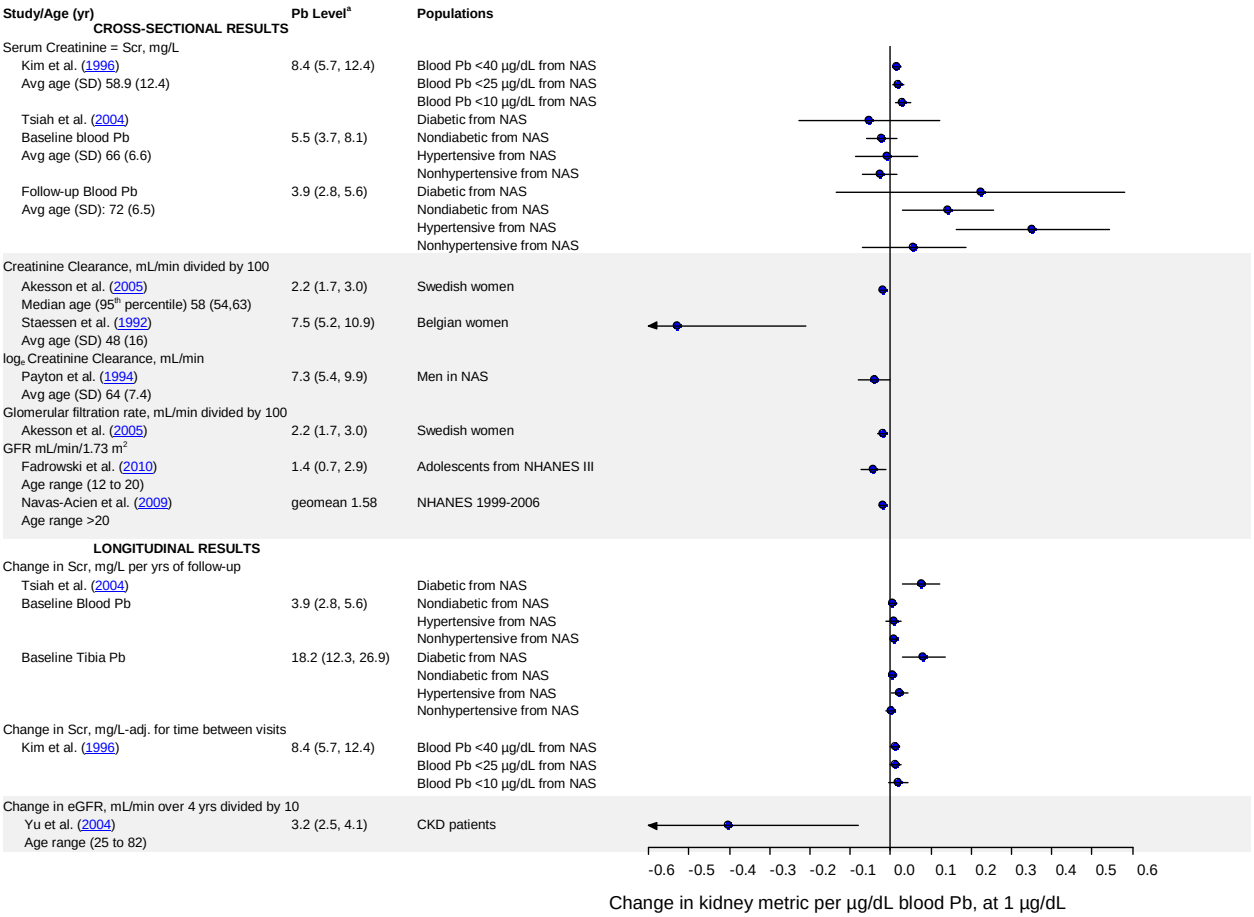
The 2006 Pb AQCD reported studies that examined the effect of Pb exposure on kidney function in general populations. This was a new approach to Pb-kidney research in the two decade time period covered by the 2006 Pb AQCD. The studies in this category provided critical evidence that the effects of Pb on the kidney occur at much lower doses than previously appreciated based on occupational exposure data. The landmark Cadmibel Study was the first large environmental study of this type that adjusted for multiple kidney risk factors (Staessen et al., 1992). It included 965 men and 1,016 women recruited from cadmium exposed and control areas in Belgium. Mean 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, 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.

Four studies assessing the kidney impact of Pb exposure have been published in the Normative Aging Study (NAS) population (R. Kim et al., 1996; Payton et al., 1994; Tsaih et al., 2004; M. T. Wu et al., 2003). Participants in this study were originally recruited in the 1960s in the Greater Boston area. Inclusion criteria included male gender, age 21 to 80 years, and absence of chronic medical conditions. Longitudinal data contained in two NAS publications remain essential, particularly in light of the dearth of prospective data on the kidney effects of Pb. The first of these 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 (R. Kim et al., 1996). Participants were randomly selected to be representative of the entire NAS population in terms of age and

follow-up. Kidney status 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 1.2 (0.2) mg/dL, respectively. In the longitudinal analysis, using random-effects modeling, ln-transformed blood Pb was associated with change in serum creatinine over the subsequent follow-up period in the 428 participants whose highest blood Pb level was ≤ 25 μg/dL (β= 0.027 [95% CI: 0.0, 0.054]); associations in the entire group and subsets with different peak blood Pb levels (≤ 10 or 40 μg/dL) had p-values between 0.07 and 0.13.

This study made two other key contributions. 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 significant positive association between blood Pb and concurrent serum creatinine remained. These authors also addressed 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, including the normal range where reverse causality would not be expected.

Cortical and trabecular bone Pb measurements were obtained in addition to whole blood Pb in evaluations performed in the Normative Aging Study 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 in a subsequent NAS publication (Tsaih et al., 2004). At baseline 6 and 26% of subjects had diabetes and hypertension, respectively. Mean blood Pb levels and serum creatinine decreased significantly over the follow-up period in the group. Pb dose was not associated with change in creatinine in all participants. However, diabetes was observed to be an effect modifier of the relations of blood and tibia Pb with change in serum creatinine. For ln blood Pb, the positive association with serum creatinine was substantially stronger in diabetics (β = 0.076 [95% CI: 0.031, 0.121]) compared to non-diabetics (β = 0.006 [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. Reverse causality was addressed in a sensitivity analysis of participants whose serum creatinine was <1.5 mg/dL; the authors reported that longitudinal associations did not materially change. These studies are depicted in either Figure 5-43 and Table 5-19.



[a]Pb level presented as median blood Pb level and (IQR) in µg/dL or bone Pb level (IQR) in µg/g unless otherwise noted.

Note: The kidney function/blood Pb curves fit by a log-linear model are depicted by their slopes at a blood Pb level of 1 µg/dL. Comparisons of the magnitude of the effect should not be made between effects having different kidney metrics. For uniform presentation, blood Pb level distributional statistics were converted to median and IQR by assuming that blood Pb is normally distributed. The white shaded areas include kidney function tests where an increase is considered impaired function. The gray shaded areas include kidney function tests where a decrease is considered impaired function.

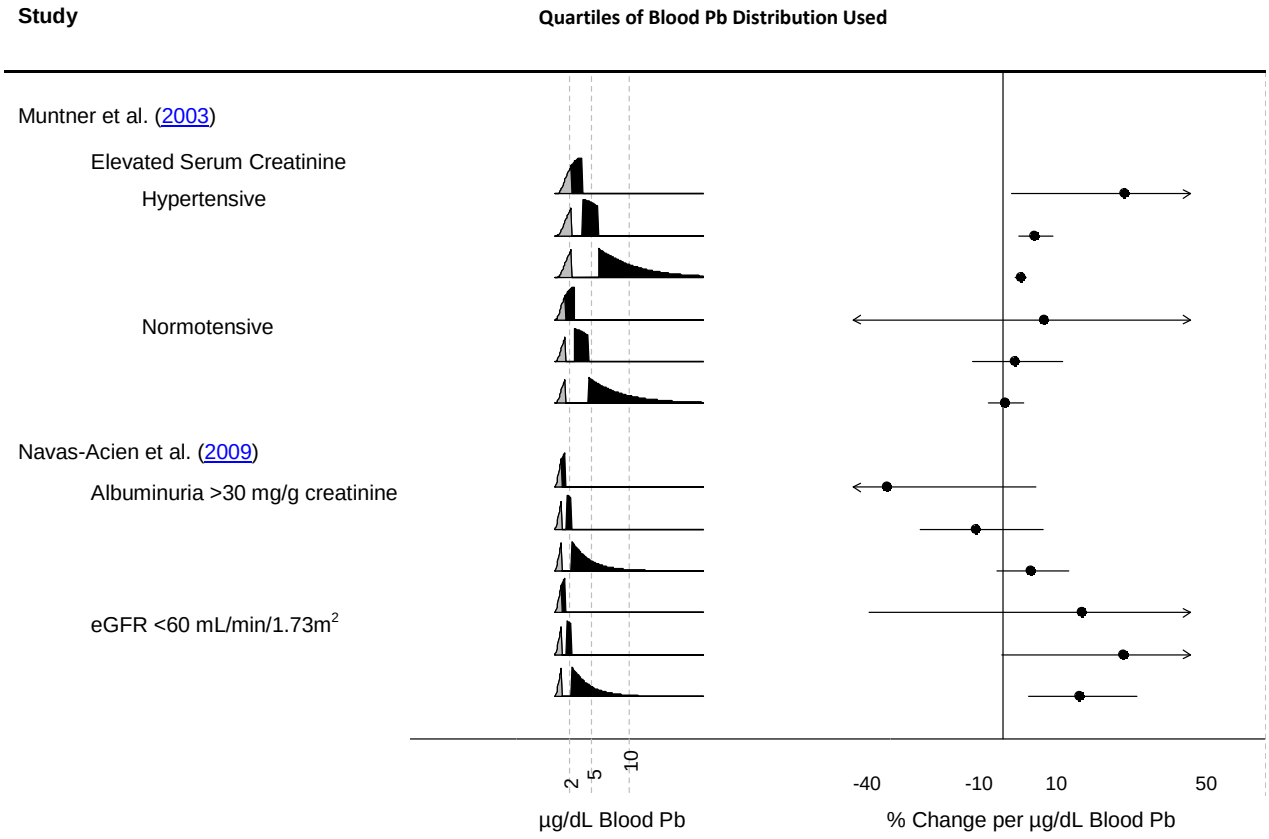
Figure 5-43. Kidney metric slopes on blood Pb or bone Pb.

NHANES data analyses benefit from a number of strengths including large sample size, ability to adjust for numerous Pb risk factors, and the fact that the study population is representative of the U.S. non-institutionalized, civilian population. As a result, the impact of Pb on the kidney has been examined in multiple NHANES datasets obtained over the last few decades (Figure 5-44 and Table 5-19). The

results of these publications, covering different time frames, have been consistent in providing support for Pb as a CKD risk factor, including NHANES III, conducted from 1988-1994, where hypertensives and diabetics were observed to be susceptible populations (Muntner et al., 2003) and NHANES 1999-2002 (Muntner et al., 2005).

A recent publication examined this relationship in NHANES data collected from 1999 through 2006 (Navas-Acien et al., 2009). The geometric mean 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 cadmium, 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 to the lowest blood Pb quartiles. Thus, in the subset of the population with the most severe kidney disease (both reduced eGFR and albuminuria), the risk from Pb was greater. Cadmium was included as a covariate and Pb remained significantly associated. In fact, the most important contribution of this recent NHANES analysis was the evaluation of joint Pb and cadmium exposure (discussed below).

An important contribution of all three 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 Pb levels during the time periods covered. Additional studies in this category have also reported worse kidney function related to Pb dose (Goswami et al., 2005; Hernandez-Serrato et al., 2006; L. H. Lai et al., 2008).



Note: 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). The blood Pb distribution of the reference group is shaded gray and the other group is shaded black. These articles reported ORs of kidney function measures by grouping by quartiles of blood Pb and then comparing each group to the quartile with the lowest blood Pb (reference group).

Figure 5-44. Percent change for kidney outcomes associated with blood Pb.

Table 5-19. Additional characteristics and quantitative data for associations of blood and bone Pb with kidney outcomes for results presented in Figures 5-43 and 5-44

Reference	Population	Study Location; Time Period	n	Pb Level	Outcome	Model	Change or % Change in Kidney Metric (95% CI)
Cross-Sectional							
Muntner et al. (2003)	NHANES III, adults	US; 1988-1994	4813	Mean (SD) blood Pb Hypertensives: 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 Normotensives: 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)	Logistic regression	Hypertensives Q1: Referent Q2: 28% (2, 54) Q3: 8% (4, 12) Q4: 5% (3, 6) Normotensives Q2: 10% (-57, 78) Q3: 3% (-9, 15) Q4: 1% (-4, 5)
Akesson et al. (2005)	WHILA, adult women	Sweden; 6/1999-1/2000	820	Median (5-95%) = 2.2 (1.1-4.6) µg/dL	Cystatin C-based eGFR (Larsson et al. 2004)	Multiple Linear regression	-2.0 (-3.2, -0.9)
					Creatinine clearance	Linear regression	-1.8 (-3.0, 0.7)
Navas-Acien et al. (2009)	NHANES III, adults	US; 1999-2006	14,778	Geometric mean = 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	Logistic regression	Q1: Referent Q2: 19% (-44, 83) Q3: 28% (0.0, 56) Q4: 19% (7, 31)
					Albuminuria and eGFR <60 mL/minute/1.73 m^2	Logistic regression	Q1: Referent Q2: -37% (-83, 8) Q3: -8% (-25, 10) Q4: 7% (-2, 16)
Fadrowski et al. (2010)	NHANES, adolescents	US; 1988-1994	769	Median = 1.5 µg/dL Q1: <1.0 Q2: 1.0 to 1.5 Q3: 1.6 to 2.9 Q4: >2.9	Cystatin C-based eGFR (mL/min/1.73 m^2; calculated using the Filler and Lepage equation)	Linear regression	Q4: -0.42 (-0.73, -0.11)
Kim et al. (1996)	Adult males	Boston, MA; 1979-1994	459	Median = 8.6 µg/dL	Serum creatinine concentrations	Random-effects model	≤ 40 µg/dL blood Pb: 0.016 (0.004, 0.028) ≤ 25 µg/dL blood Pb: 0.019 (0.006, 0.032) ≤ 10 µg/dL blood Pb: 0.030 (0.011, 0.049)
Payton et al. (1994)	Adult males	Boston, MA; 1988-1991	744	Mean (SD) = 8.1 (3.9) µg/dL	Ln creatinine clearance	Multiple linear regression	Ln blood Pb -4.0 (-8.0, -0.1)
Tsaih et al. (2004)	Adult males	Boston, MA; 8/1991-1995 with mean 6 yr follow-up	448	Mean (SD) Blood Pb = 6.5 (4.2) µg/dL Tibia Pb = 21.5 (13.5) µg/g Patella Pb = 32.4 (20.5) µg/g	Serum creatinine	Multiple linear regression	Baseline blood Pb Diabetic: -0.05 (-0.23, 0.12) Nondiabetic: -0.02 (-0.06, 0.02) Hypertensive: -0.01 (-0.09, 0.07) Nonhypertensive: -0.03 (-0.07, 0.01) Follow-up blood Pb Diabetic: 0.22 (-0.14, 0.58) Nondiabetic: 0.14 (0.03, 0.26) Hypertensive: 0.35 (0.16, 0.54) Nonhypertensive: 0.06 (-0.07, 0.19)

Reference	Population	Study Location; Time Period	n	Pb Level	Outcome	Model	Change or % Change in Kidney Metric (95% CI)
Staesson et al. (1992)	Adults	Belgium; 1985-1989	1,981	Blood Pb Mean (SD) Males: 11.4 µg/dL Females: 7.5 µg/dL	Creatinine clearance	Multiple linear regression	-52.9 (-84, 21)
Longitudinal							
Kim et al. (1996)	Adult males	Boston, MA; 1979-1994	459	Median = 8.6 µg/dL	Change in serum creatinine concentrations	Random-effects model	≤ 40 µg/dL blood Pb: 0.01 (-0.0, 0.02) ≤ 25 µg/dL blood Pb: 0.01 (-0.0, 0.03) ≤ 10 µg/dL blood Pb: 0.02 (-0.0, 0.04)
Tsaih et al. (2004)	Adult males	Boston, MA; 8/1991-1995 with mean 6 yr follow-up	448	Mean (SD) Blood Pb = 6.5 (4.2) µg/dL Tibia Pb = 21.5 (13.5) µg/g Patella Pb = 32.4 (20.5) µg/g	Change in serum creatinine	Multiple linear regression	Blood Pb Diabetics (n=26): 0.076 (0.03, 0.12) Nondiabetic (n=422): 0.006 (-0.004, 0.02) Hypertensive (n=115): 0.008 (-0.01, 0.03) Normotensive (n=333): 0.009 (-0.003, 0.021) Tibia Pb Diabetics (n=26): 0.082 (0.03, 0.14) Nondiabetic (n=422): 0.005 (-0.01, 0.02) Hypertensive (n=115): 0.023 (0.003, 0.04) Normotensive (n=333): 0.0004 (-0.01, 0.01)
Yu et al. (2004)	Adult CKD patients	Taipei, Taiwan; 48 month longitudinal study period	121	Mean (SD) = 4.2 (2.2) µg/dL	Change in MDRD eGFR	Generalized estimating equations	-4.01 ml/min/1.73 m² body surface area (p=0.0148) in the GFR over the follow-up period for each 1 µg/dL increment of blood 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 for general population studies. Publications in only three populations have reported adjusted results in occupationally exposed workers in the five years since the 2006 Pb AQCD. In a two year prospective cohort study, 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 (V. M. Weaver et al., 2009). Tibia Pb was evaluated at the beginning of each follow-up period and Pb measures were adjusted for baseline Pb dose and other covariates. In males, serum creatinine decreased 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. 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 men which may have been important factors in the effect modification observed by sex.

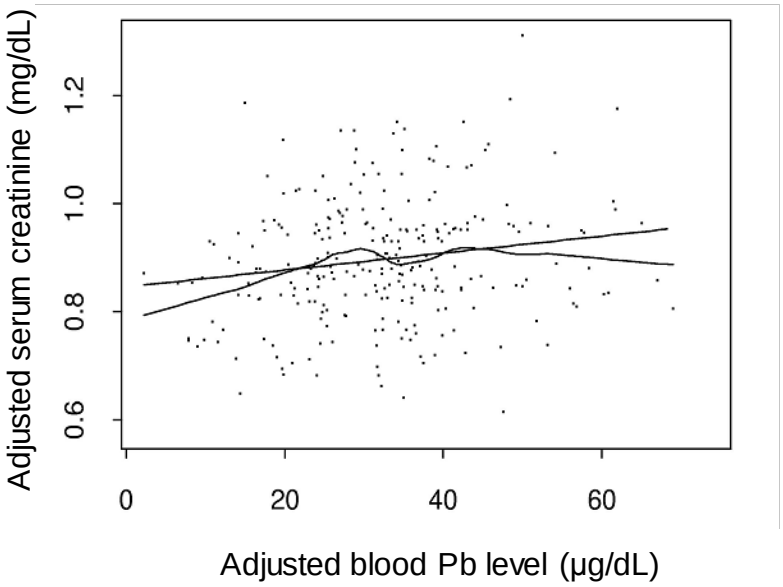
Chia and colleagues observed a significant, positive association between blood Pb and urine NAG in linear regression models after adjustment for age, gender, race, exposure duration, ALAD G177C polymorphism and the interaction between ALAD and blood Pb (Chia et al., 2006). Similar positive associations were observed between blood Pb and a wider range of EBE markers in models that adjusted for age, gender, race, exposure duration, and the HpyCH4 ALAD SNP (discussed below) (Chia 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 (Y. Sun, Sun, Zhou, Zhu, Lei, et al., 2008). An important additional study that analyzed occupational Pb exposure is discussed below under patient population studies (Evans et al., 2010).

Two studies 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 (Y. Sun, Sun, Zhou, Zhu, Lei, et al., 2008), and 25.3 μg/dL (T. A. Lin & 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 workers compared to unexposed controls (Patil et al., 2007) and/or significant correlations between higher Pb dose and worse kidney function (Alinovi et al., 2005; Garcon et al., 2007; D. A. Khan et al., 2008; T. A. Lin & Tai-yi, 2007; Y. Sun, Sun, Zhou, Zhu, Lei, et al., 2008). One small study found no significant differences (Orisakwe et al., 2007).

Overall, the occupational literature published in the last five years on the kidney impact of Pb exposure has been more consistent in reporting significant associations than 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.

Publications that include dose-response information provide evidence of Pb-related nephrotoxicity in the occupational setting across the Pb dose ranges analyzed (Ehrlich et al., 1998; V. M. Weaver, Lee, et al., 2003). Data in 267 Korean Pb workers in the oldest age tertile (mean age = 52 years) reveal no threshold for a Pb effect (beta = 0.0011, p = <0.05; regression and lowess lines shown) (V. M. Weaver, Lee, et al., 2003) (added variable plot shown in Figure 5-45).



Source: Used with permission from the BMJ Publishing Group, Weaver et al. (2003)

Note: Both the adjusted regression line and the line estimated by the smoothing method of the S-PLUS statistical software function lowess 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.

Figure 5-45. 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, increased risk for subsequent CKD occurs. This pattern has been observed in diabetes, hypertension, and obesity (Nenov et al., 2000). As discussed in the 2006 Pb AQCD, findings consistent with hyperfiltration have been observed in three occupational populations (Hsiao et al., 2001;

Roels et al., 1994; V. M. Weaver, Lee, et al., 2003), a study of adults who were Pb poisoned as children (H. 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, Gonick, Cohen, Alinovi, et al., 1992). 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 (Roels et al., 1994; Vaziri, 2008b). Whether hyperfiltration contributes to pathology in humans is unclear; longitudinal studies are needed.

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 (V. M. Weaver, Lee, et al., 2003; V. M. Weaver, Schwartz, et al., 2003), significant associations in opposite directions were observed only when relevant effect modifiers were included in the model. This is a valid concern for risk assessment, since the factors involved in these inverse associations in Pb-exposed populations are not well defined at present.

Patient Population Studies

CKD as defined by the National Kidney Foundation - Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) workgroup (National Kidney Foundation, 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 criteria and markers of kidney damage are not required for the second criteria.

Several key studies in CKD patient populations have been published in the last five years (Table 5-20). One CKD patient study, discussed in the 2006 Pb AQCD, remains the hallmark for prospective evaluation of susceptible patient populations to determine if CKD progression (kidney function decline) is greater in participants with higher baseline Pb dose. Yu et al. (2004) 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 h, 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. In a Cox multivariate regression analysis, chelatable Pb 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 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 eGFR (estimated by the MDRD equation (Levey et al., 1999) were modeled separately using GEE. 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. Two recent studies expanded the CKD patient populations in which this effect was observed to those with diabetic nephropathy (J.-L. Lin, Lin-Tan, Yu, et al., 2006) and with the lowest Pb body burdens studied to date (J.-L. Lin, Lin-Tan, Li, et al., 2006). Results of these observational studies have been summarized (V. Weaver & Jaar, 2010).

Table 5-20. 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. (2006)	87	6.5 (3.4)	108.5 (53.8)	35.1 (9.0)	1	3.87	Type II diabetics with nephropathy
Lin et al. (2006)	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: Used with permission from UpToDate.com, Weaver et al. (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. Eighty-one cases and 95 controls were judged to have had past occupational Pb exposure. Of those, 23 cases and 32 controls were thought to have been exposed to Pb levels ≥ 30 μg/m^3 (the current US OSHA limit is 50 μg/m3). Using multivariable logistic regression modeling, the adjusted OR for CKD was 0.97 (95% CI: 0.68-1.38) in Pb-exposed compared to non-exposed participants. No significant increased odds were observed for low, medium or high exposed groups using either average or cumulative exposure metrics. 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. Using mixed-effects multivariable models, eGFRs declined by 4.27 and 3.39 mL/min/1.73 m^2/y in ever and most Pb-exposed CKD patients, respectively, compared with

4.55 mL/min/1.73 m^2/y in patients without occupational Pb exposure. Thus, in this study, no adverse kidney effect of occupational Pb exposure was evident.

Strengths noted by the authors included virtually complete case ascertainment and minimal loss to follow-up. Exposure assessment was listed 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. Two case-control studies examining occupational risk factors for CKD have been published; one found Pb exposure to be a risk factor (Nuyts et al., 1995), the other did not although moonshine alcohol consumption was a risk presumably due to Pb level (Steenland et al., 1990). The prospective observational aspect of this study is similar in design to the work of Lin and colleagues but differs in several important respects. In this study, only occupational Pb exposure was considered whereas the work in Taiwan excludes occupational exposure and uses Pb dose 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) (V. M. Weaver, Lee, et al., 2003) 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 (Elinder et al., 1986; Strömberg et al., 1995), although blood Pb levels were lower than those noted during the U.S. phase-out. Finally, the severe degree of CKD in this population 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, which is characterized by the use of dialysis to perform the normal functions of the kidney. Incidence and prevalence in the US 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 (ESRD) have also been published in the past five years. One study reported much higher blood Pb levels than had been appreciated by the treating clinicians (Davenport et al., 2009). Of 271 adult patients on regular thrice weekly dialysis, 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's r = 0.38, p-value <0.001); negatively correlated

with urine output (r = -0.44, p-value <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 (B. Chen et al., 2009). 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 Pb dose 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 (B. Chen et al., 2009; 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 pilot study observed significantly 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). This study was unique in that tibia Pb levels were assessed. 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). In order to determine the potential impact of renal osteodystrophy, median blood and tibia Pb levels in the dialysis patients were examined 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 (J.-L. 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 negatively correlated with logarithmic-transformed blood Pb levels after adjustment (J.-L. 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 and highest compared to the lowest tertiles (hazard ratio= 2.1 [95% CI: 2.0-2.2] and 3.3 [95% CI: 1.3-13.5], respectively). Given other recent publications in hemodialysis patients by this group, it would be valuable to examine this risk after adjustment for serum ferritin (Jenq et al., 2009), hemoglobin A1C (Lin-Tan, Lin, Wang, et al., 2007), and blood cadmium (C. W. Hsu et al., 2009).

Clinical Trials in Chronic Kidney Disease Patients

Randomized chelation trials in CKD patients, uncommon in nephrotoxicant research, provide unique information on the kidney impact of Pb. These studies have been performed by Lin and colleagues in Taiwan and involve similar study designs. Initially, patients are observed in order to compare CKD progression prior to chelation. Then, CKD patients whose diagnostic EDTA chelatable Pb levels are within certain ranges (generally 60-600 μg/72 hours and thus below the level commonly considered for chelation) are randomized. The treated group receives weekly chelation with 1 g EDTA intravenously for up to 3 months. The control group receives placebo infusions. In the follow-up period, chelation is repeated for defined indications such as increased serum creatinine or chelatable Pb levels above specified cut-offs. Placebo infusions are repeated in the controls as well. The results of the most recent of these trials are summarized in Table 5-21 below.

Table 5-21. 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	Largest study to date
	Control	32	5.9 (3.0)	144.5 (87.9)	31.5 (9.0)		- 2.7	
Lin et al. (2006)	Chelated	15	7.5 (4.6)	148.0 (88.6)	22.4 (4.4)	15	-3.5	Type II diabetics with nephropathy
	Control	15	5.9 (2.2)	131.4 (77.4)	26.3 (6.2)		-10.6	
Lin et al. (2006)	Chelated	16	2.6 (1.0)[a]	43.1 (13.7)	41.2 (11.2)	27	+3.0	Lowest Pb exposed and treated range BLB ≥ 20- <80 μg)
	Control	16	3.0 (1.1)	47.1 (15.8)	42.6 (9.7)		-2.0	
Lin-Tan et al. (2007)	Chelated	58	5.0 (2.2)	164.1 (111.1)	36.8 (12.7)	51	-0.3	Non-diabetic
	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).

This study design requires replication in larger populations at multiple clinical centers. If confirmed, the effect may be due to removal of Pb. However, chelation may also have a direct beneficial effect on kidney function, regardless of Pb exposure. Antioxidant effects of $CaNa_2EDTA$ which may improve kidney function directly via improved blood flow to the kidneys have been reported (Jacobsen et al., 2001; Saxena & Flora, 2004). EDTA benefits in a Pb rodent model appeared to occur via reduced oxidation (Saxena & Flora, 2004). EDTA administration reduced kidney damage in a rat model of acute renal failure induced by ischemia (Foglieni et al., 2006). Similarly DMSA has been reported to prevent renal damage when co-administered during induction of nephrosclerosis in a non-Pb exposed rat model (Gonick et al., 1996). Benefits from chelation reported in rodent models of Pb-related nephrotoxicity

(Khalil-Manesh, Gonick, Cohen, Bergamaschi, et al., 1992; Sanchez-Fructuoso, Blanco, et al., 2002; Sanchez-Fructuoso, Cano, et al., 2002) did not appear to occur via reversal of structural damage (Khalil-Manesh, Gonick, Cohen, Bergamaschi, et al., 1992); again suggesting that improved hemodynamics from reduction of reactive oxidant species, which could be due to reduced Pb and/or directly from the chelating agent, may be a mechanism (Gonick et al., 1996). However, the most parsimonious explanation for the combination of Lin's observational and experimental chelation work is that Pb is the underlying reason. Moreover, if the benefit can be replicated, this could be a valid treatment regardless of the mechanism or whether Pb is involved.

The unique body of work in patient populations by Lin and co-workers, both observational and experimental, has numerous strengths including prospective study design, randomization, Pb dose assessment that includes bioavailable body burden, longitudinal statistical analysis, and control for multiple kidney risk factors. However, the generalizability of the results to broader populations is unknown. In addition, the observed effect of Pb on decline in GFR has been variable; the annual decline in eGFR per standard deviation (SD) higher Pb dose at baseline was much lower in the 2003 study than in subsequent publications (see Table 5-21 above). Small sample sizes and differences in renal diagnoses between groups may be factors in this variability. However, if confirmed in large populations at multiple centers and shown not to worsen cognition or other effects through Pb mobilization, chelation could yield important public health benefits.

5.5.2.3. Epidemiology in Children

Lead 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 (C. 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 at much lower environmental exposure levels. However, insensitivity of the clinical kidney outcome (i.e., GFR) measures for early kidney damage is a particular problem 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).

Three studies that included analysis of clinical kidney outcomes were discussed in the 2006 Pb AQCD. One found no difference in mean serum creatinine between 62 exposed and 50 control children (Fels et al., 1998). Two larger studies observed significant associations that were in opposite directions; blood Pb was positively associated with serum cystatin-C in 200 17-year-old Belgian adolescents but negatively associated with serum creatinine and cystatin C in 300-600 European children (n varied by outcome) (De Burbure et al., 2006; Staessen et al., 2001).

Therefore, one of the key gaps identified in the 2006 Pb AQCD was limited data in children and adolescents particularly with respect to GFR measures. A recently published NHANES analysis in adolescents begins to fill this gap (Fadrowski et al., 2010). Associations between blood Pb and kidney function were investigated in 769 adolescents aged 12-20 years in the US NHANES III, conducted from 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 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 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 to -5.0) lower eGFR. In contrast, the association between blood Pb and creatinine-based eGFR, although in the same direction, was not statistically significant. Additional research in children, including with longitudinal follow-up, a range of outcome assessment methods, and with exposure only after Pb was banned from gasoline, is warranted.

5.5.2.4. Associations between Lead 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 cadmium 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 blood Pb (median = 2.2 μg/dL) and cadmium (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 between cadmium dose measures were stronger for the cystatin C based outcome. Staessen et al. (2001) found a significant association between 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 European children, higher 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

very 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 3941 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.

5.5.3. Mechanisms of Lead Nephrotoxicity

5.5.3.1. Altered Uric Acid

Individuals who have been heavily exposed to Pb are at increased risk for both gout and kidney disease (Batuman, 1993; Shadick et al., 2000). Pb is thought to increase serum uric acid by decreasing its kidney excretion (Ball & Sorensen, 1969; Emmerson, 1965; Emmerson & Ravenscroft, 1975). Research during the past decade indicates that uric acid is nephrotoxic at lower levels than previously recognized (R. J. Johnson et al., 2003). Therefore, the 2006 Pb AQCD reviewed literature implicating increased uric acid as one mechanism for Pb-related nephrotoxicity (Shadick et al., 2000; V. M. Weaver et al., 2005). However, this is not the only mechanism, since associations between blood Pb and serum creatinine remained significant even after adjustment for uric acid (V. M. Weaver et al., 2005). These mechanistic relations have more than just theoretical importance. Clinically relevant therapies may be possible since EDTA chelation has been reported to improve both kidney function and urate clearance in patients with kidney insufficiency and gout, even when EDTA-chelatable Pb body burdens were low (J.-L. Lin et al., 2001).

Conterato et al. (2007) followed various parameters of kidney function after acute or chronic Pb exposure in rats. Acute exposure to Pb acetate consisted of a single i.p. injection of 25 or 50 mg/kg Pb acetate, while chronic exposure was one daily i.p. injection of either vehicle or Pb acetate (5 or 25 mg/kg) for 30 days. Acute and chronic exposure at both dose levels increased plasma uric acid levels. Conversely, Annabi Berrahal et al. (2011) found that plasma uric acid levels decreased after 65 days of Pb exposure (post-puberty; blood Pb 7.5 µg/dL) (Table 5-17). Plasma urea levels increased after 40 days of exposure (puberty; blood Pb 12.7 µg/dL). Changes in plasma urea are used as an acute renal marker of injury.

5.5.3.2. Oxidative Damage

A role for ROS in the pathogenesis of experimental Pb-induced hypertension and renal disease has been well established (Vaziri, 2008a, 2008b; Vaziri & Khan, 2007). The production of oxidative stress following Pb exposure is detailed in respect to modes of action of Pb (Section 5.2.4). Past studies have shown that acute Pb exposure can elevate kidney GST levels, affecting glutathione metabolism (Daggett et al., 1998; Moser et al., 1995; Oberley et al., 1995).

Annabi Berrahal et al. (2011) reported on the effects of age-dependent exposure to Pb on nephrotoxicity in male rats (Table 5-17). Pups were exposed to Pb lactationally (as a result of dams consuming water containing 50 ppm Pb acetate) until weaning. Thereafter the male pups were exposed to the same solution from weaning (day 21) until sacrificed at age 40 days (puberty; blood Pb 12.7 μg/dL) and at age 65 days (post-puberty; blood Pb 7.5 μg/dL). MDA concentration in kidney was significantly increased relative to controls to the same degree at both 40 and 65 days, while total sulfhydryl groups were significantly decreased only at 65 days. These changes reflect an increase in oxidative stress after exposure to Pb.

Conterato et al. (2007) examined the effect of Pb acetate on the cytosolic thioredoxin reductase activity and oxidative stress parameters in rat kidneys. Acute exposure to Pb acetate consisted of a single i.p. injection of 25 or 50 mg/kg Pb acetate, while chronic exposure 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. Acute exposure at the 25 mg Pb dose level resulted in increased SOD and thioredoxin reductase-1 activity, while exposure to the 50 mg dose level increased CAT activity and inhibited δ-ALAD activity in the kidney. Chronic exposure at the 5 mg dose level of Pb inhibited δ-ALAD and increased GST, NPSH, CAT, and thioredoxin reductase-1. Chronic exposure to 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 acute or chronic exposure. As both acute and chronic exposure to Pb increased thioredoxin reductase-1 activity, the authors suggest that this enzyme may be a sensitive indicator to exposure at low Pb dosage.

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 well as 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

El-Neweshy and El-Sayed 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. Rats treated with Pb acetate plus vitamin C exhibited relatively mild karyomegaly and eosinophilic intranuclear inclusion bodies of proximal tubules in 5 rats, while an additional 5 rats were normal. Normal glomeruli were noted in all. Thus vitamin C could be shown to ameliorate the renal histopathological effects of Pb intoxication.

Masso-Gonzalez and Antonio-Garcia (2009) studied the protective effect of natural antioxidants (zinc, 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 analyzed. Pb-exposed pups had decreased body weights. Blood Pb level in the control group was 1.43 μg/dL, in the Pb group it was 22.8 μg/dL, in the Pb plus zinc plus vitamins it was 21.2 μg/dL, and in the zinc plus vitamin group blood Pb was 0.98 μg/dL. The kidney TBARS were significantly elevated in Pb exposed pups, while treatment with vitamins and zinc returned TBARS to control levels. Kidney catalase activity was significantly increased above control with Pb treatment; however supplement with zinc and vitamins reduced catalase activity towards normal. Pb exposure inhibited kidney Mn-dependent SOD but not Cu-Zn-dependent SOD activity. Thus, supplementation with zinc and vitamins during gestation and lactation is 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 reduces 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 on male rats, two studies have appeared which compare the response of male rats with female rats (Alghazal, Lenártová, et al., 2008; Sobekova et al., 2009). 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 1000 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 1000 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. (2008) 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 response of male and female rats to Pb exposure. Differences could 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 females, so that the biological half-life of xenobiotics in the females is longer.

5.5.3.3. Lead Effect on 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, recognition and signal transduction. Perez Aguilar et al. (2008) studied changes in renal gangliosides following Pb exposure (600 ppm Pb acetate in their 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.

5.5.3.4. Role of Metallothionein

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 exerts a dual effect on MT expression in the kidney: enhancement of MT gene transcription but suppression of MT mRNA translation.

Zuo et al. (2009) explored the potential role of α-Synuclein (Scna) and MT in Pb induced inclusion body formation. They used MT-I/II double knockout (MT-null) and parental wild type (WT) cell lines to explore the formation process of Pb-induced inclusion bodies. Unlike WT cells, MT-null cells did not form inclusion bodies after Pb exposure. Western blot of the cytosol showed that soluble MT protein in WT cells was lost during Pb exposure as inclusion bodies formed. However, transfection of MT-1 into MT-null cells allowed inclusion body formation after Pb exposure. 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 exposure. Scna protein showed poor basal expression in MT-null cells, and Pb exposure increased Scna expression only in WT cells. MT transfection increased Scna transcript to WT levels. In both of these cell lines Pb-induced Scna expression rapidly increased and then decreased over 48 hours as Pb-induced inclusion bodies were formed. A direct interaction between Scna and MT was confirmed ex vivo by an antibody pull down assay, where the proteins co-precipitated with an antibody to MT. Pb exposure caused increased colocalization of MT and Scna proteins. In archival kidney samples of renal cortex from WT mice chronically treated with Pb, MT was localized to the surface of inclusion bodies. Thus, Scna may be a component of Pb-induced inclusion bodies and, with MT, may play a role in inclusion body formation.

Figure 5-46 (and Table 5-22) presents the recent animal toxicological data for studies investigating the effects of Pb (as blood Pb level) on various measures of kidney health and function. Dysfunction in kidney function measures, including urinary flow, ALP, microalbumin, and NAG, was observed at blood Pb concentrations above 19.7 µg/dL (L. Wang et al., 2010).

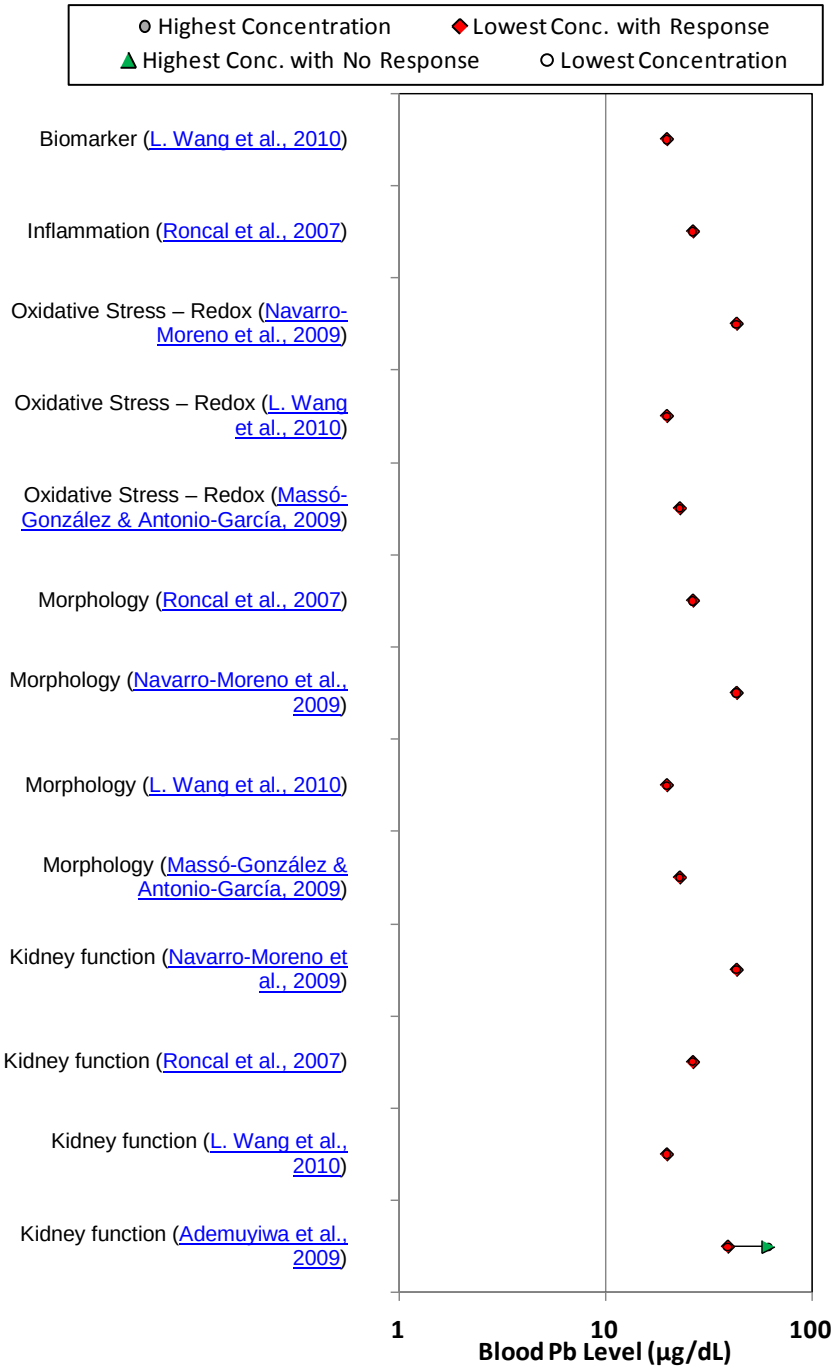


Figure 5-46. Dose-responsive representation of the effect of Pb on renal outcomes in animal toxicology studies.

Table 5-22. Additional characteristics for results of toxicological studies presented in Figure 5-46

Reference	Blood Pb Level with Response (µg/dL)	Outcome
Wang et al. (2010)	20	Biomarker - Aberrant NAG, GGT, β2-microgobulin expression in adult female mice with chronic Pb exposure.
Roncal et al. (2007)	26	Inflammation - Elevation in number of macrophages & marker MCP-1 in Pb exposed kidneys with remnant kidney surgery.
Navarro-Moreno et al. (2009)	43	Oxidative stress - Chronic Pb exposure in males increased kidney lipid peroxidation (i.e. TBARS)
Wang et al. (2010)	20	Oxidative Stress - Pb caused increased lipid peroxidation (i.e. MDA production), elevated kidney antioxidant enzymes (SOD, GPx, CAT), and depleted GSH in immature female rats.
Massó-González et al. (2009)	23	Oxidative stress - Elevated TBARS and catalase activity in weanling pups exposed to Pb during gestation and lactation
Roncal et al. (2007)	26	Morphology - Pb induced pre-glomerular vascular disease of kidney (i.e. sclerosis, fibrosis, peritubular capillary loss)
Navarro-Moreno et al. (2009)	43	Morphology - Electron micrography of chronic Pb exposure in male rats showed lumen reduction, microvilli loss, brush border loss, and mitochondrial damage
Wang et al. (2010)	20	Morphology - Electron micrography showed Pb damages mitochondria, basement membrane, and brush border in kidney tissue. Some focal tubal necrosis observed.
Massó-González et al. (2009)	23	Morphology - Pb elevated relative kidney weight at PND21 in animals with neonatal Pb exposure.
Navarro-Moreno et al. (2009)	43	Kidney function - Pb exposed males had elevated urinary pH and protein, and glucose and blood in the urine.
Roncal et al. (2007)	26	Kidney function - Remnant kidney surgery and Pb exposure induced decreased creatinine clearance and proteinuria.
Wang et al. (2010)	20	Kidney function - Elevated urinary total protein, urinary albumin, and serum urea nitrogen in immature female rats exposed to Pb.
Ademuyiwa et al. (2009)	39 and 61	Kidney Function - Renal phospholipidosis and depletion of renal cholesterol in male pups after gestational Pb exposure.

5.5.4. Effects of Exposure to Lead 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 are 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 iron deficient diet significantly increased Pb deposition in adult animals (Hashmi et al., 1989), pregnant dams, and maternally exposed fetuses (U. S. Singh et al., 1991). Dietary thiamine plus zinc slightly reduced blood and kidney Pb in exposed animals (Flora et al., 1989). Selenium, a cofactor for GPx, attenuated Pb-dependent lipid peroxidation and abrogates the Pb-dependent attenuation of GR and SOD. Concomitant exposure to the cations aluminum and Pb protects Pb-exposed animals from ensuing nephropathy (Shakoor et al., 2000). In summary, Pb is known 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.

5.5.4.1. Lead and Cadmium

Cd shares many similarities with Pb; it is a ubiquitous PT nephrotoxicant at high exposure levels and accumulates in the body. Despite this, few studies have evaluated associations between low-level Cd exposure and CKD or the impact of joint exposure of these or other metals on CKD. As discussed in the 2006 Pb AQCD, Cd, at the lower exposure levels common in the U.S. and other developed countries, has a substantial impact on associations between Pb exposure and the kidney EBE marker, NAG, even in the presence of occupational level Pb exposure. In one report, mean NAG, although higher in the Pb-exposed group compared to controls, was correlated with urine Cd and 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 a 66.9 μg/g bone mineral increase in tibia Pb (V. M. Weaver, Lee, et al., 2003).

The 2006 Pb AQCD noted that data examining the dose-response relation between environmental Cd and the kidney were too scarce to determine the impact of Cd exposure on relations between Pb exposure and other kidney outcomes. A recent publication in NHANES data collected from 1999 through 2006 addresses this need; (results pertaining solely to Pb were discussed in Section 5.5.2.2) (Navas-Acien et al., 2009). Geometric mean 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 odds ratios for albuminuria (≥ 30 mg/g creatinine), reduced eGFR (<60 mL/min/1.73 m^2), and both albuminuria and reduced eGFR were 1.92 (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 although effect modification was not observed. However, the odds ratios comparing participants in the highest with the lowest quartiles of both metals were 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 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 very recent study suggests that interpretation of low-level 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 low-level Cd exposure in workers who are occupationally exposed to other nephrotoxicants such as Pb, Cd dose was assessed 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 (V. M. Weaver et al.). After adjustment for age, sex, BMI, urine creatinine, smoking, alcohol, education, annual income, diastolic BP, current or former Pb worker

job status, new or returning study participant, and blood and tibia Pb, higher ln-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]) and ln-NAG, but lower serum creatinine. These unexpected paradoxical associations have been reported in two other publications (De Burbure et al., 2006; Hotz et al., 1999) and have been observed in two 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.

Wang et al. (2009) 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, consisting of 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 our knowledge of the synergistic effects of Pb and Cd on kidney mitochondria.

5.5.4.2. Lead, Cadmium, and Arsenic

Wang and Fowler (2008) present a general review of the roles of biomarkers in evaluating interactions among mixtures of Pb, Cd, and arsenic. Past studies have found that 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; on the other hand, animals exposed to Cd in addtion 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 & 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 either Pb or As exposure alone in kidney tubule cells. Heat shock proteins (Hsps) are commonly altered under the situation of exposure to metal mixtures. Both in vitro low dose studies and in vivo studies showed that Hsps were induced in a metal/metalloid, dose and time-specific manner (G.

Wang et al., 2005). Additive or more than additive interactions occur among Pb, Cd and As under combined exposure conditions.

5.5.4.3. Lead and Zinc

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. Zinc (10 mg/kg i.p.) and ascorbic acid (10, 20 and 30 mg/kg i.p.) treatment 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 zinc 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 (DR, 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 marginal zinc rats had higher renal Pb (35%) and lower renal zinc (16%) concentrations than control rats. On the other hand, supplemental zinc was more protective than the control diet 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 zinc but not to Pb exposure.

5.5.4.4. Lead and Mercury

Stacchiotti et al. (2009) studied stress proteins and oxidative damage in a renal derived cell line exposed to inorganic mercury and Pb. The time course of the expression of several heat shock proteins, glucose-regulating proteins and metallothioneins 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. Reactive oxygen and nitrogen species 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 heat shock proteins and metallothionein. Reverse transcription PCR was used for quantification of metallothionein. The higher doses of mercury (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 at 20 μM mercury, 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 induced a significant increase in both and reactive nitrogen species, the reactive nitrogen species maximal at 24 hours, and the ROS at 48 hours. Pb (60 or 300 µM) did not cause an increase in reactive oxygen or reactive nitrogen species beyond the levels seen in control cells. Total GSH significantly increased in cells grown in the presence of Pb; the effect was dose-dependent and GSH reached its maximal value at a dose of 300 µM Pb. The effect of mercury was biphasic, 10 µM significantly enhancing GSH by 600%, while the amount of GSH detected after 20 µM mercury only increased by 50% compared to control. GST activity was enhanced by both Pb and mercury. Heat shock proteins Hsp25 and Hsp72 were up-regulated by mercury but there was no effect on Grp78 as compared to control. On the contrary, Pb treatment only upregulated Grp78. Mercury induced a time-dependent effect on metallothionein 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 dose- 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 metallothionein synthesis clearly differ from the study of Jamieson et al. (2008) that found no increase in metallothionein following Pb exposure. This discrepancy remains to be clarified.

5.5.5. Impact of Treatment with Antioxidants on Renal Lead Accumulation and Pathology

5.5.5.1. Treatment with Antioxidants

Wang et al. (2010) assessed the protective effect of N-acetylcysteine (NAC) on experimental chronic Pb nephrotoxicity in immature female rats. NAC is a potent oxygen free radical scavenger, a metal chelator, and the precursor to the antioxidant glutathione. Sprague-Dawley rats received Pb acetate (300 ppm in drinking water) and/or NAC (100 mg/kg/day, by i.p. injection) for 8 weeks to investigate the protective effect of NAC on Pb-induced renal damage and oxidative stress. Serum and renal cortical Pb levels were markedly increased in the Pb treated animals, but reduced in the Pb plus NAC treated animals. There were time-related increases in urinary alkaline phosphatase, urinary GGT, urinary NAG, urinary total protein, urinary β-2 microglobulin, and urinary microalbumin, which were all decreased by NAC. Serum urea nitrogen was significantly increased by Pb administration and reduced towards normal by Pb plus NAC. Alterations in proximal tubular structures were observed in most kidney samples from Pb-treated rats, but animals treated with combination Pb plus NAC showed well-preserved cell structures and organelles. Indices of oxidative stress (MDA, SOD, GSH, GPx, and CAT) were altered by Pb treatment and restored to or towards normal by Pb plus NAC treatment (MDA increased and the remainder decreased). Thus NAC can be shown to have both an anti-oxidative and a chelator effect on Pb intoxication.

Saxena et al. (2005) investigated the beneficial role of monoesters of meso-2, 3-dimercaptosuccinic acid in the mobilization of Pb and recovery of tissue oxidative injury in rats. Dimercaptosuccinic acid (DMSA) is known as a Pb chelator and as an antioxidant by virtue of its possession of thiol groups. In this study, DMSA, and two of its analogues, monomethyl dimercaptosuccinic acid (MmDMSA) and mono-cyclohexyl dimercaptosuccinic acid (MchDMSA) were assessed as to their capacity to reduce Pb concentration in blood and soft tissues and to recover Pb-induced oxidative stress in male Wistar rats who were exposed to Pb acetate (0.1% in drinking water) for 20 weeks. Rats were then treated orally with five days of DMSA or its two analogues at a dose of up to 100 mg/kg once daily. Exposure to Pb caused a rise in blood Pb levels to approximately 25 μg/dL. Exposure to Pb also caused a significant decrease in blood ALAD activity and GSH levels, accompanied by inhibition of kidney ALAD and an increase in δ-aminolevulinic acid synthatase (ALAS) activity in liver and kidneys. Pb exposure also resulted in increased blood and soft tissue (brain, liver, and kidney) Pb and TBARS levels and decreased GSH levels. These were restored by treatment with DMSA and its analogues, particularly MchDMSA.

Abdallah et al. (2010) explored the effect of Pb toxicity on coenzyme Q levels in rat tissues. Coenzyme Q acts as an electron and proton carrier in mitochondria and functions as an antioxidant in its reduced form (ubiquinol). Both coenzyme Q9 and coenzyme Q10 were measured in rat tissues as coenzyme Q9 is the predominant form found in the rat. Male albino rats were injected i.p. with Pb acetate in a dose of 5 mg/kg daily for 6 weeks. No blood Pb levels were reported. TBARS were elevated above controls in serum, liver, kidney and brain while non-protein sulfhydryl groups (indicative of GSH) were decreased in serum and kidney. Both oxidized and reduced coenzyme Q9 levels were significantly reduced in kidneys from Pb-treated rats as contrasted to controls (48.6 ± 5.6 versus 95.5 ± 10.1 nmol/g tissue, oxidized, and 35.4 ± 3.0 versus 61.4 ± 5.1 nmol/g tissue, reduced). On the other hand, levels of oxidized and reduced coenzyme Q10 were unchanged. Thus the reduced levels of coenzyme Q attributable to Pb intoxication may participate in the diminished antioxidant defense mechanism.

El-Sokkary et al. (2005) evaluated the effect of melatonin against Pb-induced hepatic and renal toxicity in male rats. Melatonin is known to be efficacious as a free radical scavenger and indirect antioxidant. Three groups of animals were used: control, Pb acetate-treated (100 ppm) and Pb acetate and melatonin (10 mg/kg) given subcutaneously for 30 days. Lipid peroxidation was measured as the sum of MDA plus 4-hydroxyalkenals (4-HAD). Pb increased kidney lipid peroxidation products, but these were reduced towards normal by melatonin. Both SOD and GSH levels were reduced by Pb, and were increased by melatonin. Histological section of kidneys of Pb treated rats showed tubular degeneration with some apparently necrotic cells, while melatonin treated rats demonstrated a near normal structure. The authors conclude 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.

Ozsoy et al. studied the protective effects of L-carnitine on experimental Pb toxicity in rats. Female two-month old rats were fed 0.5 mg/kg Pb acetate alone or with daily injections of 0.5 mg/kg L-carnitine for 60 days. Control animals were injected with physiological saline. Pb caused an increase in serum creatinine and histopathological changes in the kidney, consisting of tubule dilatation, degeneration and necrosis and interstitial inflammation. In the Pb and L-carnitine group serum creatinine was reduced to control values and the histopathological changes were reversed. Immunological staining for Cu/Zn-SOD was elicited by Pb feeding and reduced by L-carnitine. The authors attribute the beneficial effects of L-carnitine to its antioxidant effect.

Reddy et al. (2010) used Sprague-Dawley rats that were treated with 10 mg/kg/day of Pb acetate and/or thiamine (25 mg/kg/day) for 7 weeks. Thiamine treatment normalized the Pb-induced alterations in blood ALAD activity and urinary NAG activity.

Kharoubi et al. (2008) described the prophylactic effects of Wormwood (*Artemisia absinthium L.*) plant extracts on kidney function on Pb-exposed animals. Male Wistar rats were exposed to Pb acetate (750 ppm in drinking water) for 11 weeks, and then received Wormwood extract (200 mg/kg) for 4 weeks. Significant differences in blood and urinary Pb concentration were observed between the Pb group and the Wormwood group (blood Pb 55.6 μg/dL compared to 22.3 μg/dL). Pb induced lipid peroxidation (TBARS and protein carbonyls in the kidney), but these levels were reduced by Wormwood extract. Wormwood extract also attenuated the effects of Pb on renal function. These results indicated that Wormwood extract had significant antioxidant activity and protected the kidney from Pb-induced toxicity.

Jayakumar et al. (2009) evaluated the effect of a methanolic extract of the Indian herb, *Achyranthes aspera*, in preventing Pb-induced nephrotoxicity in rats. Male albino Wistar rats, received Pb acetate (0.2% for 6 weeks) or Pb acetate plus *A. aspera* (200 mg/kg for 6 weeks) simultaneously. *A. aspera* partially prevented the increases in kidney weight, BUN, serum uric acid, and serum creatinine caused by Pb administration. The levels of urinary marker enzymes, GGT, β-glucuronidase, NAG, Cathepsin D, and LDH, which were reduced by Pb administration, were increased to or towards normal by *A. aspera*. Kidney histology revealed that Pb-treated animals showed tubular damage, whereas the Pb plus *A. aspera* -treated animals showed a reduction in tubular damage.

The effectiveness of various plant or bacterial extracts as antioxidants in the kidney was explored in two separate publications. El-Nekeety et al. (2009) evaluated the protective effect of an extract of the folk medicine plant *Aquilegia vulgaris* against Pb acetate-induced oxidative stress in Sprague-Dawley rats. The experimental group was treated with Pb acetate, 20 ppm, and/or an extract of *A. vulgaris*, 100 ppm, for 2 weeks prior to Pb acetate. Pb acetate increased serum urea, and decreased serum total protein and albumin. These changes were reversed by treatment with the extract. Histological examination of kidneys of rats treated with Pb showed tubular dilatation, interstitial inflammatory cells, hemorrhage, cellular debris, and hypercellularity in the glomerulus, with apoptotic nuclei in renal tubular epithelial cells. The rats treated simultaneously with Pb and the extract showed essentially normal renal tubules and

glomeruli while rats treated with Pb and then the extract showed improvement in tubular structure, but interstitial fibrosis was still present. This experiment confirmed that exposure to Pb generates free radicals, and that an extract of *A. vulgaris* resulted in restoration of the different parameters tested. The second experiment in this group was by Ponce-Canchihuaman et al. (2010) who evaluated the antioxidant activity of the cyanobacterium *Spirulina maxima* against Pb acetate-induced hyperlipidemia and oxidative damage in the liver and kidney of male rats. Male Wistar rats were exposed to Pb acetate by i.p. injection (25 mg/rat on a weekly basis for 3 weeks and a 5% supplement of Spirulina was given in food). The findings in the kidney were similar to those in the liver (see Section 5.9.1). Thus Pb-induced oxidative stress and renal damage can be attenuated by treatment with Spirulina extract.

Finally, there is a need to examine whether the chelator, $CaNa_2EDTA$, acts also as an antioxidant and promotes increased vasodilatation and thus increased renal blood flow by enhancing the delivery of NO. This question arises because of the observations of Lin et al. (2006) that repeated injections of $CaNa_2EDTA$ leads to improvement in kidney function in patients with chronic renal failure, even in individuals with very low body Pb stores. Jacobsen et al. (2001),examined the anti-oxidative effects of Gallic acid, EDTA, and an emulsifier in mayonnaise enriched with 16% fish oil. EDTA was shown to be an efficient antioxidant in the fish oil enriched mayonnaise as it strongly inhibited the formation of free radicals and volatile oxidation compounds. The authors suggest that the antioxidative effect appears to be due to its ability to chelate free iron in egg yolk at the oil-water interface.

5.5.5.2. Treatment with Antioxidants plus Chelators

Santos et al. (2006) assessed the potentiating effects of chelators (2,3-dimercaptopropanol [BAL], 2,3-dimercaptopropane-l-sulfonic acid [DMPS], and meso-2,3-dimercaptosuccinic acid [DMSA]) given simultaneously with Pb acetate on δ-ALAD activity, both in vivo and ex vivo. Ex vivo, human blood was pre-incubated with BAL or DMSA (10 μM) or DMPS (1 μM) then Pb acetate added to the reaction mixture. In vivo, mice were given daily injections of 50 mg/kg Pb acetate for 15 days and then injected with 1/3 of LD50 of the chelating agents. In human blood the inhibitory effect of Pb acetate (1 and 100 μM) was markedly increased in the presence of BAL and DMPS, whereas DMSA ameliorated the enzyme inhibition caused by 1 μM Pb acetate. In vivo Pb acetate inhibited δ-ALAD activity by 42%. Parallel to the ex vivo results, BAL and DMPS, but not DMSA, increased the inhibitory potency of Pb in blood. In the kidney, BAL and DMSA but not DMPS increased inhibitory activity. The authors conjecture that the chelators may deplete the cells of zinc, an essential element for δ-ALAD activity. Supporting the chelation effect seen is the Santos study is work by Bradberry and Vale (2009), Hamidinia et al. (2006), and Aslani et al. (Aslani et al., 2010) who found decreased kidney Pb content post-chelation.

5.5.6. Summary and Causal Determination

The 2006 Pb AQCD 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 associated with blood and bone Pb levels (U.S. EPA, 2006). Data in general and patient populations provided consistent evidence of low-level Pb nephrotoxicity (Akesson et al., 2005; R. Kim et al., 1996; Tsaih et al., 2004; C.-C. Yu et al., 2004); effects on eGFR were observed in human hypertensives at mean blood Pb level of 4.2 μg/dL (Muntner et al., 2003). These findings were substantiated by the coherence of effects observed across epidemiologic and toxicological studies. Both human and animal studies have observed hyperfiltration; in animals during the first 3 months after Pb exposure, effects were characterized by increased GFR and increased kidney weight due to glomerular hypertrophy. However, chronic exposure 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 public health significance of such effects in the general population, the implications of hyperfiltration, and reverse causality.

Recent epidemiologic studies in adult general and patient populations, with few exceptions, continue to be consistent in observing associations between blood and bone Pb levels and worse kidney function and provide important evidence that nephrotoxicity occurs at current population levels of biomarkers of Pb exposure. These studies benefit from a number of strengths that vary by study but include comprehensive assessment of Pb dose with bone Pb as a measure of cumulative body burden and chelatable Pb as a measure of bioavailable Pb; prospective study design; and statistical approaches that utilize a range of exposure and outcome measures, while adjusting for numerous kidney and Pb risk factors. Large sample sizes provide strength to the general population studies. Reexamination of a study from the 2006 Pb AQCD provided data to conclude that a 10-fold increase in blood Pb (e.g., from 1 to 10 μg/dL) would result in 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 adverse impact on eGFR as an increase of 4.7 years in age or 7 kg/m^2 in body mass index (Akesson et al., 2005). In populations with lower blood Pb levels, a downward shift in kidney function of the entire population due to Pb may not result in CKD in identifiable individuals; however, that segment of the population with the lowest kidney reserve may be at increased risk for CKD when Pb is combined with other kidney risk factors. At blood Pb levels that are common in the general U.S. population, Pb increases the risk for clinically relevant effects particularly in susceptible populations such as those with underlying

chronic medical diseases that increase CKD risk such as diabetes mellitus and hypertension and co-exposure to other environmental nephrotoxicants.

Absence of impact in the occupational setting cannot be used as a rationale for discounting Pb-related nephrotoxicity at lower environmental levels. Research in the occupational setting has traditionally been far less consistent than in environmentally exposed populations. A number of explanatory factors for this inconsistency, all due to limitations of the occupational literature, were discussed in the 2006 Pb AQCD. The observation 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 cannot be resolved solely by utilizing stronger research techniques. Irrespective of the mechanism, these associations have risk assessment implications. If associations are in opposite directions in different subgroups of the population and the relevant effect modifier is not considered, null associations will be observed.

Important data on the kidney effects of Pb on children were reported in a recent NHANES analysis in adolescents that observed an association between higher blood Pb and lower cystatin C-based eGFR (Fadrowski et al., 2010). These findings are consistent with a rodent model in which a low dose of Pb (50 ppm) administered from birth resulted in renal impairment (elevated serum creatinine as compared to control rats), but these observations require confirmation by measurement of GFR and renal pathology (Berrahal et al., 2011). These limited studies add to the strength of the association between blood Pb and altered renal function in children despite the need for additional research.

CKD results in substantial morbidity and mortality, and, even at earlier stages than those requiring kidney dialysis or transplantation, is an important risk factor for cardiac disease. 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 5.4). Interestingly, animal studies have shown Pb-induced vascular injury in the kidney associated with increased glomerular sclerosis, tubulointerstitial injury, increased collagen staining, and an increase in macrophages associated with higher levels of MCP-1 mRNA (Roncal et al., 2007). It is possible that the cardiovascular and renal effects of Pb observed are mechanistically linked and are contributing to the progression of the diseases.

Recently available animal toxicological studies strengthen the evidence regarding the mechanisms leading to these renal alterations, especially, similar to the cardiovascular system, the influence of Pb-induced oxidative stress. The mode of action of Pb in the kidneys has been extended to the field of immunology, where it was shown that low Pb exposure results in infiltration of lymphocytes and macrophages associated with increased expression of NFκB in proximal tubules and infiltrating cells. Additionally, recent evidence expands on the evidence of acute effects of Pb, including mitochondrial

dysfunction, renal cell apoptosis, and glomerular hypertrophy. These mechanisms are useful in understanding the occurrence of acute hyperfiltration followed by chronic kidney dysfunction.

Current evidence does not allow for the identification of a threshold for Pb-related nephrotoxicity; increased odds of CKD (characterized by eGFR <60 mL/min/1.73 m^2) were apparent in NHANES analyses that included data collected as recently as 2006 (Navas-Acien et al., 2009). The odds of reduced eGFR increased by 36% (95% CI: 0.99, 1.85) at blood Pb levels as low as 1.6-2.4 µg/dL and by 56% (95% CI: 1.17, 2.08) at blood Pb >.4 µg/dL.

In summary, new studies evaluated in the current review support or expand upon the strong body of evidence presented in the 2006 Pb AQCD that biomarkers of Pb exposure are associated with renal health effects. Epidemiologic studies continue to demonstrate a consistently positive relationship between blood Pb level and kidney dysfunction at blood Pb levels (mean <2 µg/dL) comparable to those occurring in the current U.S. population with no evidence for a threshold across the range of levels studied. By demonstrating Pb-induced oxidative stress and describing mechanisms of acute changes following Pb exposure, toxicological studies provide biological plausibility for the associations observed in epidemiologic studies between Pb and kidney dysfunction. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of kidney health endpoints is sufficient to conclude that there is a **causal relationship between Pb exposures and renal health effects**.

5.6. Immune System Effects

5.6.1. Introduction

With respect to studies conducted in laboratory animal and in vitro models, Pb is one of the most extensively researched and studied immunotoxicants. Experimental studies of the effects of Pb exposure on host resistance date back to the 1960s while those focusing on Pb-induced immune functional alterations, including developmental immunotoxicity (DIT), were first conducted during the 1970s. Despite the long history of Pb-associated immunotoxicity research, the immune-based effects in animals with blood Pb levels in the range of current U.S. population levels (i.e., <10 µg/dL), particularly early in life, are a relatively recent finding from within the last 10-15 years (Dietert & McCabe, 2007). Over the same time period, similar advances in the understanding of Pb-associated changes in immunological parameters in humans without occupation Pb exposures have substantiated the immunomodulatory effects of Pb.

In the 2006 Pb AQCD (U.S. EPA, 2006), both toxicological studies in animals and epidemiologic studies of humans provided strong evidence that the immune system was one of the more sensitive systems affected by Pb exposure. However, rather than producing overt cytotoxicity or pathology, Pb

exposure of experimental models and blood Pb levels in humans were found to be associated with alterations in the abundance and function of a variety of immune cells (Figure 5-47). In both toxicological and epidemiologic studies, macrophages and T lymphocytes were observed to be particularly sensitive to Pb, but Pb-associated changes were also reported in B lymphocytes and neutrophils. Several of these changes were observed at blood Pb levels <10 µg/dL or the equivalent in humans and experimental models, levels at which neurological effects also were observed.

Alterations in these aforementioned immune cells can lead to changes changes in cell-to-cell interactions, multiple signaling pathways, and inflammation that affect both innate and acquired immunity, that in turn, influence risk of developing infectious, allergic and autoimmune diseases as well as exacerbating inflammatory responses in other organ systems (Figure 5-47).

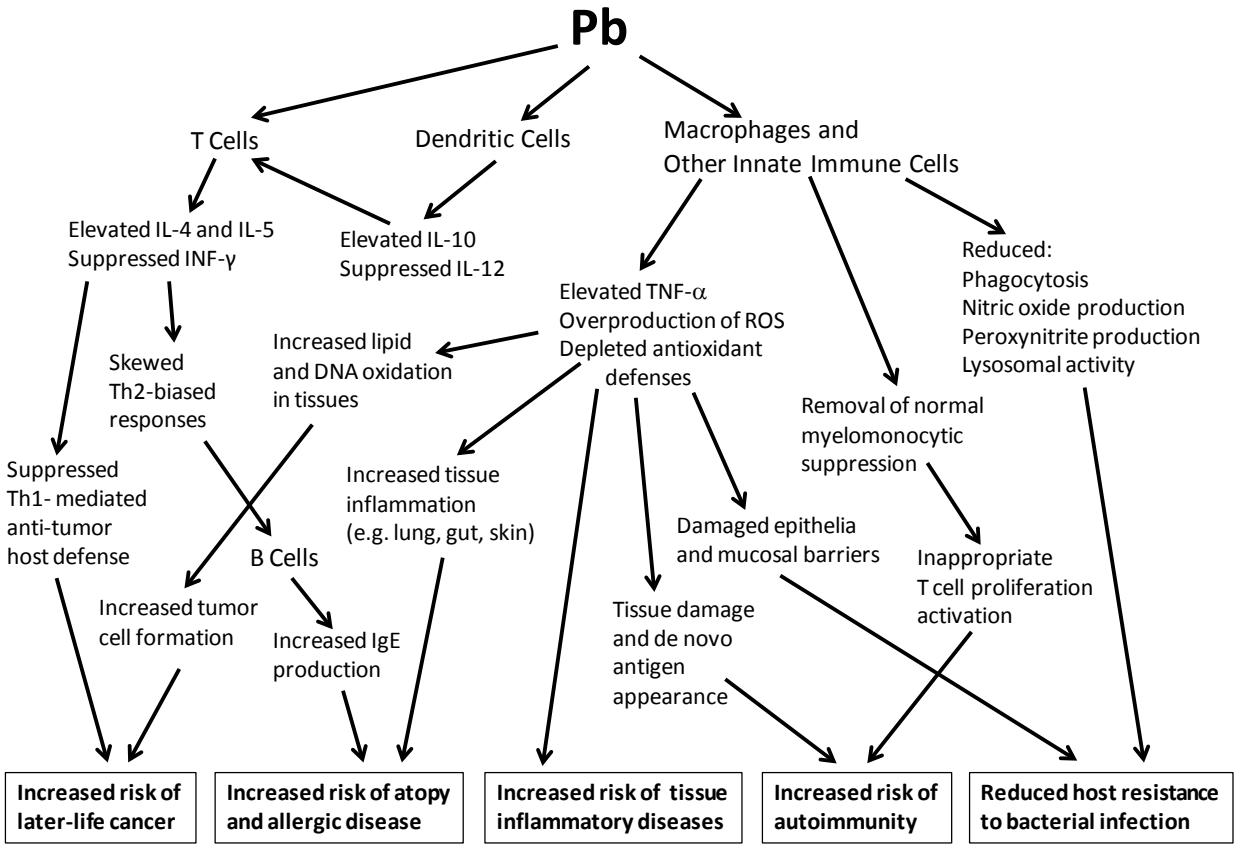


Figure 5-47. Immunological pathways by which Pb exposure may increase risk of immune-related diseases.

Studies conducted in animal and in vitro models have provided consistent evidence for Pb inducing effects on the range of immune effects presented in this continuum. Among the hallmarks reported for Pb-induced changes in functional pathways are: (1) a suppression of T-derived lymphocyte helper (Th)1-

driven cell-mediated immunity (as measured by a delayed-type hypersensitivity [DTH] response); (2) an increase in Th2-driven immunoglobulin E (IgE) antibody production; and (3) a proinflammatory shift in macrophage function. The latter was characterized by increased production of reactive oxygen intermediates reactive oxygen species (ROS), prostaglandin E_2 (PGE_2), and inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 and decreased production of IL-12 and nitric oxide (NO). In animal studies, these effects of Pb were more prominent with in utero Pb exposures and in males. In the 2006 Pb AQCD, epidemiologic evidence was available for relatively fewer immune endpoints (e.g., proinflammatory shifts in immune cell function); however, coherence was observed between toxicological and epidemiologic findings for Pb-associated changes in circulating IgE levels and T and B cell abundance and activation (U.S. EPA, 2006). Although many epidemiologic studies indicated associations between blood Pb levels and immune system effects, several limitations of study design and analytic methods were noted, including cross-sectional analyses; small sample size; inconsistent adjustment for potential confounders such as age, sex, smoking, and comorbid conditions; and limited assessment of the magnitude of association between blood Pb levels and changes in immune function.

Consistent with inhibition of Th1 activity, toxicological evidence presented in the 2006 Pb AQCD linked Pb exposure of animals to impaired host resistance and increased risk of certain infections (U.S. EPA, 2006). Consistent with inducing a hyperinflammatory state and local tissue damage, Pb exposure was found to induce generation of autoantibodies, indicating an elevated risk of autoimmune reactions. Additionally, the demonstrated shift toward a Th2 response suggested that Pb could elevate the risk of atopy and allergic responses. While toxicological studies provided the evidence for biological plausibility, the epidemiologic evidence was too sparse to draw conclusions regarding associations between blood Pb levels and these broader indicators of immune dysfunction in humans.

Studies published since the 2006 AQCD support the previous findings of Pb-induced immune effects and demonstrate similar effects at lower blood Pb levels (<2-5 µg/dL). Recent studies also expand on the array of immunological parameters affected by Pb exposure as presented in Figure 5-47. For example, new toxicological evidence indicates that Pb modulates function of dendritic cells. Results from new toxicological and epidemiologic studies strengthen the link between Pb-associated effects on immune cells and immune- and inflammatory-based diseases by providing evidence for changes in intermediary signaling and inflammatory pathways (Figure 5-47). New epidemiologic studies examine signaling molecules such as proinflammatory cytokines and NO to produce findings parallel with toxicological studies. Another important advance is the increasing knowledge of the broader role of Pb-associated immune modulation in mediating Pb effects in nonlymphoid tissues (e.g., in the neurological, reproductive, and respiratory systems). Although primarily cross-sectional in design, recent epidemiologic studies address many limitations of earlier studies through greater examination of children and adults without occupation Pb exposures with blood Pb levels more comparable to those currently measured in

the U.S. population and greater consideration of confounding by age, sex, smoking, and comorbid conditions.

5.6.2. Cell-Mediated Immunity

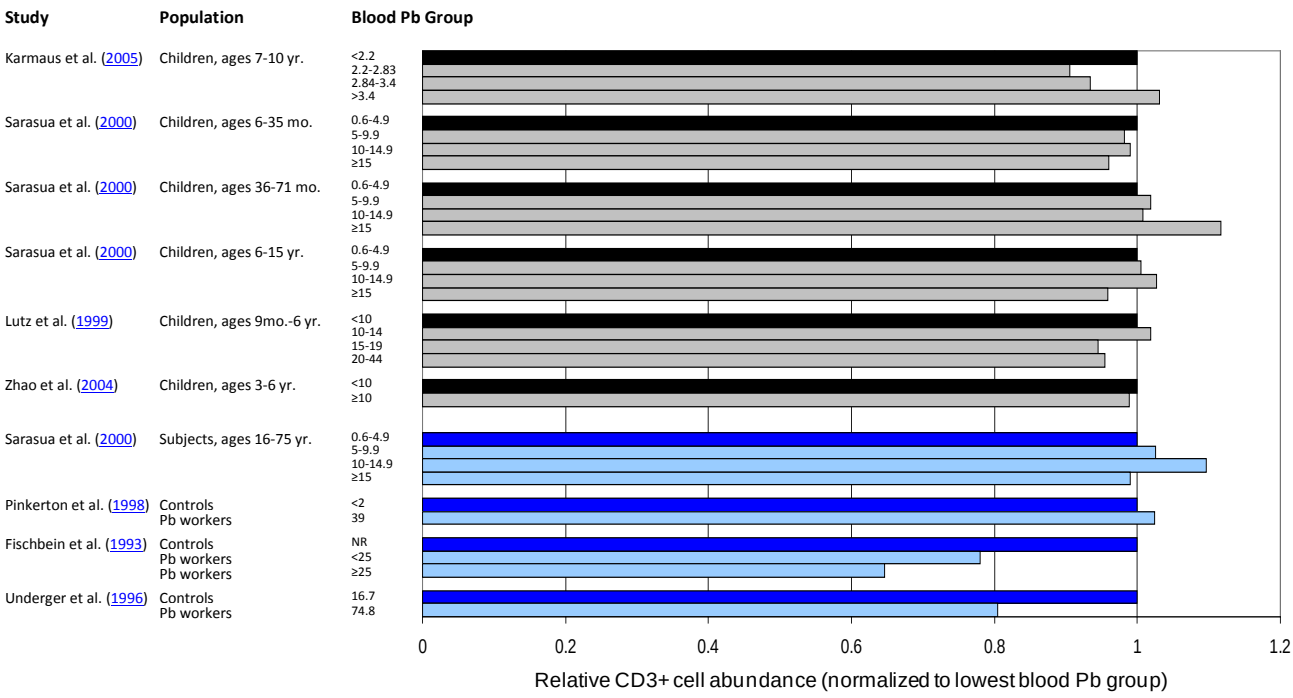
5.6.2.1. T Cells

Toxicological and epidemiologic evidence reviewed in the 2006 Pb AQCD consistently demonstrated Pb effects on decreasing T cell populations (U.S. EPA, 2006), which mediate responses to antigens and infectious agents. Consistent with previous findings, in a recent study of Wistar rats administered 200 ppm Pb acetate, the percentages of CD4+ (T helper) and CD8+ (cytotoxic T) T cells were decreased (with CD4-CD8- cells elevated) in the submaxillary lymph nodes ($p < 0.05$), with intraperitoneal (i.p.) exposure but not oral exposure (Teijon et al., 2010). The 2006 Pb AQCD also described numerous toxicological studies in which Pb exposure shifted the development and/or activation of CD4+ T cell populations such that production of Th2 cytokines was favored and production of Th1 cytokines was suppressed (U.S. EPA, 2006). Recent studies expand information on the potential mechanisms underlying T cell activation. In cultures of human CD4+ T cells, Pb (1 µM, 30 minutes) has been shown to activate transcription factor NFκB (regulates T cell activation) (Pyatt et al., 1996) and to increase, in a dose-dependent manner (10 and 50 µM $PbCl_2$, 24 hours), the expression of MHC class II surface antigens (HLA-DR), which mediates the CD4+ response to exogenous antigens (Guo et al., 1996b). Heo et al. (2007) provide evidence for the direct effects of Pb on T cells by showing that Pb (25 µM) blocked production of the Th1 cytokine interferon-γ (IFN-γ) in cultures of stimulated mouse T cells by suppressing translation of the protein. This blockage was rescued with the addition of IL-12. Kasten-Jolly et al. (2010) found that Pb may not necessarily skew towards a Th2 phenotype via a direct effect on T cells. In this study, developmental Pb exposure of mice (0.1mM Pb acetate in drinking water of dams from GD8 to PND21, resulting in pup blood Pb levels 10-30 µg/dL) induced gene expression of IL-4 and suppressed production of IFN-γ in splenic cells. These changes occurred in the absence of STAT4 or STAT6, the preferential signaling pathways for T cells and occurred with concomitant increases in adenylate cyclase 8 and phosphatidylinositol 3-kinase, indicating that Pb may promote Th2 activity via T cell-independent pathways.

Epidemiologic studies provide evidence for blood Pb levels being associated with a shift in production from Th1 to Th2 cytokines in humans (Section 5.6.5.4); however, the extant evidence for effects on T cells in humans is derived largely from older studies describing changes in the abundance of several T cell subtypes, including CD3+, CD4+, and CD8+, that may affect cell-to-cell interactions required in acquired immunity responses to antigens. Among studies of subjects without occupational Pb exposures, decreases in the abundance of CD3+ (Figure 5-48 and Table 5-23), CD4+, and CD8+ T cells

often were observed among children with blood Pb levels in the range of 5 to 44 µg/dL (Lutz et al., 1999; Sarasua et al., 2000; Z. Y. Zhao et al., 2004). However, Karmaus et al. (2005) observed decreases in several T cell subtypes in children with blood Pb levels 2.2-2.8 µg/dL compared with children with blood Pb levels <2.2 µg/dL, indicating that decreases in T cell abundance also may occur at blood Pb levels that are more comparable to those of the current U.S. general population. In association with higher blood Pb levels (≥ 10 µg/dL), Zhao et al. (2004), Fischbein et al. (1993), and Mishra et al. (2010) observed a decrease in the ratio of CD4+/CD8+ cells, indicative of a compromised response to viral infections. These findings are consistent with the documented effects of Pb exposure on diminished host resistance and associations with bacterial and viral infections (Section 5.6.4.1). Changes in the CD4+/CD8+ ratio have not been examined in populations with lower blood Pb levels. In a large multicity U.S. study conducted in subjects living near a Pb smelting operation plus demographically-matched controls, Sarasua et al. (2000) indicated that the associations between blood Pb levels and T cells may vary with age. Investigators analyzed blood Pb level as a continuous variable and found that among children 6-35 months in age, a 1 µg/dL increase in blood Pb level was associated with decreases in the percentage of CD3+ (-0.18 [95% CI: -0.34, -0.02]), CD4+ (-0.10 [95% CI: -0.24, 0.04]), and CD8+ (-0.04 [95% CI: -0.15, 0.07]) T cells; however, in older children (36-71 months, 6-15 years) and adults (16-75 years), many effect estimates were positive.

Despite the consistency of findings across studies, it is not clear what may be the impact of the small magnitudes of change in T cell abundance on the cell-to-cell interactions that mediate downstream acquired immune responses. For example, in populations without occupational Pb exposures, Pb-associated decreases in the relative abundance of CD3+ cells range between 1 and 9% (Table 5-23). Larger decreases (20-35%) are observed in studies of occupationally-exposed males with higher blood Pb levels than those expected in the general population (>25 µg/dL) (Fischbein et al., 1993; Undeger et al., 1996).



Note: Bars represent the abundance of CD3+ cells normalized to the level measured in the lowest blood Pb group (depicted as black or dark blue). Bars in black or gray represent results in subjects without occupational Pb exposures, and bars in dark or light blue represent results in subjects with occupational Pb exposures.

Figure 5-48. Comparisons of the relative abundance of CD3+ T cells among groups with increasing blood Pb level (µg/dL).

Table 5-23. Comparison of serum abundance of T-cell subtypes[a] among various blood Pb groups.

Study	Population	Blood Pb group (µg/dl)	CD3+	CD4+	CD8+	CD4+:CD8+	CD45RO+	CD45RA+
				Children				
Karmaus et al. (2005)[b]	331 children, ages 7-10 yr Hesse, Germany	<2.2 2.21-2.83 2.84-3.41 >3.41	2118 1919 1979 2184	1214 1106 1128 1123	712 634 661 662			358 321 348 351
Sarasua et al. (2000)[c]	382 children, ages 6-30 mo Multiple U.S. locations	0.6-4.9 5-9.9 10-14.9 ≥ 15	67.8 66.6 67.1 65.1	46.6 45.5 47.1 45.2	19.9 20.2 19.1 18.8			
Sarasua et al. (2000)[c]	562 children, ages 36-71 mo Multiple U.S. locations	0.6-4.9 5-9.9 10-14.9 ≥ 15	68.1 69.4 68.6 76.1	42.0 44.0 44.2 41.6	23.5 23.1 21.4 24.4			
Sarasua et al. (2000)[c]	675 children ages 5-16 yr Multiple U.S. locations	0.6-4.9 5-9.9 10-14.9 ≥ 15	69.2 69.5 71.1 66.3	43.0 43.0 44.5 44.0	24.7 25.4 24.5 20.1			
Lutz et al. (1999)[d]	279 children, ages 9 mo-6 yr Springfield, MO	<10 10-14 15-19 20-44	68.4 69.7 64.6 65.3					

Study	Population	Blood Pb group (µg/dl)	CD3+	CD4+	CD8+	CD4+:CD8+	CD45RO+	CD45RA+
Zhao et al. (2004)	75 children, ages 3-6 yr Zhejiang Province, China	<10 ≥ 10	55.2 54.6	27.1 23.7[e]	20.6 23.2[e]	1.41 1.09[e]		
Adults without Occupational Pb Exposures								
Sarasua et al. (2000)[c]	433 children and adults, ages 16-75 yr Multiple U.S. locations	0.6-4.9 5-9.9 10-14.9 ≥ 15	71.6 73.4 78.5 70.9	50.3 53.3 54.9 49.6	24.9 23.9 24.1 25.3			
Adults with Occupational Pb Exposures								
Fischbein et al. (1993)	36 unexposed controls, mean age 47 yr 51 firearms instructors, mean age 48 yr	NR <25 ≥ 15	72.6 57 46.9[e]	45.6 29.6 21.4[e]	25.7 23 25.4	1.95 1.38 0.95[e]		
Pinkerton et al. (1998)[f]	84 unexposed controls, mean age 30 yr 145 Pb smelter workers, mean age 33 yr	<2 39	73.1 74.9	44.7 44.6	23.0 23.3	1.9 1.9		45.6 44.1
Underger et al. (1996)[g]	25 unexposed controls, ages 22-56 yr 25 Pb battery plant workers, ages 22-55 yr	16.7 74.8	2044.7 1644	1140.3 858.8[e]	977.6 829.1	1.3 1.1		
Yucesoy et al. (1997b)	10 unexposed controls, ages 25-42 yr 47 Pb battery plant workers, ages 19-49 yr	4.0 59.4, 58.4		30.8 30.1				
Mishra et al. (2010)	21 unexposed controls, median age 27 yr 26 three-wheel drivers, median age 31 yr 33 Pb battery workers, median age 27 yr	4.5 6.7 132 (103)		55 37 31[e]	26 27 26	2.6 1.4 1.3[e]	44 43 40	61 73 70[e]

[a]Data are presented as the percentage of T cell subtype among all T cells unless otherwise specified.
[b]Data represent the number of cells/µL serum. Means are adjusted for age, sex, ETS, number of infections in the previous 12 months, serum lipid concentration, and organochlorine exposures.
[c]Means are adjusted for age, sex, and location of study.
[d]Means are adjusted for age.
[e]p<0.05 for difference among groups.
[f]Means adjusted for age, race, current smoking status, and workshift.
[g]Data represent the number of cells/µL serum.

5.6.2.2. Lymphocyte Activation

Pb exposure produces an expansion of alloreactive T and B lymphocytes. This occurs as a result of reversing the normal suppression that is mediated by a macrophage-like subpopulation. As discussed in Section 5.6.5.2, changes in NO production appear to be involved in this process (Farrer et al., 2008). Additionally, Pb alters antigen processing that occurs in antigen presenting cells (APCs, e.g., primarily dendritic cells and macrophages), which appear to shift signals to T cells skewing both the nature of the subsequent response and the spectrum of activities among those expanded populations of lymphocytes (Farrer et al., 2005). Gao et al. (2007) reported that dendritic cells that matured in the presence of Pb promoted enhanced alloreactive T cell proliferation compared to control dendritic cells. An additional effect of Pb has been described relative to activation of T cells. Using the local lymph node assay (LLNA), Carey et al. (2006) found that $PbCl_2$ was able to provide a costimulatory signal to antigens that could activate T cells. The exact mechanistic basis for this is not known.

Epidemiologic findings are mostly limited to studies of occupationally-exposed adults and to associations with blood Pb level >10 µg/dL. In the only study of children in Missouri, the percentage of activated T cells (as indicated by the cell surface marker HLA-DR) was higher in children with blood Pb level 15-19 µg/dL than in children with blood Pb level <15 µg/dL; however, activated cells were not elevated in children with blood Pb levels 20-44 µg/dL (Lutz et al., 1999). In contrast with toxicological findings, the direction of lymphocyte proliferation (in response to mitogens and/or to specific antigens) in association with occupational Pb exposure is unclear. Pb promotes the activation of Th2 cells and suppresses Th1 cells, therefore, the differential activation of specific subtypes may be not discernable in studies that measure overall lymphocyte proliferation. Whereas some studies have reported similar levels of lymphocyte proliferation (≤ 1% difference) between Pb-exposed workers and unexposed controls (N. Cohen et al., 1989; Queiroz, Perlingeiro, et al., 1994), others have reported lower lymphocyte proliferation among Pb-exposed workers (8-25%) (Alomran & Shleamoon, 1988; Fischbein et al., 1993; Kimber et al., 1986; Mishra et al., 2003). With the exception of Fischbein et al. (1993), these latter studies included subjects with high blood Pb levels (>60 µg/dL). Additionally, with much of the evidence limited to comparisons of mean proliferative responses between Pb-exposed and -unexposed workers, it is difficult to apply findings to populations with lower blood Pb levels. In one of the few analyses of correlation between blood Pb levels and lymphocyte proliferation, Mishra et al. (2003) found a lack of correlation, suggesting the influence of another occupationally-related factor on lymphocyte proliferation.

5.6.2.3. Delayed-type Hypersensitivity

The DTH assay is commonly used as an indicator of the T cell-mediated adaptive immune response, i.e., induration and erythema resulting from the activation of T cells and recruitment of monocytes to the site of antigen deposition. The DTH response is largely Th1-dependent in that Th1 cytokines drive the production of antigen-specific T cells directed against the antigen (sensitizing phase) and the recruitment of antigen-specific T cells and monocytes to the site of antigen deposition (elicitation phase). In the 2006 Pb AQCD and several recent reviews, a suppressed DTH response was identified as one of the most consistently observed and well-established immunomodulatory effects of Pb exposure in animal models (Dietert & McCabe, 2007; Mishra, 2009; U.S. EPA, 2006). A majority of the evidence for Pb suppressing the DTH response is provided by the historical literature in which effects were observed in association with both gestational (Bunn, Ladics, et al., 2001; Bunn, Parsons, et al., 2001a, 2001b; S. Chen et al., 2004; S. Chen et al., 1999; Faith et al., 1979; J.-E. Lee et al., 2001; Miller et al., 1998) and postnatal (Laschi-Loquerie et al., 1984; M. J. McCabe, Jr. et al., 1999; Muller et al., 1977) Pb exposures of animals. In some studies, the suppressed DTH response was accompanied by a decreased production of IFN-γ (S. Chen et al., 1999; J.-E. Lee et al., 2001), which is the primary cytokine that stimulates recruitment of macrophages, a key component of the DTH response. The concomitant decrease in IFN-γ demonstrated

further that Pb-induced suppression of the DTH response reflects the inhibition of Th1 functional activities.

One of the most salient findings collectively was that DTH was suppressed in animals with blood Pb levels ranging from <2 to 5 µg/dL (Bunn, Ladics, et al., 2001; Bunn, Parsons, et al., 2001a, 2001b; Miller et al., 1998; Muller et al., 1977). In some studies that examined Pb exposures at different stages of gestation, exposures later in gestation suppressed DTH (Bunn, Parsons, et al., 2001b; J.-E. Lee et al., 2001). These latter findings may reflect the status of thymus and T cell development. A recent study contributed to the robust evidence 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 $PbCl_2$ (25 µM, 10 days) then the antigen ovalbumin (OVA) and injected the cells into naïve mice. The Pb-exposed dendritic cells inhibited the OVA-specific DTH footpad response in mice compared with mice exposed to control dendritic cells.

The capacity of Pb to suppress the DTH response is strongly supported by mechanistic studies in which Pb suppresses Th1 cytokine production (Section 5.6.5.4). Further, coherence is provided by associations observed between Pb exposure or blood Pb levels and other responses related to the inhibition of Th1-driven adaptive immune responses, including increased susceptibility to developing certain infections and tumors (Section 5.6.4.1).

5.6.2.4. Macrophages and Monocytes

Macrophages and monocytes, the blood form of tissue macrophages, are among the most sensitive targets of Pb-induced immune effects. The 2006 Pb AQCD emphasized the large number of toxicological studies showing the effects of Pb on a wide range of alterations in macrophage function to promote a hyperinflammatory phenotype (U.S. EPA, 2006). These changes include enhanced production of ROS, suppressed production of NO, enhanced production of TNF-α, excessive metabolism of arachidonic acid into immunosuppressive metabolites (e.g., prostaglandin E_2), impaired phagocytic activity and lysosomal function, and potentially altered receptor expression [e.g., toll-like receptors]). These studies are described in detail in Section 5.6.5.2. Because macrophages are major resident populations in most tissues and organs and are also 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 and organs (Section 5.6.4.5). Recent studies by Bussolaro et al. (2008), Kasten-Jolly et al. (2010), and Mishra et al. (2006) using mouse macrophages exposed in vitro to Pb reinforced the capacity of Pb to induce a broad spectrum of functional alterations in macrophages such as lipopolysaccharide (LPS)-induced production of NO. Although not examined widely, epidemiologic evidence suggests that Pb exposure may be associated with changes in macrophage function in humans. Pineda-Zavaleta et al.

(2004) examined children in Lagunera, Mexico, attending schools at varying distances from an active Pb smelter (range of blood Pb levels: 3.5-47.5 µg/dL) and found that blood Pb levels were associated with a hyperinflammatory state in macrophages as indicated by a decrease in NO production and increase in superoxide anion production (Section 5.6.5.2). Studies of occupationally-exposed adults mostly observe that Pb exposure is associated with impaired function of macrophages. Adjusting for age, race, smoking, and workshift, Pinkerton et al. (1998) found lower abundance of monocytes among Pb smelter workers (7.8%) than among unexposed controls (8.5%). Fischbein et al. (1993) found lower abundance of HLA-DR+ cells in firearms instructors with blood Pb level <25 µg/dL (8.8%) and ≥ 25 µg/dL (8.7%) than among unexposed controls (15.2%). HLA-DR+ is an indicator of activated function state of APCs and is upregulated in response to cell signaling.

5.6.2.5. Neutrophils

Although neutrophils were found not to be a significant direct target of Pb in the 2006 Pb AQCD (U.S. EPA, 2006), the modulation of their activity by Pb may have important consequences on the dysregulation of inflammation and ability to response to infectious agents. Studies of cultured human polymorphonuclear cells (PMNs) (Governa et al., 1987) and occupationally-exposed adults (Bergeret et al., 1990; Queiroz et al., 1993; Queiroz, Costa, et al., 1994; Valentino et al., 1991) have found reduced PMN functionality, as indicated by reduced chemotactic response, phagocytic activity, respiratory oxidative burst activity, or reduced ability to kill ingested antigen, among Pb workers compared with controls. Important limitations to applying these epidemiologic findings broadly include male-only study populations, relatively high blood Pb levels of workers (range of mean levels: 33.1-71 µg/dL) and the lack of direct examination of associations between blood Pb level and neutrophil function.

In both studies of animals and occupationally-exposed adults, Pb exposure has been associated with an increase in neutrophil counts, which has been interpreted as a compensatory response to Pb-induced impairment in neutrophil chemotactic activity and a hyperinflammatory response. In a study by Kibayashi et al. (2010) to investigate host responses to gunshot wounds in the brain, neutrophils were a major responding cell. Implantation of Pb spheres (compared with glass spheres in the controls) in male Wistar rats led to major neutrophil infiltration with inflammatory-related damage that included apoptosis and indications of neurodegeneration.

In a group of 68 ceramic, Pb recycling, or bullet manufacturing workers and 50 controls selected among food plant workers, DiLorenzo et al. (2006) observed that a 1 µg/dL increase in blood Pb level was associated with an increase in ANC of 21.8 cells/µL (95% CI: 11.2, 32.4 cells/µL) adjusted for age, BMI, and smoking status. The geometric mean (range) of blood Pb levels was 20.5 µg/dL (3.2-120) among workers and 3.5 µg/dL (1-11) among controls. Eight workers with medium to high Pb exposures (exact blood Pb levels not reported) had neutrophilia (n >7,500 cells/mm^3) versus no controls, suggesting

that chronic, higher-level Pb exposures can lead to a biologically meaningful excess of circulating neutrophils. Additionally, in analyses comparing three blood Pb level groups, controls, workers with blood Pb levels ≤ 30 µg/dL, and workers with blood Pb levels >30 µg/dL, ANC was observed to increase monotonically, supporting a blood Pb dose-dependent relationship. When the three blood Pb groups were further stratified by current smoking, two-way ANOVA indicated positive interaction between blood Pb level and current smoking. Higher blood Pb level was associated with higher ANC only when current smokers were compared. Among nonsmokers, ANCs were similar across blood Pb groups.

Coherence for the effects of Pb exposure on neutrophils is provided by findings that blood Pb level is associated with mediators of neutrophil proliferation, survival, maturation, and functional activation. These mediators include cytokines such as TNF-α (Section 5.6.5.4) and complement. The complement system is a component of the innate immune system that controls various cell-mediated immune responses such as chemotaxis of macrophages and neutrophils and phagocytosis of antigens. The effects of Pb exposure on complement have not been widely examined; however the limited data suggest Pb may suppress complement activity. Both Ewers et al. (1982) and Undeger et al. (1996) measured lower complement C3 protein among Pb-exposed workers compared with unexposed controls, with Ewers et al. (1982) additionally observing an inverse association between blood Pb level and C3 among workers. However, the implications of these findings are limited due to the high blood Pb levels in these occupationally-exposed groups (range of blood Pb levels: 18.6-85.2 µg/dL and 38-100 µg/dL, respectively) and by the lack of adjustment for potential confounding variables.

5.6.2.6. Dendritic Cells

Since the 2006 Pb AQCD (U.S. EPA, 2006), new evidence from both an ex vivo and in vitro model suggests that the effects of Pb exposure 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). Prior research on the effects of Pb in favoring Th2 over Th1 activity emphasized the direct measurement of Th1 vs. Th2 T-cell populations and cytokine profiles. But new research techniques (D. Gao & Lawrence, 2010) have provided an opportunity to look upstream at how dendritic cells may be involved in mediating the effects of Pb on acquired immunity. Gao et al. (2007) used bone marrow cultures exposed to Pb to examine the impact of Pb on dendritic cell maturation and function. They found that Pb (25 µM, 10 days) altered the course of dendritic cell maturation by changing the ratio of cell surface markers, such as the CD86/CD80 ratio, that promote Th2 cell development. 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 control cells but the same amount of IL-10 (inhibits production of Th1 cytokines). The effect of Pb in altering the cytokine expression profile of dendritic cells, in particular, the lower IL-12/IL-10 ratio, may serve as an important signal to shift naïve T cell populations towards a Th2 phenotype. Strengthening the role of

dendritic cells in mediating Pb immune effects were ex vivo results from the same study that Pb-naïve Balb/c mice implanted with Pb-treated dendritic cells were skewed towards Th2 activity as indicated by inhibited DTH (Section 5.6.2.3) and IgG2a antibody (Section 5.6.3) responses (D. Gao et al., 2007).

5.6.2.7. Natural Killer (NK) Cells

The collective body of toxicological and epidemiologic evidence indicates that the innate immune NK cells are not affected to a large extent by Pb exposure. This conclusion is underscored by epidemiologic observations that blood Pb levels are associated with T or B cell abudance but are not associated with NK cell abundance or the level of functional activity (Karmaus et al., 2005; Pinkerton et al., 1998; Sarasua et al., 2000). Likewise, similar means of NK cell abundance or functional activity have been observed in Pb-exposed workers and unexposed controls (Fischbein et al., 1993; Kimber et al., 1986; Mishra et al., 2003; Pinkerton et al., 1998; Undeger et al., 1996; Yucesoy et al., 1997b). Consistent with epidemiologic findings, in a recent in vitro study comparing the toxicity of metals for different populations of immune cells, Fortier et al. (2008) found that $PbCl_2$ (7.5-20.7 µg/dL), did not significantly affect NK cytotoxicity compared with the DMSO vehicle; however, $PbCl_2$ did not affect other immune parameters (e.g., monocyte phagocytic activity or lymphocyte proliferation as well.

5.6.3. Humoral Immunity

The 2006 Pb AQCD indicated that Pb exposure was associated with enhanced humoral immune responses as characterized by the proliferation of B cells and increased production of Ig antibodies (U.S. EPA, 2006). Both toxicological and epidemiologic studies (Figure 5-49 and Table 5-24) have demonstrated Pb-associated increases in IgE production, which is strongly implicated in mediating allergic responses and inflammation in allergic asthma. In animal studies, Pb exposures induced concomitant increases in IgE and IL-4 production by T cells (S. Chen et al., 1999; J. E. Snyder et al., 2000), consistent with the hypothesis that Th2-mediated mechanisms can induce class switching of B cells for the production of IgE. Additional coherence and biological plausibility for Pb effects on humoral immunity have been provided by epidemiologic observations for increases in B cell abundance in association with increasing blood Pb level or occupational Pb exposure (Figure 5-50 and Table 5-24). Earlier toxicological and epidemiologic studies also found similar associations of Pb with increases in other classes of Igs including IgG, IgM, and IgA.

Recent toxicological evidence continues to support the role of T cell-mediated mechanisms in Pb-induced activation of B cells and production of Ig antibodies. Carey et al. (2006) treated Balb/c mice with subsensitizing doses of a T cell-independent [Trinitrophenyl-Ficoll (TNP-Ficoll)] or T cell-dependent [TNP-ovalabumin (TNP-OVA)] hapten-protein conjugate with or without co-exposures to $PbCl_2$. Seven days later, they examined the effects of $PbCl_2$ on the LLNA response to TNP-Ficoll or TNP-OVA. $PbCl_2$

exposure (25-50 µg, injected) increased the numbers of T and B cells in the lymph node against both TNP-Ficoll and TNP-OVA. Further, in a dose-dependent manner, $PbCl_2$ induced statistically significant elevations of IgM, IgG2a, and IgG1 antibody producing cells in the lymph node. While the increase in IgM-producing cells against TNP-Ficoll indicated a T-cell independent mechanism, the increases in IgG2a- and IgG1- producing cells against both antigens indicated a Th1- and Th2-mediated mechanism, respectively. Despite seeing increases in both IgG1- and IgG2a-producing cells, the authors concluded that Pb skewed the response toward Th2 and had considerable potential for promoting allergic sensitization against T-dependent antigens.

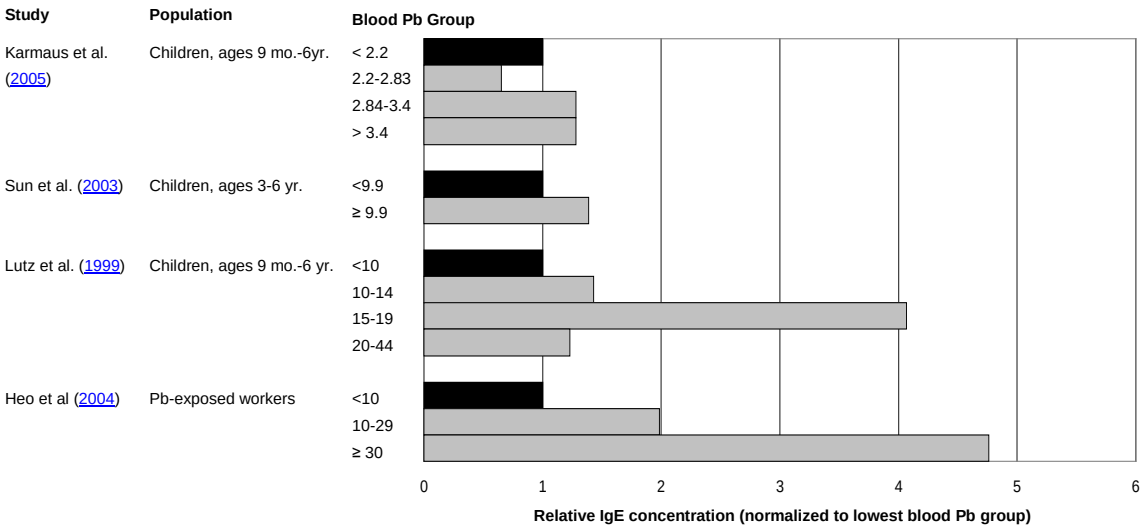
Other animal studies provided strong support for Pb exposure stimulating humoral responses preferentially via Th2-mediated mechanisms. Gao et al. (2006) found that Pb exposure (50 µg, i.p., 3 times/week, 3 weeks) of Balb/c mice elevated IgG1 over that of Th1-driven IgG2a. Similar results were reported when Pb-exposed dendritic cells were used to initiate antibody production (D. Gao & Lawrence, 2010). In a highly-specialized strain of IFN-γ knockout mice (lacking the capacity to produce IFN-γ), Pb exposure had the reverse effect of increasing the IgG2a/IgG1 ratio. These results were 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 (D. Gao et al., 2006). In a microarray study examining the DIT of Pb in Balb/c mice, Kasten-Jolly et al. (2010) found that early-life Pb exposure (0.1mM Pb acetate in drinking water of dams from GD8 to PND21, resulting in pup blood Pb levels 10-30 µg/dL) produced statistically significant increases in the expression of genes encoding Ig antibodies or those involved in B lymphocyte function and activation. These genes included those 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.

In epidemiologic studies, associations between increasing blood Pb level and increasing serum IgE level are demonstrated primarily in children (Annesi-Maesano et al., 2003; Karmaus et al., 2005; Lutz et al., 1999; L. Sun et al., 2003). Most studies indicate that the association between blood Pb level and IgE is nonmonotonic (Figure 5-49 and Table 5-24). Whereas many studies were focused on examining differences between children with blood Pb levels below and above 10 µg/dL (Figure 5-49 and Table 5-24), Karmaus et al. (2005) demonstrated 28% increases in IgE among children with blood Pb levels 2.84-3.41 and >3.4 µg/dL compared with children with blood Pb level <2.2 µg/dL. Another strength of this study was the adjustment for potential confounding by various organochlorine compounds, age, number of infections in the previous 12 months, and serum lipids. A clear blood Pb dose-dependent relationship was not observed as the mean IgE was lower among children in the second quartile of blood Pb levels (2.2-2.8 µg/dL) than among children in the lowest quartile (<2.2 µg/dL). Annesi-Maesano et al. (2003) provided additional information on potential critical developmental windows of Pb exposure. Cord blood IgE was associated with infant hair Pb level but not with cord or placental Pb level. From these findings,

the authors inferred a stronger effect of Pb exposure integrated over the entire gestational period rather than exposures closer to birth. Additionally, the magnitude of association was larger in the subgroup with nonallergic mothers, pointing to the possible stronger effect of family history of allergy on IgE levels at birth compared with blood Pb level. Sarasua et al. (2000) was unique among studies of children in examining associations of blood Pb level with IgA, IgG, and IgM. Associations were consistently positive (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 increase in IgA, IgG, and IgM, respectively, per 1 µg/dL increase in blood Pb level, adjusted for age, sex, and location) and tended to be larger in magnitude in the youngest age group (6-35 months), suggesting an increased susceptibility of exposure at younger ages. In this study, associations with IgE were not examined.

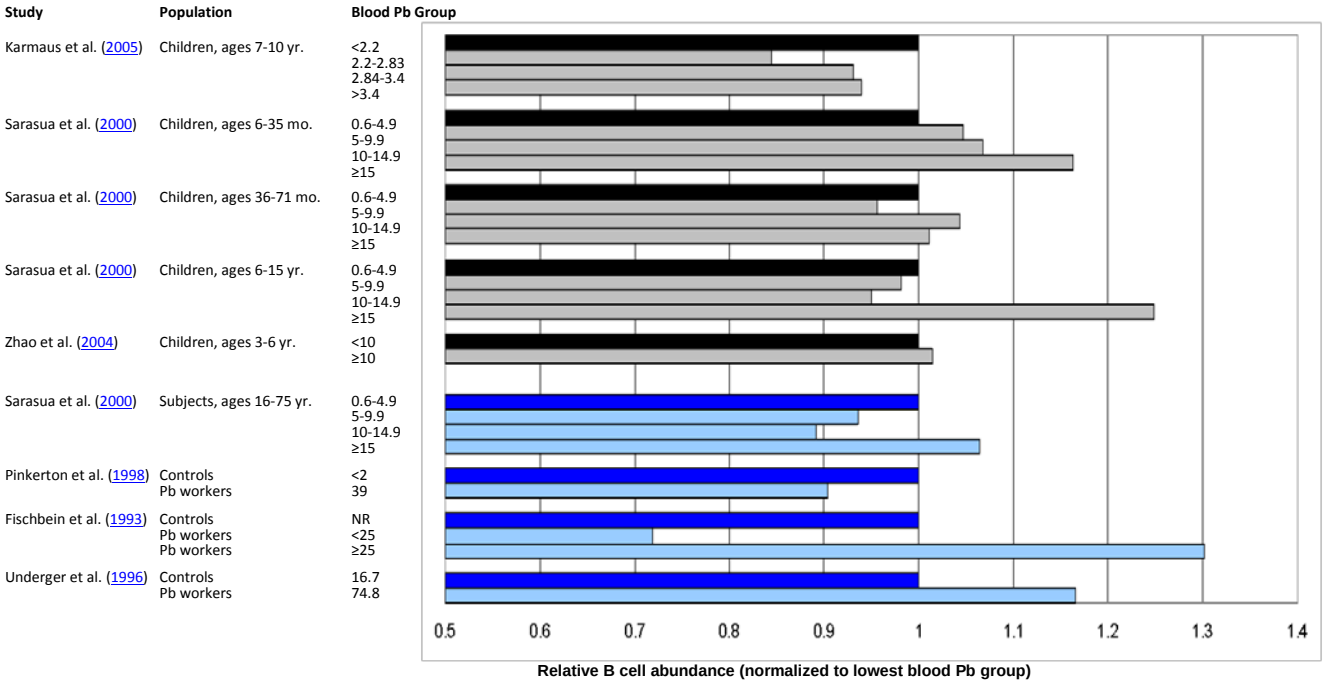
In studies of adults, one without (Pizent et al., 2008) and one with occupational Pb exposure (Heo et al., 2004), investigators reported higher IgE levels in association with higher blood Pb levels. In a study of urban adults in Zagreb, Croatia with blood Pb levels between 0.56 and 7.4 µg/dL, a statistically significant, positive association between blood Pb level and IgE was found in women but not men (Pizent et al., 2008). Several covariates were considered in a stepwise multiple regression, including age, smoking intensity, and alcohol consumption. Among women not on hormone replacement therapy or oral contraceptives, a 1 µg/dL increase in blood Pb level was associated with a 0.60 increase in log IgE (95% CI: 0.58, 1.18). Investigators did not report an effect estimate in men because it did not attain statistical significance. The study included 166 women and 50 men, thus, it was difficult to ascertain whether there was suggestion of association in men but lack of power to indicate statistical significance. Although blood Pb levels were lower in women (mean: 2.16 µg/dL, range 0.56-7.35 µg/dL) than in men (mean: 3.17 µg/dL, range 0.99-7.23), both groups had levels similar to those reported in studies of children. Among battery manufacturing workers (mostly males), Heo et al. (2004) found a monotonic increase in IgE levels among workers with blood Pb levels <0, 10-29, and ≥ 30 µg/dL.

A majority of the epidemiologic evidence for the effects of Pb on humoral immunity comprises comparisons of serum IgA, IgG, and IgM levels between Pb-exposed and -unexposed workers (Alomran & Shleamoon, 1988; Anetor & Adeniyi, 1998; Ewers et al., 1982; Kimber et al., 1986; Pinkerton et al., 1998; Queiroz, Perlingeiro, et al., 1994; Sarasua et al., 2000; Undeger et al., 1996). In contrast with toxicological findings, epidemiologic evidence reflects more mixed associations, with studies reporting higher, lower, and similar Ig levels in Pb-exposed workers compared with -unexposed controls. Among studies reporting lower Ig levels in Pb-exposed workers were a few with high mean blood Pb levels among exposed workers (56.3 and 74.8 µg/dL) (Anetor & Adeniyi, 1998; Undeger et al., 1996).



Note: Bars represent the IgE normalized to the level measured in the lowest blood Pb group (depicted as black).

Figure 5-49. Comparison of IgE levels among groups with increasing blood Pb level (µg/dL).



Note: Bars represent the abundance of B cells normalized to the level measured in the lowest blood Pb group (depicted as black or dark blue). Bars in black or gray represent results in subjects without occupational Pb exposures, and bars in dark or light blue represent results subjects with occupational Pb exposures.

Figure 5-50. Comparison of the relative abundance of B cells among groups with increasing blood Pb level (µg/dL).

Table 5-24. Additional characteristics and quantitative data[a] for results presented in Figures 5-49 and 5-50

Study	Population	Blood Pb group (µg/dL)	IgE	IgG	IgM	IgA	B cells
Children							
Karmaus et al. (2005)[b]	331 children, ages 7-10 yr Hesse, Germany	<2.2 2.21-2.83 2.84-3.41 >3.41	46 30 59 59[c]	1210 1214 1241 1201	150 143 153 148	123 121 133 136	418 353 389 393
Sun et al. (2003)	Zeijiang Province, China 217 children, ages 3-6 yr.	<9.9 ≥ 9.9					
Lutz et al. (1999)	279 children, ages 9 mo-6 yr Springfield, MO	<10 10-14 15-19 20-44	51.8 74.0 210.7 63.7[d]				13.4 12.6 16.9 11.1
Sarasua et al. (2000)[e]	382 children, ages 6-30 mo Multiple U.S. locations	0.6-4.9 5-9.9 10-14.9 ≥ 15		609 666[f] 680[f] 630	103 108 105 124[f]	50.1 55.0 58.2 61.4[f]	19.1 20 20.4 22.2
Sarasua et al. (2000)[e]	562 children, ages 36-71 mo Multiple U.S. locations	0.6-4.9 5-9.9 10-14.9 ≥ 15		817 813 856 835	120 116 125 121	88.6 90.9 96.3 94.1	18.4 17.6 19.2 18.6
Sarasua et al. (2000)[e]	675 children ages 5-16 yr Multiple U.S. locations	0.6-4.9 5-9.9 10-14.9 ≥ 15		1031 1094[f] 1048 1221	128 131 136 106	140 143 140 108	16.1 15.8 15.3 20.1
Zhao et al. (2004)	75 children, ages 3-6 yr Zhejiang Province, China	<10 ≥ 10					16.58 16.82
Adults without Occupational Pb Exposures							
Sarasua et al. (2000)[e]	433 children and adults, ages 16-75 yr Multiple U.S. locations	0.6-4.9 5-9.9 10-14.9 ≥ 15		1099 1085 1231 1169	175 175 262[f] 139	252 242 283 193	13.9 13.0 12.4 14.8
Adults with Occupational Pb Exposures							
Heo et al. (2004)	Korea 606 Pb battery plant workers	<10 10-29 ≥ 30	112.5 223.3 535.8[g]				
Pinkerton et al. (1998)[h]	84 unexposed controls, mean age 30 yr 145 Pb smelter workers, mean age 33 yr	<2 39		1090 1110	94.5 106.2	180 202	14.6 13.2
Fischbein et al. (1993)	36 unexposed controls, mean age 47 yr 51 firearms instructors, mean age 48 yr	NR <25 ≥ 15					8.6 10.5 11.2[g]
Underger et al. (1996)	25 unexposed controls, ages 22-56 yr 25 Pb battery plant workers, ages 22-55 yr	16.7 74.8		1202.1 854.6[g]	140.4 93.3[g]	210.3 168.1	635.9[i] 545.5

[a]IgE data are presented as IU/mL. Other Ig data are presented as mg/dL. B cell data are presented as the percentage of B cells among all lymphocytes unless otherwise specified.
[b]All means are adjusted for age, sex, ETS, number of infections in the previous 12 months, serum lipid concentration, and organochlorine exposures. B cell data represent the number of cells/µL serum.
[c]p<0.05 for difference among groups.
[d]p<0.05 for differences among groups adjusted for age.
[e]Means are adjusted for age, sex, and location of study.
[f]p≤0.05 in comparison with mean in lowest blood Pb group.
[g]p<0.05 for differences among groups.
[h]Means adjusted for age, race, current smoking status, and workshift.
[i]Data represent the number of cells/µL serum.

5.6.4. Immune-based Diseases

5.6.4.1. Host Resistance

The capacity of Pb to reduce host resistance to bacteria has been known for almost 40 years and was supported by several toxicological studies described in the 2006 Pb AQCD (U.S. EPA, 2006). Biological plausibility has been provided by observations of Pb affecting mechanisms underlying diminished host resistance, e.g., decreased capacity for Th1-driven acquired immune antiviral responses (indicated by the overproduction of PGE_2) and increased inflammatory responses in target tissue resulting in further damage to host protective barriers. Gupta et al. (2002) demonstrated that elevated Pb exposure of mice (>125 mg/kg Pb, 13.0 µg/dL blood Pb) reduced host resistance to viral infections as indicated by an increased viral titre and increased mortality. Host resistance to bacteria such as Listeria requires effective Th1-driven responses including the production of IL-12 and IFN-γ (Lara-Tejero & Pamer, 2004), but these are suppressed by Pb (D. Gao et al., 2007). The lack of IFN-γ can inhibit appropriate and timely macrophage activation. However, beyond this, suppression of NO production and along with it, the microbicidal metabolite peroxynitrite, can compromise host resistance to some bacteria (U.S. EPA, 2006). Recently, mechanisms through which Pb impacts both innate immune cells and natural host defense barriers to increase the likelihood of serious bacterial infections have been delineated further. Kasten-Jolly et al. (2010) showed that developmental exposure of mice to Pb resulted in an upregulation of splenic RNA of caspase-12, a cysteine protease that inhibits the clearance of bacteria both systemically and in the gut mucosa (Saleh et al., 2006).

With limited investigation, the effect of Pb on host resistance to parasitic agents is uncertain. The 2006 Pb ACQD described one study in which Pb-exposed (30-100 mM) mouse macrophages had diminished ability to kill *Leishmania enrietti* parasites (Mauel et al., 1989); however, given the well-established effect of Pb in promoting Th2 activity, it is plausible that Pb could enhance host resistance to parasites that require robust Th2 responses (e.g., helminths) (U.S. EPA, 2006). In a recent study, Pb enhanced host resistance to malaria (Koka et al., 2007). However, this was related to the capacity of Pb to induce eryptosis and the rapid removal of malaria-infected erythrocytes and not to Pb-induced alterations in immune function.

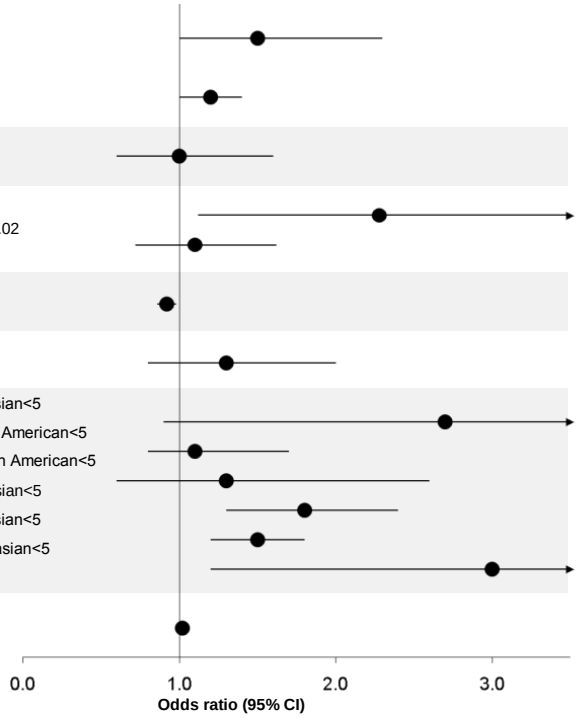
The collective body of epidemiologic data is sparse; however, several studies have found an association between blood Pb levels and respiratory infections. In a Boston-area study of 1978 children (ages 4-8 years), Rabinowitz et al. (1990) reported that compared with children with blood Pb levels <10 µg/dL, children with blood Pb levels ≥ 10 µg/dL were more likely to have parental report “other respiratory infections” (not defined by authors) (OR: 1.5 [95% CI: 1.0, 2.3]), severe ear infections (OR: 1.2 [95% CI: 1.0, 1.4]), and illnesses other than cold or influenza (OR: 1.3 [95% CI: 1.0, 1.5]) (Figure 5-51 and Table 5-25). Analyses did not adjust for any potential confounders. Likewise, without considering

confounders, Karmaus et al. (2005) found that children with more than 10 infections in the previous 12 months had higher blood Pb levels (mean: 3.3 µg/dL) compared with children with 1 to <5 infections (mean: 2.8 µg/dL) or 5-10 infections (mean: 2.6 µg/dL) in the same time period. A unique study was conducted in children in Cordoba, Argentina, in which Pb exposure was assessed by measuring Pb in total deposition samples and in lichen biomonitors from sites near 4 city health clinics (Carreras et al., 2009). Lichen has been recognized for the uptake, accumulation, and sequestration of environmental chemicals from the air. Pb content in lichen and colocated total deposition samples were highly correlated, indicating that the lichen was a suitable indicator of environmental concentrations. In an ecologic analysis that did not consider potential confounders, clinics near sites with higher levels of Pb in total deposition and lichen samples had higher frequency of visits by children for pharyngitis, tonsilitis, and laryngitis. Because other metals, including manganese, iron, copper, and nickel were also associated with respiratory illnesses, it was difficult to characterize the independent effects of Pb. Similar to studies in children, among adults, frequency of self-reported colds or influenza was greater among Pb battery or smelter plant workers (28.8%) than among unexposed controls (16.1%); however, a statistical analysis was not performed on the data (Ewers et al., 1982). Thus, while several studies in humans indicate associations between indicators of Pb exposure and infectious illnesses, they are limited by weak analytic methods and lack of consideration for potential confounders. Evaluation of the relationship between Pb exposure and host resistance in humans would be improved by more longitudinal investigation and rather than group comparisons, analyses of associations between blood Pb levels and viral or bacterial infections.

5.6.4.2. Asthma and Allergy

Toxicological studies and to varying degrees, epidemiologic studies, have demonstrated Pb effects on multiple immunological pathways, including elevated production of Th2 cytokines such as IL-4, increased IgE antibody production (Figure 5-49), and Pb-induced inflammatory cell dysfunction. These are well-recognized pathways in the development of allergy and allergic disease, including asthma. It has been suggested that low exposure to Pb exerts immunostimulating effects in contrast to higher exposure, which has been implicated in suppressing immune function (Mishra et al., 2006). Coherent with the mechanistic evidence, several epidemiologic studies indicate associations between blood Pb levels and asthma and allergy (Figure 5-51 and Table 5-25). In univariate analyses of children near Boston, MA, Rabinowitz et al. (1990) reported a positive association between high blood Pb level (>10 µg/dL) and asthma (RR: 1.3 [95% CI: 0.8, 2.0]) but not eczema (RR: 1.0 [95% CI: 0.6, 1.6]).

Reference	Population	Outcome	Blood Pb Level Mean or Group
Rabinowitz et al. (1990)	Children	Other respiratory infection	≥10 vs. <10
		Severe ear infection	
Rabinowitz et al. (1990)	Children	Eczema	≥10 vs. <10
Jedrychowski et al. (2011)	Children	Positive SPT	Prenatal: 1.16
			Concurrent: 2.02
Pizent et al. (2008)	Adult males	Positive SPT	21.6
Rabinowitz et al. (1990)	Children	Prevalent asthma	≥10 vs. <10
Joseph et al. (2005)	Children, Caucasian	Incident asthma requiring	≥5 vs. Caucasian<5
	Children, African American	medical care	≥5 vs. African American<5
	Children, African American		≥10 vs. African American<5
	Children, African American		<5 vs. Caucasian<5
	Children, African American		≥5 vs. Caucasian<5
	Children, African American		≥10 vs. Caucasian<5
Min et al. (2004)	Adults	BR	2.9



Note: Odds ratios are are standardized to a 1 µg/dL increase in blood Pb level or 1 unit in log of blood Pb. SPT = skin prick test, BR = bronchial responsiveness.

Figure 5-51. Associations of blood Pb levels with immune-based conditions.

Table 5-25. Additional characteristics and quantitative data for results presented in Figure 5-51.

Reference	Population	Blood Pb level mean or group (µg/dL)	Outcome	Odds ratio (95% CI)[a]
Rabinowitz et al. (1990)	Boston area, MA 1768 children	≥ 10 vs. <10[b]	Other respiratory infection Severe ear infection	1.5 (1.0, 2.3) 1.2 (1.0, 1.4)
Rabinowitz et al. (1990)	Boston area, MA 1768 children.	≥ 10 vs. <10[b]	Eczema	1.0 (0.6, 1.6)
Jedrychowski et al. (2011)	Krakow, Poland 224 children followed prenatally to age 5 yr	Prenatal: 1.16 Concurrent: 2.02	Positive SPT	2.3 (1.1, 4.6) 1.1 (0.7, 1.6)
Pizent et al. (2008)	Zagreb, Croatia 50 males, ages 21-67 yr	21.6	Positive SPT	0.92 (0.86, 0.98)
Rabinowitz et al. (1990)	Boston area, MA 1768 children.	≥ 10 vs. <10[b]	Prevalent asthma	1.3 (0.8, 2.0)
Joseph et al. (2005)	Southeastern MI 4634 children followed prospectively from age 1-3 yr	Caucasian ≥5 vs. Caucasian <5[c] African American ≥5 vs. African American <5[d] African American ≥10 vs. African American <5[d] African American <5 vs. Caucasian <5[c] African American ≥5 vs. Caucasian <5[c] African American ≥10 vs. Caucasian <5[c]	Incident asthma requiring medical care	2.7 (0.9, 8.1) 1.1 (0.8, 1.7) 1.3 (0.6, 2.6) 1.8 (1.3, 2.4) 1.5 (1.2, 1.8) 3.0 (1.2, 7.1)
Zhao et al. (2004)	75 children, ages 3-6 yr Zhejiang Province, China	<10 ≥ 10	BR	1.02 (1.00, 1.03)

SPT = skin prick test, BR = bronchial responsiveness.
[a]Odds ratios are standardized to a 1 µg/dL increase in blood Pb level, except in studies in which Blood Pb is analyzed as a categorical variable.
[b]Odds ratio in children with blood Pb level ≥ 10 µg/dL with children with blood Pb level <10 µg/dL as the reference group. No additional covariates included in model.
[c]Relative risk in each specified subgroup with Caucasian children with blood Pb level <5 µg/dL as the reference group. Model covariates include sex, birth weight, and annual income.
[d]Relative risk in each specified subgroup with African American children with blood Pb level <5 µg/dL as the reference group. Model covariates include sex, birth weight, and annual income.

Positive associations between blood Pb level and asthma- and allergy-related conditions also were observed in large studies with multivariate analyses. An additional common strength of these studies was the prospective follow-up of subjects that allowed investigators to establish temporality between the measurement of blood Pb level and onset of disease. In a study of 4,634 children (ages 1-3 years) in southeastern Michigan that controlled for annual income, birth weight, and sex, an elevated risk of incident asthma requiring a doctor visit or medication (indicator of severe asthma) was reported in association with blood Pb levels ≥ 5 µg/dL most strongly among Caucasian children (relative risk [RR]: 2.7 [95% CI: 0.9, 8.1]) (Joseph et al., 2005) (Figure 5-51 and Table 5-25). In analyses restricted to African American children, blood Pb level was weakly associated with asthma requiring medical care (Figure 5-51 and Table 5-25). In analyses that used Caucasian children with blood Pb level <5 µg/dL as the reference group, blood Pb level was associated with statistically significant increases in the risk of asthma among African children in all blood Pb level categories, which indicated a racial/ethnic effect rather than a Pb effect. However, among African American children, the RR was much higher in the ≥ 10 µg/dL blood Pb category (RR: 3.0 [95% CI: 1.2, 7.1]) than in the ≥ 5 µg/dL (RR: 1.5 [95% CI: 1.2, 1.8]) or <5

µg/dL blood Pb categories (RR: 1.8 [95% CI: 1.3, 2.4]), which pointed to a possible race/ethnicity by blood Pb level interaction. These findings should be interpreted with caution due to the small number of asthmatics requiring medical care in the high blood Pb level categories (5 Caucasian children with blood Pb ≥ 5 µg/dL and 9 African American children with blood Pb level ≥ 10 µg/dL).

While the aforementioned studies examined concurrent blood Pb levels, findings from a recent prospective birth cohort study demonstrated that prenatal blood Pb levels (cord blood Pb level mean: 1.16 µg/dL [95% CI: 0.12, 1.22]) was associated with an increased risk of allergic sensitization at age 5 years (Jedrychowski et al., 2011). In models that adjusted for sex, parity, maternal age, maternal education, maternal atopy, and environmental tobacco smoke exposure, measures of prenatal Pb exposure (cord or maternal blood Pb) were associated with greater risk of positive skin prick test (SPT) to dust mite, dog, or cat allergen compared with concurrent blood Pb levels (Figure 5-51 and Table 5-25). The greater risk associated with prenatal blood Pb measures was substantiated by the weak correlation observed between umbilical cord and age 5-year blood Pb levels (r = 0.29). Larger risks were estimated for Pb than for other risk factors, including blood levels of mercury, polycyclic aromatic hydrocarbon DNA adducts, and residential levels of dust mite or pet allergen.

Interestingly, in a study of 216 adults without occupational Pb exposures, Pizent et al. (2008) found that among women, the association between blood Pb level and total IgE was statistically significant, whereas the association with positive SPT to common inhaled allergens was not. An increase in IgE mediates the acute inflammatory response to allergens. Among men, not only was the opposite observed, but the association between blood Pb level and positive SPT was negative (OR: 0.92 [95% CI: 0.86, 0.98] in stepwise regression models that considered age, smoking intensity, and alcohol consumption). Interpretation of the findings is difficult because only statistically significant effect estimates were reported; thus it is not possible to ascertain whether there were suggestions of association in women or whether there were discrepant findings for the related outcomes of IgE and positive SPT.

5.6.4.3. Other Respiratory Effects

Increased bronchial responsiveness (BR) is a characteristic feature of asthma and other respiratory diseases and can result from activation of innate immune responses and increased airway inflammation. Compared with findings for IgE or asthma, evidence of association between blood Pb levels and BR is weak (J. Y. Min et al., 2008; Pizent et al., 2008). In a study of 525 middle-aged adults in Seoul, Korea, Min et al. (2008) found an association between blood Pb levels and BR. Study subjects had a mean (SD) blood Pb level of 2.90 (1.59) µg/dL. A 1 µg/dL increase in blood Pb level was associated with an increase in BR index (log [% decline in forced expiratory volume in 1 second (FEV_1)/log of final methacholine concentration in mg/dL]) of 0.018 (95% CI: 0.004, 0.03), adjusting for age, sex, height, smoking, lung function, and asthma diagnosis (J. Y. Min et al., 2008). In contrast to Min et al. (2008), Pizent et al. (2008)

observed a negative association between blood Pb level and nonspecific BR in men (2.4% decrease [95% CI: -4.2, -0.52%] in percent change FEV_1 post-histamine challenge per 1 µg/dL increase in blood Pb level). Although this counterintuitive finding was not discussed by investigators, it was consistent with the negative association found between blood Pb level and SPT among men in this study.

In less rigorous analyses that compared occupational groups with relatively low Pb exposures, associations of blood Pb levels with lung function were less clearly indicated (A. Y. M. Jones et al., 2008; A. Y. M. Jones et al., 2006). In a comparison of male drivers of buses with (mean blood Pb level: 5.0 µg/dL) and without (mean blood Pb level: 3.7 µg/dL) air conditioning in Hong Kong, China, drivers of non-air conditioned buses had lower exposures to PM_{10}, lower blood Pb levels but lower forced vital capacity and similar FEV_1 (A. Y. M. Jones et al., 2006). In this study, the authors attributed the slightly higher blood Pb levels among air conditioned bus drivers to the poor efficiency in the filters and higher PM_{10} levels measured on those buses versus the non-air conditioned buses. In a comparison of roadside vendors and adjacent shopkeepers, blood Pb levels and various lung function parameters were similar between groups (A. Y. M. Jones et al., 2008). Neither study directly examined associations between blood Pb levels and lung function.

In addition to blood Pb, several recent epidemiologic studies have used Pb measured in PM_{10} and $PM_{2.5}$ air samples to represent Pb exposures. Some studies have analyzed the Pb component individually, whereas others have applied source apportionment techniques to analyze Pb as part of a group of correlated components. A majority of air-Pb studies has found associations with asthma-related morbidity in children and respiratory-related hospitalizations and mortality in older adults (Table 5-26). Despite the concordance between the findings of air-Pb and blood Pb studies, a common limitation of air-Pb studies is the variable size distribution of Pb-bearing PM (Section 3.5.3) and its relationship with blood Pb levels. Additionally, in these air-Pb studies, other PM components such as elemental carbon, copper, and zinc also were associated with respiratory effects. In the absence of detailed data on correlations among components or results adjusted for copollutants, it is difficult to exclude confounding by these other components.

Table 5-26. Associations of air-Pb with respiratory effects

Study	Population	Air-Pb Data	Statistical analysis	Outcome	Effect Estimate (95% CI)[a]
Gent et al. (2009)	CT, MA 149 asthmatic children, ages 4-12 yr 1-yr follow-up 2000-2003	2-day avg Pb-$PM_{2.5}$ Mean: 5.1 ng/m^3	Generalized estimating equations with one-day lag autoregressive structure, adjusted for season, day of week, and date	Wheeze Shortness of breath Fast-acting inhaler use	OR per 5 ng/m^3 increase[b] 1.07 (p = 0.13) 1.12 (p = 0.01) 1.04 (p = 0.10)
Hong et al. (2007)	Dukjeok Island, Korea 43 children, grades 3-6 6-wk follow up March-May 2004	Lag 1 Pb-PM_{10} Mean (SD): 51 ng/m^3 (31)	Linear mixed effects models with random effect for subject adjusted for age, sex, height, weight, asthma history, passive smoking exposure at home, GST genotype, temperature, relative humidity, air pressure, day of week	Morning PEF Daily average PEF	Change per 1 log increase[b] -6.83 L/min (p <0.01) -6.37 L/min (p <0.01)
Ostro et al. (2007)	6 California counties Adults, ages ≥ 65 yr 2000-2003	Lag 3 Pb-$PM_{2.5}$ Mean (IQR): 4 (4) ng/m^3	County-specific poisson regression adjusted for day of week, smoothing splines for temperture and humidity (3 df), smoothing spline for time (4 df). County-specific estimates combined using random effects model	Respiratory mortality All-year Summer-only	RR per 4 ng/m^3 increase 1.011 (0.99, 1.033) Statistically significant, quantitative results not reported
Bell et al. (2009)	106 U.S. counties Adults, ages ≥ 65 yr 1999-2005	Lag 0 Pb-$PM_{2.5}$ Mean (SD): 4.89 ng/m^3 (31)	Bayesian hierarchical regression to combine county-specific estimates adjusted for day of week, seasonality, smooth function of time, daily temperature, previous 3-day's temperature and dew point temperature	Respiratory hospital admissions	Results were reported in a figure. RR per IQR increase was negative with wide 95% CI
Andersen et al. (2007)	Copenhagen, Denmark Adults, ages ≥ 65 yr Children, ages 5-18 yr 1999-2004	Lag 3 "vehicle" PM_{10} factor[c] comprising Pb, copper, iron, and other trace metals	Generalized additive Poisson regression adjusted for smoothed splines for temperature and dew point temperature (4-5 df), smoothed spline for calendar time (3-5 df), influenza, day of week, public holidays, school holidays, and pollen (only for asthma models)	Respiratory hospital admissions (adults ages ≥ 65 yr) Asthma (children ages 5-18 yr)	RR per 0.6 µg/m^3 increase 0.95 (0.89, 1.02) 1.20 (0.98, 1.47)
Sarnat et al. (2008)	20 Atlanta, GA-area counties 1998-2002 All ages	"Woodsmoke" $PM_{2.5}$ factor[c] comprising Pb (minor contribution), potassium, organic carbon, ammonium Mean: 1.6 mg/m^3 (cool season), 0.8 mg/m^3 (warm season)	Generalized linear Poisson regression adjusted for day of week, holidays, hospital, cubic splines for time (monthly knots), temperature, and mean dew point temperature (knots at 25th and 75th percentiles)	Respiratory hospital admissions	Increment NR 0.999 (0.993, 1.004)

OR = odds ratio, PEF = peak expiratory flow, RR = relative risk, NR = not reported.
[a]Effect estimates are reported as given in the study and are not standardized because of variability in exposure metrics among studies.
[b]Investigators did not provide sufficient information to calculate 95% CIs.
[c]Souce apportionment techniques were applied to group correlated components into common source categories.

5.6.4.4. Autoimmunity

The 2006 AQCD described animal studies in which Pb exposure induced the generation of autoantibodies (Bunn et al., 2000; El-Fawal et al., 1999; Hudson et al., 2003; Waterman et al., 1994). Whereas some evidence linked this risk of autoimmunity to a shift towards Th2 responses, other evidence pointed to a shift towards Th1 responses. While recent studies did not examine Pb-induced production of autoantibodies, some provided indirect evidence by indicating that the changes induced by Pb had broader implications for increasing risk of autoimmunity. For example, Kasten-Jolly et al. (2010) examined the

impact of developmental Pb exposure of mice on changes in gene expression in the spleen. They found that Pb upregulated digestive and catabolizing enzymes that could lead to the generation of self-peptides, which in conjunction with other Pb-induced immunomodulatory effects, had the potential to induce the generation of autoantibodies. In Carey et al. (2006), the activation of neoantigen-specific T cells in $PbCl_2$-exposed mice also indicated the potential for autoantibody generation. Evidence of Pb-associated autoimmune responses in humans is limited to a study of male Pb battery workers with blood Pb levels ranging from 10 to 40 µg/dL (El-Fawal et al., 1999). In this study, the Pb-exposed workers had higher levels of IgM and IgG autoantibodies to neural proteins compared with unexposed controls (blood Pb levels not reported) (El-Fawal et al., 1999).

5.6.4.5. Specialized Cells in Other Tissues

Resident macrophages in tissues represent a significant target for Pb-induced immune effects, and alteration in the function of these cells can contribute to organ/tissue dysfunction, cell death, tissue pathology and tissue-specific autoimmune reactions. Among the specialized populations are microglia and astrocytes in the brain, Kupffer cells in the liver, alveolar macrophages in the lung, keratinocytes and Langerhans cells in the skin, osteoclasts in the bone, and preadipocytes in adipose tissue. The effects of Pb on these cells are important as they may contribute to disease in nonlymphoid tissues (Figure 5-52). Because these specialized cells are not always recognized as macrophages, the resulting diseases and conditions are not always recognized as being linked with Pb-induced immune dysfunction.

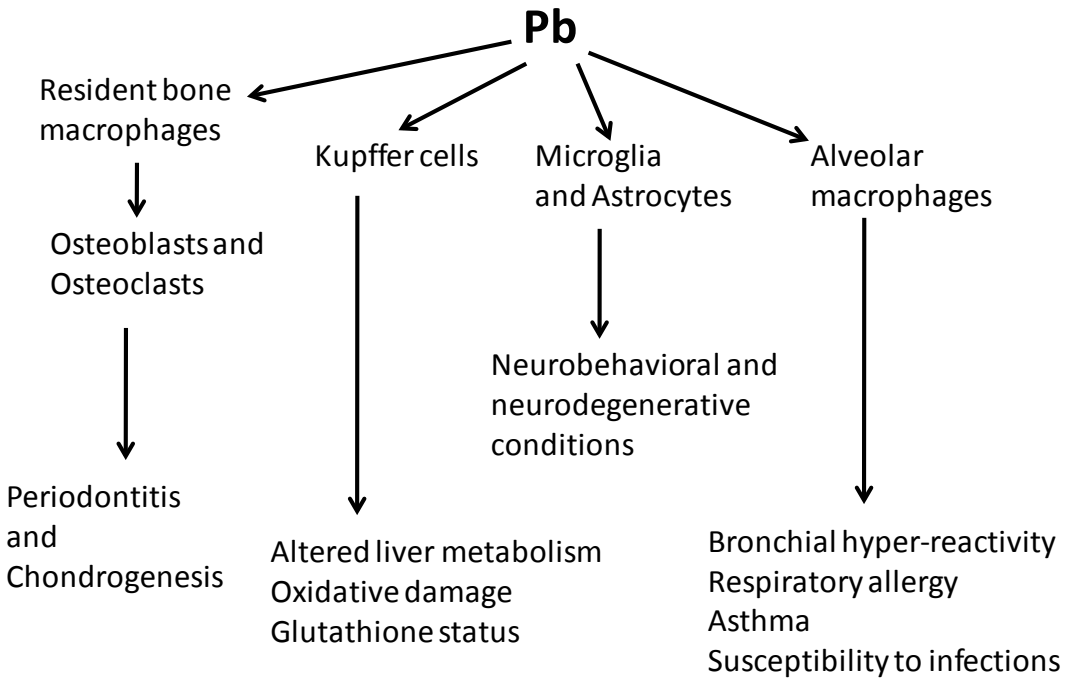


Figure 5-52. Specialized macrophages in nonlymphoid tissue may serve as a significant link between Pb and disease in multiple organ systems.

Fan et al. (2009) reported that Kupffer cells undergo significant changes in phenotypic expression (e.g., CD68 and ferritin light chain), organization, and functional activity connected to Pb-induced apoptosis in the liver. In the central nervous system, subacute exposure of Wistar rats to Pb (15 mg/kg of Pb acetate, i.p.) during early postnatal maturation was observed to produce chronic glial activation with coexisting features of inflammation and neurodegeneration (Struzynska et al., 2007). Among the cytokines detected in the brains of these Pb-treated rats were IL-1β, TNF-α and IL-6. In bone, resident tissue macrophages regulate osteoblast function (M. K. Chang et al., 2008), and osteoblasts are known targets of Pb. This can contribute to later life diseases such as arthritis [reviewed in Zoeger et al. (2006)]. Pb-induced elevation of TGF-β production is also involved in chondrogenesis in bone.

Resident immune cells in reproductive organs are also potential targets of Pb. Pace et al. (2005) reported that Pb exposure in mice contributed to poor reproductive performance that was concomitant with altered homeostasis of the testicular macrophage population in that organ. The authors proposed that increased oxidative damage and apoptosis among these macrophages and reduced potential to maintain organ homeostasis contributed to the observed pattern of male sterility.

5.6.4.6. Tumors

While toxicological evidence supports high doses of Pb directly promoting tumor formation or inducing mutagenesis and genotoxicity (Section 5.10), evidence for involvement of the immune system is limited. Kerkvliet and Baecher-Steppan (1982) observed that male C57Bl/6 mice exposed to 130 and 1300 ppm of Pb acetate in drinking water had enhanced moloney sarcoma virus-induced tumor growth compared with control animals. These findings indicated that Pb-induced immunomodulation affecting tumors likely results from a combination of suppressed Th1 responses and increased inflammation leading to excessive release of ROS into tissues. The promotion of cancer is a relatively common outcome in chemical-induced immuntoxicology, particularly when early life exposures are involved (Dietert).

5.6.5. Mechanisms of Lead-Induced Immunomodulation

5.6.5.1. Inflammation

Misregulated inflammation represents one of the major immune-related effects of Pb and a major mode of action for Pb effects in multiple organ systems (Section 5.2.5). It is important to note that inflammation can manifest in any tissue where immune cell mobilization and tissue insult occurs (as with an infection). Enhanced inflammation and tissue damage occurs through the modulation of inflammatory cell function and production of proinflammatory cytokines and metabolites. Among the problems presented by this immunomodulation are the overproduction of ROS and an apparent depletion of antioxidant protective enzymes and factors (e.g., selenium). Chetty et al. (2005) reported that Pb-induced inflammatory damage involves the depletion of glutathione. 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 5.2.4).

Traditional immune-mediated inflammation can be seen with asthma, respiratory infections, and BR in association with Pb exposure. Nonetheless, inflammation also is a general response to tissue injury whether mediated by infection, toxic insult, or other stresses. Thus, Pb-induced misregulation of inflammation could exacerbate disease and damage in almost any organ given the distribution of immune cells as both permanent residents and infiltrating cell populations. As described in Section 5.2.5, misregulation of inflammation represents a potential mode of action for Pb induced effects on the liver, kidney, and vasculature.

In epidemiologic studies, whereas Pb-associated changes in proinflammatory cell function (Section 5.6.2) and cytokine production (Section 5.6.5.4) have been demonstrated, less certain are the effects of Pb exposure on nonspecific indicators of inflammation that may be related to multisystemic effects as have been demonstrated in toxicological and in vitro studies. Using 1999-2004 NHANES data for adults 40

years of age or older, Songdej et al. (2010) examined the relationship between blood Pb levels (mean: 1.89 µg/dL) and the inflammation markers, C-reactive protein (CRP), fibrinogen, and white blood cell (WBC) count. Adjusting for age, sex, race/ethnicity, education, income, body mass index (BMI), physical activity, smoking status, diabetes status, inflammatory disease status, and cardiovascular disease status, the researchers found that men appeared to be more susceptible than women to Pb-associated inflammation. Among women, most odds ratios for associations between quintiles of blood Pb level and tertiles of CRP, fibrinogen, and WBC count were less than 1.0 whereas corresponding odds ratios in men tended to be greater than 1.0. Additionally, compared with men with blood Pb levels less than 1.16 µg/dL, men with blood Pb levels of 1.16-1.63 µg/dL, 2.17-3.09 µg/dL, and ≥ 3.09 µg/dL had statistically significant increases in CRP (ORs: 2.22, 2.12, 2.85, respectively). For all inflammation markers, although the highest quintile of blood Pb level was associated with the largest odds ratio, a blood Pb dose-dependent association was not observed. Consistent with these findings, among men in Incheon, Korea without occupational Pb exposures, Kim et al. (2007) reported positive associations of blood Pb level with WBC as well as with IL-6. These findings of associations between blood Pb levels and inflammatory mediators are consistent with Pb effects on promoting a Th2 phenotype. Th2 cells produce IL-6 which is the primary stimulus for expression of CRP and fibrinogen (Fuller & Zhang, 2001; Hage & Szalai, 2007).

In a genome-wide association study that included 37 autistic and 15 nonautistic children (ages 2-5 years; blood Pb level range: 0.37 to 5.2 µg/dL) in California, in models that included age, sex, and autism diagnosis, blood Pb level was associated with the expression of several genes related to immune function and inflammation, including human leukocyte antigen genes (HLA-DRB) and MHC Class II-associated invariant chain CD74 (involved in antigen presentation) (Y. Tian et al., 2011). Although blood Pb levels were similar between autistic and nonautistic children and correlations were observed in both groups, they were in opposite directions (positive among autistic and negative among nonautistic). These results are consistent with findings that Pb increases MHC molecule surface expression in mouse and human HLA antigen presenting cells (Guo et al., 1996a; M. J. McCabe & Lawrence, 1991) and also suggest that Pb-associated changes in the expression of immune genes may be modified by underlying autism.

5.6.5.2. Increased Prostaglandin E_2 and Decreased Nitric Oxide

Consistent with the findings presented in the 2006 Pb AQCD (U.S. EPA, 2006), recent studies continue to show that Pb exposure alters the levels of signaling molecules. These signaling molecules are involved in mediating inflammation and host resistance (Figure 5-47). For example, Pb exposure increases arachidonic acid metabolism, elevating the production of prostaglandin E_2 (PGE_2) (Chetty et al., 2005). Additionally, production of nitric oxide (NO) by macrophages is decreased at low-moderate exposure levels (Farrer et al., 2008; Pineda-Zavaleta et al., 2004). Decreases in NO can impact not only innate host defenses, but also, acquired immunity. This is proposed to occur via the Pb-induced release of

myeloid cell (CD11b+)-mediated suppression of CD4+ T cell proliferation (Farrer et al., 2008). The result is that Pb exposure leads to an increased production of alloreactive T and B cells. Farrer et al. (2008) found that Pb decreases inducible nitric oxide synthease function in myeloid cells without decreasing its abundance. The resulting loss of NO production also leads to a reduction in the production of the microbicidal metabolite peroxynitrite, thus weakening host defenses against bacteria. When this is 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 signal against pathogenic challenge, multiple arms of the host defense against infectious challenge can be compromised. The loss of NO production in innate immune cells such as macrophages would be expected to affect other physiological systems (e.g., neurological, cardiovascular, endocrine) that require NO signaling cascades.

Relative to studies in animal and in vitro models, fewer epidemiologic studies have examined the effects of Pb on signaling molecules; however the limited data support associations of blood Pb level with suppressed NO production (Barbosa et al., 2006; Mishra et al., 2006; Pineda-Zavaleta et al., 2004; Valentino et al., 2007) and increased ROS production (Pineda-Zavaleta et al., 2004). Among children, in Pineda-Zavaleta et al. (2004), with increasing residential proximity to the Pb smelter, mean blood Pb levels increased (7.02 to 20.6 to 30.38 µg/dL) as did superoxide anion release from macrophages (directly activated by IFN-γ/LPS) isolated from children. NO release from macrophages (indirectly activated by phytohemagglutinin, PHA) decreased with increasing blood Pb level. After adjusting for age and sex, a 1 µg/dL increase in blood Pb level was associated with a decrease in NO of 0.00089 (95% CI: -0.0017, -0.00005) nmol/µg protein and an increase in superoxide anion of 0.00389 (95% CI: 0.00031, 0.00748) µmol/mg protein. Because PHA activates macrophages indirectly through the activation of lymphocytes and IFN-γ directly activates macrophages, these results suggest that Pb suppressed T cell-mediated macrophage activation and stimulated cytokine-induced macrophage activation. Results also demonstrated a larger magnitude of association between blood Pb levels and superoxide anion release in males. Although not described in detail, the association between blood Pb level and NO was reported to be not negative in girls. Barbosa et al. (2006) also observed negative associations between blood Pb level and plasma NO (quantitative results not provided) in a group of adults in Sao Paolo, Brazil residing near a battery plant, although it was not possible to identify immune cells as the specific sources of NO.

In contrast to studies of populations without occupational Pb exposures, studies of occupationally-exposed groups provided less clear indication of association of blood Pb level with NO and ROS. Among 30 male Pb recycling plant workers and 27 unexposed controls, despite large differences in blood Pb levels, levels of ROS released from neutrophils (indicators of respiratory burst) were similar between workers and controls (Mishra et al., 2006). NO production of neutrophils after stimulation with zymosan-A was lower in controls, but the difference was not statistically significant. In a study of male foundry workers (mean blood Pb level: 21.7 µg/dL), pottery workers (mean blood Pb level: 9.7 µg/dL), and unexposed workers (mean blood Pb level: 3.9 µg/dL), Valentino et al. (2007) also found lower plasma NO

levels in controls compared with Pb-exposed workers. The means (ranges) of plasma NO levels in controls, pottery workers, and foundry workers were 23.73 µM (11.21 to 55.71 µM), 28.44 µM (15.23 to 57.65 µM), and 25.30 µM (15.03 to 61.98 µM), respectively. Further, although quantitative results were not reported, blood Pb level was reported to be not correlated with NO.

5.6.5.3. Cellular Death (Apoptosis, Necrosis)

In a study in mice, Bishayi and Sengupta (2006) found that Pb exposure elevated DNA fragmentation in splenic macrophages. Using mouse resident peritoneal macrophages, Gargioni et al. (2006) found that inorganic Pb induced both necrosis and apoptosis in vitro. While the exact pathways involved were not determined, the authors concluded that activation of the Bax pro-apoptotic protein was not the key effect of Pb on inducing macrophage apoptosis. In an in vivo study in mice, Xu et al. (2008) found that a 4-week administration of Pb acetate (50-100 mg/kg, oral) significantly elevated both ROS and malondialdehyde (an indicator of ROS-induced peroxidation) levels in peripheral blood lymphocytes. The Pb-induced DNA damage, determined by the comet assay, was accompanied by elevations in p53 and Bax expression with no change in Bcl-2 expression (creating a Bax/Bcl-2 imbalance). The authors propose that this is a likely route to Pb-induced apoptosis and tumorigenesis.

5.6.5.4. Cytokine Production

Toxicological studies have indicated that Pb affects immune cytokine production via action on T cells, macrophages, and dendritic cells (Section 5.6.2). The combination of these three pathways of cytokine changes induced by Pb creates a hyperinflammatory state among innate immune cells, and acquired immunity is skewed toward Th2 responses. As illustrated in Figure 5-47, downstream effects include altered IgE production, ROS production, and inflammation. Cheng et al. (2006) found that Pb exposure affected TNF-α production in macrophages by affecting signaling pathways. This can result in local tissue damage during the course of immune responses affecting such targets as the liver. In a study involving macrophage-mediated liver injury in mice (A/J), substimulatory levels of Pb (10 µM) coadministered with LPS stimulated the phosphorylation of p42/44 mitogen-activated protein kinase (MAPK) and TNF-α expression (Y.-J. Cheng et al., 2006). Blocking protein kinase C or MAPK reduced TNF-α production of macrophages in vitro, which in turn, protected against Pb + LPS-induced liver injury in vivo. These findings are consistent with those of Gao et al. (2007), which showed that treatment of mouse dendritic cells with Pb produced an increased phosphorylation of the Erk/MAPK signaling molecule.

The most consistent immunomodulatory effect of Pb is the skewing of immune responses away from Th1 and toward Th2. Pb was observed to skew toward Th2 cytokine production in both dendritic cells (5.6.2.6) and T cells. Lynes et al. (2006) observed that Pb suppressed the production of Th1 cytokine

IFN-γ. Gao et al. (2007) observed that Pb exposure elevated production of Th2 cytokines such as IL-4, IL-5 and IL-6. This shift to a Th2 phenotype also was demonstrated in cultures of human blood monocytes activated with *Salmonella enteritidis* or with monoclonal antibodies of CD3, CD28, and CD40 and exposed to inorganic Pb. Pb exposure 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 (Hemdan et al., 2005).

In a recent study conducted across a lifetime (developmental through adulthood) using a broad range of dietary Pb concentrations in Swiss mice (females and males), Iavicoli et al. (2006) suggested a nonlinear hierarchical cytokine response. At the lowest dietary Pb concentration (0.8 µg/dL), IL-2 and IFN-γ were elevated over the controls, indicating an enhanced Th1 response. However, as dietary Pb exposure 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 low Pb exposures and that compensatory mechanisms may be stimulated at low Pb exposures. Other studies have found variable Pb-induced changes in IL-2, with no change or elevated production, depending upon the protocol used. Recently, Gao et al. (2007) found that Pb-treated dendritic cells promoted a slight but statistically significant increase in IL-2 production among lymphocytes. A recent study on bone (in vivo and in vitro) chondrogenesis found Pb-induced increases in production of TGF-β (Zuscik et al., 2007).

Consistent with toxicological studies, epidemiologic studies also find higher blood Pb levels in humans to be associated with a shift towards production of Th2 cytokines relative to Th1 cytokines. One consequence of an increase in the Th2 cytokine IL-4 is the activation of B cells to induce B cell class switching to IgE. In particular, Lutz et al. (1999) provided evidence for Pb affecting this pathway by finding that children with blood Pb levels greater than 10 µg/dL had both higher levels of IL-4 and IgE (Section 5.6.3). Among adults in Incheon, Korea without occupational Pb exposures with blood Pb levels ranging from 0.337 to 10.47 µg/dL, Kim et al. (2007) found associations of blood Pb level with serum levels of TNF-α and IL-6 that were larger among men with blood Pb levels ≥ 2.51 µg/dL (median). 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 in log of IL-6 (95% CI: 0, 55%). The associations between levels of blood Pb and plasma TNF-α were 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 metabolism of ROS, may confer increased susceptibility to Pb-associated immune effects. The results for the TNF-α polymorphism were 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.

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, analysis were mostly limited to comparisons of mean cytokine levels among different blood Pb groups or Pb-exposure groups (Di Lorenzo et al., 2007; Valentino et al., 2007; Yucesoy et al., 1997a). The exception was the study of male foundry workers, pottery workers, and unexposed workers by Valentino et al. (2007). Multiple regression analyses were performed with age, BMI, smoking, and alcohol consumption included as covariates. Although effect estimates were not reported, statistically significant associations were observed between blood Pb level and IL-10 and TNF-α. Levels of IL-2, IL-6, and IL-10 showed an increasing trend 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). Excluded from this study were exposed workers from the highest end of the distribution of blood Pb levels in DiLorenzo et al. (2006). Mean TNF-α levels showed a monotonic increase from the low to high blood Pb level group. A synergistic effect was observed between blood Pb levels and smoking. Among current smokers, a 12- to 16-fold difference in TNF-α levels was observed among blood Pb groups, whereas a less than twofold difference was observed among nonsmokers. In Yucesoy et al. (1997a), levels of the Th1 cytokines, IL-1β and IFN-γ, were lower in workers than in controls; however, differences were not observed in levels of the Th2 cytokines, IL-2 and TNF-α.

5.6.6. Immune Effects of Lead within Mixtures

One of the most striking observations regarding Pb effects on the immune system since the 2006 Pb AQCD concerns the effects of metal mixtures. Recent studies indicate that immune effects may be observed with lower levels of Pb exposure when they occur in conjunction with other metals. Information on interactions among metal mixtures may improve risk assessment methods because most human exposures to chemicals involve mixtures at low environmental levels. In a study of mice exposed to Pb acetate (10 mg/kg by weight), As (0.5 mg/kg by weight), or both, Bishayi and Senguta (2006) reported a greater than additive effect of coadministered Pb and As on macrophages in producing a decrease in bacterial resistance, myeloperoxidase (MPO) release, and NO production. Investigators assessed the Pb-As interaction using the multivariate ANOVA for the experimental results of MPO release and constructing an isobologram by running an ordinary least squares regression between effects (% MPO release) and dose levels of metals (single and multimetal) in log-linear form.

Institoris et al. (2006) reached a similar conclusion after observing that lymph node weight decreased with exposure to 20 mg/kg Pb plus a second metal (Cd or Hg) but not with 20 mg/kg of Pb alone. Another study conducted in rats (Jadhav et al., 2007) found that mixtures of Pb, Hg, and other

metals at 10 to 100 times the concentrations of the individual components typically measured in drinking water in India suppressed lymphocyte counts and antibody titers and increased neutrophil counts. In contrast with these aforementioned studies, Fortier et al. (2008) found that $PbCl_2$-exposed (7.5-20.7 µg/dL) human leukocytes did not have alterations in lymphocyte proliferation, monocytic phagocytic activity, or NK cell activity. Although the combination of 20.7 µg/dL $PbCl_2$ plus 12.0 µg/dL methylmercuric chloride (MeHgCl) decreased lymphocyte proliferation, these effects were attributed to MeHgCl, which had a stronger suppressive effect individually. The majority of evidence indicating synergism between Pb and metals such as As, Cd, and Hg suggests that a threshold for producing Pb-induced immune effects may be lower if additional metals are present.

In a study designed to mimic exposure to particles associated with urban traffic, Wei et al. exposed human endothelial cells in culture (EA.hy926) to urban fine particles ($PM_{2.5}$ enriched with Pb and other metals). Investigators found that the particle mixture increased ROS production and mitochondrial-mediated apoptosis; however, they did not test metals individually and could not attribute findings to Pb individually or interactions between Pb and other metals within the mixture. Similarly, in two studies of mice exposed to Pb and Cd in drinking water (1 ppm of each metal in drinking water for 28 days), Pb and Cd altered antibody titres (Massadeh et al., 2007). The main aims of these studies were to demonstrate that the effects of Pb and Cd could be reversed with administration of Nigella sativa L (black cumin) or garlic extract. However, investigators did not test each metal individually to assess interactions between metals.

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 5.6.5.2) reported interactions between Pb and As. In addition to Pb, As contamination of drinking water was a concern in the study area; however, urinary As levels were higher in children who had lower blood Pb levels. In multiple regression analyses, urinary As was negatively associated with NO (similar to Pb), and a statistically significant negative interaction was observed between Pb and As, indicating that high internal doses of both metals were associated with a larger decrease in NO than that associated with either metal alone. Urinary As was negatively associated with superoxide anion (opposite direction of Pb), and the Pb by As interaction was positive. Thus, although higher urinary As was associated with a lower superoxide anion level, higher internal doses both Pb and As were associated with a larger increase in superoxide anion than that associated with blood Pb level alone.

5.6.7. Summary and Causal Determination

The collective body of evidence integrated across epidemiologic and toxicological studies consistently demonstrates that the immune system is a major target of Pb. Rather than resulting in overt cytotoxicity to lymphoid tissues, Pb exposure has been associated predominantly with more subtle

changes in a spectrum of immune mediators and functions. The importance of Pb as an immunomodulator is particularly evident when one considers: (1) the relative sensitivity of the immune system to Pb-induced modulation; (2) the lifetime health ramifications of exposure to Pb in early development, particularly given the recognized sensitivity of the developing immune system to environmentally-induced programming; and (3) the consequent broad spectrum of diseases and illnesses in multiple physiological systems potentially related to Pb-associated immune dysfunction. The majority of results from animal studies indicates that immune changes are observable at blood Pb levels of <8.0 µg/dL, with new evidence in mice demonstrating that nonmonotonic cytokine changes occur at blood Pb levels of 2-3 µg/dL. Consistent with the animal studies, in the newly expanded body of epidemiologic studies in children and adults without occupational Pb exposures, changes in immune parameters are commonly demonstrated in association with mean blood Pb levels in the range of <2 µg/dL to 10 µg/dL.

The strength of evidence for Pb-associated immune effects is derived not only from the consistency of associations but also from the coherence of findings between toxicological and epidemiologic studies and coherence of findings among the spectrum of immune changes operating within particular pathways. A large body of toxicological evidence demonstrates Pb-induced suppression of T cell proliferation, and epidemiologic evidence in children consistently links elevated blood Pb levels (as low as 2.2-3.4 µg/dL) with decreases in T cell abundance. These changes can affect cell-to-cell interactions that mediate acquired immunity required in subsequent memory responses to antigen exposures. Despite the consistency of evidence for some T-cell subtypes (e.g., CD3+, CD4+), it is unclear what effect the observed magnitudes of decrease may have in attenuating acquired immunity.

The prominent effect of Pb exposure on T cells, in terms of coherence with effects on other immune endpoints and implications for developing immune-based diseases, is the skewing of immune function away from a Th1 phenotype towards a Th2 phenotype. In toxicological studies, this shift is well-established by suppressed production of Th1 cytokines (e.g., IFN-γ) and increased production of Th2 cytokines (e.g., IL-4). A recent toxicological study builds on this extant evidence by indicating that Pb may promote Th2 responses by acting directly on dendritic cells, the major effector in antigen response. Findings from recent epidemiologic studies strengthen the evidence with observations of similarly skewed cytokine profiles in association with blood Pb levels in humans in the range of 2.5-10 µg/dL. Further, toxicological and epidemiologic studies link the Pb-associated predominance of Th2 cytokines with downstream effects on humoral immunity by demonstrating Pb-associated changes in B cell abundance and changes in circulating antibody levels. An increase in IL-4 from activated Th2 cells induces differentiation of B cells into antibody-producing cells, thereby amplifying B cell expansion to secrete IgE, IgA, and IgG. IgE is the primary mediator for type 1 hypersensitivity resulting in various allergic conditions and asthma. In support of this well-established mechanism, toxicological studies describe Pb-induced (blood Pb levels 10-30 µg/dL) changes in IgA, IgG, and IgM. Additionally, epidemiologic studies in children consistently demonstrate that blood Pb levels are positively associated

with B cell abundance (blood Pb levels in the range of 5-10 µg/dL) and increases in IgE (blood Pb levels as low as 2.8-3.4 µg/dL). Observations of Pb-associated increases in Th2 responses and circulating IgE levels provide biological plausibility for epidemiologic observations in children of associations of blood Pb levels (in the range of 1.16-10 µg/dL) with asthma and allergic conditions. Such epidemiologic data are limited, and additional studies with more rigorous methodology (e.g., longitudinal design to establish temporality, improved assessment of Pb exposures, adjustment for potential confounders such as smoking, SES, and exposures to other metals) are needed to substantiate the findings.

Suppression of Th1 function by Pb places individuals at greater risk of certain infectious diseases and cancer. Compared with the relationships between Pb-suppressed Th1-dependent antitumor processes and effects on tumor formation, Pb effects on decreasing host resistance are relatively well-established. Pb exposure of animals (resulting in blood Pb levels <2-5 µg/dL) suppresses the DTH response, and a recent in vitro study indicates such effects may be mediated by dendritic cells. Further evidence of Pb-associated suppressed Th1 activity is provided by toxicological and epidemiologic observations that Pb exposure and blood Pb levels, respectively, are associated impaired phagocytic and chemotactic activity of macrophages and neutrophils (blood Pb levels >25 µg/dL in humans). Th1-dependent IFN-γ normally enhances the killing capacity of macrophages. Epidemiologic studies additionally demonstrate that higher blood Pb levels are associated with lower neutrophil respiratory burst (superoxide anion release), indicative of diminished degradation of phagocytosed particles. Toxicological and epidemiologic evidence for suppressed Th1 activity and effects on macrophage and neutrophil functional activities provide biological plausibility for observations in animals and humans for associations between blood Pb levels (in the range of 3-10 µg/dL in humans) and increased risk of infection.

Toxicological studies demonstrate that Pb induces macrophages into a hyperinflammatory state as characterized by enhanced production of ROS, suppressed production of NO, enhanced production of TNF-α, and excessive metabolism of arachidonic acid into immunosuppressive metabolites (e.g., PGE_2). Although examined in fewer studies, epidemiologic studies find higher blood Pb levels (in the range of 6-20 µg/dL) to be associated with higher serum levels of ROS, TNF- α and lower serum levels of NO. These proinflammatory effects of Pb exposure on macrophages provide support for associations of Pb with elevated risk of disease in multiple physiological systems. The strongest evidence comprises Pb-induced changes in specialized macrophages such as alveolar macrophages, testicular macrophages, and microglia, whose altered homeostasis or function may contribute to documented associations of blood Pb levels with asthma and bronchial reactivity, poor reproductive performance, and neurodegeneration, respectively. Although limited mostly to toxicological studies, Pb has been shown to induce the generation of autoantibodies, suggesting that Pb exposure may increase the risk of developing autoimmune conditions. These findings are supported by Pb-induced inflammation and tissue damage and Pb-induced T cell activation in response to new antigens.

In summary, recent toxicological and epidemiologic studies support the strong body of evidence presented in the 2006 Pb AQCD that Pb exposure is associated with changes in immune cell abundance and function that subsequently lead to a broad spectrum of changes in both cell-mediated and humoral immunity to promote a Th2 phenotype and hyperinflammatory state. Toxicological and epidemiologic findings of Pb-associated decreases in T cells, inhibition of Th1-type responses, and impaired phagocytic activity of macrophages and neutrophils provide biological plausibility for evidence linking Pb exposure with increased risk of bacterial and viral infection. Additionally, toxicological and epidemiologic evidence of a Pb-associated promotion of the Th2 phenotype, increased B cell abundance, increased synthesis of IgE, and increased inflammation support observations in children for associations of blood Pb levels with asthma and allergic conditions. Although not widely examined in humans, toxicological findings indicate that Pb-induced immunomodulation may have broader implications for autoimmunity and mediating Pb effects in other physiological systems such as the nervous, cardiovascular, and reproductive systems. Animal studies and to a limited extent, epidemiologic studies, demonstrate increased susceptibility from prenatal exposures and enhanced responses with co-exposures to other metals. The consistency of findings among toxicological and epidemiologic studies and the coherence of findings between these disciplines and across the continuum of related immune responses are sufficient to conclude that there is a **causal relationship between Pb exposures and immune system effects.**

5.7. Effects on Heme Synthesis and Red Blood Cell Function

5.7.1. Summary of Findings from 2006 Pb AQCD

The 2006 Pb AQCD reported that Pb affects developing RBCs (red blood cells [RBC]) as noted by anemia observed with blood Pb >40 µg/dL. Pb-induced anemia is thought to occur due to decreased RBC life span and effects on hemoglobin (Hb) synthesis. The exact mechanism for these effects was not known, although Pbinduced changes on iron uptake or inhibition of enzymes in the heme synthetic pathway may be responsible.

The 2006 Pb AQCD indicated that Pb crosses RBC membranes through passive (i.e., energy-independent) carrier-mediated mechanisms including a vanadate-sensitive Ca^{2+} pump. Once Pb enters the cells, it is predominantly found in protein-bound form, with Hb and aminolevulinic acid dehydratase (ALAD) both identified as targets. Pb poisoning decreases RBC survival, as well as alters RBC mobility and morphology, although the precise mechanisms by which it does so are not known. Pb exposure has been found to significantly decrease several hematological parameters including Hb, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular

hemoglobin concentration (MCHC). Pb has also been observed to exert multiple 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 potentially lead to RBC fragility, abnormal cellular function, RBC destruction, and ultimately anemic conditions. Pb exposure also results in increased activation of RBC scramblase, an enzyme responsible for the expression of phosphotidylserine (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 affects heme synthesis through the inhibition of multiple key enzymes, most notably ALAD, the enzyme that catalyzes the second, rate-limiting step in heme biosynthesis (See Figure 5-53 for a schematic representation of the heme biosynthetic pathway). The 2006 Pb AQCD further reported that decreased RBC ALAD activity is the most sensitive measure of Pb exposure with a concentration-response change in the ratio of activated/nonactivated ALAD activity that is not dependent on the method of Pb administration. The inhibition of the ALAD enzyme was observed in RBCs from multiple species, including birds, Cynomolgous 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 iron 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 possible biomarker of Pb exposure. Inhibition of P5N results in an intracellular increase in pyrimidine nucleotides leading to hemolysis. 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.

Pb was found to alter the activity of membrane-bound ion pumps. Potassium (K^+) permeability was found to be increased by Pb due to altered sensitivity of the membrane calcium (Ca^{2+})-binding site that caused selective efflux of K^+ ions from the RBC membrane. Inhibition of RBC sodium (Na^+)-K^+ adenosine triphosphate synthase (ATPase), acetylcholinesterase (ACh), and NADH dehydrogenase was also observed. In human RBCs, Na^+-K^+ ATPase activity was more sensitive to Pb exposure than Ca^{2+} or magnesium (Mg^{2+}) ATPases.

The 2006 Pb AQCD document identified oxidative stress as an important potential mechanism of action resulting from Pb exposure. Increased lipid peroxidation and inhibition of antioxidant enzymes (e.g., superoxide dismutase [SOD], catalase [CAT]) was observed following exposure to Pb.

5.7.2. Effects on Red Blood Cell Functions

As stated in the 2006 Pb AQCD, Pb poisoning is associated with anemia resulting from shortened RBC life span and Pb effects on Hb synthesis. As of 2006, the mechanism for this was not clear, but it was determined not to be due to iron deficiency, which can be found to occur independently of Pb exposure. However, Zimmerman et al. (2006) found that blood Pb levels were statistically significantly reduced in non- or mildly anemic, iron-deficient children in India fed an iron-fortified diet for 30 weeks ($p < 0.02$); blood Pb levels were not reduced in the children not receiving the iron-fortified diet. Although a number of studies find decreases in RBCs and/or Hct levels associated with Pb, it is not known whether this is due to reduced cell survival or a decrease in RBC cell production. However, 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.

5.7.2.1. Lead Uptake, Binding, and Transport into Red Blood Cells

The 2006 Pb AQCD reports 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 leaves 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 was 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. (1997) suggested that ALAD was the primary Pb binding protein and not Hb. The 2006 Pb AQCD also reports 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 4 (Section 4.2).

Although no studies were indentified that examined transport of Pb into RBCs, Lind et al. (2009) recently observed that several zinc (Zn) ionophores (8-hydroxyquinoline derivatives and Zn and Na pyrithione) were able to effectively transport Pb out of RBCs into the extracellular space.

5.7.2.2. Red Blood Cell Survival, Mobility, and Membrane Integrity

Although it is known that Pb exposure shortens the RBC life span and alters 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 in free Ca^{2+}. There are also newer studies that

examine the relationship between Pb and RBC survival, mobility, and membrane integrity. Pb-exposed workers in a recycled automobile battery factory in Mexico had 7.5 times as much blood Pb as was found in unexposed workers (74.4 ± 21.9 µg/dL in 15 exposed workers versus. 9.9 ± 2 µg/dL in 15 unexposed workers, $p < 0.01$) (Quintanar-Escorza et al., 2007). Intracellular RBC free calcium levels $[Ca^{2+}]_i$ were significantly higher in the Pb-exposed workers than in the unexposed workers (79 ± 13 versus. 30 ± 9 nM, $p < 0.05$). The level of $[Ca^{2+}]_i$ was highly correlated ($R^2 = 0.754$) with blood Pb levels in exposed workers; $[Ca^{2+}]_i$ was also elevated in the unexposed workers who had low blood Pb levels ranging from 7.9 µg/dL to 11.9 µg/dL. The observed elevation in $[Ca^{2+}]_i$ was related to an increased uptake and a decreased efflux due to reduced Ca^{2+}-Mg^{2+}-ATPase activity. In the RBCs of Pb-exposed workers, the activity of Ca^{2+}-Mg^{2+}-ATPase was 28 ± 8 nmol Pi/mg protein/min versus. 40 ± 9 in unexposed workers ($p < 0.05$). These changes were associated with increased fragility of the RBCs and dramatic morphological alterations, including the increased presence of ecinocytes (cells without normal biconcave shape) and crenocytes (speculated cells) in Pb-exposed workers. Similar dose-dependent effects were observed when RBCs from healthy subjects were incubated with Pb at concentrations from 0.2 to 6.0 µM (Quintanar-Escorza et al., 2010). Abam et al. (2008) also observed a decrease in the activity of RBC membrane bound Ca^{2+}-Mg^{2+}-ATPase in workers exposed to Pb in a number of occupations. While the authors did not observe an increase in $[Ca^{2+}]_i$ or Mg in RBCs from exposed workers, the RBC membrane $[Ca^{2+}]_i$ and Mg^{2+} concentration were increased. Abam et al. (2008) did not report on morphological changes in erythrocytes from exposed workers. Ciubar et al. (2007) found that RBC morphology was disrupted with 50% or more of RBCs exposed to Pb concentrations of 0.5 µM or higher for 24 hours at 37°C having lost the typical discocytic morphology and displaying moderate to severe echinocytosis. Exposure of RBCs to higher concentrations of Pb nitrate resulted in cell shrinkage. Ademuyiwa et al. (2009) observed that the cholesterol content of RBC plasma membranes, but not the phospholipid content, was statistically significantly higher in rats exposed to 200 ppm Pb acetate (blood Pb = 40.63 ± 9.21 µg/dL) through drinking water compared to controls. Further, the cholesterol/phospholipid ratio was increased in the rats with increased cholesterol, indicating that RBC membrane fluidity was decreased.

A number of studies have investigated the effect of occupational exposure to Pb on various hematological parameters. (Ukaejiofo et al., 2009) studied hematological effects of Pb in 81 male subjects exposed to Pb at three different manufacturing companies in Nigeria. Two control groups were used for comparison (30 individuals from the same industries not involved in handling Pb and 20 individuals from the same locality but not involved in Pb handling). The exposed individuals had an average blood Pb level of 7.00 µg/dL compared to 3 µg/dL in controls drawn from industries not involved in Pb handling (control group I) and 2 µg/dL in controls drawn from the general population (control group II) ($p < 0.05$). Exposed subjects had significantly reduced Hb and packed cell volume (PCV) levels and increased percentage of reticulocytes. Although the differences were significant between the exposed and control subjects, the study authors state that the results in the exposed subjects were at the lower range of normal for

Nigerians. The percent cell lysis did not 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 control group II ($p < 0.01$). Patil et al. (2006) examined hematology effects in jewelry workers in India occupationally exposed to Pb. Blood Pb was significantly higher in the jewelry workers (48.56 ± 7.39 µg/dL) compared to individuals not occupationally exposed (12.52 ± 4.08 µg/dL). There was no significant difference in the Hb, MCV, or MCH; however, jewelry workers had a significant decrease in PCV and total RBCs, accompanied by a significant increase in MCHC. In addition, the exposed workers had a significant increase in total leukocyte levels. In battery manufacturing workers in India, significant decreases in Hb, PCV, MCV, MCH, MCHC, and total RBCs were observed (Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006) with similar blood Pb levels (53.63 ± 16.98 µg/dL). Industrial workers in Pakistan occupationally exposed to Pb (blood Pb = 29.1 (range 9.0-61.1) µg/dL) had a significant increase (3.5-fold higher median) in blood Pb levels compared to age and gender matched controls (blood Pb = 8.3 (range 1.0-21.7) µg/dL) (D. A. Khan et al., 2008). The industrial workers had a significantly lower Hb, but not a significant difference in the number of RBCs. There were no significant differences in the number of white blood cells (WBCs) or in platelet counts. Karita et al. (2005) examined the relationship between hematological parameters in Pb-exposed workers (blood Pb = 26.9 µg/dL) in a variety of occupational settings. Blood Pb was found to be negatively correlated with Hb, Hct, and total RBC count ($p < 0.01$). Fonte et al. (2007) described a case-report in which a 47-year old male exposed to Pb fumes and vapors at a recycling plant (blood Pb = 148 µg/dL) experienced normocytic, normochromic anemia, along with reticulocytosis and RBC basophilic stipplings. Following chelation therapy, the hematological symptoms improved. Taken together, these studies provide consistent evidence that occupational exposure to Pb reduces the number of RBCs in circulation. Although this decrease in RBCs may be explained by both decreased cell 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.

Studies in children were more equivocal than those in adults. Riddell et al. (2007) found that 21% of children 6 months to 5 years of age living in rural Philippines had blood Pb levels greater than 10 µg/dL (mean = 6.9 µg/dL). Hb levels were inversely related to blood Pb, with a decrease of 3% blood Pb associated with every 1 g/dL increase in Hb. However, Huo et al. (2007) found that children living near an area where electronic waste was recycled in China had significantly higher blood Pb levels than children in the neighboring town with no waste recycling (15.3 versus 9.94 µg/dL), but no difference in the Hb levels in the children in the two towns was detected (127.55 g/L in children with the higher blood Pb levels versus 123.46 g/L). Ahamed et al. (2006) studied male urban adolescents in India. The 39 adolescents were separated into groups according to their blood Pb level (group 1: <10 µg/dL, group 2: >10 µg/dL). Although the groups were similar in their age, height, weight, and body mass index, group 2 had a significantly lower PCV compared to group 1. The equivocal findings in studies investigating

hematological effects in children may be due to the comparatively shorter time period and magnitude of exposure versus. those seen in occupational studies.

Baranowska-Bosiacka et al. (2009) examined the effects of Pb on RBC hemolysis both in vitro measuring lysate in human RBCs incubated with Pb at concentrations ranging from 100 nM to 100 µM for 5-30 minutes, and in vivo using a rat RBC lysate from rats exposed to Pb acetate (0.1 %) in drinking water for 9 months. Rats exposed to Pb in the in vivo portion of the study achieved a blood Pb concentration of 7.1 µg/dL. Both studies demonstrated that Pb exposure resulted in increased hemolysis, observed as a significant increase in extracellular Hb. Pb-induced hemolysis in these experiments may be due to inhibition of RBC phosphoribosyltransferases (Section 5.7.5.1). The in vitro studies indicated a concentration-dependent increase in the amount of hemolysis, with a significant (threefold) increase even at the lowest concentration tested (i.e., 100 nM). Lee et al. (2005) observed that rats orally administered Pb (25 mg/kg) once a week for 4 weeks had an average plasma Pb level of 6.5 µg/dL (9.6-fold higher than controls, $p < 0.05$), and had significant decreases in Hct, Hb, and RBCs ($p < 0.05$) (M. K. Lee et al., 2005). Male mice administered 50 mg/kg Pb nitrate in distilled water via gavage for 40 days had significantly reduced total RBC counts, total leukocyte counts, Hb, lymphocytes, and monocytes (Sharma et al., 2010). Rats exposed to 2 g/L Pb acetate 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). Microcytic and basophilic erythrocytic granulations were commonly seen in Pb-exposed animals. An indication that the decrease in RBC count was related to decreased survival, and not a disruption of hematopoiesis, was the observation of significantly increased reticulocyte density and total count compared to controls ($p < 0.05$). Mice exposed to 1 g/L Pb acetate 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 ($p < 0.05$) (C. C. Marques et al., 2006). Spleen weights were also observed to be increased relative to body weight in animals exposed to Pb for 45 days. Male rats administered Pb acetate in the drinking water for 4 weeks at concentrations ranging from 100 to 1,000 ppm had a dose-dependent increase in blood Pb (range: 6.57 to 22.39 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 (M. Y. Lee et al., 2006). Slight, nonsignificant, increases in PS expression on RBC membranes were also observed. 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.

Khaĭrullina et al. (2008) observed that the surface profiles of RBC membrane shadows incubated with 0.5-10 µM/l Pb acetate for three hours were much smoother than untreated RBC membranes when examined by atomic force microscopy. The authors postulate that the observed smoothing in treated RBC membranes may be due to clusterization of band 3 protein. Band 3 (anion exchanger 1 [AE1]), is a chloride/bicarbonate (Cl^{-1}/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 AE1's normal activity.

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, Kempe et al. (2005) found that exposing human RBCs to Pb at concentrations ranging from 0.3 to 3 µM caused increased activation of K^+ channels that lead to cell shrinkage and scramblase activation. The activation of scramblase increased the exposure to PS on the cell membrane, which causes an increase in destruction of the RBCs by macrophages.

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 increase in expression of PS was 26.8 ± 3.15% (compared to deionized water) after incubation with 5 µM Pb for four hours. The expression of PS in RBCs is considered to be regulated through a Ca^{2+} dependent mechanism and, correspondingly, $[Ca^{2+}]_i$ was observed to increase with exposure to Pb (0.24 ± 0.21 µM in controls to 6.88 ± 1.13 µM in RBCs treated with 5 µM Pb for one hour). Consistent with this finding, Shin et al (2007) also observed that scramblase activity, which is important for induction of PS exposure and is activated by $[Ca^{2+}]_i$, was increased in Pb-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 dose-dependent manner following exposure to Pb. To confirm these findings in vivo, Shin et al. (2007) exposed male rats i.p. to 25, 50, or 100 mg/kg Pb acetate. 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 and in vivo experiments. Ciubar et al. (2007) also found that exposure to Pb nitrate (0.5-2 µM) resulted an increase in PS exposure and cell shrinkage, which they stated were indicators of cell apoptosis. As reported above, Khaĭrullina et al. (2008) observed 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 $AE1^{-/-}$ mice, PS exposure was much greater than in wild type mice. Decreased RBCs and increased reticulocytes were also observed, an indication of high cell turnover.

5.7.2.3. Red Blood Cell Hematopoiesis

Erythropoietin is a glycoprotein hormone excreted by the kidney to promote the development of RBCs in the bone marrow. Sakata et al. (2007) examined the relationship between blood Pb level and serum erythropoietin levels in Pb-exposed tricycle taxi drivers (n = 27) working at Kathmandu who were not anemic. The average blood Pb level in the taxi drivers was 6.4 µg/dL compared to 2.4 µg/dL in nondrivers. Drivers had a significantly lower level of serum erythropoietin (12.7 versus 18.8 mU/mL) compared to the nondrivers and there was a statistically significant inverse relationship between the level of serum erythropoietin and blood Pb ($r = -0.68$, $p < 0.001$). No other hematological effects were observed. The Sakata et al. (2007) study demonstrated that serum erythropoietin levels are affected by Pb even at levels low enough not to cause anemia. While this is generally considered a measure of kidney toxicity, it can also be considered an indicator that Pb could possibly affect the level of RBCs through decreased levels of serum erythropoietin.

Celik et al. (2005) observed that exposure of female rats to 140, 250, or 500 mg/kg Pb acetate once per week for 10 weeks resulted in statistically significantly decreased numbers of polychromatic RBCs (PCE) and increased numbers of micronucleated PCEs, compared to controls ($p < 0.001$). Alghazal et al. (2008) exposed male and female rats to 100 mg/L Pb acetate daily for 125 days and observed statistically significant 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, a significant 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. (2008) indicate that Pb is cytotoxic in male rats only, but is genotoxic in both sexes, Celik et al. (2005) indicates Pb is cytotoxic in female rats as well. Mice exposed to 1 g/L Pb acetate in drinking water for 90 days had statistically significant increases in micronucleated PCEs; a small, but not statistically significant decrease in the PCE/NCE ratio was also observed (C. C. Marques et al., 2006). Cyto- and genotoxicity in RBC precursor cells is a strong indication of altered hematopoiesis in bone marrow.

5.7.2.4. Membrane Proteins

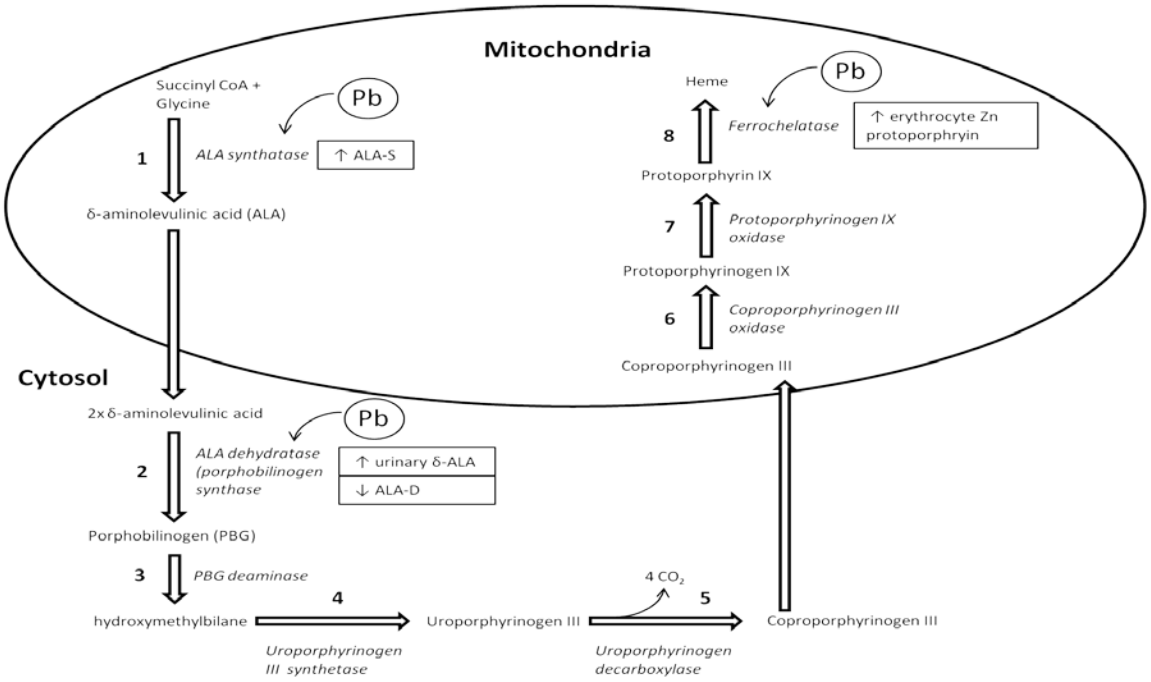
There have been few studies examining the effects of Pb on membrane proteins since the 2006 Pb AQCD. According to the 2006 Pb AQCD report, Pb has been found to affect RBC membrane polypeptides in exposed workers (Fukumoto et al., 1983);(Apostoli et al., 1988). Fukumoto et al. (1983) found decreased levels of polypeptides in band 3, which Apostoli et al. (1988) suggested may represent an anion channel protein, and increases in the level of polypeptides in bands 2, 4, 6, and 7. Fukumoto et al. (1983) suggested that the changes in the RBC membrane polypeptides may cause changes in membrane permeability. Apostoli et al. (1988) found that the changes in membrane polypeptides occurred at blood Pb levels greater than 50 µg/dL. Exposure to Pb acetate at concentrations above 100 nM for 60 minute

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.

Huel et al. (2008) found that newborn hair and cord blood Pb levels (1.22 ± 1.41 µg/g and 3.54 ± 1.72 µg/dL) were negatively associated with Ca-ATPase activity in plasma membranes of RBCs isolated from cord blood; newborn hair Pb levels were more strongly associated with cord Ca pump activity than cord blood Pb ($p < 0.0001$ versus. $p < 0.05$). Maternal Pb levels were not correlated with Ca pump activity in maternal or cord blood. Pb-induced disruptions in Ca homeostasis in RBCs can lead to cytotoxicity and necrosis, and these effects may be representative of cellular dysfunction in other organ systems.

5.7.3. Effects on Red Blood Cell Heme Metabolism

Pb has been found to inhibit several enzymes involved in heme synthesis, namely ALAD (cytoplasmic enzyme catalyzing the second, rate-limiting, step of the heme biosynthesis pathway), coporphyrinogen oxidase (catalyses the sixth step in heme biosynthesis converting coporphyrinogen III into protoporphyrinogen IX), and ferrochelatase (catalyses the terminal step in heme synthesis converting protoporphyrin IX into heme) (Figure 5-53). The observation of decreased Hb (measured as total Hb, MCH, or MCHC) in occupationally exposed adults (Karita et al., 2005; D. A. Khan et al., 2008; Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006; Ukaejiofo et al., 2009), children (Riddell et al., 2007), and experimental animal models (M. K. Lee et al., 2005; C. C. Marques et al., 2006; Sharma et al., 2010; Simsek et al., 2009) is a direct measure of decreased heme synthesis due to Pb intoxication.



Note: Steps in the pathway potentially affected by Pb are indicated with curved arrows pointing to the affected enzyme, and effects are represented by ↑ and ↓ arrows.

Figure 5-53. Schematic representation of the enzymatic steps involved in the heme synthetic pathway.

5.7.3.1. 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 has been used 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.

Patil et al. (2006) examined jewelry workers (blood Pb = 48.46 ± 7.39 µg/dL) in India occupationally exposed to Pb. In the study both the activated and nonactivated ALAD activities were measured. The study authors state that this is because decreases in ALAD activity reached a plateau and anemia can result in increased ALAD levels. Therefore, they considered the ratio of activated/nonactivated ALAD to be a good indicator of Pb toxicity. As described in the 2006 Pb AQCD, Scheuhammer (1987) studied the usefulness of this ratio in avian RBCs and found it to be a sensitive, dose-dependent measure of Pb exposure regardless of the route of exposure. They found that the activated/nonactivated ALAD ratio could be used to determine the oral exposure due to the highly positive correlation between Pb exposure in the 5–100 ppm range and ALAD activity ratio. Patil et al. (2006) observed a statistically significant decrease in nonactivated ALAD and an increase in the ratio of activated to nonactivated ALAD, compared to nonexposed controls ($p < 0.05$ and 0.001, respectively) .

The study authors state that this indicates the inhibition of heme synthesis. Urinary excretion of both ALA and PBG was statistically significantly increased in jewelry workers, a further indication of RBC ALAD inhibition. Similar results were seen in battery manufacturing workers in India (Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006). Pb-exposed workers (blood Pb = 74.4 ± 21.9 µg/dL) in a recycled automobile battery factory in Mexico had a significant decrease (~90%) in RBC ALAD activity compared to unexposed workers (9.9 ± 2 µg/dL) (Quintanar-Escorza et al., 2007). Painters in India with an average blood Pb level of 21.92 µg/dL had a significant decrease in ALAD levels compared to controls with an average blood Pb level of 3.06 µg/dL ($p < 0.01$) (Mohammad et al., 2008). Stoleski et al. (2008) observed that workers in a Pb smelter in Macedonia (blood Pb = 16.4 ± 8.5 µg/dL) had significantly decreased ALAD activity ($p < 0.001$) and increased ALA levels ($p < 0.0005$) compared to workers with no exposure to (blood Pb = 7.0 ± 5.4 µg/dL). Lastly, Ademuyiwa et al. (2005) observed that workers in mechanic workshops (blood Pb = 27.0 ± 1.1 - 48.9 ± 19.1 µg/dL µg/dL) had significant decreases in ALAD activity compared to controls (15.8 ± 2.8 µg/dL, $p < 0.001$). Petrol station workers had the highest degree of ALAD inhibition relative to controls (77%), whereas welders only had a 36% decrease in ALAD activity compared to controls. A case report by Fonte et al. (2007) described a worker occupationally exposed to Pb vapors (blood Pb = 148 µg/dL) with ALAD levels substantially decreased compared to normal levels (3 versus >25 U/L). Zinc protoporphyrin (ZPP) levels were also described to be greatly increased. Following chelation therapy, the patient's clinical picture improved.

Wang et al. (2010) found that even with low to moderate blood Pb levels, there was a concentration-dependent decrease in ALAD activity in both children and adults (blood Pb = 7.1 and 6.4 µg/dL, respectively) in rural southwest China. Further, Wang et al. (2010) observed that the relationship between blood Pb and ALAD activity was nonlinear and exponential, with more significant decreases in ALAD activity occurring with blood Pb levels greater than 10 µg/dL. No correlation was observed between urinary ALA levels and blood Pb. Ahamed et al. (Ahamed et al., 2006) studied male urban adolescents in India. The 39 adolescents were separated into groups according to their blood Pb levels (group 1: <10 µg/dL, group 2: >10 µg/dL). Although the groups were similar in their age, height, weight, and body mass index, group 2 had a significantly lower ALAD activity than group 1 ($p < 0.001$). When all 39 adolescents were examined together, an inverse relationship was found between blood Pb and ALAD activity. Ahamed et al. (2005) also observed decreased ALAD activity in Indian children (aged 4-12) with a mean blood Pb level of 11.39 ± 1.39 µg/dL compared to children with mean blood Pb levels of 3.93 ± 0.61 µg/dL. Similar decreases were also observed in children 3-6 years of age with >10 µg/dL, compared to children <10 µg/dL (Y. P. Jin et al., 2006).

Rats administered 500 ppm Pb acetate in drinking water for 15 or 30 days had decreased blood ALAD activity that was related to duration of exposure and blood Pb (Rendón-Ramirez et al., 2007). 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 were associated with significant decreases (approximately 50% lower than control levels) in RBC ALAD activity (M. K. Lee et al., 2005).

5.7.4. Other Heme Metabolism Enzymes

The 2006 Pb AQCD report indicates Pb affects RBC PBG synthase (Farant & Wigfield, 1987); (Farant & Wigfield, 1990; Simons, 1995), PBG deaminase (Tomokuni & Ichiba, 1990), and TF endocytosis and iron transport across membranes (Z. M. Qian & Morgan, 1990), all of which are directly or indirectly involved in heme synthesis. Although there are no new studies that measure the effect Pb has on other heme metabolism enzymes' activities, a number of studies investigated the effect blood Pb had on concentrations of various intermediate products in the heme biosynthetic pathway.

Pb intoxication is known to inhibit the function of ferrochelatase, the enzyme that catalyzes the last step in the heme biosynthetic pathway. Under normal conditions, ferrochelatase incorporates ferrous iron (Fe^{2+}) into protoporphyrin IX, converting it into a heme molecule. However, Pb inhibits this insertion of Fe^{2+} into the protoporphyrin ring and instead, Zn is inserted into the right creating ZPP. A number of recent studies have shown that blood Pb is statistically significantly associated with increased RBC ZPP levels in humans (Ademuyiwa, Ugbaja, Ojo, et al., 2005; Counter et al., 2007; Mohammad et al., 2008; Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006) and animals (Rendón-Ramirez et al., 2007). Patil et al. (2006) observed a small, but not statistically significant increase in RBC ZPP levels among silver jewelry workers exposed to Pb, compared to nonexposed controls. Interestingly, Q. Wang et al. (2010) found that in children and adults living in a rural area of Southwest China, ZPP levels were decreased at the low blood levels of Pb and were only increased with higher blood Pb levels. The authors suggest that this may be representative of ALAD activities at low Pb levels, which contributes to lower ZPP levels. Scinicariello et al. (2007) performed a meta-analysis and observed that Pb-exposed individuals that carried the ALAD2 allele had slightly, but not statistically significant, lower concentrations of blood ZPP levels compared to carriers of the ALAD1 allele.

5.7.5. Effects on Other Hematological Parameters

5.7.5.1. Energy Metabolism

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 via either an adenine pathway, which requires adenine phosphoribosyltransferase (APRT), or an adenosine pathway, which requires adenosine kinase. The 2006 Pb AQCD reports 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 report, 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) due to in vitro and in vivo exposures. For the in vitro exposure, APRT and HPRT were measured in lysate of human RBCs after exposure to Pb at concentration range from 100 nM to 100 µM for 5-30 minutes of exposure. In vivo tests measured APRT and HPRT in rat RBC lysate from rats exposed to Pb acetate (0.1 %) 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 were significantly decreased in vitro after only 5 minutes of exposure to the 100 nM concentration, but the decrease was also dose-dependent. However, the study authors considered the inhibition moderate (30-35%) even with the highest levels used in vitro. Shin et al. (2007) found a dose-dependent decrease in intracellular ATP in human RBCs in vitro with significant decreases found even with the lowest concentration (i.e., 1 µM).

5.7.5.2. Other Enzymes

The 2006 Pb AQCD reports that K^+ permeability was increased by Pb 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 the inhibition of Ca^{2+}-Mg^{2+} ATPase. Only two studies since the 2006 report were found that examined the effects of Pb exposure on other enzymes. Ekinci et al. (2007) tested the effects of Pb on two carbonic anhydrase isozymes (I and II) isolated from human RBCs. Carbonic anhydrases are metalloprotein that use Zn to catalyze the equilibrium between carbon dioxide and bicarbonate in the cells of higher invertebrates. Although they found Pb nitrate inhibited both carbonic anhydrase isozymes in a concentration-dependent manner, the concentrations used (i.e., 2×10^{-4} to 1×10^{-3} M) 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 manganese for normal activity. Pb was observed to bind directly to the enzyme's active site in a different position than the manganese, thus possibly resulting in improper protein folding and inhibition of activity.

5.7.6. 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) (Ahamed & Siddiqui, 2007; Nemsadze et al., 2009). Many studies have found an association between the level of blood Pb and lipid peroxidation, antioxidant levels, or indicators of ROS production.

5.7.6.1. Oxidative Stress, Lipid Peroxidation, and Antioxidant Enzymes

Malondialdehyde (MDA) is an end product of lipid peroxidation and is commonly used as an indicator of lipid peroxidation. Patil et al. (2006) found significantly higher blood Pb in the jewelry workers (48.56 ± 7.39 µg/dL) compared to individuals not occupationally exposed (12.52 ± 4.08 µg/dL) to Pb. These workers had significantly higher plasma MDA, along with significantly lower levels of RBC SOD, catalyses, and plasma ceruloplasm, all of which are indicators of oxidative stress in the RBCs. Patil et al. (2006) found similar effects in a group of battery manufacturing workers in India. Levels of MDA were significantly positively correlated with blood Pb in workers occupationally exposed to Pb, but no correlation was observed in controls. These effects were also demonstrated in vitro by Ciubar et al. (2007), but only with concentrations of 2 µM (highest concentration tested). In this study, RBCs from nine volunteers were incubated with Pb at concentrations ranging from 0.1-2 µM for 24 hours. Evidence of lipid peroxidation was also observed in auto repair apprentices in Turkey with blood Pb levels as low as 7.9 µg/dL (compared to 2.6 µg/dL in controls) (Ergurhan-Ilhan et al., 2008), including increases in glutathione peroxidase (GPx) and MDA, as well as decreases in α-tocopherol and β-carotene. Decreases were observed in SOD and CAT, but the results did not achieve statistical significance. Decreased glutathione reductase (GR) activity was observed in human RBCs incubated with 5-18 µM Pb in vitro (Coban et al., 2007). Industrial workers in Pakistan occupationally exposed to Pb had a significant increase in blood Pb levels (mean: 29.1 µg/dL, range: 9.0 to 61.1 µg/dL) compared to age and gender matched controls (mean: 8.3 µg/dL, range: 1.0 to 21.7 µg/dL) (D. A. Khan et al., 2008). The industrial workers also had increased oxidative stress as measured by increased levels of serum MDA and C-reactive protein (CRP). In painters in India with an average blood Pb level of 21.92 µg/dL (compared to 3.06 µg/dL in controls), there was a significant decrease in SOD, glutathione (GSH), and CAT accompanied by a significant increase in oxidized GSH (i.e., GSSG) and thiobarbituric acid reactive species (TBARS, expressed in terms of MDA) measured in plasma and RBC lysate (Mohammad et al., 2008). Quintanar-Escorza et al. (2007) found elevated RBC lipid peroxidation measured as increased MDA levels in Pb-exposed workers in a recycled automobile battery factory in Mexico. There was a correlation in the MDA levels and blood Pb even in the unexposed workers who had low (i.e., <12 µg/dL) blood Pb levels, although the observed correlation in exposed workers was greater. Similar effects were seen when RBCs from healthy volunteers with no Pb exposure were incubated with 0.4 µM Pb for 24 hours (Quintanar-Escorza et al., 2010). MDA concentrations, SOD and GPx activities were observed to be elevated in normotensive exposed workers compared to controls (S. Kasperczyk et al., 2009). The concentration of MDA was statistically significantly greater in workers with hypertension compared to both controls and Pb-exposed normotensive workers, whereas the activity of GR in hypertensive workers decreased to levels comparable to those seen in the control group. Exposure related increases in lipid peroxidation were also observed in occupationally exposed workers in Poland (S. Kasperczyk et al.,

2005). The concentrations of MDA and 7-ketocholesterol (oxidized cholesterol) were significantly greater in exposed hypertensive workers compared to normotensive workers.

Ahamed et al. (2005) investigated the relationship between blood Pb levels and antioxidant enzyme levels and lipid peroxidation in children aged 4-12 years in Lucknow, India. A total of 62 children, with a mean blood Pb level of 7.47 ± 3.06 µg/dL, were included in the study; children were separated into three groups based on their blood Pb levels: group I, 3.93 ± 0.61 µg/dl; group II, 7.11 ± 1.25 µg/dL; and group III, 11.39 ± 1.39 µg/dL. Lipid peroxidation, measured as blood MDA, was statistically significant greater in group III, compared to group II and I, whereas GSH was decreased in group III relative to groups II and I. Catalase activity was the only measure of oxidative stress that was statistically significantly elevated in group II compared to group I. Additionally, blood Pb levels were found to be statistically significantly positively correlated with MDA and CAT and negatively correlated with GSH. Ahamed et al. (2006) additionally studied male urban adolescents in India. The 39 adolescents were separated into groups according to their blood Pb level (group 1: <10 µg/dL, group 2: >10 µg/dL). Although the groups were similar in their age, height, weight, and body mass index, group 2 had significantly higher levels of CAT and MDA compared to group 1. There were no significant differences in blood GSH levels. Examining all the study subjects together, there was a correlation between the blood Pb level and blood MDA and RBC CAT levels, as well as an inverse relationship between ALAD activity and MDA and CAT levels. In a similar study, Ahamed et al. (2008) examined oxidative stress in Indian children with neurological disorders. There was a significantly higher blood Pb level in the study population compared to the control population (18.60 versus 10.37 µg/dL). In addition, the following indicators of oxidative stress were observed in the study population: 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 and) 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 demonstrated a significant increase in plasma MDA compared to the children with blood Pb levels<10 µg/dL. However, levels of RBC SOD, GSH, and GPx were not different from controls (Y. P. Jin et al., 2006).

Administration of Pb (25 mg/kg) to rats once a week for 4 weeks, which was related to a blood Pb level of about 6.5 µg/dL, caused a significant increase in RBC MDA levels (M. K. Lee et al., 2005). Other indications of oxidative stress included significant increases in RBC SOD and CAT levels accompanied by significant decreases in GSH and GPx. Exposure of rats to 750 mg/kg Pb acetate in drinking water for 11 weeks resulted in decreased concentrations of plasma vitamin C, vitamin E, nonprotein thiol, and RBC-reduced glutathione, while simultaneously increasing the activity of SOD and GPx (Kharoubi, Slimani, Krouf, et al., 2008). CAT activity was also slightly elevated in Pb-exposed rats, but the increase failed to reach statistical significance. SOD activity was significantly decreased in rats injected with 15 mg/kg Pb i.p. for seven days, but not rats exposed to 5 mg/kg Pb (Berrahal et al., 2007). GPx activity and

MDA concentrations were slightly elevated in the exposure groups, but failed to reach statistical significance.

5.7.6.2. Antioxidant Defense

In addition to the studies listed above that examine lipid peroxidation and oxidative stress, there have been studies that indicate that the use of antioxidants and free radical reactions were protective against Pb-induced RBC oxidative stress. Rats treated with 500 ppm Pb acetate in drinking water for 15 or 30 days had an increase in free RBC protoporphyrin and TBARS that was related to length of exposure and blood Pb (Rendón-Ramirez et al., 2007). Vitamin E administration after exposure to Pb significantly reduced the 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. (2006), found that hemolysis and 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 (iron chelator), trolox (chain breaking antioxidant), and mannitol and Na formate ($^{\bullet}OH$ scavengers). Results suggested the role of singlet oxygen in Pb-mediated membrane damage and hemolysis of exposed RBCs. In rats exposed to 2000 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 statistically significantly different from controls, but statistically smaller than concentrations observed with Pb alone. Exposure to L-methionine an N-acetylcysteine also reduced Pb-induced depletion of vitamin E.

5.7.7. Summary and Causal Determination

There is consistent toxicological and epidemiologic evidence that exposure to Pb induces adverse effects on hematological endpoints, including altered heme synthesis, decreased RBC survival and function, and increased RBC oxidative stress. Pb preferentially partitions into RBCs following exposure, with RBC concentrations approximately 100-fold greater than those observed in the plasma (C. Jin et al., 2008; Timchalk et al., 2006).

Multiple occupational epidemiologic studies have observed that Pb affects several hematological parameters such as Hb, PCV, MCV, MCH, and MCHC (Karita et al., 2005; D. A. Khan et al., 2008; Patil, Bhagwat, Patil, Dongre, Ambekar, & Das, 2006; Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006; Ukaejiofo et al., 2009). Although the majority of occupationally exposed adults had blood Pb levels in excess of 20 µg/dL, decreases in Hb and PCV were observed in adults with blood Pb levels of 7 µg/dL.

In addition, Pb exposure was shown to reduce Ca^{2+}- and Ca^{2+}-Mg^{2+}-ATPase activity in RBC membranes, which leads to an increase in RBC $[Ca^{2+}]_i$, increased membrane fragility, and abnormal morphological changes (Ciubar et al., 2007; Huel et al., 2008; Quintanar-Escorza et al., 2010; Quintanar-Escorza et al., 2007). Heul observed a reduction of RBC Ca^{2+}-Mg^{2+}-ATPase activity at a cord blood Pb level of 3.54 µg/dL. Studies in children are more equivocal than those investigating occupationally exposed adults; this may due to the comparatively shorter duration of and magnitude of exposure experienced by children. Toxicological studies have also observed decreases in Hct and Hb and increases in hemolysis and reticulocyte density in rats and mice with blood Pb levels as low as 6.6-7.1 µg/dL (Baranowska-Bosiacka et al., 2009; M. K. Lee et al., 2005; Sharma et al., 2010; Simsek et al., 2009). Pb exposure has also been observed to increase PS expression on RBC membranes, leading to cell shrinkage, eyrptosis, and destruction of the RBCs by macrophages (Ciubar et al., 2007; Shin et al., 2007). Suggestive evidence of disrupted hematopoiesis evidenced by decreased serum erythropoietin was observed in occupationally exposed adults with a blood Pb level of 6.4 µg/dL; toxicological studies in rats also indicate that Pb is cytotoxic to RBC progenitor cells. Taken together, these studies provide consistent evidence that exposure to Pb adversely effects RBC function and survival, and leads to the reduction of RBCs in circulation. Although this decrease in RBCs may be explained by both decreased cell survival and/or disruption of hematopoiesis, the observation of increased reticulocytes seems to represent compensation for decreased RBC survival due to Pb exposure.

Recently, numerous epidemiologic studies have confirmed that decreases in RBC ALAD levels and activity are strongly associated with Pb exposure in adults and children at blood Pb levels as low as 6.4 and 7.1 µg/dL, respectively (Ademuyiwa, Ugbaja, Ojo, et al., 2005; Mohammad et al., 2008; Patil, Bhagwat, Patil, Dongre, Ambekar, & Das, 2006; Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006; Quintanar-Escorza et al., 2007). Decreases in blood ALAD activity were also seen in rats with blood Pb levels of 6.5 µg/dL (M. K. Lee et al., 2005). In addition to ALAD, recent studies have shown that Pb exposure inhibits the activity of ferrochelatase, leading to increased RBC ZPP in humans (Ademuyiwa, Ugbaja, Ojo, et al., 2005; Counter et al., 2007; Mohammad et al., 2008; Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006) and animals (Rendón-Ramirez et al., 2007). Pb has also been shown to inhibit the activities of other enzymes in RBCs, including those involved in nucleotide scavenging, energy metabolism, and acid-base homeostasis (Baranowska-Bosiacka et al., 2009; Ekinci et al., 2007).

Lastly, Pb exposure induces lipid peroxidation and oxidative stress in RBCs. Epidemiologic studies have observed increases in MDA in occupationally-exposed adults with blood Pb levels as low as 7.9 µg/dL (Ergurhan-Ilhan et al., 2008; D. A. Khan et al., 2008; Mohammad et al., 2008; Patil, Bhagwat, Patil, Dongre, Ambekar, & Das, 2006; Patil, Bhagwat, Patil, Dongre, Ambekar, Jailkhani, et al., 2006; Quintanar-Escorza et al., 2007). Other measures of oxidative stress observed included lowered activities of SOD, GR, and CAT, and increased CRP. Indices of RBC oxidative stress were also seen in adolescents

and children exposed to Pb (Ahamed et al., 2008; Ahamed et al., 2006; Y. P. Jin et al., 2006). In vitro and vivo studies have also demonstrated that prior, concurrent, or subsequent treatment with various antioxidants has been shown to at least partially ameliorate Pb-induced oxidative stress in RBCs (Casadoa et al., 2006; Cecil et al., 2008; Rendón-Ramirez et al., 2007).

Similar to the epidemiologic and 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 aquatic and terrestrial invertebrates and vertebrates (Section 7.4.5). 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 on serum enzyme levels and white blood cell counts in birds and mammals.

In conclusion, the recent epidemiologic and toxicological literature provides strong evidence that exposure to Pb is associated with numerous deleterious effects on the hematological system, including altered heme synthesis mediated through decreased ALAD and ferrochelatase activities, decreased RBC survival and function, decreased hematopoiesis, and increased oxidative stress and lipid peroxidation. The consistency of findings in the epidemiologic and toxicological literature and coherence across the disciplines is sufficient to conclude that there is a **causal relationship between Pb exposures and effects on heme synthesis and red blood cell function.**

5.8. Reproductive Effects and Birth Outcomes

The effect of Pb on reproductive outcomes has been of interest for years, starting in cohorts of occupationally exposed individuals. More recently, researchers have begun to focus on reproductive effects in people with environmentally relevant Pb exposure. In the toxicological and epidemiologic literature, research on reproductive effects of Pb include female and male reproductive function (hormone levels, fertility, puberty, and effects on reproductive organs and estrus), birth defects, spontaneous abortions, infant mortality, preterm birth, low birth weight/fetal growth, and other developmental effects. In epidemiologic studies, various biological measures of Pb are used including Pb measured in blood and bone; toxicological studies only report exposure using blood Pb. Bone Pb is indicative of cumulative Pb exposure. Blood Pb can represent more recent exposure, although it can also represent remobilized Pb occurring during times of bone remodeling. More detailed discussion of these measures and Pb transfer via umbilical cord blood Pb, across the placenta, and via lactation is given in Section 4.3.5 on Pb Toxicokinetics. A few studies of pregnancy-induced hypertension and eclampsia have been conducted and are reported on in the section on hypertension (Section 5.4.2.1). Briefly, the relatively small number of

studies found consistently positive associations between recent Pb exposure and pregnancy-induced hypertension.

Overall, the recent reproductive literature continues to support associations reported in earlier AQCDs between Pb exposure and adverse outcomes on various parameters of sperm (function, motility, count, integrity, histology). The toxicological and epidemiologic literature also support the finding that Pb exposure is consistently associated with delayed onset of puberty in both males and females. The new information from epidemiologic and toxicological studies and conclusions from previous AQCDs are summarized below.

5.8.1. Effects on Female Reproductive Function

The epidemiologic studies presented on Pb and female reproductive function in the 2006 AQCD (U.S. EPA, 2006) provided little evidence on the possible associations between Pb exposure and female reproduction and fertility. However, the 1986 and 2006 Pb AQCDs (U.S. EPA, 1986, 2006) reported toxicological findings that Pb exposure is 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 AQCD, many epidemiologic studies have been published regarding Pb levels in women and their effects on reproduction. In addition, recent toxicological studies add further knowledge of Pb-related effects on the female reproductive system.

5.8.1.1. Effects on Female Sex Endocrine System and Estrus Cycle

Multiple studies have examined the association between Pb and its effects on hormones and the estrus cyclce. Epidemiologic studies support the toxicological findings, which have the majority of the evidence.

An epidemiologic study using the NHANES III database and including women aged 35-60 years old examined the relationship between blood Pb levels (mean 2.8 µg/dL) and serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) (E. F. Krieg, Jr., 2007). Deviation from normal FSH and LH levels may indicate endocrine disruption related to ovary functioning. Researchers determined that as blood Pb levels increased, serum FSH and LH increased among both post-menopausal women and women with both ovaries removed. There was also an increasing trend for pre-menopausal women who were not menstruating or pregnant, although the association was not statistically significant for LH. Increasing blood Pb levels were associated with decreasing 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 found that the lowest level of blood Pb for which

a statistically significant association could be observed between blood Pb and FSH was 1.7 µg/dL among women with their ovaries removed. A limitation 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. Another epidemiologic study was performed in Kaohsiung City, Taiwan among two groups of women aged 23-44: 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 (S. H. Chang et al., 2006). The mean blood Pb in this study was 3.12 µg/dL (SD 0.19 µg/dL). The study reported a positive association between increased blood Pb levels and serum estradiol concentrations, which reflects ovary activity.

The effect of Pb exposure on the female endocrine system has been demonstrated in toxicological studies in the 1986 and 2006 Pb AQCD (U.S. EPA, 1986, 2006). 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 previously, Pine et al. (2006) observed that maternal Pb exposure causes a decrease in basal LH levels in pre-pubertal female Fisher 344 rat pups when compared to non-Pb exposed pups during gestation and lactation. Dumitrescu et al. (2008) observed alteration of hormone levels in female Wister rats after ingesting Pb acetate (50, 100, 150 ppb) in drinking water for six months. The authors reported decreases in FSH, estradiol, and progesterone levels with increases in LH and testosterone levels. Nampoothiri and Gupta (2008) administered Pb acetate at a concentration that did not affect reproductive performance, implantation or pregnancy outcome (0.05 mg/kg body weight) to Charles Foster female rats 5 days before mating and during the gestational period. They observed a decrease in steroidogenic enzymes, 3β-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.

Kolesarova et al. (2010) conducted an in vitro study to examine the secretory activity of porcine ovarian granulose cells after Pb administration. The results of the study showed that Pb acetate concentrations of 0.046 mg/mL and 0.063 mg/mL statistically significantly inhibited IGF-1 release, but concentrations of 0.25 mg/mL and 0.5 mg/mL 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 exposure (Nampoothiri & Gupta, 2006).

No recent toxicological studies were found that addressed Pb-induced effects on the estrus cycle.

Overall, toxicological studies report alterations in hormone levels related to Pb concentration. This was also observed in epidemiologic studies. Although these changes are observed, there are discrepancies

about the direction of the hormone changes related to Pb. One explanation is that the direction of effect could vary based on current hormonal and reproductive status.

5.8.1.2. Effects on Fertility

Previous studies indicated that Pb exposure does not produce total sterility, but it can disrupt female fertility (U.S. EPA, 2006). Recent epidemiologic studies and studies in experimental animals support this finding. The epidemiologic studies are summarized in Table 5-27.

Table 5-27. Summary of recent epidemiologic studies of effects on fertility for females

Reference	Study, Location, and Years	Outcome	Study population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Al-Saleh et al. (2008)	Riyadh, Saudi Arabia 2002-2003	Achieving pregnancy and/or fertilization	Women aged 19-50 undergoing IVF	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)
Chang et al. (2006)	Kaohsiung City, Taiwan 1999, 2000-2001	Fertility	Women receiving care at a infertility clinic in 2000-2001 or delivering a normal infant at a nearby medical center in 1999	Blood Pb	3.12 (0.19)	OR (95% CI) Fertility ≤2.5 µg/dl: 1.00 (Ref) >2.5 µg/dl: 2.94 (1.18, 7.34)
Silberstein et al. (2006)	Providence, RI NS	Achieving pregnancy	Women undergoing IVF at the study hospital	Follicular fluid Pb	Not given quantitatively 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, study only included 9 women

Epidemiologic studies examined women having difficulty conceiving by performing studies among patients of fertility clinics or undergoing in vitro fertilization (IVF). The first of these was performed in Kaohsiung City, Taiwan among women aged 23-44 (S. H. Chang et al., 2006). 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. 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. Another study examining fertility reported on women in Saudi Arabia aged 19-50 years who were undergoing IVF treatment (Al-Saleh et al., 2008). Women were categorized as having achieved a pregnancy versus not achieved a pregnancy and achieved fertilization versus not achieving fertilization. The majority of women had follicular Pb levels that were below the level 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 an inverse association was detected between blood Pb and 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 non-pregnant women. One limitation present in these studies is that the participants, especially in the later two studies, are women who are seeking help for fertility problems. The studies are not a sample of the general population and therefore cannot be generalized to all women of childbearing age.

Several studies observed a decrease in litter size when females were exposed to Pb before mating or during pregnancy (Dumitrescu, Alexandra, et al., 2008; Iavicoli, Carelli, Stanek, Castellino, Li, et al., 2006; Teijon et al., 2006). Pups in Teijon et al.'s study receiving 400 ppm Pb acetate in drinking water had blood Pb of 97 µg Pb/dL blood at 1 wk post-weaning and 18.2 µg Pb/dL blood at 2 wk post-weaning. Dumitrescu et al. observed a modification in sex ratio of pups born to dams exposed to Pb before mating and during 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. (1998), who did not observe differences in sex ratio if liters from females 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 dose-dependent decline in viability rate, maturation, fertilization, and cleavage rates of buffalo oocytes cultured in medium containing 1-10 µg/mL Pb acetate. Karaca and Şimşek (2007) observed an increase in the number of mast cells in ovary tissue after Pb exposure (2 g/L in drinking water) 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 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.

Epidemiologic and toxicological studies on the effect of Pb on fertility outcomes have generated inconsistent results. However, there is some indication that increased Pb exposure may decrease fertility.

5.8.1.3. Effects on Puberty

Recent toxicological studies of rodents have examined the effects of Pb on pubertal and reproductive organ development and on biomarker development. There have also been recent epidemiologic studies examining Pb levels and onset of puberty, which are summarized in Table 5-28 and in the text below.

Table 5-28. Summary of recent epidemiologic studies of effects on puberty for females

Reference	Study Location and Years	Outcome	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Denham et al. (2005)	Akwesasne Mohawk Nation (boundaries of New York, Ontario, and Quebec NS	Age at menarche	10- to 16.9-yr-old girls in the Akwesasne community	Blood Pb	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%
Gollenberg et al. (2010)	U.S.A. 1988-1994	Luteinizing hormone (LH) and inhibin B	Girls ages 6-11 from the NHANES III study	Blood Pb	Median 2.5 (range 0.07, 29.4) blood Pb >10 µg/dL: 5%	OR (95% CI) for exceeding pubertal inhibin B cutoff (>35pg/mL) <1 µg/dl: 1.00 (Ref) 1-4.9 µg/dl: 0.38 (0.12, 1.15) ≥ 5µg/dl: 0.26 (0.11, 0.60) OR (95% CI) for 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) ≥ 5µg/dl: 0.83 (0.37, 1.87) *a sensitivity analysis including only those with blood Pb <10 µg/dl had similar results but ORs were slightly attenuated

Reference	Study Location and Years	Outcome	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Naicker et al. (2010)	Johannesburg/Soweto, South Africa Born in 1990	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	Blood Pb at 13 yr of age	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) ≥ 5µg/dl: 2.34 (1.45, 3.79) Delay in pubic hair development at age 13 <5 µg/dl: 1.00 (Ref) ≥ 5µg/dl: 1.81 (1.15, 2.84) Delay in attainment of menarche at age 13 <5 µg/dl: 1.00 (Ref) ≥ 5µg/dl: 2.01 (1.38, 2.94)
Selevan et al. (2003)	U.S.A. 1988-1994	Tanner staging and age at menarche	Girls ages 8-18 from the NHANES III study	Blood Pb	Geometric mean NHWhites: 1.4 NHBlacks: 2.1 Mexican-Americans: 1.7 Blood Pb levels>5µg/dL: NHWhites: 2.7% NHBlacks: 11.6% Mexican-Americans: 12.8% Blood Pb levels>10µg/dL: NHWhites: 0.3% NHBlacks: 1.6% Mexican-Americans: 2.3%	OR (95% CI) Breast development NH Whites: 1 µg/dl: 1.00 (Ref) 3µg/dl: 0.82 (0.47, 1.42) NH Blacks: 1 µg/dl: 1.00 (Ref) 3µg/dl: 0.64 (0.42, 0.97) Mexican Americans: 1 µg/dl: 1.00 (Ref) 3µg/dl: 0.76 (0.63, 0.91) Pubic hair development NH Whites: 1 µg/dl: 1.00 (Ref) 3µg/dl: 0.75 (0.37, 1.51) NH Blacks: 1 µg/dl: 1.00 (Ref) 3µg/dl: 0.62 (0.41, 0.96) Mexican Americans: 1 µg/dl: 1.00 (Ref) 3µg/dl: 0.70 (0.54, 0.91) HR (95% CI) *included only girls 8-16 Age at menarche NH Whites: 1 µg/dl: 1.00 (Ref) 3µg/dl: 0.74 (0.55, 1.002) NH Blacks: 1 µg/dl: 1.00 (Ref) 3µg/dl: 0.78 (0.63, 0.98) Mexican Americans: 1 µg/dl: 1.00 (Ref) 3µg/dl: 0.90 (0.73, 1.11)
Tomoum et al. (2010)	Cairo, Egypt 2007	Hormones and pubertal development	Healthy children aged 10-13 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)	Blood Pb	NS 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% ≥ 10µg/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% ≥ 10µg/dl: Stage 2: 77.8% Stage 3: 22.2% Chi-square p-value>0.05 *Quantitative results for hormones not provided
Wolff et al. (2008); Wolf et al.(2007)	New York City, NY 1996-1997	Pubertal stages defined using standard drawings	9-yr old girls from the study hospital and nearby pediatric offices	Blood Pb	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)

Reference	Study Location and Years	Outcome	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Wu et al. (2003)	U.S.A. 1988-1994	Tanner staging and age at menarche	Girls ages 8-16 from the NHANES III study	Blood Pb	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)

Several epidemiologic studies investigated the association between blood Pb and indicators of puberty onset. A study performed in NYC among 9 year old girls reported no association between Pb levels and pubertal development (Wolff et al., 2008). However, a study among girls aged 10-13 (median age 12) reported decreased 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. A study of girls aged 10-16.9 years of age in the Akwesasne Mohawk Nation reported a non-linear positive association between blood Pb and age at menarche (Denham et al., 2005). No association was observed below blood Pb of 0.49 µg/dL. A study conducted in South Africa reported a positive association between blood Pb levels and age at menarche and pubertal development (Naicker et al., 2010). Blood Pb levels were associated with delayed pubertal development and later age at menarche. This study illustrates not only the association between Pb and pubertal development, but that delays can occur at low Pb levels. Multiple studies have been performed examining blood Pb levels and puberty using the NHANES III database (Gollenberg et al., 2010; Selevan et al., 2003; T. Wu et al., 2003). One study included girls aged 8-16 and reported an positive association for delayed attainment of menarche and pubic hair development, but not for breast development (T. Wu et al., 2003). The associations were observed even at low levels of blood Pb. Another NHANES III study included girls 8-18 years of age and reported the results stratified by race (Selevan et al., 2003). Blood Pb levels were inversely associated with Tanner stage of breast and pubic hair development and age at menarche among African Americans and with breast and pubic hair development among Mexican Americans. The associations were in the same directions for whites, but none of the associations reached statistical significance. A third study using the NHANES III database examined the association between Pb and reproductive hormones among girls 6-11 years old (Gollenberg et al., 2010). Blood Pb levels were inversely associated with inhibin B, a protein that inhibits FSH

production, but no association was observed for LH. The inverse association between blood Pb and inhibin B was greater among girls with iron deficiency compared to those with high Pb but sufficient iron levels. Inhibin B and LH were chosen for this study because these hormones are, "believed to be relevant for younger girls… near the onset of puberty and…serve as markers for hypothalamic-pituitary-gonadal functioning."

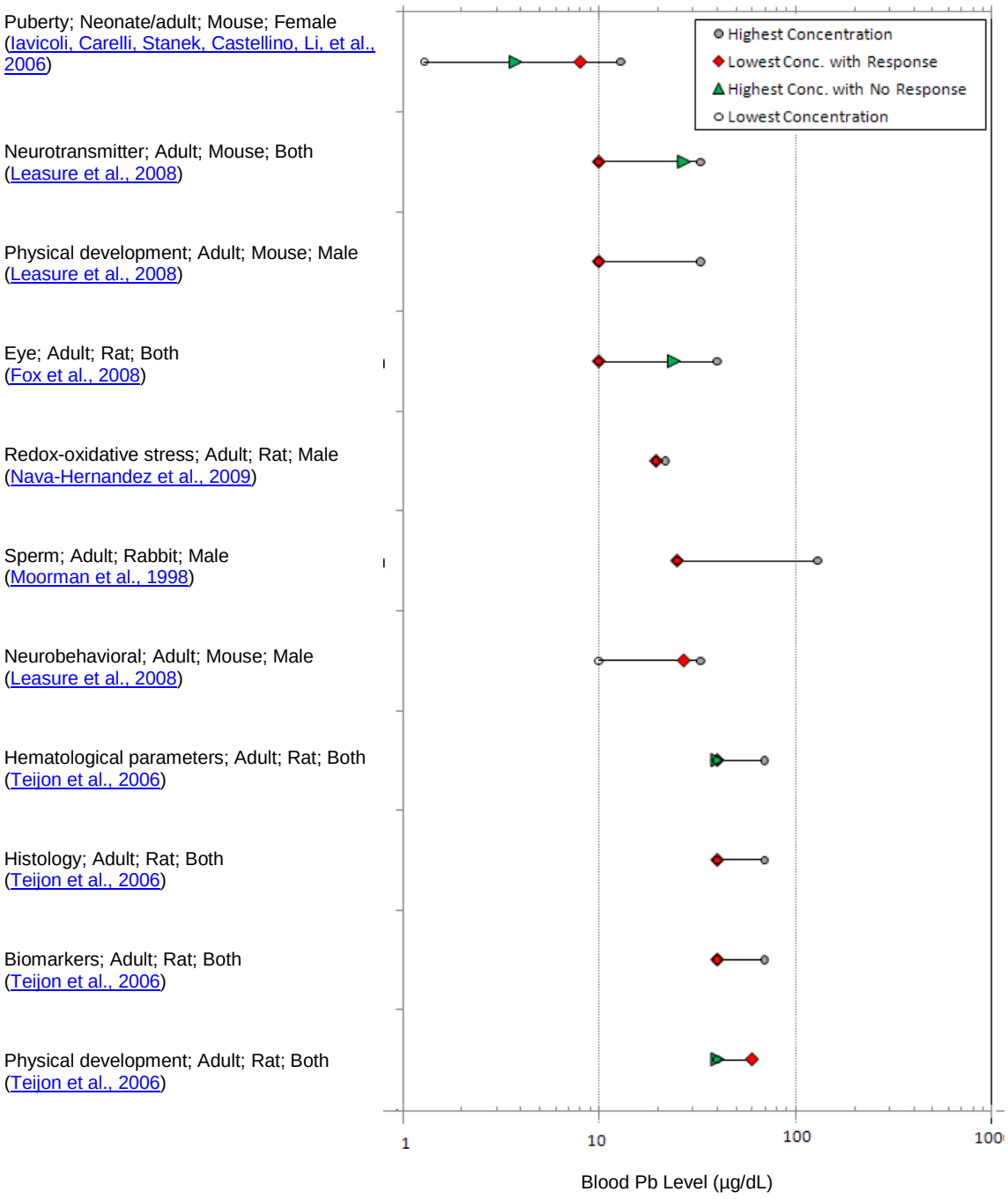


Figure 5-54. Toxicological Exposure-Response Array for Reproductive Effects of Pb.

Table 5-29. Toxicological Exposure-Response Array Summaries for Reproductive Effects of Pb presented in Figure 5-54

Reference	Blood Pb level with Effect (µg/dl)	Altered Outcome
Iavicoli I. et al. (2006)	8 & 13	Delayed onset female puberty
Leasure et al. (2008)	10 & 42 10, 24 &42 10 & 42	Neurotransmitter, Dopamine homeostasis Physical Development, Adult obesity (males) Aberrant response to amphetamine
Fox et al. (2008)	12	Retinal aberrations
Nava-Hernandez et al. (2009)	19.5	Sperm affected via redox imbalance
Moorman et al. (1998)	25-130	Semen quality affected
Teijon et al. (2006)	40 &100 40 &100 40 &100 100	Hematology Histology-Offspring renal & hepatic Biomarker-Offspring renal function Physical development: birth weight
Fox et al. (2008)	12	Retinal aberrations
Nava-Hernandez et al. (2009)	19.5	Sperm affected via redox imbalance
Moorman et al. (1998)	25-130	Semen quality affected
Teijon et al. (2006)	40 &100 40 &100 40 &100 100	Hematology Histology-Offspring renal & hepatic Biomarker-Offspring renal function Physical development: birth weight
Fox et al. (2008)	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 confirm these findings and show that puberty onset is one of the more sensitive markers of Pb exposure as is demonstrated in the exposure response array (Table 5-29 and Figure 5-54). Dumitrescu et al. (2008) exposed adult Wister 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 dose-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 dose-dependent relationship.

In another recent study, Iavicoli et al. (2006) reported a statistically significant delay in several biomarkers of sexual maturity in offspring (F_1 generation) born to dams that ingested 3.5-40 ppm in their daily diet. Maternal ingestion of Pb at the various doses resulted in female pup blood Pb levels of 3.5-13 µg/dL. For all diet groups, there was a delay in age at vaginal opening, age of first estrus, age of vaginal plug formation, and age of first parturition. A novel finding in the Iavicoli study was that very low dose Pb (blood Pb of 0.7 µg/dL, food concentration of 0.02 ppm) induced statistically significant acceleration of markers of sexual maturation in female offspring versus background Pb level animals (blood Pb of 2 µg/dL) animals. There were statistically significant increases in time of vaginal opening (30% increased), first estrous, first vaginal plug formation, and first parturition at the very low Pb exposure versus 2 µg/dL animals. Thus, the timing of puberty is delayed in a dose-dependent fashion with very low dose Pb having

a statistically significant earlier onset of puberty than the background Pb animals. Also, the animals exposed to the higher dose of Pb (blood Pb up to 13 µg/dL) had statistically significant delays in onset of puberty when compared to the other dose groups.

In addition, Pb-induced shifts in sexual maturity were observed in the subsequent generation (F2 generation) across that dose range. These animals continued to be exposed to same concentrations of Pb over multiple generations through the diet. Data results of the F2 generation closely resembled those of the F_1 generation, as both generations received Pb exposure. The authors concluded that a modest elevation in blood Pb level (13 µg/dL) over background (2-3 µg/dL) can result in a profound delay in the onset of puberty (15-20%). In the F_2 generation, reduction in blood Pb (0.7 µg/dL) below background (2-3 µg/dL) was associated with an earlier onset of sexual maturity (30% increase) above background.

In the 2006 Pb AQCD (U.S. EPA, 2006), 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 in Fisher 344 pups. Subsequently, Pine et al. (2006) evaluated whether IGF-1 replacement could reverse the effects of Pb on female puberty. The authors reported that offspring exposed to Pb during gestation and lactation (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 $ng^3/µL$) resulting in restored timing of vaginal opening such that they were the same as control. It should be noted that, IGF-1 replacement in Pb-exposed animals did not cause advanced puberty 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, 2006) 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 sum, epidemiologic studies consistently show a positive association between blood Pb and delayed pubertal development in girls. This association is apparent even at low blood Pb levels. New evidence from the toxicology literature continues to support 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 to be IGF-1-dependent.

5.8.1.4. Summary of Effects on Female Reproductive Function

In summary, Pb exposure affects female reproductive function as demonstrated by both epidemiologic and toxicological studies. At low Pb levels, associations are observed with delayed puberty. Some evidence is also available regarding Pb levels and altered hormone levels as well as decreased fertility, although studies reported inconsistent findings for the later.

5.8.2. Effects on Male Reproductive Function

The 2006 Pb AQCD (U.S. EPA, 2006) reported on male Pb exposure/levels and reproductive functions as measured by sperm count/motility/morphology, time to pregnancy, reproductive history, and chromosomal aberrations. Despite limitations in many of the studies, most of the studies found slight associations between high Pb levels (i.e. ≥ 45 µg/dL) and reduced male fecundity or fertility (U.S. EPA, 2006). Evidence provided in the 1986 Pb AQCD (U.S. EPA, 1986) 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 reproductive function in males. These studies are described in the sections below.

5.8.2.1. 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 5-30.

Table 5-30. Summary of recent epidemiologic studies of effects on sperm and semen

Reference	Study Location and Years	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Hsu et al. (2009)	Taiwan NS	Men working at a battery plant	Blood Pb Categorized into 3 groups: <25 µg/dl, 25-45 µg/dl, >45 µg/dl	40.2	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)

Reference	Study Location and Years	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Kasperczyk et al. (2008)	Poland NS	Healthy, non-smoking, fertile men that worked at the Zn and Pb Metalworks	Blood Pb; seminal fluid Pb Categorized as high exposure workers (blood Pb 40-81 µg/dl), low exposed workers (blood Pb 25-40 µg/dl), and controls (office workers with no history of occupational Pb exposure)	Blood Pb High exposure workers: 53.1 (2.05) Low exposure workers: 34.7 (0.83) Controls: 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
Meeker et al. (2008)	Michigan NS	Men aged 18-55 going to infertility clinics (distinction not made between clinic visits for male or female fertility issues)	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)

Reference	Study Location and Years	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Naha and Chowdhury (2006)	Kolkata, India NS	Men aged 31-45 that were non-occupationally exposed controls and occupationally exposed workers)	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
Naha and Manna (2007)	Bangalore, India NS	Non-occupationally exposed controls and occupationally exposed workers	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
Slivkova et al. (2009)	NS	Men aged 22-48 undergoing semen analysis at an infertility clinic	Semen Pb	1.49 mg/kg (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
Telisman et al. (2007)	Croatia 2002-2005	Men aged 19-55, never occupationally exposed to metals and going to a clinic for infertility examination or for semen donation to be used for artificial insemination	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

International epidemiologic studies of men occupationally exposed to Pb have reported on associations between Pb levels and semen/sperm count and quality. Most of these studies included individuals occupationally exposed to Pb and have reported blood Pb levels over 40 µg/dL. For example, two studies performed in India (Naha & Chowdhury, 2006; Naha & Manna, 2007) 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 in one study (Naha & Chowdhury, 2006) and 68.26 in the other study (Naha & Manna, 2007). Control groups in these studies (those without occupational Pb

exposure) had 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 increased hyploidy of sperm DNA and morphologic abnormalities (Naha & Chowdhury, 2006; Naha & Manna, 2007). Decreased viability and increased lipid peroxidation were detected (Naha & Chowdhury, 2006). A study performed in Taiwan reported that men with greater blood Pb levels had increased sperm head abnormalities, increased sperm DNA denaturation, and increased sensitivity to denaturation compared to men with lower blood Pb levels (P. C. Hsu et al., 2009). No difference was detected between three Pb exposure groups and semen volume, sperm count, motility, velocity, and reactive oxygen species production. A similar study in Poland included employees exposed to Pb and compared them with a group of male office workers (A. 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 compared to both the control and moderate exposure groups. Lipid peroxidation, which induces tissue damage in sperm via reactive oxygen species, was greater in the highest exposure group compared to the controls.

One study performed in Croatia recruited men who had never been occupationally exposed to metals (Telisman et al., 2007). Increased blood Pb was associated with increased percent 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 biomarkers for Pb (erythrocyte protoporphyrin and δ-aminolevulinic acid dehydratase [ALAD]) were used instead.

Two studies examined Pb levels and semen quality of men at infertility clinics (Meeker et al., 2008; Slivkova et al., 2009). Meeker et al. (2008) detected no associations between increases in blood Pb and semen concentration, morphology, or motility (although a slight positive trend was observed between increasing Pb levels and motility in unadjusted models). In models that include multiple metals, blood Pb was associated with being below the WHO's 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. 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.

An abundance of evidence in the toxicological literature demonstrates that Pb exposure is detrimental to the quality and overall health of testicular germ cells. Earlier studies showed that chronic Pb exposure (15 weeks) in adult male rabbits induced statistically significant effects on semen quality and testicular pathology at blood Pb of 16-24 µg/dL (Moorman et al., 1998). Recent studies confirm earlier studies that Pb alters sperm parameters such as sperm count, viability, motility, and morphology. Oliveira et al. (2009) observed a negative correlation between Pb dose and intact acrosomes. Rubio et al (2006), Biswas and Ghosh (2006), and Salawu et al. (2009) observed a decrease in absolute testicular weight after

Pb exposure. Rubio et al (2006) and Biswas and Ghosh (2006) also observed a Pb-induced decrease in seminal vesicle and ventral prostate weights and Rubio et al. (2006) reported that Pb acetate, in a dose-dependent fashion (8-24 mg/kg body weight), reduced the length of certain stages of the spermatogenic cycle of rat seminiferous tubules and thus affected spermatogenesis. Reshma Anjum et al. reported decreased testicular and epididymal weights, sperm count, and viable sperm of male rats exposed to Pb acetate (273 mg/L or 819 mg/L in drinking water). Pb induced morphological abnormalities in sperm in a dose-dependent manner (Allouche et al., 2009; Massanyi et al., 2007; Oliveira et al., 2009; Salawu et al., 2009; Shan et al., 2009; Tapisso et al., 2009; C. H. Wang et al., 2006). Sperm abnormalities reported after Pb exposures are amorphous sperm head, abnormal tail, and abnormal neck. Dong et al. (2009) reported decreased epididymis and body weights in mice after exposure to 0.6% Pb acetate in drinking water. 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 the above 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 suggest that a component of Pb-induced toxicity is the generation of reactive oxygen species (ROS) which can then affect antioxidant defense systems of cells (Pandya et al., 2010). 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 catalse (CAT) in plasma and testes of adult male Sprague Dawley rats after administration of 1% Pb acetate in drinking water for 8 weeks. Supplementation with tomato paste (used as a source of antioxidants) reduced ROS production and prevented the 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 reduction in sperm count, sperm motility, and sperm viability (Madhavi et al., 2007; Rubio et al., 2006; Salawu et al., 2009; Shan et al., 2009; C. H. Wang et al., 2006).

Recent studies also demonstrate that Pb may be directly toxic to mature spermatozoa (Hernandez-Ochoa et al., 2006; Tapisso et al., 2009) as well as primary spermatocytes (Nava-Hernandez et al., 2009; Rafique et al., 2009). Nava-Hernandez et al. had two Pb exposure groups via drinking water. 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 because the L2 group drank less water than the L1 group. Piao et al. (2007) report Pb exposure caused DNA damage to sperm; the Pb exposure group had blood Pb of 67 µg/l. Piao et al. (2007) looked at 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 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) report that Pb reaches the sperm nucleus in the epididymis of mice chronically exposed to Pb (mean blood Pb 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 mice administered 21.5 mg/kg body weight Pb acetate by intraperitoneal injection. Nava-Hernandez (2009) reported a dose-dependent increase in DNA damage in rat primary spermatocytes after a 13-week exposure period to Pb acetate in drinking water (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.

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 (C. H. Wang et al., 2006). Dong et al. (2009) reported a dose-related increase in apoptosis in spermatogonia and spermatocytes of Kunming mice after exposure to 0.15-0.6% 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. (2006) observed a dose-dependent increase in the expression of apoptotic markers TGFβ1 and caspase-3 in spermatogenic cells, Sertoli cells, and Leydig cells. Shan et al. (2009) also reported a statistically significant increase in mRNA expression and protein levels of Fas, Fas-L and caspase-3 after Pb exposure. Supplementation with ascorbic acid inhibited or reduced the Pb-induced apoptosis in germ cells and protected testicular structure and function (Shan et al., 2009; C. H. Wang et al., 2006) suggesting ROS generation is a major contributing factor in decreased male fertility observed after chronic Pb exposure.

Similar to the results summarized in previous AQCDs, recent epidemiologic and toxicological studies report negative effects of high levels of Pb on sperm and semen. Future studies will aid in determining whether this association is observed at lower Pb levels.

5.8.2.2. Hormone Levels

The 2006 Pb AQCD (U.S. EPA, 2006) provided evidence that Pb acts as an endocrine disruptor in males at various points along the hypothalamic-pituitary-gonadal axis. The 2006 document also reported inconsistencies in the reported effects of Pb exposure on circulating testosterone levels. Recent epidemiologic and toxicological studies are reported below. Epidemiologic studies are summarized in Table 5-31.

Table 5-31. Summary of recent epidemiologic studies of effects on hormones for males

Reference	Study Location and Years	Outcome	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Meeker et al. (2008)	Michigan NS	FSH, LH, Inhibin B, testosterone, SHBG, FAI, testosterone/LH	Men aged 18-55 going to infertility clinics (distinction not made between clinic visits for male or female fertility issues)	Blood Pb	Median: 1.50 (IQR 1.10, 2.00)	Regression coefficients (95% CI) FSH 1st quartile: 1.00 (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: 1.00 (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: 1.00 (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) Testosterone 1st quartile: 1.00 (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: 1.00 (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: 1.00 (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)

Reference	Study Location and Years	Outcome	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Naha and Manna (2007)	Bangalore, India NS	FSH, LH, testosterone	Non-occupationally exposed controls and occupationally exposed workers	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)	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
Telisman et al. (2007)	Croatia 2002-2005	FSH, LH, testosterone, estradiol, prolactin	Men aged 19-55, never occupationally exposed to metals and going to a clinic for infertility examination or for semen donation to be used for artificial insemination	Blood Pb	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) Coefficients and p-values not given if not statistically significant (LH, FSH)

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 was associated with increasing serum testosterone and estradiol but decreasing serum prolactin (Telisman et al., 2007). In addition, the interaction term of blood Pb and blood cadmium levels demonstrated a synergistic effect on increasing serum testosterone levels. No association was observed between blood Pb and FSH or LH. Another study of men with high blood Pb levels reported no difference in serum FSH, LH, and testosterone among the three groups (Naha & Manna, 2007). 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, InhibinB, sex hormone-binding globulin (SHBG), free androgen index (FAI) or a measure of Leydig cell function (T/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.

In a recent toxicological study, Rubio et al. (2006) observed a decrease in testosterone levels in Pb acetate-treated rats in a dose-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). Pandya et al. (2010) reported altered hepatic sterodogenic enzyme activity. 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 adrenal

steroidogenic enzyme, Δ5-3β-HSD activity and serum corticosterone levels indicating disruption of the adrenocortical process. Dose-dependent decreases in serum testosterone were reported in Pb-exposed male rats (Reshma Anjum et al.). In contrast, Salawu et al. (2009) did not observe a decrease in serum testosterone between control animals and animals administered 1% 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 0.05-0.3% Pb acetate treatment in drinking water (only statistically significant in animals administered 0.05% 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, 2006).

Overall, recent epidemiologic and toxicological studies report mixed outcomes regarding hormone aberrations associated with Pb exposure. These results are similar to those from the 2006 Pb AQCD on the effects of Pb exposure on circulating testosterone levels.

5.8.2.3. Fertility

Epidemiologic studies have been performed comparing Pb and infertility in men. A study conducted in Turkey reported blood and seminal plasma Pb levels were different in fertile and infertile men [mean blood Pb 23.16 µg/dL (SD 5.59 µg/dL) for fertile men and 36.82 µg/dL (SD 12.30 µg/dL) for infertile men (p-values for ANOVA <0.001)] (Kiziler et al., 2007). Another study examined occupational Pb exposure (determined by self-report of occupational exposure) and detected no difference in reported exposure for infertile versus fertile men [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. As with the fertility studies among women, a limitation present in these studies is that the cases included are men who are seeking help at fertility clinics; the studies are not a sample of the general population regarding fertility.

5.8.2.4. Puberty

Research has also been published examining the association between blood Pb and onset of puberty in males. These studies are summarized in Table 5-32.

Table 5-32. Summary of recent epidemiologic studies of effects on puberty for males.

Reference	Study Location	Outcome	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Hauser et al. (2008)	Chapaevsk, Russia 2003-2005	Pubertal stages defined using standard drawings	Healthy boys aged 8-9	Blood Pb	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)
Tomoum et al. (2010)	Cairo, Egypt 2007	Hormones and pubertal development	Healthy children aged 10-13 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)	Blood Pb	NS 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% ≥ 10µg/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% ≥ 10µg/dl: Stage 1: 33.3% Stage 2: 66.7% Stage 3: 0% Chi-square p-value<0.05 Penile staging <10 µg/dl: Stage 1: 11.1% Stage 2: 44.4% Stage 3: 44.4% ≥ 10µg/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) ≥ 10µg/dl: 1.84 (SD 1.04) *Quantitative results for LH and FSH not provided

Williams et al. (2010)	Chapaevsk, Russia 2003-2008	Pubertal stages defined using standard drawings	Healthy boys aged 8-9 at enrollment who had annual follow-up evaluations	Blood Pb at ages 8-9	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)

Studies were performed among a cohort of Russian boys enrolled between ages 8-9 (Hauser et al., 2008; P. L. Williams et al., 2010). Both the cross-sectional study (Hauser et al., 2008) and the prospective study with annual follow-ups (P. L. Williams et al., 2010) demonstrated an association between increased blood Pb levels and later onset of puberty. In addition, in a study of boys and girls in Egypt boys with higher blood Pb had delayed pubertal development compared to those with lower levels (median age in the high blood Pb group was 12.5 years compared to 13.0 years in the low blood Pb group) (Tomoum et al., 2010). In addition, compared to the low Pb group, those boys with higher blood Pb had lower testosterone, FSH, and LH levels.

Thus, recent studies have demonstrated a negative effect of Pb on pubertal development among boys that exists even at low 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, 2006) supported earlier findings that Pb exposure may result in delayed onset of male puberty and altered reproductive function later in life in experimental animals.

5.8.2.5. Effects on Morphology and Histology of Male Sex Organs

Recent toxicological studies further support historical findings that showed an association between Pb exposure and histological changes in the testes as well as germ cells. Histological changes of testes in Pb-treated animals included seminiferous tubule atrophy, Sertoli cell and Leydig cell shrinkage with pyknotic nuclei (Shan et al., 2009; C. H. Wang et al., 2006), dilatation of blood capillaries in the interstitium, undulation of basal membrane, and occurrence of empty spaces in seminiferous epithelium (Massanyi et al., 2007).

5.8.2.6. Summary of Effects on Male Reproductive Function

Associations between Pb exposure and male reproductive function vary by outcome. The strongest evidence of an association is the relationship observed between high levels of Pb and negative effects on sperm and semen in both recent epidemiologic and toxicological studies and previous AQCDs. Recent toxicological studies also reported an association between Pb exposure and histological changes in the testes and germ cells. In addition, recent epidemiologic studies found Pb exposure to be associated with

delayed pubertal development at low blood Pb levels. This is supported by earlier toxicological studies. Similar to the 2006 Pb AQCD, 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.

5.8.3. Effects on Ovaries, Embryo Development, Placental function, and Spontaneous Abortions

The 2006 Pb AQCD (U.S. EPA, 2006) included studies of Pb exposure among men and women and their associations with spontaneous abortions. The 2006 AQCD concluded that overall there was little evidence to support an association between Pb levels among women and spontaneous abortion (U.S. EPA, 2006). Most of the studies examined in the 2006 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 AQCD to suggest an association with paternal Pb levels (U.S. EPA, 2006), and no recent studies have been performed to examine paternal Pb levels and spontaneous abortion. Since the 2006 AQCD, multiple epidemiologic studies have been published that examine Pb levels in women and their possible association with spontaneous abortion. Additionally, toxicological studies have studied the effects of Pb on fetal loss and the contribution of the ovaries and placenta to fetal loss.

Table 5-33. Summary of recent epidemiologic studies of effects on spontaneous abortions.

Reference	Study Location	Outcome	Study population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Gundacker et al. (2010)	Vienna, Austria 2005	Previous miscarriage	Women recruited during the second trimester of pregnancy	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)
Lamadrid-Figueroa et al. (2007)	Mexico City, Mexico 1997-1999, 2001-2004	Previous miscarriage	Women who had a previous pregnancy and were currently pregnant with gestational age of ≤14 wks	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)	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)

Yin et al. (2008)	Shanxi Province, China 2004-2006	Anembryonic pregnancy	Women age 25-35 yr old 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	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

Table 5-33, above, provides a summary of the recent epidemiologic studies examining the association between Pb levels and past and current spontaneous abortion. 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. Of note, among cases Pb was inversely correlated with folate and vitamin B12, but this correlation was not observed among those who delivered at term; no models examining Pb levels were controlled for nutrient status. A study in Turkey reported on groups of women who either had a spontaneous abortion before the 20th week of gestation or who had a viable pregnancy (Faikoglu et al., 2006). No difference was detected between the blood Pb levels of the two groups (Pb levels not reported here due to calculation errors discovered in the paper; errors do not appear to affect conclusions). 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 higher rates of previous spontaneous abortions than women in the lowest tertile. The authors state that the plasma/whole blood ratio represents the availability of Pb capable of crossing the placental barrier for a given blood concentration. No association was observed when examining 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 although the number of women included in the study was small (only 8 women reported previously having a miscarriage) (Gundacker et al., 2010).

Isolated 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 a dose-dependent decline in embryo development of fertilized buffalo oocytes cultured 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 understand to Pb-dependent 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, 2006). Wang et al. (2009) observed that elevated maternal blood Pbs (0.6-1.74 μM) compared to control (0.04 μM) were 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 dose-dependent increasing pathology of cytoarchitecture and cytoplasmic organelles. The authors also reported a 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 Pb level exposure condition which correlated to blood levels.

Pb-exposed (273 mg/L or 819 mg/L in drinking water, 0.05 or 0.15% Pb Acetate, respectively) male rats from Reshma Anjum et al. that had dose-dependent decreases in serum testosterone, decreased male reproductive organ weight and decreased sperm were mated to untreated females. These untreated dams had dose-dependent decreased implantation rate and higher pre- and post-implantation loss, indicating paternally mediated fetal loss.

As observed in sperm cells, Pb stimulates changes in antioxidant enzyme activity in rat ovaries indicating that a contributing factor in Pb-induced ovarian toxicity may be oxidative stress. 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 (0.05 mg/kg body weight).

Previous studies demonstrated that Pb accumulates in the ovaries and causes histological changes, thus contributing to Pb-induced effects on female fertility (U.S. EPA, 2006). In support of historical studies, recent studies demonstrate histological changes in ovarian cells of pigs (Kolesarova et al., 2010) and rats (Nampoothiri et al., 2007; Nampoothiri & Gupta, 2006). Kolesarova et al. (2010) observed a reduction of the monolayer of granulosa cells after Pb addition (0.5 mg/mL). Nampoothiri and Gupta (2006) reported that Pb exposure caused a decrease in cholesterol and total phospholipid content in the membranes of granulosa cells which resulted in increased membrane fluidity. 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, 2006).

Overall, the recent studies support the conclusions of the last Pb AQCD that there is insufficient evidence among epidemiologic studies to suggest an association between Pb and spontaneous abortions. In addition, studies of spontaneous abortions are difficult to conduct. The majority of spontaneous abortions are 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 levels do lead to

increased risk of early spontaneous abortions. Toxicological data provide mechanistic understanding of the contribution of Pb 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 up to the current date provide mixed effects on the role of Pb in spontaneous abortions.

5.8.4. Infant Mortality and Embryogenesis

The 2006 AQCD (U.S. EPA, 2006) 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. 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; Uzbekov et al., 2007; Y.-Y. Wang et al., 2009). No recent epidemiologic studies have reported on the relationship between Pb levels and infant mortality.

5.8.5. Birth Defects

The 2006 Pb AQCD (U.S. EPA, 2006) reported the possibility of small associations between high Pb exposure and birth defects, but many of the studies used occupational histories instead of actual measures of blood Pb levels. Among the studies included in the 2006 AQCD, two studies reported possible associations between parental exposure to Pb and neural tube defects (Bound et al., 1997; Irgens et al., 1998). Recent studies also examined Pb levels and neural tube defects (Table 5-34). No other recent studies of Pb levels/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 AQCD toxicological studies demonstrated associations between exposure to high doses of Pb and increased incidences of teratogenic effect in experimental animals.

Table 5-34. Summary of recent epidemiologic studies of effects on neural tube defects.

Reference	Study Location	Study Population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Brender et al. (2006)	Texas 1995-2000	Infants of Mexican-American women	Maternal blood Pb taken 5-6 wks post-partum	Cases: 2.4 (1.9) Controls: 2.5 (1.6)	Blood Pb<6.0 µg/dL: 1.0 (Ref) Blood Pb≥6.0 µg/dL: 1.5 (95% CI: 0.6, 4.3)

Zeyrek et al. (2009)	Turkey NS	Infants with gestational age of at least 20 wks	Maternal and umbilical cord blood Pb taken 0.5h after birth	Cases: Maternal: 15.5 (15.0) Umbilical cord: 18.2 (17.8) 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

Among the recent epidemiologic studies (described in Table 5-34), 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. These measurements were taken 5-6 weeks postpartum, which is a limitation of this study because the levels may be different than during the developmental period of gestation. The OR comparing those with at least 6 µg/dL Pb to those with less than 6 µg/dL Pb was 1.5 (95% CI: 0.6, 4.3). This increased after adjusting for breast feeding, although this variable was not a confounder due to its inability to be associated with neural tube defects. For these women neither occupational exposure to Pb or proximity of residence to a facility with Pb air emissions at the time of conception were associated with increased odds of neural tube defects.

Overall, in contrast to studies from the 2006 AQCD, recent studies of Pb and neural tube defects observed no associations.

5.8.6. Preterm Birth

Research on preterm birth included in the 2006 Pb AQCD (U.S. EPA, 2006) reported inconsistent findings regarding the relationship between Pb and gestational age. Recent studies have examined this potential association and again mixed results were reported (Table 5-35). 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 towards the null.

Table 5-35. Summary of recent epidemiologic studies of effects on preterm birth.

Reference	Study Location	Outcome	Study Population	Exposure Measurement	Mean Pb (SD) in g/dL	Adjusted Effect estimates
Berkowitz et al. (2006)	Idaho 1970-1981	Preterm birth (<37wk)	Singleton infants with 28-45 wk gestation	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"	NS	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)
Patel and Prabhu (2009)	Nagpur, India NS	Gestational age	Consecutive births at the study hospital	Umbilical cord blood Pb	Umbilical cord blood Pb: 4.7 (12.1)	>5 µg/dL: mean gestational age 38 wks ≤5 µg/dL: mean gestational age 39 wks Linear regression: gestational age decreased 1 wk with every 1 µg/dL increase in umbilical cord blood Pb (exact values and 95% CI not given)
Jelliffe-Pawlowski et al. (2006)	California 1995-2002	Preterm birth (<37 completed wk)	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	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) ≥ 40µg/dl: 4.7 (1.1, 19.9) <10 µg/dl: 1.00 (Ref) ≥ 10µg/dl: 3.2 (1.2, 7.4)
Jones et al. (2010)	Tennessee 2006	Gestational Age: preterm (<37wk) , term (37-40 wk), post-term (>40 wk)	Singleton births ≥ 27 wk gestation from mothers aged 16-45 living in the Shelby County area for at least 5 mo during pregnancy	Umbilical cord blood Pb	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
Vigeh et al. (2011)	Tehran, Iran 2006	Preterm birth (20-37 wk)	Singleton births from non-smoking, non-obese mothers aged 16-35 and referred for prenatal care during the 8th-12th week of gestation	Maternal blood Pb	3.8 (2.0)	Mean blood Pb (SD): 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)

One study of preterm birth included women living in two different residential areas over three different time periods (Berkowitz et al., 2006). One residential area was consistently unexposed but the other had a period of high Pb emissions due to damage at a local factory. The three time periods examined preterm birth rates before, during, and after the time of high exposure. No association was observed between women living in the high exposure area compared to those in the low exposure area during any of the time periods, but the number of preterm infants born during the period of high exposure was small. In another study, measurements of umbilical cord blood were taken after birth at a hospital in Nagpur,

India (Patel & 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 varied between infants with >5 µg/dL Pb and infants with ≤ 5 µg/dL 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 (E. A. Jones et al., 2010). Mean levels of umbilical cord blood Pb were slightly higher among infants born preterm but the difference was not statistically significant. In a study taking place in California, women with information on blood Pb levels 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) 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 the majority being <5 µg/dL. Preterm birth was associated with increased blood Pb when comparing women with maximum blood Pb levels ≥ 10 µg/dL to women with blood Pb levels <10 µg/dL in adjusted analyses. In analyses of maximum Pb levels refined into further categories, when compared to maximum blood Pb levels ≤ 5 µg/dL the positive association between maximum blood Pb measurement and preterm birth was present at 20 µg/dL and higher. Finally, a study in Iran reported higher maternal blood Pb for preterm births than term births (Vigeh et al., 2011). A positive association between maternal blood Pb levels and preterm birth was observed.

In sum, as in the 2006 Pb AQCD, recent epidemiologic studies report inconsistent findings for the relationship between Pb and preterm birth.

5.8.7. Low Birth Weight/Fetal Growth

The 2006 Pb AQCD reported inconsistent study results examining the associations between Pb and birth weight/fetal growth and concluded that there could be a small effect of Pb exposure on birth weight and fetal growth (U.S. EPA, 2006). Since then, multiple 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 5-36 below. Additionally, there have been a few recent toxicological studies evaluating the effect of Pb exposure during gestation on birth weight.

Table 5-36. Summary of recent epidemiologic studies of effects on low birth weight and fetal growth.

Reference	Study Location	Outcome	Study population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Berkowitz et al. (2006)	Idaho 1970-1981	Low birth weight (<2,500 g and ≥37 wk) Small for gestational age (birth weight ≤5th percentile of sex- and gestational wk weights for singletons in Idaho)	Singleton infants with 28-45 wk gestation	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"	Not specified	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)
Gundacker et al. (2010)	Vienna, Austria 2005	Birth length, birth weight, head circumference	Infants of women recruited during their second trimester	Maternal blood Pb between wk 34-38 of gestation, whole placentas and umbilical cord Pb shortly after birth, meconium samples in first five days after birth	Median (IQR): Maternal blood Pb: 2.5 (1.8, 3.5) Umbilical cord blood Pb: 1.3 (0.8, 2.4) Placenta Pb: 25.8 µg/kg (21.0, 36.8 µg/kg) Meconium Pb: 15.5 µg/kg (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)
Iranpour et al. (2007)	Isfahan, Iran 2005	Low birth weight (≤ 2,500g, >37wk)	Full-term infants born at a hospital affiliated with Isfahan University	Umbilical cord and maternal blood Pb within 12 h of delivery	Maternal blood Pb: Cases: 12.5 (2.0) Controls: 13.5 (2.7) Umbilical cord blood Pb: Cases: 10.7 (1.7) Controls: 11.3 (1.9)	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 P-values for correlations: Umbilical cord blood Pb and birth weight: Low birth weight: 0.84 normal birth weight: 0.26
Janjua et al. (2009)	Karachi, Pakistan 2005	Low birth weight (≤ 2,500g)	Infants of randomly selected women who planned to deliver between 37-42 wk	Umbilical cord blood Pb	Umbilical cord blood Pb: 10.8 (0.2)	Prevalence ratio: <10 µg/dl: 1.00 (Ref) ≥ 10µg/dl: 0.82 (0.57, 1.17)

Reference	Study Location	Outcome	Study population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Jelliffe-Pawlowski et al. (2006)	California 1995-2002	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	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: -- 10-19 µg/dl: 2.7 (0.5, 14.8) 20-39 µg/dl: 1.5 (0.3, 7.7) ≥ 40µg/dl: -- <10 µg/dl: 1.00 (Ref) ≥ 10µg/dl: 3.6 (0.3, 40.0) Small for gestational age ≤5 µg/dl: 1.00 (Ref) 6-9 µg/dl: -- 10-19 µg/dl: 2.3 (0.6, 9.2) 20-39 µg/dl: 2.1 (0.7, 6.7) ≥ 40µg/dl: -- <10 µg/dl: 1.00 (Ref) ≥ 10µg/dl: 4.2 (1.3, 13.9)
Jones et al. (2010)	Tennessee 2006	Low birth weight (<2.500g)	Singleton births ≥27 wks gestation from mothers aged 16-45 living in the Shelby County area for at least 5 mo during pregnancy	Umbilical cord blood Pb	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
Kordas et al. (2009)	Mexico City, Mexico 1994-1995	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)	Umbilical cord and maternal blood Pb within 12 h of delivery; maternal tibia Pb	Maternal tibia Pb: 9.9 µg/g (9.8 µg/g) Maternal blood Pb ≥ 10µg/dl: 27% Umbilical cord blood Pb ≥ 10µg/dl: 13.7%	Regression coefficients (SE) (adjusted for maternal BMI, maternal height, infant gestational age, and other variables) 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 with 4th quartile tibia Pb (15.6-76.5 µg/g) delivered infants 140 g less than women with tibia Pb in the lowest quartile
Lamb et al. (2008)	Mitrovica and Pristina, Yugoslavia 1985-1986	Height and BMI at birth	Participants of the Yugoslavia Study of Environmental Lead Exposure, Pregnancy Outcomes, and Childhood Development	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 Mitrovica: -0.18 (-0.69, 0.33) Pristina: -0.14 (-0.69, 0.42) Height Mitrovica: 0.43 (-0.83, 1.69) Pristina: 0.35 (-0.64, 1.34)-
Llanos and Ronco (2009)	Santiago, Chile NS	Fetal growth restriction (1,000-2,500g) *note normal birth weights were >3,000g	Term births (37-40 wks) from non-smoking mothers	Placenta Pb	Fetal growth restricted: 0.21 µg/g (0.04 µg/g) Controls: 0.04 µg/g (0.009 µg/g)	P-value for Mann-Whitney U-test <0.01
Williams et al. (2007)	Tennessee 2002	Birth weight	Infants from singleton births or the firstborn infant in a set of multiples	Air Pb levels during first trimester of pregnancy	0.12 µg/m3 (0.04 µg/m3)	p-value for multilevel regression of Pb with birth weight: 0.002 Increase of Pb from 0 to 0.04 relates to a 38g decrease in birth weight Increase of Pb from 0 to 0.13 (maximum) relates to a 124g decrease in birth weight

Reference	Study Location	Outcome	Study population	Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Zentner et al. (2006)	Santo Amaro, Brazil 2002	Birth weight and length	Singleton births with maternal residence within 5 km of Pb smelter	Umbilical cord blood Pb from delivery	Umbilical cord blood Pb: 3.9 (3.6)	Linear regression coefficient with umbilical cord blood Pb as the dependent variable in model with only length and weight (unit not given, assume per 1 µg/dL): Length -0.46 (p-value 0.003) and Weight -0.275 (0.048)

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 blood Pb levels were greater in 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, no differences in the associations were detected. Another study using maternal blood Pb was conducted in California (Jelliffe-Pawlowski et al., 2006). Women's blood Pb measurements during pregnancy were matched with the corresponding birth certificates. 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 subsequently large 95% CI. An association was detected for high blood Pb and having an infant who was small of his/her gestational age (SGA; defined as birth weight <10th percentile of normal weight for population-based singleton race and gender specific infants of the same gestational age). A study of term births in Iran reported no difference in blood Pb of women giving birth to a normal weight infant and women giving birth to an infant with low birth weight (Iranpour et al., 2007). Finally, 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).

One study examining the association between Pb levels and birth weight used tibia bone measurements from the mothers living in Mexico City (Kordas et al., 2009). Pb tibia 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 increased among women with certain genotypes (statistical tests not reported).

Multiple studies examined the relationship using Pb measured from the placenta or umbilical cord. First, the study by Iranpour et al. (2007) discussed above investigated the association between umbilical cord blood Pb levels in addition to their examination of whole blood. They again report no difference in levels between term infants of normal and low birth weight. 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 & 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 Pb level was found to be inversely correlated with length and weight of the infants. A third study recruited women in Pakistan (Janjua et al., 2009). Umbilical cord blood Pb levels were not associated with 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 among infants born to women living primarily in urban areas of Memphis, TN (E. A. Jones et al., 2010). 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.

Two studies examined air exposures and reported inverse associations between air Pb concentrations and birth weight. 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 µg/m^3 (i.e., one standard deviation) increase in Pb concentration. The other 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 a local factory that lead to high Pb emissions during one of the time periods under study (Berkowitz et al., 2006). No levels of Pb are provided. Mean birth weight for term births was decreased among women living in high exposure areas during the period of high exposure compared to those living in unexposed areas. 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 exposure, the odds of low birth weight among term births was increased among those living in the exposed area compared to those in the unexposed 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 fifth percentile of birth weight for their sex and age) was also associated with living in the exposed area during the time period of 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 unexposed area.

Evidence from previous toxicological studies has shown an association between gestational Pb exposure and reduced birth weight and impaired postnatal growth (U.S. EPA, 2006). More recent studies have reported conflicting results. Wang et al. (2009) demonstrated a statistically significant decrease in fetal body weight and body length of Wistar rats after maternal exposure to 0.025% 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, female pups had a decreased birth weight when compared to male pups of the same litter (only statistically significant at the 400 ppm dose). This effect did not persist in the postnatal growth of the rats. 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 (mean blood Pb level 22.8 µg/100mL), but no changes in body length were reported. In contrast, Leasure et al. (2008) reported a statistically significant inverse relationship between Pb exposure and body weight for male mice exposed to low-and high-levels of Pb during gestation. Male mice exposed to the low and high Pb concentrations during gestation were 26% and 13% heavier than controls at 1 year of age, respectively. In this study, dams were administered 27 ppm (low), 55 ppm (moderate), and 109 ppm (high) Pb in drinking water beginning 2 weeks before mating and continuing until PND10. Resulting blood Pb levels ranged from 10 µg/dL or less in the low-exposure offspring to 42 µg/dL in the high-exposure offspring at PND10. The authors also reported that when dams received low or moderate levels of Pb in drinking water from birth to weaning neither male nor female offspring exposed to Pb postnatally exhibited a difference in body weight when compared to control offspring.

In summary, associations were observed between Pb and low birth weight in a study of maternal bone Pb and studies of Pb air exposures and birth weight. However, the associations were less consistent when using maternal blood Pb or umbilical cord and placenta Pb as the exposure measurement. Previous toxicological studies observed an association between gestational Pb exposure and reduced birth weight with moderate to high dose Pb. More recent findings using low dose Pb exposure reported increased offspring body weight after developmental Pb exposure.

5.8.8. Toxicological Studies of Developmental Effects

5.8.8.1. Developmental Effects on Blood and Liver

The 1986 and 2006 AQCD 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 report an increase in the number of erythrocytes; however their size was diminished by 62%. Pb produced microcitic anemia as evidenced by decreased hemoglobin content and hematocrit values without changes in mean corpuscular hemoglobin (MCH) concentration. No changes were observed in alkaline phosphatase (ALP) activity, CAT activity, or

thiobarbituric acid reactive substances (TBARS) production in pups at postnatal 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 21.

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 reported increases in TBARS and CAT activity in the liver after Pb exposure. Intoxication with Pb also resulted in decreased liver protein concentrations and manganese-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 in 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 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). Pb-induced histological changes observed include massive fatty degeneration in hepatocytes, large vacuoles in cytoplasm, appearance of pycnotic nuclei, and infiltration of lymphocytes in the liver. 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.

5.8.8.2. Developmental Effects on Skin

The 2006 Pb AQCD (U.S. EPA, 2006) reported one study that demonstrated Pb-induced abnormalities in skin development. No current studies were identified that addressed Pb-induced skin alterations.

5.8.8.3. Developmental Effects on the Retina

The 2006 AQCD concluded that Pb exposure during early postnatal development (blood Pb ~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 low (27 ppm), moderate (55 ppm), and high (109 ppm) levels of Pb acetate in drinking water beginning 2 weeks before mating, throughout gestation, and until PND10. blood Pb levels in pups on postnatal days 0-10 exposed to Pb during gestation were 10-12 µg/dL (low), 21-24 µg/dL (moderate), and 40-46 µg/dL (high). Results of the study demonstrated supernormal persistent rod photoreceptor-mediated (scotopic) electroretinograms (ERG) in adult rats similar to ERG findings in male and female children after gestational exposure to low- and moderate-levels of Pb. Low- 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. High-level Pb exposure (109 ppm) statistically significantly decreased the number of rods in central and peripheral retina Pb-exposure induced dose-dependent decreases in adult rat retinal dopamine synthesis and utilization/release.

5.8.8.4. 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. The authors concluded that additional research is needed on the intracellular uptake of Pb during tooth development to fully understand the spatial distribution of Pb in teeth.

5.8.9. Summary and Causal Determination

Many epidemiologic and toxicological studies of the effects of Pb on reproductive outcomes have been performed since the 2006 AQCD. These studies covered outcomes such as female and male reproductive function, birth defects spontaneous abortions, infant mortality, preterm birth, low birth weight, and developmental effects. There is an abundance of evidence in the literature demonstrating that

Pb induces reproductive and developmental effects in laboratory animals exposed to Pb during gestation and/or lactation. Many of the Pb-induced effects occur in a dose-dependent manner and have been observed at maternal blood Pb levels that do not result in clinical toxicity in the dams. Additionally, epidemiologic studies have demonstrated strong evidence of an association between Pb and delayed puberty as well as decrements to sperm/semen quality and function.

Many of the animal toxicology studies included in the 2006 AQCD explored the effect of Pb on reproduction and development at blood Pb levels greater than 40 µg/dL, a dose where maternal toxicity can develop during pregnancy. Data from the 2006 AQCD on male fertility showed perturbed semen quality. Recent studies have shown the effects of Pb exposure during early development to include disruption of endocrine function; delay in the onset of puberty and alteration in reproductive function later in life; and changes in morphology or histology in sex organs and placenta. Additionally, epidemiologic studies of reproductive factors among males and females investigated whether Pb levels were associated with hormone levels, fertility, and onset of puberty. Epidemiologic studies showed associations between blood Pb and hormone levels for females. Studies of Pb and fertility are limited and inconsistent for females and males. Strong and consistent associations were observed between Pb levels in males in occupational settings with blood Pb levels as low as 20-45 µg/dL and sperm count and quality. Multiple studies of Pb and puberty have shown associations between blood Pb levels and delayed pubertal development for girls and boys. These associations are consistently observed in multiple epidemiologic studies and demonstrate effects on pubertal development at blood Pb levels <10ug/dL.

Pb-mediated changes in levels or function of reproductive and growth hormones have been demonstrated in past and more recent toxicological studies; however the findings are inconsistent. More data are needed to determine whether Pb exerts its toxic effects on the reproductive system by affecting the responsiveness of the hypothalamic-pituitary-gonad axis or by suppressing circulating hormone levels. More recent toxicological studies suggest that oxidative stress is a major contributor to the toxic effects of Pb on male and female reproductive systems. 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 an association between increased generation of ROS and germ cell injury as evidenced by destruction of 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.

Overall, results of pregnancy outcomes were similar to those of the 2006 AQCD; inconsistent evidence of a relationship with Pb was available for preterm birth and little evidence was available to study the associations with spontaneous abortions. The previous AQCD included a few studies that reported possible associations between Pb and neural tube defects, but the recent epidemiologic studies found no association. Possible associations were observed between Pb and low birth weight when

epidemiologic studies used measures of maternal bone Pb or air exposures, but the associations were less consistent when using maternal blood Pb or umbilical cord and placenta Pb. Effects of Pb exposure during early development on toxicological studies included reduction in litter size, implantation, birth weight and postnatal growth.

Toxicological studies demonstrated that the effects of Pb exposure during early development include impairment of retinal development and alterations in the developing hematopoietic and hepatic systems. Negative developmental outcomes were also noted including effects on the skin and teeth.

Similar to toxicological and epidemiologic studies that observed Pb to be associated with delayed puberty, delays of dynamic changes in the HPT axis are seen in the ecological literature, i.e., delayed metamorphosis in Pb exposed frogs. Additionally, Pb exposure has been shown to have detrimental effects on sperm, albeit often at higher blood Pb levels in epidemiology studies but in lower doses in the toxicology literature. Again, these findings agree with the ecological literature where Pb-dependent sperm effects are seen in rotifers, earthworms, and trout.

In conclusion, the recent toxicological and epidemiologic literature provides strong evidence that Pb exposure is related to delayed onset of puberty in both males and females. Additionally, Pb exposure has been shown to have detrimental effects on sperm (at high blood Pb levels in epidemiologic studies and in low doses in the toxicological literature). The data on preterm birth, low birth weight, spontaneous abortions, birth defects, hormonal influences, and fecundity are a bit more mixed and less consistent between the toxicological and epidemiologic literature. The collective body of evidence integrated across epidemiologic and toxicological studies with a focus on the strong relationship observed with negative effects on sperm and delayed pubertal onset is sufficient to conclude that there is a **causal relationship between Pb exposures and reproductive effects and birth outcomes.**

5.9. Effects on Other Organ Systems

5.9.1. Effects on the Hepatic System

Hepatotoxic effects of Pb in various animal and human models include alterations in hepatic metabolism, hepatic cell proliferation, changes in cholesterol metabolism, as well as oxidative stress-related injury. Animal studies have also shown that exposure to Pb causes a decrease in Phase I along with a simultaneous increase in Phase II enzymes following exposure to various forms of Pb. Induction of oxidative stress is well supported by an increase in lipid peroxidation along with a decrease in glutathione (GSH) levels and catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities

5.9.1.1. Summary of Key Findings of the Effects on the Hepatic System from the 2006 Lead AQCD

The 2006 Pb AQCD stated that some of the frequent toxicological effects in the liver following exposure to Pb included increased hepatic cell proliferation, cholesterol synthesis, DNA synthesis and glucose-6-phosphotase dehydrogenase (G6DP) activity resulting in Pb-induced hyperplasia. The AQCD concluded that cytochrome (CYP) P450 levels decreased following single exposures to Pb, primarily Pb nitrate. Inhibition of induced and constitutive expression of microsomal CYP 1A1 and 1A2 was observed among various P450 isozymes. Inhibition of Phase I 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 causing a biochemical phenotype similar to hepatic nodules. Studies relating to Pb-induced hepatic hyperplasia suggested alterations in the gluconeogenic mechanism, DNA hypomethylation along with changes in proto-oncogene expression as well as cholesterol synthesis. Cholesterol metabolism changes following exposure to Pb were reportedly mediated as a result of induction of several enzymes related to cholesterol metabolism as well as a decrease in the cholesterol catabolizing enzyme, 7 α-hydroxylase. Tumor necrosis factor α (TNF-α) was reported to be one of the major mitogenic signals that mediated Pb nitrate-induced hepatic hyperplasia in studies using inhibitors to block TNF-α activity. Other Pb-related effects presented in the 2006 Pb AQCD include liver cell apoptosis mediated by Kupffer cell derived signals and Pb-induced oxidative stress in vitro cell cultures. More recent Pb exposure experiments suggested that alterations in liver heme metabolism may involve changes in 5-aminolevulinic acid dehydrogenase (ALAD) activity, porphyrin metabolism, Transferrin (TF) gene expression and changes in iron metabolism.

In humans, the 2006 Pb AQCD stated that nonspecific liver injury generally observed as increases in liver enzymes in the serum was reported in occupational studies. In addition, similar to effects noted in animal studies, cytochrome P450 activity was also suppressed in humans following exposure to Pb under various conditions. The 2006 Pb AQCD concluded that hepatic effects occurred only at high Pb exposure levels.

5.9.1.2. New Epidemiologic Studies

A few studies examined liver biochemical parameters effects on antioxidant status and oxidative stress resulting from occupational exposures. Patil et al. (2007) examined the effect of occupational Pb exposure to liver and kidney function in silver jewelry workers (SJW), battery manufacturing workers (BMW) and spray painters (SP) in western Maharashtra, India. Blood Pb was statistically significantly increased in all three groups: 53.63 ± 16.98 (BMW), 48.56 ± 7.39 (SJW), and 22.32 ± 8.87 µg/dL (SP), compared to controls (12.52 ± 4.08). Liver function enzymes including serum glutamic oxaloacetic transaminase (SGOT)/AST, and serum glutamic pyruvic transaminase (SGPT)/ALT levels were only

increased in the SP group compared to the concurrent control group but not in the SJW and BMW groups. Total serum protein levels were decreased in all three groups, while serum albumin levels were decreased in SJW and SP groups and increased in the BMW group. Serum globulin levels were decreased and the albumin/globulin levels were increased in the BMW and SJW groups compared to controls. In addition, bilirubin levels were increased only in the BMW group. In a study examining the impact of Pb exposure in an occupational setting, Khan et al. (2008) reported that workers in Pakistan occupationally exposed to Pb (blood Pb = 29.1 [range 9.0 to 61.1] µg/dL) had a significant increase (3.5-fold higher median) in blood levels compared to age and gender matched controls (blood Pb = 8.3 [range 1.0 to 21.7] µg/dL). Oxidative stress markers such as MDA, and gamma-glutamyl transpeptidase (GGT) were significantly increased in workers as were ALT levels. Serum albumin and total protein levels were significantly decreased in the examined workers, compared to controls. Based on these results, study authors concluded that Pb exposure causes oxidative stress and changes in liver enzymes that may lead to hepatic toxicity in exposed workers. Can et al. (2008) also reported changes in liver function enzymes among battery workers and muffler repair workers exposed to Pb in an occupational setting. Blood Pb was elevated in both worker groups (36.83 ± 8.13 and 26.99 ± 9.42 µg/dL for battery workers and muffler repair workers, respectively) versus. controls (14.81 ± 3.01 µg/dL). The study authors reported that total protein, globulin, and lactate dehydrogenase (LDH) levels were within or very close to the normal range, but were statistically significantly higher in both worker groups compared to controls. Additionally, increases in cholesterol and ALP were increased only in battery workers and muffler repair workers, respectively. Though an increase in LDH levels among the workers was observed, the study authors stated that this increase was not related to liver injury. Total protein, globulin, ALP, and LDH were also observed to be significantly correlated to blood Pb levels in workers. Though liver enzyme function changes were nominal, the study authors concluded that in an occupational setting, exposure to Pb may lead to liver injury. While Can et al. (2008) did consider the impact of smoking in their analysis, it is not clear whether Patil et al. (2007) and Khan et al. (2008) considered the impact on these factors in their analysis. In a single case study report of a 40-year old Iranian male accustomed to using opium as a pain reliever, Verheij et al. (2009) reported that a liver biopsy taken following elevated liver function enzymes exhibited bile intracytoplasmic pigmentation in the hepatocytes. The study authors reported that the blood Pb levels were highly elevated in the patient (86.0 µg/dL), and attributed exposure to Pb from Pb-contaminated opium consumption. The liver parenchyma also revealed disrupted architecture along with regenerated nodules. Pathomorphological changes, rarely seen in humans, were also reported in the form of active hepatitis along with microvesicular and macrovesicular steatosis, hemosiderosis, and cholestatis as well as lymphocytic cholangitis. The study authors stated that following chelation therapy, liver enzymes returned to normal suggesting reversal of the histological findings. However, the reversibility was not confirmed with another liver biopsy. A case report by Fonte et al. (2007) described a worker occupationally exposed to Pb vapors (blood Pb = 148 µg/dL) with hypersideremia, mixed bilirubinemia, and elevated levels of

ALT and AST. Following chelation therapy, the patient's clinical symptoms resolved, indicating the reversibility of the Pb-induced effects on the liver.

5.9.1.3. New Toxicological Studies

Hepatic Metabolism

As stated in the AQCD 2006, acute exposures to Pb nitrate and other Pb compounds causes a decrease in Phase I enzymes accompanied by a simultaneous increase in Phase II enzymes. The conclusions presented in the AQCD 2006 were also reviewed and corroborated by Mudipalli (2007).

Changes in biochemical parameters, suggestive of liver damage, in male Wistar rats treated with 500 ppm Pb acetate in drinking water over a 10 month period included decreases in serum protein and albumin levels as well as an increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), and gamma glutamyl transpeptidase (GGT) levels (S. et al., 2009). In treated animals, the blood Pb levels steadily increased throughout the study period, reaching a maximum of approximately 110 µg/dL. The study authors reported that similar biochemical changes were not observed in animals treated with Pb acetate as well a mineral rich diet and concluded that nutritional management is important in managing Pb-related poisoning. Swarup et al. (2007) investigated serum biochemical changes in cows living in Pb-contaminated environments. Serum levels of ALT, AST, alkaline phosphatase, total protein, albumin, globulin, and A/G ratio were statistically significantly altered in cows living near Pb-Zn smelters (blood Pb = 86 ± 6) compared to control cows (blood Pb = 7 ± 1 µg/dL). Significant positive correlations were found between blood Pb and ALT and AST, whereas a negative correlation was observed between blood Pb and total lipids, protein, and albumin. Upadhyay et al. (2009) investigated the effects of Pb exposure on biochemical alterations in Sprague-Dawley rats exposed to 35 mg/kg via i.p. injection for 3 days. The activities of ALT, AST, serum ALP, and acid phosphatase were all significantly increased over control in exposed animals, whereas alkaline phosphatase activity was decreased in exposed animals. Concomitant treatment with zinc and varying levels of ascorbic acid were observed to ameliorate the toxic effects of Pb.

Pillai et al. (2009) investigated gestational and lactational exposure to Pb on hepatic phase I and II enzymes in male and female rats. Pregnant rats were exposed to 50 µg/kg Pb acetate via subcutaneous injection daily throughout gestation, and continuing until PND21. The female and male pups were then allowed to reach sexual maturity (PND55-56) to assess continuing exposure to bioaccumulated Pb. The activities of hepatic phase I enzymes NADPH- and NADH-cytochrome c reductase were statistically significantly reduced in Pb-exposed male and female rats on PND56, compared to controls. In rats treated with 25 µg/kg Pb and Cd, the effect on phase I enzymes was increased. Pb exposure 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 in Pb-exposed rats regarding serum glutamate pyruvate dehydrogenase or ALP activities in males or females. Histological observations in both male and female rats demonstrated fatty degeneration, vacuolization, and pycnotic nuclei, indicating general hepatotoxicity following Pb exposure. In a similar study, Teijon et al. (2006) exposed Wistar rats to 200 or 400 ppm throughout gestation, lactation, and 3 months postweaning, or only 1 month postweaning. In the animals exposed continuously throughout gestation and lactation, the concentrations of Pb in the liver were elevated in the 200- and 400-ppm groups 1 and 3 months postweaning. Liver concentrations of Pb were greater in the 200 ppm animals compared to the 400 ppm animals at one month postweaning, but were similar between the 2 dosing regimens at 3 months postweaning. ALP activity was increased at 2 weeks postweaning in animals continuously exposed to Pb throughout gestation and lactation, whereas ALT activity was decreased only at 2 and 3 months postweaning. In animals exposed only for 1 month postweaning, serum ALP activity was significantly increased, although in a non-dose dependent manner. ALT and AST activities were not affected.

Cheng et al. (2006) studied the mechanism of Pb effects on bacterial lipopolysaccharide (LPS)-induced TNF-α expression. A/J mice were injected via i.p with 100 μmol/kg Pb, with or without 5 mg/kg LPS. Pb alone did not affect liver function (measured as AST or ALT activity) or the level of TNF-α in the serum. In comparison, exposing the mice to 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 an in vitro study, the authors reported that 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) and concluded that monocytes/macrophages (rather than hepatocytes) were primarily responsible for Pb increased LPS- induced TNF-α levels via the protein kinase C (PKC)/MAPK pathway.

Lipid Metabolism

In a lipid metabolism study, Ademuyiwa et al. (2009) reported that male albino Sprague Dawley rats exposed to 200, 300 and 400 ppm Pb in drinking water had 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 had liver Pb concentrations of 10.04 ± 1.14 μg/g, compared to 3.24 ± 1.19 and 2.41 ± 0.31 in animals exposed to 300 or 400 ppm Pb. Animals exposed to Pb exhibited increased hepatic cholesterogenesis at all doses tested compared to controls. Additionally, a decrease in triglyceride was observed at 300 and 400 ppm, a decrease in phospholipid levels was observed at 400 ppm. The authors also reported a positive correlation between tissue cholesterol and phospholipids compared to Pb accumulation in liver across all doses. In contrast, the association between tissue triglyceride levels and Pb accumulation was negative. In a related study,

Khotimchenko and Kolenchenko (2007) reported that adult male albino rats treated with 100 mg/kg Pb acetate for as little as 14 days exhibited disorders in lipid metabolism that were supported by increased levels of total cholesterol and triglyceride levels in the liver tissue. Pillai et al. (2009) observed decreases in total liver cholesterol in PND56 male and female rats that had been exposed to 50 µg/kg Pb acetate continuously throughout gestation and lactation. These results suggest that 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 the gender-related differences in the hepatic sterol regulatory element binding protein-2 (SREBP-2) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) gene expressions in male and female Sprague Dawley rats injected with 100 µmol/kg body weight of Pb nitrate intravenously. The SREBP-2 expression, which is a transcription factor for the HMGR gene, was significantly increased in males and females with the increase occurring earlier in male rats (6-12 hours, compared to 24-36 hours in females). In contrast, expression of the HMGR gene, a rate limiting enzyme in cholesterol biosynthesis, was significantly increased in both males and females at earlier time frames (3-48 hours in males; 12-48 hours in females) compared to the SREBP-2 gene expression. Significant increases in total liver cholesterol were also observed in males and females at 3-48 and 24-48 hours, respectively. These results suggest that the SREBP-2 and HMGR gene expressions and increase in total cholesterol levels in the liver occur earlier in males compared to females and also suggest that the HMGR gene expression and increase in total cholesterol levels in the liver occur before an increase in the SREBP-2 gene expression in either sex.

Hepatic Oxidative Stress

A number of studies pertaining to hepatic oxidative stress as a result of exposure to various Pb compounds were identified. Adegbesan and Adenuga (2007) reported that protein undernourished male Wistar rats injected with 100 µmol/kg Pb nitrate exhibited increased lipid peroxidation, increased CAT activity, decreased SOD activity, and increased GSH levels, compared to undernourished rats not exposed to Pb. Increased lipid peroxidation and decreased CAT and SOD activity were also observed when comparing undernourished Pb-exposed rats to wellnourished control rats. Study authors concluded that malnutrition exacerbated Pb exposure effects on liver lipid peroxidation and the involvement of free radicals in Pb toxicity. Male Foster rats exposed to 0.025 mg/kg Pb via i.p. injection also exhibited statistically significant increases in lipid peroxidation levels and decreases in SOD, CAT, and glucose-6-phosphatase dehydrogenase (G6PD) levels in liver mitochondrial and postmitochondrial fraction (Pandya et al., 2010). Nonstatistically significant decreases were also observed in GSH levels and GPx and GR activities in exposed animals. Yu et al. (2008) reported similar dose-dependent increases in lipid peroxide levels and decreases in GSH levels and CAT, SOD and GPx activities in castrated boars that received a supplemental diet with 0, 5, 10, or 20 mg/kg Pb. The level of hepatic CuZnSOD mRNA was also reduced

in treated animals. The study authors suggested that this decrease in SOD mRNA expression and activity of antioxidant enzymes may lead to a reduction free radical scavenging capability, along with increased lipid peroxidation, potentially causing serious damage to hepatic function and structure. Khotimchenko and Kolinchenko (2007) also reported an increase in lipid peroxidation and development of hepatitis in male albino rat liver parenchyma following treatment with 100 mg/kg Pb acetate for as little as 14 days. Lipid peroxidation was confirmed by increases in malondialdehyde (MDA) levels along with decreases in GSH and thiol groups indicating injury in the liver antioxidant system. In another experiment, Jurczuk et al. (2007) reported that male Wistar rats treated with 500 mg/L Pb in drinking water exhibited decreases in liver vitamin E and GSH levels along with an increase in lipid peroxidation. The study authors hypothesized that vitamin E is involved in the mechanism of peroxidative action of Pb in the liver, and concluded that the suggested protective role of vitamin E in the potential toxicity by Pb may be related to scavenging of free radicals that are generated either directly or indirectly by Pb. In a study examining the effects of Pb exposure to fetuses, Massó et al. (2007) exposed pregnant Wistar rats with 300 mg/L Pb in drinking water starting at day 1 of pregnancy to parturition or until weaning to determine the effects of Pb exposure in the fetal liver. Pups exhibited liver damage that was supported by an increase in thiobarbituric acid-reactive species (TBARS) production and increased CAT activity compared to controls. In addition, increased ALP and acid phosphatase activity was also observed. Uzbekov et al. (2007) exposed female Wistar rats to 0.3 and 3.0 mg/L Pb nitrate for 1 and 5 months prior to, and continuing during pregnancy, and measured fetal hepatic SOD activity on GD20. In the fetuses from dams exposed for 1 month prior to pregnancy, an dose-dependent increase in liver SOD activity was observed, whereas SOD activity was decreased in the 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 mg/L Pb nitrate for one month suggests that 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. In a study examining the role of low molecular weight thiols on peroxidative mechanism, Jurczuk et al. (2006) stated that male Wistar rats treated with 500 mg/L Pb acetate in drinking water exhibited a decrease in blood ALAD as well as decreases in GSH and nonprotein sulfhydryl (NPSH) levels in the liver. Metalothionine levels were also reported to be higher in the liver following exposure to Pb. Levels of hepatic lipid peroxidation were observed to be significantly increased in rats exposed to 35 mg/kg Pb via i.p. injection, whereas hepatic GSH was significantly decreased (Upadhyay et al., 2009). No effects on GSH or MDA levels were observed in PND56 male and female rats following continuous exposure to 50 µg/kg Pb acetate throughout gestation and lactation (Pillai et al., 2009).

The studies presented above all confirm the possible oxidative stress impacts following exposure to various doses of Pb administered in various forms and the potential for hepatotoxicity as a result of oxidative stress.

5.9.2. Effects on the Gastrointestinal System

Gastrointestinal effects of Pb exposure primarily include abdominal pain, constipation, and internal paralysis in humans. In animals, degeneration of the intestinal epithelial mucosa and a decrease in duodenal motility has been reported.

5.9.2.1. Summary of Key Findings on the Effects on the Gastrointestinal System from the 2006 Lead AQCD

The 2006 Pb AQCD states that a number of factors influence the gastrointestinal absorption of Pb including the chemical and physical form of Pb, the age at Pb intake, as well as various nutritional factors. Potential malabsorption of Pb as a result of degeneration of the intestinal epithelial mucosa has been observed in rats exposed to Pb. Casein micelles incidences were reported as a result of Pb present in bovine and rat milk and in infant milk formula. Pb ingestion through water was more toxic compared to Pb ingestion via milk. Pb ingested in milk was reported to be taken up by the ileal tissue, whereas Pb administered intragastrically as a soluble salt was primarily accumulated in the duodenum irrespective of vehicle used for administration. Decreases in duodenal motility and the amplitude of contractility in the intestinal tract were observed in rats following Pb exposure. Nutritional studies examining different dietary levels of Pb, calcium, and vitamin D indicated competition in absorption between Pb and calcium. Diet supplement with vitamin D Pb to an increase in intestinal absorption of Pb and calcium. In instances where severe calcium deficiency was noted, ingestion of Pb caused a clear decrease in 1,25-dihydroxy vitamin D (1,25-(OH)2D3) levels. Overall, the 2006 Pb AQCD states that studies in rat intestine have shown that the largest amount of 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 AQCD also states that the 1986 Pb AQCD reported that common gastrointestinal effects in humans following exposure to Pb include early symptoms of abdominal pain, constipation, and internal paralysis with these symptoms generally observed at blood Pb range of 30–80 μg/dL.

5.9.2.2. New Epidemiologic Studies

The 2006 Pb AQCD reported that in humans, gastrointestinal effects generally include abdominal pain, constipation, and internal paralysis. In a case study, Cabb et al. (2008) reported similar symptoms in a 3-year-old child diagnosed with elevated blood Pb (19 μg/dL). The child was reported to be complaining of nonlocalized abdominal pain along with vomiting, nausea, constipation, lack of appetite, joint pains, fatigue, irritability as well as headaches. Based on detailed nutritional information obtained from the mother, the study authors inferred that the child was regularly ingesting candy contaminated with Pb. Following this the child was treated by a folk healer with “greta” an orange powder that

worsened the abdominal symptoms. When analyzed, it was determined that "greta" contained 14,000 mg/kg Pb monoxide. When the child was taken off "greta" his GI symptoms began to resolve. Based on this study report, it may be concluded that Pb is capable of causing severe GI trauma and the GI effects may be reversible once exposure to Pb is ceased. In a similar case study, Fonte et al. (2007) reported that a 47-year-old male exposed to Pb fumes and vapors at a recycling plant experienced similar symptoms reported by Cabb et al. (2008) in the 3-year-old child. The male patients' Pb levels were elevated with a blood Pb level of 148 µg/dL. Once exposure to Pb had ceased and the patient was treated with EDTA, his condition improved and the blood Pb dropped to 16 µg/dL. Kuruvilla et al. (2006) also reported gastrointestinal effects including stomach pain and gastritis along with other Pb-related clinical manifestations in battery workers and painters (blood Pb = 42.40 ± 25.53 and 8.04 ± 5.04 µg/dL, respectively) occupationally exposed to Pb in India.

5.9.2.3. New Toxicological Studies

Two studies pertaining to gastrointestinal effects of Pb exposure were identified. Santos et al. (2006) examined the impact of Pb exposure on nonadrenergic noncholinergic (NANC) relaxations in rat gastric fundus. Male Wistar rats treated with 0.008% Pb acetate via drinking water for 15, 30, and 120 days exhibited significant difference in NANC relaxations in the gastric fundus following electrical field stimulus (EFS). While frequency-dependent relaxations were observed in all groups, including the control group, the relaxations were significantly inhibited in rats treated with Pb acetate at all three durations. When gastric fundus strips from rats were incubated with L-nitroarginine (L-NOARG), a nitric oxide synthase (NOS) inhibiter, no additional inhibition in relaxations was observed. In contrast, incubation with sodium nitroprusside and 8-Br-GMPc (a Cyclic guanosine monophosphate [cGMP] analog), exhibited at dose-dependent relaxation in strips in the control group and group exposed to Pb acetate for 120 days. Study authors concluded that chronic 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 another study examining Pb-induced oxidative stress in the gastric mucosa, Olaleye et al. (2007) treated Albino Wistar rats with 100 or 5,000 mg/L of Pb acetate for 15 weeks. Exposure to Pb acetate caused a significant increase in gastric mucosal damage caused by pretreatment with acidified ethanol. Study authors reported that though the basal gastric acid secretory rate was not altered, stomach response to histamine was significantly higher in animals treated with Pb acetate compared to the controls. Additionally, there was a significant increase in gastric lipid peroxidation in both the 100 and 5,000 mg/L dose levels. In contrast, CAT, and SOD activities and nitrite levels were significantly decreased in the gastric mucosa. Study authors concluded that exposure to Pb may increase the formation of gastric ulcers as a result of changes in the oxidative metabolism in the stomach.

5.9.3. Effects on the Endocrine System

Endocrine processes that are most commonly impacted by Pb exposure include changes in the thyroid, such as changes in the thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4). In addition changes in the male sex hormone levels have also been reported following exposure to Pb.

5.9.3.1. Summary of Key Findings of the Effects on the Endocrine System from the 2006 Lead AQCD

Endocrine processes that may be impacted by Pb include thyroid hormone levels, changes in male sex hormone levels, as well as changes in the production of 1,25-(OH)2D3 levels. However, these effects were reported to be observed only at blood Pb levels exceeding 30–40 µg/dL. Summary of key findings pertaining to reproductive hormones in females is presented in the section on reproductive effects and birth outcomes (Section 5.8.1).

5.9.3.2. New Epidemiologic Studies

Thyroid hormone levels were reported to be impacted following exposure to various environmental contaminates, including Pb, by Abdelouahab et al. (2008), Croes et al. (2009), and Dundar et al. (2006). Abdelouahab et al. (2008) performed a cross-sectional study in a Canadian population exposed to various environmental contaminants, including Pb, following consumption of freshwater fish. The median blood Pb level of men included in this study was 3.1 µg/dL, whereas for women the median was 1.7 µg/dL. It is important to note that the median blood Pb level for women is lower than the limit of detection for Pb in the blood (2.1 µg/dL), effectively meaning that greater than 50% of women in the study had nondetectable levels of Pb in their blood. The study authors conducted a stratified analysis and concluded that TSH levels were negatively correlated with blood Pb in women that consumed fish contaminated with Pb and other environmental pollutants. No impacts in T3 and T4 levels were reported in women. TSH, T3 and T4 levels were not observed to be correlated with blood Pb in males. However, study authors stated that occupational exposure to Pb in men can affect pituitary thyroid axis homeostasis and the relation between low level Pb exposure thyroid hormone homeostasis in men and women needs to be investigated further. Dundar et al. (2006) examined the effects of blood Pb on thyroid function in 42 male adolescent auto repair workers exposed long term to Pb. A control group comprised of 55 healthy subjects was also used for comparison purpose. Mean blood Pb levels were reported to be higher in the auto repair workers compared to the control subjects (7.3 ± 2.92 versus. 2.08 ± 1.24 µg/dL). Free T4 (FT4) levels were significantly lower in the study group compared to the control group, which had no abnormal FT4 levels reported. In contrast, free T3 (FT3) and TSH levels were comparable between the study and control group. Blood Pb level was reported to be negatively correlated with FT4 levels. Based on the study

outcome, the study authors reported that long-term exposures that result in the studied blood Pb levels may lead to lower FT4 levels without impact on T3 and TSH levels in adolescents. The study authors stated that this effect is likely secondary to the toxic effects of Pb on the pituitary-thyroid axis and to the earlier findings of primary hypothyroidism as a result of impaired production of peripheral thyroid hormones. Similar findings were reported by Croes et al. (2009) in a study conducted in Belgium. Croes et al. (2009) examined the hormone levels and degree of sexual maturity in 1679 adolescents residing in nine study areas with varying exposures to multiple industrial pollutants including Pb. The median blood Pb of the participants from the nine different regions ranged from 1.6 to 2.8 µg/dL. The study authors reported that, after correction for confounding, significant interregional differences were observed in the levels of sex hormones including total and free testosterone, estradiol, aromatase, and luteinizing hormone as well as FT3 hormone levels. When individual neighborhoods were analyzed within the larger study areas, altered levels of testosterone, aromatase, and FT3 levels were also observed. Altered sexual maturation was also observed among boys and girls of individual study areas, compared to the sexual maturation of the entire cohort. Though varying levels of sex hormones and rates of sexual maturation was observed, the study authors reported that these changes are not wholly due to exposure to various pollutants, including Pb that were measured in the study and stated that other pollutants and environmental factors may also contribute to the effects noted.

Gump et al. (2008) examined cortisol response to acute stress in children (aged 9.5 years) whose prenatal and postnatal blood Pb levels had been determined prior to the study at birth (from cord blood) and at age 2.62 ± 1.2 years, respectively. For prenatal blood Pb, the children were broken 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. The study authors reported that blood Pb was not associated with initial salivary cortisol levels. However following an acute stressor, which was comprised of a gallon of one part ice to one part water into which a child was asked to submerge his or her dominant arm for a minute, increasing prenatal and postnatal blood Pb levels were statistically significantly associated with increases in salivary cortisol responses. Children in the 2nd, 3rd and 4th prenatal blood Pb quartiles and in the 4th postnatal quartile appeared to have 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 significantly correlated to salivary cortisol reactivity. Based on these results, the study authors reported that relatively low prenatal and postnatal blood Pb levels, notably those well below 10 µg/dL, can alter children's adrenocortical responses following acute stress and the health impact and behavioral aspects of this Pb-induced HPA deregulation in children needs to be further examined.

In another study on the impact of Pb in children, Kemp et al. (2007) examined the blood levels in 142 young, urban African-American and Hispanic children in winter and summer to determine the seasonal increase in blood Pb and its association with vitamin D, age and race. There was a

winter/summer (W/S) increase in blood Pb levels in children aged between 1 and 3 years (winter blood Pb = 4.94 ± 0.45 (SE) µg/dL, summer blood Pb = 6.54 ± 0.82 (SE) µg/dL), with a smaller W/S increase observed in children aged between 4 and 8 years (winter blood Pb = 3.68 ± 0.31 (SE) µg/dL, summer blood Pb = 4.16 ± 0.36 (SE) µg/dL). Additionally, the winter and summer blood Pb levels were highly correlated with one another. The percentage of African-American children with blood Pb levels ≥ 10 µg/dL increased from 12.2% in winter to 22.5% in summer. Summer and winter concentrations of 1,25-(OH)2D3 were observed to differ in children aged 4-8 years and the correlation between the serum 1,25-(OH)2D3. No difference in 1,25-(OH)2D3 was observed in children 1-3 years old. There was a significant correlation between seasonal differences in blood Pb and serum 1,25-(OH)2D3 in all children and African-American children between 4 and 8 years. Based on these results, the study authors concluded that higher summertime increase in serum 1,25-(OH)2D3 levels in children between 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.

5.9.3.3. New Toxicological Studies

A single study examining the impact of Pb exposure on the endocrine system was identified. In a study examining the effects of Pb and cadmium in adult cows reared in a polluted environment in India, Swarup et al. (2007) stated that the mean plasma T3 and T4 levels were significantly higher in cows near Pb and zinc smelters (blood Pb = 86 ± 6 µg/dL) and near closed Pb and operational zinc smelters (blood Pb = 51 ± 9 µg/dL) when compared to cows in unpolluted areas (blood Pb = 7 ± 1 µg/dL). Regression analyses from 269 cows examined in the study showed a significant positive correlation between blood Pb and plasma T3 and T4 levels, whereas the correlation between blood Pb and plasma cortisol was nonsignificant. Mean plasma estradiol level was significantly higher in cows near closed Pb and operational zinc smelter industries compared to the control group. Based on these results, the study authors concluded that endocrine profile in animals can be impacted following exposure to Pb in polluted environments.

Biswas and Ghosh (2006) investigated the effect of Pb exposure on adrenal and male gonadal functions in Wistar rats exposed to 8.0 mg/kg Pb acetate via i.p. injection for 21 days. Exposure to Pb was observed to significantly increase adrenal steroidogenic enzyme activity and serum corticosterone levels. Accessory sex organ (prostate and seminal vesicle) weights were decreased in Pb-exposed animals, whereas adrenal weights were increased. Spermatogenesis was decreased and the percent of spermatid degeneration was increased in animals exposed to Pb. Lastly, serum concentrations of testosterone, FSH, and LH, were decreased in Pb-exposed animals. Supplementation with testosterone during the last 14 days of exposure to Pb was observed to ameliorate these effects.

5.9.4. Effects on Bone and Teeth

Primary effects on bone as a result of Pb exposure include 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. Similar to bone, calcium in the teeth is easily substituted by Pb following Pb exposure. Exposure to high levels of Pb may result in the formation of "Pb line" and Pb can also cause a decrease in cell proliferation, procollagen type I production, intracellular protein, and osteocalcin in human dental pulp cell cultures. Accumulation of Pb was also associated with tooth loss and higher incidence of periodontitis.

5.9.4.1. Summary of Key Findings of the Effects on Bone and Teeth from the 2006 Lead AQCD

The 2006 Pb AQCD reported many effects on bone and some in teeth following exposure to Pb. Calcium in bone was easily substituted by Pb and taken up by the bone causing changes in bone cell function. Exposure to Pb during gestation and immediate postnatal period was reported to significantly depress early bone growth with the effects showing a dose-dependent trend, though this effect was not manifested below certain exposure thresholds. Bone effects following short-term exposure to Pb were not reported in mature animals; however, long-term exposures to Pb along with poor nutrition had an adverse effect on bone growth as well bone density. Systemic effects of Pb exposure include 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 1,2-[OH]2D3 and impact on calcium binding proteins and increases in calcium and phosphorus concentrations in the blood stream. Bone cell cultures exposed to Pb were shown to impact vitamin D-stimulated production of osteocalcin accompanied by inhibition of secretion of bone-related proteins such as osteonectin and collagen. In addition, Pb exposure also 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).

Like the bone, Pb can easily substitute for calcium in the teeth and is taken and incorporated into developing teeth in experimental animals. Since teeth do not undergo remodeling like the bone during growth, most of the Pb in the teeth remains in a state of permanent storage. High dose exposure of Pb to animals has lead to 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 are 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 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 enamel areas that were calcium deficient. The AQCD also reported that a number of epidemiologic and animal studies have both separately suggested that Pb is a caries-promoting element.

5.9.4.2. New Toxicological and Epidemiologic Studies

As reported in the 2006 Pb AQCD, Pb is capable of causing significant toxicological effects in bones of humans and animals following short-term and long-term exposure. The association between Pb exposure and osteoporosis was examined in three different epidemiologic studies. Campbell and Auigner (2007) examined subjects ≥ 50 years of age using the Third National Health and Nutrition Examination Survey (NHANES III) for association between Pb exposure and osteoporosis. The study authors used the bone mineral density (BMD) in the hip as the primary variable to examine groups comprised of non-Hispanic white men (mean blood Pb = 4.9, range: 0.7 to 48.1 µg/dl), non-Hispanic white women (mean blood Pb = 3.6, range: 0.7 to 28.7 µg/dL), African-American men (mean blood Pb = 7.7, range: 0.7 to 52.9 µg/dL) and African-American women (mean blood Pb = 4.5, range: 0.7 to 23.3 µg/dL). The results indicated that the adjusted mean total hip BMD in the non-Hispanic white males who had the lowest blood Pb levels was statistically significantly higher than the males with higher blood Pb levels. Similar associations, although not statistically significant, were reported among white females. Due to the small sample size, similar results were not observed among African-American men and women. No association was observed between blood Pb and osteoporotic fractures in any gender/race. Since the NHANES data were comprised of a cross-sectional design, no inferences could be made regarding the temporal sequence of the observed association. The study authors concluded that further inquiry was needed to study the possible causal association between Pb exposure and osteoporosis. In a similar study, Sun et al. (2008) examined the association between Pb exposure and osteoporosis in 192 (155 males; 37 females) Chinese individuals occupationally-exposed to Pb (blood Pb = 20.22 and 15.5 µg/dL, respectively). BMD was reported to be statistically significantly lower in exposed females compared to exposed males. When all participants (including 36 male and 21 female unexposed controls) were divided into groups according to blood Pb and urinary Pb levels, the study authors reported that there were significant decreases in BMD in groups that had high urinary Pb levels (≥ 5 µg/g creatinine) compared to groups with low urinary Pb in both genders. In contrast a significant difference was only observed between blood Pb and BMD in males with blood Pb >30 µg/dL. Prevalence of osteoporosis was reported to increase significantly with increasing blood Pb in a linear manner. Khalil et al. (2008) reported similar associations between Pb exposure and osteoporosis in older women. The study authors conducted a prospective study using 533 women aged 65-78 years with a mean blood Pb of 5.3 ± 2.3 µg/dL to determine the association between blood Pb and recurring fractures. The BMD was 7% lower in the total hip ($p < 0.02$) and 5% lower in the

femoral neck ($p < 0.03$) in the highest blood Pb group (≥ 8 µg/dL) compared to the lowest blood Pb group (≤ 3 µg/dL). The trend across all dose groups was also observed to be statistically significant for hip and femoral neck BMD. In addition, hip, femoral neck, and calcaneus bone loss was observed to be greater in the medium (blood Pb = 4-7 µg/dL) and high Pb groups compared to the low Pb group, but the observed trend was only significant for calcaneus bone loss. Multivariate analysis indicated that women with high blood Pb levels had an increased risk of nonspine fracture and women with medium or high blood Pb levels had a higher risk of falls compared to the low blood Pb level group. Based on these results, the study authors concluded that blood Pb is associated with an increased risk of falls and fractures leading to negative health consequences including osteoporosis-related fractures.

The effect of Pb exposure on bone development in younger children has been studied before and an association has been found between Pb exposure and bone development. Ignasiak et al. (2006) conducted a study on school children aged 7-15 years (463 males, 436 females) living close to copper smelters and refineries in Poland to assess the impact of Pb exposure on their growth status. The mean blood Pb for all participants was 7.7 ± 3.5 µg/dL (range: 2.0 to 33.9). Blood Pb levels were similar between males and females except at age 14.8 years, when females had lower blood Pb than males. Study results indicated that there was a statistically significant and linear relationship between blood Pb and reduced weight, height, and trunk, leg and arm lengths. Regression analysis revealed that, per every 10 µg/dL increase in blood Pb levels, height decreased 2.1 and 2.9 cm for males and females, respectively. This decrease in height was more influenced by decreases in leg length than trunk length. Based on these results, the study authors concluded that linear skeletal growth was reduced with increases in blood Pb levels and these effects were seen even at levels below 10 µg/dL. These results also indicated that there was attenuation in osteoblast activity as a result of Pb exposure.

To understand the significance of bone as a target tissue of Pb toxicity, Jang et al. (2008) studied the effect of Pb on calcium release activated calcium influx (CRACI) using primary cultures of human osteoblast-like cells (OLC). When cells were incubated with 1 or 3 mM Pb, a dose-dependent impact on the CRACI observed, as was a dose-dependent increase in the influx of Pb into human OLC. These results suggest that Pb interferes with CARCI in human OLCs by initiating the CRACI (i.e. the measurable influx of calcium upon re-addition of calcium is partially inhibited by Pb) and the influx of Pb was enhanced after CRACI had been induced. Since skeletal growth in Pb-exposed children is stunted, Zuscik et al. (2007) conducted a study using murine limb bud mesenchymal cells (MSCs) to test the hypothesis that Pb alters chondrogenic commitment of mesenchymal cells and also assessed the effects of Pb on various signaling pathways. Exposure to 1 µM Pb caused increased basal and TGF-β/BMP induction of chondrogenesis in MSCs which was supported by nodule formation and upregulation of Sox-9, type 2 collagen, and aggrecan which are all key markers of chondrogenesis. The study authors also observed enhanced chondrogenesis during ectopic bone formation in mice that had been pre-exposed to Pb in drinking water (55 or 233 ppm, corresponding to 14 or 40 µg/dL blood Pb). MSCs exposed to Pb

exhibited an increase in TGF-β, but BMP-2 signaling was inhibited. Pb was also reported to induce NFκB and inhibit AP-1 signaling. Based on these results, the study authors concluded that chondrogenesis following exposure to Pb most likely involved modulation and integration of multiple signaling pathways including TGF-β, BMP, AP-1, and NFκB.

Effects of Pb exposure on teeth were examined in three epidemiologic studies. Since individuals previously exposed to Pb may be impacted due to the endogenous release of Pb stored in their skeletal compartments, Arora et al. (2009) examined the association between bone Pb concentrations and loss of natural teeth in 333 male participants of the Normative Aging Study. Tooth loss in men was categorized as 0, 1-8 or ≥ 9. Individuals with ≥ 9 teeth missing had significantly higher bone Pb concentrations compared to those with no tooth loss; no significant difference in blood Pb levels was observed between the categories of teeth loss. Following adjustment for different variables (age, education, smoking status, pack-years of smoking, and diabetes), men with the highest tibia Pb concentrations (>23μg/g) had higher odds of tooth loss (OR: 3.03 [95% CI: 1.60,5.75]) compared to men with tibia Pb ≤ 15 μg/g. Men with the highest patellar Pb (>36 μg/g) also had higher odds of tooth loss (OR: 2.41 [95% CI: 1.30,4.49]) compared to men with patellar Pb ≤ 22.0 μg/g. Odds of tooth loss were not statistically associated with blood Pb levels. Based on these results, the study authors concluded that long-term cumulative exposure to Pb is associated with increased odds of tooth loss. In a study examining the effects of Pb exposure on periodontitis in the United States (US), Saraiva et al. (2007) used data from NHANESIII by analyzing data for 2,500 men and 2,399 women aged between 30 and 56 years. The analysis took into account various covariates including age, NHANESIII phase, cotinine levels, poverty ration, race/ethnicity, education, BMD, diabetes, calcium intake, dental visits, and menopause in women. After adjusting for these covariates and comparing individuals with a blood Pb level of >7 μg/dL to those with a blood Pb level of <3 μg/dL, the prevalence ratios of periodontitis was 1.70 (95% CI: 1.02,2.85) for men and 3.80 (95% CI: 1.66,8.73) for women. Based on these results, the study authors concluded that there was a positive and statistical association between periodontitis and Pb levels for both men and women. In a similar study, Yetkin et al. (2007) recruited 60 male subjects (30 apprentices with Pb exposure, 30 controls), to examine the impact of occupational exposure to Pb on periodontal status and association between periodontitis and blood Pb or oxidative stress. The results of their analysis indicated that blood Pb was significantly higher in apprentices exposed to Pb compared to controls (7.38 ± 4.41 versus. 2.27 ± 1.49 μg/dL). No clinical periodontal or oxidative stress parameters were significantly difference between apprentices and controls. While the correlation between blood Pb and periodontal parameters was not reported, significant correlations between plaque index and CAT, probing depth and SOD, clinical attachment level and SOD, and clinical attachment level and malondialdehyde in Pb-exposed apprentices was observed. These results demonstrate that there is significant association between clinical periodontal parameters and oxidative stress/damage indices in Pb-exposed apprentices. Following multiple regression

analysis, a statistically significant association between gingival index and working status, family income and either probing depth or clinical attachment level was noted.

5.9.5. Effects on Ocular Health

Ocular effects most commonly observed following exposure to Pb include formation of cataract, impaired vision, edema and retinal stippling.

5.9.5.1. Summary of Key Findings of the Effects on Ocular Health from the 2006 Lead AQCD

The 2006 Pb AQCD stated that various changes in the visual system were observed with Pb poisoning including retinal stippling and edema, cataract, ocular muscle paralysis and impaired vision. The AQCD reported that retinal responses were observed in children of mothers with a blood Pb range of 10.5 to 32.5 µg/dL during pregnancy, while cataracts were noted in middle-aged male with tibia bone Pb levels of 31-126 µg/g.

5.9.5.2. New Toxicology and Epidemiology Studies

New animal studies pertaining to the ocular effects of Pb have investigated endpoints such as retinal progenitor cell proliferation and neurogenesis, and have observed effects at exposures that resulted in blood Pb levels as low as <10 µg/dL (Section 5.3.4.3). One human study pertaining to ocular effects of Pb was identified. Mosad et al. (2010) studied the association between subcapsular cataract and Pb, cadmium, vitamin C, vitamin E, and beta carotene blood levels in middle-aged male smokers compared to nonsmokers. Blood Pb was statistically significantly elevated in light (14.5 ± 0.41 µg/dL), moderate (14.5 ± 0.41 µg/dL), and heavy smokers (18.7 ± 1.24 µg/dL) compared to nonsmokers (12.2 ± 0.21 µg/dL). Pb concentrations were also observed to be statistically higher in the cataracts of smokers versus. nonsmokers. Similar associations were also observed for cadmium blood and lens levels, while vitamins C, E, and beta carotene levels were significantly decreased in smokers. Based on these results, the study authors concluded that the Pb and cadmium present in high concentration in smokers were associated with cataracts due to oxidative stress which was indicated by reduced levels of antioxidants such as vitamins C, and E and beta carotene.

5.9.6. Effects on the Respiratory System

The collective body of toxicological and epidemiologic studies demonstrates Pb-induced effects on multiple immunological pathways, including a shift from a Th1 to a Th2 phenotype, increased IgE antibody production, and increased inflammatory responses (Sections 5.2.5.1. and 5.6). These are well

recognized pathways that contribute to increased susceptibility to infections and also to the development of respiratory diseases such as asthma. Recent investigation of the respiratory effects of Pb exposure has been limited; however, cross-sectional studies have indicated an association of increasing blood Pb level with increased prevalence of respiratory tract illnesses (Section 5.6.4.1) and asthma in children (Section 5.6.4.2). As described in Section 5.2.4, Pb 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. Studies investigating these airway responses also are limited in number and collectively do not provide strong evidence of an association with blood Pb (Section 5.6.4.3). Collectively, panel and time-series studies demonstrate associations between Pb measured in $PM_{2.5}$ or PM_{10} air samples and decreases in lung function and increases in respiratory symptoms, hospitalizations, and mortality (Section 5.6.4.3). However, common limitations of these air-Pb studies are the variable size distribution of Pb-bearing PM (Section 3.5.3) and its relationship with blood Pb levels as well as the lack of adjustment for other correlated PM chemical components.

5.9.7. Summary

There is evidence from epidemiologic and toxicological studies that exposure to Pb results in altered liver function and hepatic toxicity. Biochemical changes indicative of liver injury, including decreases in serum protein and albumin levels and increased AST, ALT, ALP, and GGT activities, have been observed in humans (Can et al., 2008; D. A. Khan et al., 2008; Patil et al., 2007) and animals (Y.-J. Cheng et al., 2006; S. et al., 2009). Increased hepatic cholesterogenesis, altered triglyceride and phospholipid levels, and disorders in lipid metabolism supported by increased levels of total cholesterol and triglycerides has been reported in the animal literature (Ademuyiwa et al., 2009; Khotimchenko & Kolenchenko, 2007). These results suggest that induction of cholesterogenesis and phospholipids in the liver may cause subtle effects at the cellular level, leading to hepatic injury. Multiple studies in humans and animals have observed hepatic oxidative stress, generally indicated by an increase in lipid peroxidation along with a decrease in GSH levels and CAT, SOD, and GPx activities following exposure to Pb (Adegbesan & Adenuga, 2007; D. A. Khan et al., 2008; Khotimchenko & Kolenchenko, 2007; D. Y. Yu et al., 2008). Indices of oxidative stress were additionally observed in the livers of fetuses exposed throughout gestation (Uzbekov et al., 2007).

Relatively few human studies have been conducted on the gastrointestinal toxicity of Pb since the completion of the 2006 Pb AQCD. Two cases studies reporting on GI symptoms in a child and adult report that elevated blood Pb was associated with nonlocalized abdominal pain, vomiting, nausea, constipation, lack of appetite, fatigue, and headaches (Cabb et al., 2008; Fonte et al., 2007). The adult's blood Pb level was reported as 148 µg/dL. Both subjects' symptoms were reported to diminish following cessation of exposure or chelation therapy. Similar GI symptoms (stomach pain and gastritis) were

observed in battery works and painters exposed to Pb in India with blood Pb levels ranging from 0.4-116.6 µg/dL (Kuruvilla et al., 2006). Toxicological evidence for Pb-induced GI health effects in rats includes inhibition of NANC relaxations in the gastric fundus and the observation of oxidative stress (lipid peroxidation, decreased SOD and CAT) in the gastric mucosa (Olaleye et al., 2007; M. R. V. Santos et al., 2006). The observation of oxidative stress was accompanied gastric mucosal damage.

The endocrine processes most impacted by exposure to Pb include changes in thyroid function, as well as alteration in sex and stress hormone profiles. TSH was negatively associated with blood Pb in women that ate contaminated fish (median blood Pb = 1.7 µg/dL) but not men, and FT4, but not FT3, was decreased in adolescent male auto repair workers (Abdelouahab et al., 2008; Dundar et al., 2006). Alterations in the levels of sex hormones, including total and free testosterone, estradiol, aromatase, and luteinizing hormone, were observed in Belgian adolescents environmentally exposed to Pb (Croes et al., 2009). Toxicological evidence for similar effects was observed in adults cows reared in a contaminated environment and exposed to Pb. A positive correlation was reported between blood Pb and plasma T3 and T4 levels (Swarup et al., 2007). Mean plasma estradiol levels were also significantly higher in Pb-exposed cows. In children challenged with an acute stressor, increasing blood Pb was associated with significant increases in salivary cortisol responses, even at blood Pb levels less than 10 µg/dL (Gump et al., 2008).

Numerous epidemiologic studies investigated the association between Pb exposure and osteoporosis in adults. High blood Pb has been observed to be associated with decreased BMD in non-Hispanic white males (Campbell & Auinger, 2007), whereas urinary Pb, but not blood Pb, was seen to be associated with decreased BMD in Chinese individuals occupationally exposed to Pb (Y. Sun, Sun, Zhou, Zhu, Zhang, et al., 2008). In elderly women, blood Pb levels were associated with an increased risk of falls and fractures, including osteoporosis-related falls (Khalil et al., 2008). Linear skeletal growth in children exposed to Pb was reduced with increased blood Pb; these effects were seen at blood levels <10 µg/dL (Ignasiak et al., 2006). In vitro studies indicate that Pb interferes with CARCI in human OLCs and that Pb perturbs multiple signaling pathways during murine limb bud growth, potentially resulted in altered skeletal development (Jang et al., 2008; Zuscik et al., 2007). Epidemiologic studies investigating Pb exposure and tooth loss report that long-term, cumulative exposure to Pb is associated with increased odds of tooth loss, periodontitis in men and women, and that periodontitis is associated with oxidative stress/damage in individuals exposed in an occupational setting (Arora et al., 2009; Saraiva et al., 2007; Yetkin-Ay et al., 2007).

New toxicology studies have reported ocular effects (i.e., retinal progenitor cell proliferation) at blood Pb levels as low as <10 µg/dL (Section 5.3.4.3), and one human study reports and association between heavy smoking, increased blood Pb levels, and cataracts (Mosad et al., 2010). Investigation of the respiratory effects of Pb exposure has been limited; however, cross-sectional studies have indicated an association of increasing blood Pb with increased prevalence of respiratory tract illnesses (Section

5.6.4.1) and asthma in children (Section 5.6.4.2). Additionally, Pb-induced production of ROS is implicated in increased BR and decrements in lung function (Section 5.6.4.3).

In summary, recent toxicological and epidemiologic evidence regarding the effects of Pb exposure on the liver, GI tract, endocrine system, bone and teeth, eyes, and respiratory tract largely are confirmatory of those effects noted in the 2006 Pb AQCD. However, recent evidence of these effects is relatively limited in number, and therefore no causal determinations are made regarding Pb-induced effects in these organ systems.

5.10. Cancer

The previous epidemiologic studies included in the 2006 Pb AQCD (U.S. EPA, 2006) "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) has recently classified inorganic Pb compounds as probable human carcinogens (Group 2A of IARC classifications) based on stronger evidence in animal studies than human studies, and organic Pb compounds as not classifiable (Group 3 of IARC classifications) (IARC, 2006; Rousseau et al., 2005). Additionally, the National Toxicology Program has listed Pb and Pb compounds as "reasonably anticipated to be human carcinogens" (NTP, 2004).

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 and DNA and cellular damage, as well as epigenetics studies, are summarized. In epidemiologic studies, various biological measures of Pb are used including Pb measured in blood and bone. Bone Pb is indicative of cumulative Pb exposure. Blood Pb can represent more recent exposure, although it can also represent remobilized Pb occurring during times of bone remodeling. Toxicological studies only report exposure by blood Pb. More detailed discussion of these measures is given in Section 4.3.5.

5.10.1. Cancer Incidence and Mortality

Recent studies have included epidemiologic evaluations of the associations between Pb and both specific cancers, such as lung cancer and brain cancer, and overall cancer. Table 5-37 provides an overview of the study characteristics and results for the epidemiologic studies that reported effect estimates. This section also presents toxicological evidence on the carcinogenicity of Pb.

Table 5-37. Summary of epidemiologic studies of cancer incidence and mortality

Reference	Study Location	Cancer Outcome	Study Population	Exposure Measurement	Mean Pb (SD)	Adjusted Effect Estimates
(Menke et al., 2006)	U.S.A.	Overall cancer mortality	NHANES III cohort with Blood Pb measures in 1988-1994 - at least 12 years of follow-up - blood Pb <10 µg/dL	Blood Pb	2.58 µg/dL (geometric mean) Tertile 1: <1.93 µg/dL Tertile 2: 1.94-3.62 µg/dL Tertile 3: >=3.63 µg/dL	Tertile 1: 1.00 Tertile 2: 0.72 (95% CI: 0.46, 1.12) Tertile 3: 1.10 (95% CI: 0.82, 1.47)
Schober et al. (2006)	U.S.A.	Overall cancer mortality	NHANES III cohort – at least 40 years of age	Blood Pb	Blood Pb<5 µg/dL: 67.7% Blood Pb 5-9 µg/dL: 26.0% Blood Pb>=10 µg/dL: 6.3%	Blood Pb<5 µg/dL: 1.00 Blood Pb 5-9 µg/dL: 1.44 (95% CI: 1.12, 1.86) Blood Pb≥10 µg/dL: 1.69 (95% CI: 1.14, 2.52) Note:Modification by age assessed and associations varied slightly
Weisskopf et al. (2009)	Boston, MA	Overall cancer mortality	Normative Aging Study -included men only - mean follow-up period for this study: 8.9 yr	Blood Pb, patella Pb	Blood Pb: 5.6 µg/dl (3.4) Tertile 1 of Blood Pb: <4 µg/dL Tertile 2 of Blood Pb: 4-6 µg/dL Tertile 3 of Blood Pb: >6 µg/dL Tertile 1 of patella Pb: <22µg/g Tertile 2 of patella Pb: 22-35µg/g Tertile 3 of patella Pb: >35 µg/g	Blood Pb Tertile 1: 1.00 Blood Pb Tertile 2: 1.03 (95% CI: 0.42, 2.55) Blood Pb Tertile 3: 0.53 (95% CI: 0.20, 1.39) Patella Pb Tertile 1: 1.00 Patella Pb Tertile 2: 0.82 (95% CI: 0.26, 2.59) Patella Pb Tertile 3: 0.32 (95% CI: 0.08, 1.35)
Khalil et al. (2009)	Baltimore, MD, and Monongahela Valley, PA	Overall cancer mortality	Subgroup of the Study of Osteoporotic Fractures cohort - included white women aged 65-87 12 yr (+/- 3 yr) follow-up		Blood Pb Level 5.3 (2.3) µg/dL	Blood Pb<8 µg/dL: 1.00 Blood Pb≥ 8 µg/dL: 1.64 (95% CI: 0.73, 3.71)
Lundstrom et al. (2006)	Sweden	Lung cancer (incidence and mortality)	Pb smelter workers first employed for ≥3 months between 1928 and 1979 Followed up for mortality from 1955 -1987	Median peak blood Pb Level Median number of yr with at least one blood sample obtained Median cumulative blood Pb index (sum of annual blood Pb Level)	Median peak blood Pb Level: cases 2.4 µmol/L, controls 2.7 µmol/L Median number of yr with at least one blood sample obtained: cases 4.5 yr, controls 6.0 yr Median cumulative blood Pb index: cases 9.0 µmol/Pb, controls 11.9 µmol/Pb	Median peak blood Pb Level: 1.00 (95% CI: 0.71, 1.42) Median number of yr with at least one blood sample obtained: 0.96 (95% CI: 0.91, 1.02) per µmol/L Median cumulative Blood Pb index: 0.99 (95% CI: 0.96, 1.02) per µmol/L Note: similar results were observed when restricted to smokers only

Reference	Study Location	Cancer Outcome	Study Population	Exposure Measurement	Mean Pb (SD)	Adjusted Effect Estimates
Jones et al. (2007)	Humberside, UK	Lung cancer mortality	Male tin smelter employees	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 yr/m^3	NA	RR for Pb exposure weighted age and time since exposure: 1.54 (90% CI: 1.14, 2.08) Note: Similar results for other exposure determination scenarios.
Rousseau et al. (2007)	Montreal, Canada	Lung cancer and other cancer incidence	Men aged 35-79	Interview of job history and exposure matrix	Ever exposed to: Organic Pb 3.0% Inorganic Pb 17.0% Pb in gasoline emissions 38.6%	Never exposed is referent group Organic Pb: Esophageal 1.7 (95% CI: 0.5, 6.4) Stomach (3.0 (95% CI: 1.2, 7.3) Colon 1.5 (95% CI: 0.7, 3.6) Rectum 3.0 (95% CI: 1.2, 7.5) Pancreas 0.9 (95% CI: 0.1, 5.2) Lung 1.3 (95% CI: 0.5, 3.1) Prostate 1.9 (95% CI: 0.8, 4.6) Bladder 1.7 (95% CI: 0.7, 4.2) Kidney 2.3 (95% CI: 0.8, 6.7) Non-Hodgkin's lymphoma 0.4 (95% CI: 0.1, 2.2) Inorganic Pb: Esophageal 0.6 (95% CI: 0.3, 1.2) Stomach 0.9 (95% CI: 0.6, 1.5) Colon 0.8 (95% CI: 0.5, 1.1) Rectum 0.8 (95% CI: 0.5, 1.3) Pancreas 0.9 (95% CI: 0.4, 1.8) Lung 1.1 (95% CI: 0.7, 1.7) Prostate 1.1 (95% CI: 0.7, 1.6) Bladder 1.1 (95% CI: 0.7, 1.5) Kidney 1.0 (95% CI: 0.6, 1.7) Melanoma 0.4 (95% CI: 0.2, 1.0) Non-Hodgkin's lymphoma 0.7 (95% CI: 0.4, 1.2) Pb in gasoline emissions: Esophageal 0.6 (95% CI: 0.4, 1.1) Stomach 1.0 (95% CI: 0.7, 1.4) Colon 0.8 (95% CI: 0.6, 1.1) Rectum 1.0 (95% CI: 0.7, 1.4) Pancreas 0.9 (95% CI: 0.5, 1.4) Lung 0.8 (95% CI: 0.6, 1.1) Prostate 0.9 (95% CI: 0.7, 1.2) Bladder 0.8 (95% CI: 0.6, 1.1) Kidney 1.0 (95% CI: 0.7, 1.5) Melanoma 0.8 (95% CI: 0.5, 1.4) Non-Hodgkin's lymphoma 0.7 (95% CI: 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

Reference	Study Location	Cancer Outcome	Study Population	Exposure Measurement	Mean Pb (SD)	Adjusted Effect Estimates
van Wijngaarden and Dosemeci (2006)	U.S.A.	Brain cancer mortality	National Longitudinal Mortality Study – included individuals with occupational information -included follow-up from 1970-1989	Interview about current or most recent job within the past 5 years and a job exposure matrix	NA	Any Pb exposure compared to no exposure 1.56 (95% CI: 1.00, 2.43) Note: HRs were greatest among those with high probabilities of exposure and medium/high exposure intensity
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 wk before hospitalization; frequency-matched controls were individuals admitted to the same hospitals for non-neoplastic conditions	Interviews of lifetime work history and exposure databases	NA	Meningioma: Ever exposure to Pb 0.8 (95% CI: 0.5, 1.3) Glioma: Ever exposure to Pb 0.8 (95% CI: 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 genotypes; these associations were not observed for glioma incidence
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 wk before hospitalization; frequency-matched controls were individuals admitted to the same hospitals for non-neoplastic conditions	Interviews of lifetime work history and exposure databases	Glioma: 70.5 µg/m^{3}y (193.8 µg/m^{3}y) Glioblastoma multiform: 97.5 µg/m3y (233.9 µg/m^{3}y) Meningioma: 101.1 µg/m^{3}y (408.7 µg/m^{3}y) Controls: 69.7 µg/m^{3}y (248.8 µg/m^{3}y)	Per 100 µg/m^{3}y increase in cumulative Pb exposure Glioma: 1.0 (95% CI: 0.9, 1.1) Glioblastoma multiform: 1.0 (95% CI 0.9, 1.1) Meningioma: 1.1 (95% CI: 1.0, 1.2) Note: modification by SNPs was conducted and associations varied by SNP
Absalon and Slesak (2010)	Silesia province, Poland	Overall cancer incidence	Children living in this province at least five years	Pb-related air pollution measures	NA	Reported correlations between changes in Pb and cancer incidence – no/low correlations observed (correlation coefficients between -0.3 and 0.2)
Obhodas et al. (2007)	Island of Krk, Croatia	Incidence rates for neoplasms	Individuals living in the Island of Krk from 1997-2001	Soil and vegetation samples, household potable water samples, children's hair samples	NA	No association observed between Pb in the samples and incidence of neoplasm (numerical results not provided)

Reference	Study Location	Cancer Outcome	Study Population	Exposure Measurement	Mean Pb (SD)	Adjusted Effect Estimates
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	Interviews to determine occupational history and a job exposure matrix	NA	All esophageal cancers: Unexposed: 1.00 Low workplace Pb exposure (≤0.237 µmol/L): 0.79 (95% CI: 0.43, 1.46) High workplace Pb exposure (>0.237 µmol/L): 1.69 (95% CI: 0.57, 5.03) Esophageal squamous cell carcinoma: Unexposed: 1.00 Low workplace Pb exposure (≤0.237 µmol/L): 0.70 (95% CI: 0.34, 1.43) High workplace Pb exposure (>0.237 µmol/L): 0.91 (95% CI: 0.22, 3.75) Adenocarcinoma: Unexposed: 1.00 Low workplace Pb exposure (≤0.237 µmol/L): 0.95 (95% CI: 0.32, 2.82) High workplace Pb exposure (>0.237 µmol/L): 5.30 (95% CI: 1.39, 20.22) *associations not changed or slightly increased when restricted to occupational exposures ≥15yr

5.10.1.1. Overall Cancer Mortality

Recent studies have been performed examining the association between biologically measured Pb levels and cancer mortality. The Third National Health and Nutrition Examination Survey (NHANES III) included a nationally representative sample of U.S. adults who had blood Pb measurements taken and were followed up for 12 years. Mean blood Pb levels were 2.58 µg/dL (individuals with blood Pb levels greater than 10 µg/dL were excluded from the study). No association was observed between blood Pb and cancer mortality (HR of highest tertile compared to lowest tertile: 1.10 [95% CI: 0.82, 1.47]) (Menke et al., 2006). Another analysis of the NHANES III population, restricted to individuals 40 years and older at the time of blood Pb collection, included individuals with all blood Pb levels (including those greater than 10 µg/dL) (Schober et al., 2006). Overall, 68% of the study population had blood Pb levels less than 5 µg/dL and 6% had blood Pb levels greater than 10 µg/dL. Among individuals who died of cancer during the study period, 52% had blood Pb levels less than 5 µg/dL and 12% had blood Pb levels greater than 10 µg/dL. In this study, median follow-up time was 8.6 years and a positive association was observed between blood Pb and cancer mortality. 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 categorized by age groups, 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. A positive association for blood Pb levels of 10 µg/dL and greater was present among those 40-74 years old and 85 years and older. A study of men from the greater Boston area enrolled in the Department of Veterans Affairs Normative Aging Study reported mean blood Pb levels of 5.6 µg/dL (SD 3.4 µg/dL) but this measure was poorly correlated with measured bone Pb (Weisskopf et al., 2009). No association was detected between either measure of Pb and cancer

mortality in adjusted analyses. As part of the Study of Osteoporotic Fractures study, White women aged 65-87 were included in a sub-study of blood Pb levels and cancer mortality and were followed for approximately 12 years (Khalil, Wilson, et al., 2009). Mean blood Pb levels were 5.3 µg/dL (SD 2.3 µg/dL) and no association was detected between blood Pb and cancer mortality in the study population.

5.10.1.2. Lung Cancer

A study was conducted among smelter workers to examine the relationship between Pb and lung cancer (incidence and mortality combined) (Lundstrom et al., 2006). Pb exposure was measured with three different variables (peak blood Pb values, number of years Pb samples were obtained, and cumulative blood Pb index) but none showed an association with lung cancer. Median follow-up in the study was about 30 years and the peak blood Pb values during employment were 49.7 µg/dL for lung cancer cases and 55.9 µg/dL for controls. A study in the UK of tin smelters reported no association 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 (S. R. Jones et al., 2007). Pb exposure was calculated in this study by combining historical air sampling data and personnel record cards, which specified work histories. The median Pb exposure was estimated to be approximately 2 mg-year/m^3 and the smelters were exposed to other metals as well, such as arsenic and antimony. A population-based case-control study performed among men in Montreal, Canada assessed Pb exposure via interviews regarding job histories and calculated 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.

Other studies of Pb and lung cancer were performed by comparing the lung tissue of 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). One study reported increased Pb concentrations were observed 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) (De Palma et al., 2008), but no statistical difference in Pb levels was reported for lung tissue of individuals with lung cancer compared to controls in the other study (Kuo et al., 2006).

5.10.1.3. Brain Cancer

A few studies of brain cancer examined the association between cancer and Pb using exposures determined via exposure databases and patient interviews about past jobs and known exposures. The National Longitudinal Mortality Study, a study that included a national sample of the U.S. population, estimated Pb exposure based on current/most recent employment among individuals (van Wijngaarden &

Dosemeci, 2006). Although not all estimates 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, glioma was reported to have no association with any Pb exposure metric; however, positive associations were observed between high cumulative Pb exposure and meningioma among individuals with *ALAD2* genotypes (Rajaraman et al., 2006). The association was not present among individuals with *ALAD1* genotypes. A third study of the association between Pb exposure and brain tumors reported none or slight overall associations with types of brain tumors; however, positive associations were observed among individuals with certain single nucleotide polymorphisms (SNPs) (Bhatti et al., 2009). After control for multiple comparisons, individuals with *GPX1* variants had positive associations between cumulative Pb exposure and glioblastoma multiforme and meningioma, whereas individuals without *RAC2* variants showed a positive association between Pb and glioblastoma multiforme and individuals without *XDH* variants displayed a positive association between Pb and meningioma.

5.10.1.4. Breast Cancer

A few studies examined Pb levels and breast tumors among individuals with and without breast tumor and/or cancer present. One study of newly diagnosed breast cancer patients and controls examined Pb levels in blood and hair samples and reported higher levels in both for cancer cases, although the difference in the hair samples was not statistically significant (Alatise & 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 also reported no statistical difference in Pb levels (Pasha, Malik, et al., 2008b). However, one 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 bisphostphonates (a combination responsible for bone loss) were excluded from the analysis (McElroy et al., 2008).

Overall, these studies demonstrate the possibility that women with breast cancer may have increased Pb levels in blood measurement, whereas the results for actual breast tissue are mixed. However, these studies are limited by their study design. The samples are taken after cancer is already present in the cases, leading to issues of temporality for the Pb levels. Additionally, the sample sizes are often small and the studies may be underpowered.

5.10.1.5. Other Cancers

Studies of multiple cancers or cancers not listed above have also been performed. An ecologic analysis compared levels of Pb in the air from 1990 to 2005 with incidence rates of cancer (cancer sites not specified) among children during this time period (Absalon & Slesak, 2010). The highest Pb levels were measured in 1990 when over 50% of the study area exceeded the limit of 1 $\mu g/m^2 \cdot$year. No correlation was observed both overall and in sex-specific analyses. A similar study examined correlations between Pb concentrations in soil, water, vegetation, and hair samples with incidence of neoplasms (Obhodas, 2007). No correlations were reported.

A study performed among men evaluated multiple cancer outcomes and determined exposures to organic Pb, inorganic Pb, and Pb in gasoline emissions via interviews regarding job histories and then subsequent exposure approximations by chemists and hygenists (Rousseau et al., 2007). Organic Pb exposure was positively associated with stomach cancer. 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 esophageous, colon, pancreas, prostate, bladder, kidney, melanoma, or non-Hodgkin's lymphoma. None of the cancers were associated with exposure to inorganic Pb. When occupational exposure to Pb in gasoline was categorized as unexposed, nonsubstantial level, and substantial level, a positive association with stomach cancer was observed when cancer controls were used (association not present when population controls were employed as the control group). Another case-control study using participant interviews and a job exposure matrix, including only men, reported no association between Pb exposure and esophageal squamous cell carcinomas, but an association was present between high Pb exposure and adenocarcinmoa of the esophagus (Santibanez et al., 2008).

Several studies compared Pb levels in blood, tissue, and urine of individuals who have cancer with individuals who are cancer-free. Compared to control groups, increased 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., 2007; Pasha, Malik, & Shah, 2008), and the hair of individuals with benign tumors (Pasha, Malik, 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 (C. N. 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 examine temporality; the presence 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 who have not undergone certain treatments. Additionally, studies are limited by their small sample size 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.

5.10.1.6. Toxicological Models of Carcinogenicity

Carcinogenicity in Animal Models

Inorganic Pb has been shown to act as a carcinogen in animal toxicology models. Most commonly the kidneys (Azar et al., 1973; Kasprzak et al., 1985; Koller et al., 1985; Van Esch & Kroes, 1969) are targeted but the testes, brain, adrenals, prostate, pituitary, and mammary gland have also been affected (IARC, 2006). More recently it has been shown that early life transplacental and lactational exposure of laboratory rodents to inorganic Pb induces carcinogenicity in adulthood (Waalkes et al., 1995). Chronic, lifetime exposure to Pb is also associated with carcinogenicity in laboratory rodents (Koller et al., 1985). One recent study considered Pb-dependent carcinogenesis in laboratory animals. Tokar et al. (2010) considered tumorigenesis in rodents. Homozygous metallothionein I/II knockout mice and their corresponding wild type controls (groups of ten mice each) were exposed by drinking water to 2,000 or 4,000 ppm $Pb(Ac)_2$ and compared to untreated controls. Study animals were exposed in utero, through birth and lactation, and then directly to drinking water until 8 weeks old. The metallothionein I/II knockout mice had increased testicular teratomas and renal and urinary bladder preneoplasia. Pb exposed wild-type mice were not statistically significantly different than controls. The data suggest metallothionein can protect against Pb-induced tumorigenesis. Concerns with the study are that the doses are at levels of Pb humans would not likely be exposed to 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 has any impact on Pb carcinogenesis.

Neoplastic Transformation Studies, Human Cell Cultures

Three studies considered Pb-dependent carcinogenesis in human cells. Xie et al. (2007) treated BEP2D cells (human papilloma virus- immortalized human bronchial cells) with 0, 1, 5, or 10 µg/cm2 $PbCrO_4$ for 120 h. $PbCrO_4$ 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 $PbCrO_4$ for 120 h. $PbCrO_4$ induced foci formation in a concentration-dependent manner in the Mre11 deficient 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 $PbCrO_4$ for 24 or 120 h. Mre11 was required to prevent $PbCrO_4$-induced neoplastic transformation.

Immune Modulation of Tumorogenesis by Pb

As described in the prior AQCD for Pb (U.S. EPA, 2006), Pb-induced immunotoxicity affecting tumors results from a combination of suppressed Th1 responses and misregulated inflammation. The intersection of these two general Pb-induced alterations that elevate the risk of cancer. First, Pb-induced misregulation of inflammation involving innate immune cells results in chronic insult to tissues. Decades of excessive lipid and DNA oxidation production by overproduction of ROS and weakened anti-oxidant defenses increase the likelihood of mutagenesis, cellular instability, and tumor cell formation. In support of this, Xu et al. (J. Xu et al., 2008) found evidence that supports the association with Pb exposure and DNA damage and concluded that it is a 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.

5.10.2. Cancer Biomarkers

A study of men aged 21-40 years never occupationally exposed to metals examined prostate specific antigen (PSA), a biomarker for prostate cancer. This study reported a positive association between blood Pb and PSA levels in adjusted analyses (Pizent et al., 2009). The median 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 older men.

5.10.3. 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.

5.10.3.1. Epidemiologic Evidence for DNA and Cellular Damage

Multiple studies examined the relationship between Pb and sister chromatid exchange (SCE). A study of male policeman reported mean blood Pb levels for the study population of 43.5 µg/dL

(Wiwanitkit et al., 2008). When categorized as having 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 levels of 40.14 µg/dL) and office workers (mean blood Pb levels of 9.77 µg/dL) (Duydu et al., 2005). The exposed workers had higher SCE levels and also a greater number of cells with the SCEs per cell higher than the 95th percentile of the population. Finally, a study of children aged 5-14 years old (mean blood Pb levels of 7.69 µg/dL [SD 4.29 µg/dL]) reported no correlation with SCE (Mielzyńska et al., 2006). However, the study did report a positive association between blood Pb and micronuclei 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 DNA basal damage or repair ability after a peroxide challenge (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 or oxidative DNA damage (De Coster et al., 2008). A study conducted among workers exposed to Pb (mean blood Pb levels of 30.3 µg/dL) and unexposed controls (mean blood Pb levels of 3.2 µg/dL) reported positive associations between the exposed group and cytogenetic damage (measured by micronuclei frequency), chromosomal aberrations, and DNA damage (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 mean blood Pb levels of 21.56 µg/dL (SD 6.43 µg/dL) among painters who reported painting houses for 8-9 hours/day for 5-10 years (M. I. Khan et al., 2010); the mean blood Pb levels were 2.84 µg/dL (SD 0.96 µg/dL) for healthy workers who had not been occupationally exposed to Pb. Cytogenetic damage was increased among the painters compared to the healthy controls. Another study compared the blood Pb of metal workers and office workers and reported greater blood Pb levels (both current and 2 year average) among the metal workers (blood Pb levels at least 20 µg/dL for metal workers compared to blood Pb levels less than 10 µg/dL for the office workers) (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 & Jamil, 2009). Higher chromosomal aberrations, micronuclei frequency, and DNA damage were reported for the battery workers as compared to the controls.

5.10.3.2. Toxicological Evidence for DNA and Cellular Damage

Sister Chromatid Exchanges

One study, Tapisso et al. (2009), considered sister chromatid exchanges (SCE) in rodents. Algerian mice (groups of six mice each) were exposed by intraperitoneal injection to 5 or 10 doses of 0.46 mg/kg

$Pb(Ac)_2$ and compared to untreated controls. The SCE in bone marrow were elevated after Pb exposure alone, which increased with time. Co-exposure with cadmium or zinc further increased SCE levels.

Two studies considered SCE in cultured human cells. In one study, Ustundag and Duydu (2007), considered the ability of N-acetylcysteine and melatonin to reduce led nitrate-induced SCE in a single human donor. Cells were treated with 0, 1, 5, 10, or 50 µM $Pb(NO_3)_2$. 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. Exposure times were not provided. 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. In the other study, Turkez et al. considered the ability of boron compounds 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. Exposure times were not provided. 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

Two studies considered MN in rodents. Alghazal et al. (2008), considered the ability of $Pb(Ac)_2$ trihydrate to induce MN in bone marrow of 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 than unexposed controls. The second study, Tapisso et al., (2009), considered Pb alone, Pb plus zinc and Pb plus cadmium-induced MN in rodents. Algerian mice were exposed by intraperitoneal injection to 5 or 10 doses of 0.46 mg/kg $Pb(Ac)_2$ and compared to untreated controls. The MN in bone marrow were elevated after Pb exposure, which increased with time. Co-exposure with cadmium or zinc did not further increase MN levels.

Three studies considered MN in cultured human cells. In one study, Ustundag and Duydu (2007) considered the ability of N-acetylcysteine and melatonin to reduce led nitrate-induced MN in a single human donor. Cells were treated with 0, 1, 5, 10, or 50 µM $Pb(NO_3)_2$. MN 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. Exposure times were not provided. 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. The second 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 significantly attenuation of these levels. Exposure times were not provided. 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. The third study, Gastaldo et al. (2007), evaluated the ability of Pb to induce MN. Human endothelial HMEC cell line was treated with 1–1000 µM $Pb(NO_3)_2$ for 24 h. MN increased in a statistically significant, concentration-dependent manner.

HPRT Mutations

Two studies evaluated HPRT mutations in human cell cultures. Li et al., (2008), evaluated $Pb(Ac)_2$-induced HPRT in the non-small-cell lung carcinoma tumor cell line, CL3 and normal human diploid fibroblasts (specific tissue source not provided). All cells were exposed to 0, 100, 300 or 500 µM $Pb(Ac)_2$ 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. A second study, Wang et al. (2008), investigated $Pb(Ac)_2$-induced HPRT mutations in CL3 cells. All cells were exposed to 0, 100, 300 or 500 µM $Pb(Ac)_2$ for 24 hours in serum-free medium ± a 1-hour pretreatment with a PKC-alpha inhibitor or siRNA fpr PKC-alpha. Pb alone did not induce HPRT mutations. Inhibiting the PKC-alpha via either inhibitor statistically significantly increased Pb-induced mutagenesis.

One study considered HPRT in animal cell culture. McNeill et al. (2007) considered $Pb(Ac)_2$ induced HPRT mutations in Chinese hamster ovary AA8 cells and AA8 cells overexpressing human Ape1. Cells were treated with 5 µM $Pb(Ac)_2$ for 6 hours. No increases in HPRT mutations were observed after Pb exposure in either cell line.

Chromosomal Aberrations

Only one study (El-Ashmawy et al., 2006) considered Pb in laboratory rodents. The study focused on dietary exposure to $Pb(Ac)_2$ administered as a single dose of 0.5% w/w. Male Swiss albino mice, 30 per group, were studied. In addition, the authors considered the protective effects of turmeric and myrrh powder. The study reported statistically significant levels of chromosomal aberrations in the Pb treatment alone group, particularly with respect to fragments, deletions, ring chromosomes, gaps, and end-to-end associations. The turmeric and myrrh powders were protective. Concerns with the study include the use of only a single dose of $Pb(Ac)_2$ 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 common in this study raising questions about the quality of the metaphase preparations. One 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 or why they only used one dose or analyzed fewer metaphases per dose.

Seven studies considered the ability of Pb to induce chromosomal aberrations in cultured human cells. One study (Pasha Shaik et al., 2006) considered the ability of $Pb(NO_3)_2$ to induce chromosomal

aberrations in primary human peripheral blood lymphocytes obtained from healthy, non-smoking donors. Cells were treated with 0, 1.2 or 2 mM $Pb(NO_3)_2$ for 2 h. 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. (2006), treated WHTBF-6 cells (hTERT-immortalized human lung cells) with 0, 0.1, 0.5, or 1 μg/cm^2 $PbCrO_4$ for 24-120 hours or with 0, 0.1, 0.5, 1, 5 or 10 μg/cm^2 PbO for 24 or 120 hours. $PbCrO_4$ induced statistically significant, concentration-dependent increases in centrosome abnormalities and aneuploidy. Wise et al. (2006) treated BEP2D cells with 0, 0.5, 1, 5, or 10 μg/cm^2 $PbCrO_4$ for 24 hours. $PbCrO_4$ induced statistically significant concentration-dependent increases in chromosomal aberrations. Holmes et al. (2006), treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 μg/cm^2 $PbCrO_4$ for 24-72 hours. $PbCrO_4$ induced statistically significant, concentration-dependent increases in chromosomal aberrations. The effects were attributed to the chromate anion. Wise et al. (2006), treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 μg/cm^2 $PbCrO_4$ for 24-120 hours. $PbCrO_4$ induced statistically significant, concentration-dependent increases in spindle assembly checkpoint disruption, effects of mitosis and aneuploidy. By contrast, chromate-free PbO did not induce centrosome amplification. The effects were attributed to the chromate anion. Xie et al. (2007) treated BEP2D cells with 0, 1, 5, or 10 μg/cm^2 $PbCrO_4$ for 24 hours. $PbCrO_4$ 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 $PbCrO_4$ for 24 hours in a study comparing 4 chromate compounds. $PbCrO_4$ induced statistically significant, concentration-dependent increases in chromosomal aberrations, but was the least potent chromate based on administered concentration.

Five studies considered the ability of $PbCrO_4$ to induce chromosome aberrations in rodent cell cultures. All focused on $PbCrO_4$. Duzevik et al. (2006) treated Chinese hamster ovary (CHO) cells with 0, 0.1, 0.5, or 1 μg/cm^2 $PbCrO_4$ for 24 h. Specific CHO lines used included AA8 (wildtype) EM9 (XRCC1-deficient), and H9T3 (EM9 complemented with human XRCC1 gene). $PbCrO_4$ induced statistically significant, concentration-dependent increases in chromosomal aberrations that were statistically significantly increased by XRCC1 deficiency. 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 $PbCrO_4$ (as pigment yellow) for 18 h. 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 $PbCrO_4$ for 24 h. Specific CHO lines used included AA8 (wildtype) KO40 (Fancg-deficient), and 40BP6 (Fancg complemented). $PbCrO_4$ induced statistically significant, concentration-dependent increases in chromosomal aberrations that were increased by Fancg deficiency. Camrye et al., (2007) treated CHO cells with 0, 0.1, 0.5, 1, 5, or 10 μg/cm^2 $PbCrO_4$ for 24 hours. Specific CHO lines

used included CHO-K1 (parental), xrs-6 (Ku80 deficient), and 2E (xrs-6 complemented with Ku80 gene). $PbCrO_4$induced statistically significant, concentration-dependent increases in chromosomal aberrations that were not affected by Ku80 deficiency. Stackpole et al. (2007) treated CHO and Chinese hamster lung (CHL) cells with 0, 0.1, 0.5, or 1 µg/cm^2 $PbCrO_4$ for 24 hours. Specific CHO lines used included AA8 (wildtype), irs1SF (XRCC3-deficient), and 1SFwt8 (XRCC3 complemented). CHL lines used included V79 (wildtype), irs3 (Rad51C deficient) and irs3#6 (Rad51C complemented). $PbCrO_4$ induced statistically significant, concentration-dependent increases in chromosomal aberrations that were statistically significantly increased by both XRC3 and Rad51C deficiency.

Three studies considered the ability of $PbCrO_4$ to induce chromosome aberrations in marine mammal cell cultures. All focused on $PbCrO_4$. 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 $PbCrO_4$ 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 $PbCrO_4$ 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 $PbCrO_4$ for 24 hours. In all three studies, $PbCrO_4$ induced statistically significant, concentration-dependent increases in chromosomal aberrations.

COMET Assay

Three studies considered the ability of Pb to induce DNA single strand breaks measured by the comet assays in laboratory animals. Xu et al. (2008) considered the ability of $Pb(Ac)_2$ to induce DNA damage measured by the comet assay in lymphocytes in male ICR mice. Animals (5 per group) were given $Pb(Ac)_2$ 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 dose-dependent manner. Nava-Hernandez et al. (2009) considered the ability of $Pb(Ac)_2$ to induce DNA damage measured by the comet assay 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) considered the ability of $Pb(NO3)_2$ to induce DNA damage measured by the comet assay in liver tissue of adult male Wistar rats. Animals (8 per group) were treated for 60 days with doses of 0, 0.5 or 1% $Pb(NO_3)2$ in their drinking water. There were no statistical differences between treated groups and controls.

Two studies considered the ability of Pb to induce DNA strand breaks measured by the comet assay in cultured human cells. Only, one study (Pasha Shaik et al., 2006) considered the ability of Pb to induce DNA single strand breaks using the comet assay in primary human peripheral blood lymphocytes obtained from healthy, non-smoking donors. Cells were treated with 0, 2.1, 2.4, 2.7, 3.0, 3.3 $Pb(NO_3)_2$ for 2 hours. Increased comet tail length with increased dose was reported.

Concerns with the study are that apparently no untreated 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. 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 $PbCrO_4$ for 24 hours. $PbCrO_4$ induced a concentration-dependent increase in DNA double strand breaks measured by the comet assay.

Two studies considered Pb-induced DNA single strand breaks in rodent cell cultures using the comet assay. Xu et al. (2006), treated PC12 cells with 0, 0.1, 1 or 10 μM $Pb(Ac)_2$. 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 $Pb(Ac)_2$ for 48 hours. There was an increase in several comet assay measurements including tail length.

Other Assays

Three studies considered the ability of Pb to induce DNA double strand breaks measured 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 $PbCrO_4$ for 24 hours. $PbCrO_4$ induced a concentration-dependent increase in DNA double strand breaks measured by gamma-H2A.X foci formation. Gastaldo et al. (2007) evaluated the ability of Pb to induce DNA double strand breaks measure with gamma-H2A.X foci formation and by pulse-field gel electrophoresis in cultured human cells. Human endothelial HMEC cell line was treated with 1 to 1,000 μM $Pb(NO_3)_2$ 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 $PbCrO_4$ for 24 hours in a study comparing 4 chromate compounds. $PbCrO_4$ induced statistically significant, concentration-dependent increases in DNA double strand breaks measured by gamma-H2A.X foci formation, at a similar level to the other 3 compounds.

Four studies considered Pb and DNA repair. All were done in cultured cells. Li et al., (2008), evaluated $Pb(Ac)_2$-induced effects on nucleotide excision repair efficiency in CL3 cells. All cells were exposed to 0, 100, 300 or 500 μM $Pb(Ac)_2$ 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. Human endothelial HMEC cell line was treated with 100 μM $Pb(NO_3)_2$ for 24 hours. Pb inhibited non-homologous end joining (NHEJ) repair, over activates 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 $PbCrO_4$ for 24 or 120 hours. Mre11 was required to prevent $PbCrO_4$-induced

DNA double strand breaks. McNeill et al, (2007) considered $Pb(Ac)_2$ effects on Ape1. Chinese hamster ovary cells (AA8) were treated with 0, 0.5, 5, 50, or 500 µM $Pb(Ac)_2$ and then whole cell extracts were used to determine AP site incision activity. The data show that Pb reduced AP endonuclease function. Finally, two studies considered Pb-induced cellular proliferation in laboratory animals. Fortoul et al. (2005) exposed adult male CD1 mice (24 animals per group) to 0.01 M $Pb(Ac)_2$, 0.006 M ca.dmium chloride or a mixture of the two chemicals for 1 h twice a week for 4 weeks by inhalation. The lungs were then examined by electron microscopy for changes. Pb induced cellular proliferation in the lungs. Kermani et al. (2008) exposed mouse bone marrow-mesenchymal stem cells to 0-100 µM $Pb(Ac)_2$ for 48 hours. As measured by the MTT assay, Pb decrease cell proliferation at all concentrations tested.

5.10.3.3. Mechanisms of Action

The carcinogenic mechanism of action of Pb is poorly understood. Three well-accepted general paradigms of carcinogenesis include multistage carcinogenesis (including initiation, promotion, and progression), genomic instability, and epigenetic modification. Of the aforementioned paradigms, it is unclear which of these best fit Pb. 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 chromosomal mutation and DNA damage, i.e. clastogenicity. However, the ability of Pb to alter gene expression (epigenetic effects) and to interact with proteins may be a means by which Pb induces its carcinogenicity. It is known that Pb can replace zinc in zinc-binding (zinc-finger) proteins, which include hormone receptors, cell-cycle regulatory proteins, the Ah receptor, estrogen receptor, p53, DNA repair proteins, protamines, and histones. These zinc-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(s). These modifications then provide the cancer cells with a selective growth advantage. In this schematic, Pb appears to contribute to epigenetic changes, and chromosomal aberrations.

The genomic instability paradigm requires a cascade of genome-wide changes caused by interfering with DNA repair, kinetochore assembly, cellular checkpoints, centrosome duplication, microtubule dynamics or a number of cell maintenance processes. There are some data that suggest Pb may interfere with some of these processes, but the data are few as these areas are rarely studied for Pb. Furthermore, the bulk of the literature in this area involves $PbCrO_4$ and it is unclear if the effects are due to Pb or chromate. Epigenetic modifications lead to cancer by altering cellular functions without altering the genetic material. The most commonly studied epigenetic change is methylation alterations. Data show

Pb can induce epigenetic changes, but studies are still missing to clearly tie these effects to Pb-induced carcinogenesis and genotoxicity. Thus, the mechanism is difficult to define but, if Pb is a human carcinogen, the mechanism likely involves either genomic instability or epigenetic modification paradigms or some combination of the two, but it not likely to occur by a multistage carcinogenesis paradigm. More work is needed to determine the mechanism.

No recent studies of the protective role of calcium or zinc in Pb-dependent carcinogenesis or genotoxicity were found. There were some data suggesting that metallothionein protects rodents from Pb-induced cancers. There were some data suggesting that boron, melatonin, N-acetylcysteine, turmeric and myrrh protecting cells against Pb-induced genotoxicity. There were some data suggesting that Pb mimics or antagonizes selenium in rodents. These data are discussed in more detail elsewhere in the cancer section.

5.10.3.4. Effects of Lead within Mixtures

Three studies considered the impact of mixtures with Pb. All considered genotoxicity. Mendez-Gomez et al., (2008), considered 65 children from Mexico exposed to both arsenic and Pb. DNA damage and decreased DNA repair were seen using the comet assay and other assays, but did not correlate with arsenic or Pb levels. Tapisso et al., (2009), considered Pb alone, Pb plus zinc and Pb plus cadmium-induced MN in rodents. Algerian mice (groups of six mice each) were exposed by intraperitoneal to 5 or 10 doses of 0.46 mg/kg $Pb(Ac)_2$ compared to untreated controls. The MN in bone marrow were elevated after Pb alone exposure, which increased with time. Co-exposure with cadmium or zinc increased SCE levels, but did not further increase MN levels. Glahn et al., (2008) performed a gene array study in primary normal human bronchial epithelial cells from 4 donors treated with 550 µg/l Pb chloride, 15 µg/l cadmium sulphate, 25 µg/l cobalt chloride or all three combined for 72 hours. There was a clear interaction of all three metals impacting RNA expression.

One new publication details the interaction of Pb and selenium in virus-dependent mammary tumor formation. No recent studies of the protective role of calcium in Pb-dependent carcinogenesis or genotoxicity were found. There were some data suggesting that boron, melatonin, N-acetylcysteine, turmeric and myrrh protect cells against Pb-induced genotoxicity (Sections 5.10.3.5, 5.10.3.7 and 5.10.3.10.).

One study considered Pb and selenium interactions in carcinogenesis in laboratory animals. Schrauzer (2008) considered the impact of selenium on carcinogensis by studying 4 groups of weanling virgin female C3H/St mice infected with murine mammary tumor virus (groups of 20-30 mice). One set of two groups were fed a diet containing 0.15 ppm selenium and then were exposed via drinking water to acetic acid (control group) or 0.5 ppm $Pb(Ac)_2$ (treated group). The second set of two groups were fed a diet containing 0.65 ppm selenium and then similarly exposed to acetic acid or 0.5 ppm $Pb(Ac)_2$. The

effects of selenium and Pb on the tumors caused by the virus were studied. The study is primarily focused on the general effects of a low selenium diet. The data suggest that selenium is anticarcinogenic as in the control groups the animals exposed to the higher selenium levels had fewer mammary tumors and these tumors had a delayed onset. Pb exposure with low selenium caused the same delayed onset as the higher dose of selenium and also caused some reduction in the tumor frequency. Pb exposure with higher selenium 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 selenium, but they suggest that Pb mimics or antagonizes selenium. They do not suggest that selenium is protective of Pb-induced toxicity or carcinogenesis.

5.10.4. Epigenetics

Epigenetic studies have been conducted to examine the associations between Pb levels and global DNA methylation markers [Alu and long interspersed nuclear element-1 (LINE-1)] (Pilsner et al., 2009; R. O. Wright et al., 2010). Wright et al. (2010) utilized a sample of participants from the Normative Aging Study with mean Pb levels of 20.5 g/g (SD 14.8 g/g) for tibia measures, 27.4 g/g (SD 19.7 g/g) for patella measures, and 4.1 µg/dL (SD 2.4 µg/dL) for blood measures. In both crude and adjusted analyses, patella Pb levels were inversely associated with LINE-1 methylation but not with Alu. When examining the relationship between patella Pb and LINE-1 more closely, a non-linear trend was observed with leveling off at higher Pb levels. No associations were observed for tibia or blood Pb and either LINE-1 or Alu. The second study included maternal-infant pairs from the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study and measured LINE-1 and Alu methylation in umbilical cord blood samples (Pilsner et al., 2009). In unadjusted models, maternal tibia Pb levels [mean 10.5 µg/g (SD 8.4 µg/g)] were inversely associated with Alu methylation and maternal patella Pb levels [12.9 µg/g (SD 14.3 µg/g)] were inversely associated with LINE-1 methylation. The associations persisted in adjusted models although the association between patella Pb and LINE-1 was only apparent when the adjusted models also included umbilical cord blood Pb levels. No association was detected between umbilical cord Pb levels and the DNA methylation markers. Overall, the studies consistently demonstrate an association between patella Pb levels and LINE-1 methylation.

Toxicological studies have been performed examining Pb-dependent 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 4 donors after in vitro treatment of the cells with 550 µg/l Pb chloride, 15 µg/l cadmium sulphate, 25 µg/l cobalt 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 epigentic

changes, but full interpretations of the data as epigenetic changes are complicated by the absence of a measure to determine if these changes were a result of genotoxic effects.

5.10.5. Summary and Causal Determination

In summary, the toxicology literature on the genotoxic, mutagenic, and carcinogenic potential of Pb have strong evidence of effects in laboratory animals. In laboratory studies, high-dose Pb has been demonstrated to be an animal carcinogen. There are data to suggest Pb is a human carcinogen among toxicological studies, but they are not definitive. The three toxicological studies of neoplastic transformation in cultured cells both were positive, but both focused on $PbCrO_4$ and attributed the positive response to the CrO4 and not the Pb. Mechanistic understanding of the carcinogenicity of Pb is expanding with work on the antioxidant selenium and metallothionein, a protein that binds Pb and reduces its bioavailability. In separate studies, selenium and metallothionein are 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 zinc, Pb replaces zinc at many zinc binding or zinc finger proteins. This substitution has the potential to induce indirect effects that can contribute to carcinogenicity via interactions at hormone receptors, at cell-cycle regulatory proteins, with tumor suppressor genes like p53, with DNA repair enzymes, with histones, etc. These indirect effects may act at a post-translational level to adversely alter protein structure and DNA repair. Also, epigenetic changes associated with Pb exposure are beginning to appear in the literature. These modifications may further alter DNA repair or change the expression of a tumor suppressor gene or oncogene in an adverse fashion. Thus, the animal toxicology literature provides a strong base for understanding the potential contribution of Pb exposure to cancer in laboratory animals.

Multiple epidemiologic studies have been performed examining cancer incidence and mortality, determined with biological measures and exposure databases. Mixed results have been reported for cancer mortality studies; one strong epidemiologic study demonstrated a positive association between blood Pb and cancer mortality, but the other studies reported null results. Although the previous Pb AQCD reported some studies demonstrating an association between Pb exposure and lung cancer, current studies mostly include studies of occupational exposure 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 genotypes. A limited amount of research on other types of cancer has been performed. The previous AQCD reported evidence that suggested an association between Pb exposure and stomach cancer, but recent studies of this association are lacking. One study examining Pb and stomach cancer has been performed since the last AQCD and the results of the study are mixed.

Among epidemiologic studies, positive associations were observed between high Pb levels and SCE among adults but not children. Other epidemiologic studies of DNA damage reported inconsistent results. Consistent with previous toxicological findings, Pb does appear to have genotoxic activity inducing SCE, MN and DNA strand breaks, but continues to not produce chromosomal aberrations except for $PbCrO_4$; this again is likely due to the chromate. Pb does not appear to be very mutagenic as the HPRT assays were typically negative unless a cell signaling pathway was disturbed.

Epigenetic effects, particularly with respect to methylation and effects on DNA repair were also consistently seen. Epigenetic studies examining Pb and LINE-1 and Alu consistently demonstrated an inverse association between patella Pb and LINE-1 methylation. Toxicological studies do show that Pb can activate or interfere with a number of signaling and repair pathways, though it is unclear whether these are in response to epigenetics or to genotoxicity. Thus, an underlying mechanism is still uncertain, but likely involves either genomic instability or epigenetic modifications or both.

Overall, there is some epidemiologic evidence supporting associations between Pb and cancer. Strong evidence from toxicological studies demonstrates an association between Pb and cancer, genotoxicity/clastogenicity or epigenetic modification. The collective body of evidence integrated across epidemiologic and toxicological studies is sufficient to conclude that there is a **likely causal relationship between Pb exposures and cancer.**

5.11. Overall Summary

The evidence reviewed in this chapter describes the recent findings regarding the health effects of Pb. Table 5-38 provides an overview of the causal determinations for each of the health categories evaluated.

Table 5-38. Summary of causal determinations for Pb.

Health Category	Causal Determination
Neurological Effects	Causal relationship
Cardiovascular Effects	Causal relationship
Renal Effects	Causal relationship
Immune System Effects	Causal relationship
Effects on Heme Synthesis and Red Blood Cell Function	Causal relationship
Reproductive Effects and Birth Outcomes	Causal relationship
Cancer	Likely causal relationship

Chapter 5. References

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Chapter 6. Susceptible Populations and Lifestages

Interindividual variation in human responses to air pollution exposure suggests that some populations are at increased risk for detrimental effects of ambient exposure to an air pollutant. The NAAQS are intended to provide an adequate margin of safety for both the population as a whole and those individuals potentially at increased risk for health effects in response to ambient air pollution (Section 1.1). Several studies have evaluated factors to identify populations at greater risk for Pb-related health effects. Many studies have termed such increased risk as the susceptibility and/or vulnerability of an individual to Pb. The definition for both of these terms has been found to vary across studies, but in most instances susceptibility refers to biological or intrinsic factors (e.g., lifestage, sex) while vulnerability refers to non-biological or extrinsic factors (e.g., socioeconomic status [SES]) (U.S. EPA, 2009, 2010). Additionally, in some cases, the terms "at-risk" and sensitive populations have been used to encompass these concepts more generally. However, in many cases, a factor identified that increases an individual's risk for morbidity or mortality effects from exposure to an air pollutant cannot be easily categorized as either a susceptibility or vulnerability factor.

As developed in previous ISAs and reviews (Sacks et al.; U.S. EPA, 2009, 2010), an all encompassing definition for "susceptible population" is used to circumvent the need to distinguish between susceptible and vulnerable, and to identify the populations at greater risk for Pb-induced health effects. This definition identifies susceptible populations as the following:

> Individual- and population-level characteristics that increase the risk of Pb-related health effects in a population including, but not limited to: genetic background, birth outcomes (e.g., low birth weight, birth defects), race, sex, lifestage, lifestyle (e.g., smoking status, nutrition), preexisting disease, SES (e.g., educational attainment, reduced access to health care), and characteristics that may modify exposure to Pb (e.g., time spent outdoors).

To examine whether Pb differentially affects certain populations, epidemiologic studies 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 more susceptible to Pb. Toxicological studies provide support and biological plausibility for factors that may lead to increased susceptibility for Pb-related health effects through the study of animal models of disease. Therefore, the results from these studies, combined with those results obtained through stratified analyses

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Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

in epidemiologic studies, comprise the overall weight of evidence for the increased susceptibility of specific populations to Pb-related health effects.

The first section of this chapter summarizes susceptibility of population groups related to differential Pb body burden. The studies presented in this section supplement the material provided in Chapters 3 and 4 by examining how susceptibility factors, such as age, race, ethnicity, and SES may affect Pb body burden, as measured by blood Pb or bone Pb. These biomarkers are influenced to varying degrees by exposure, absorption, biokinetics, and diet. The second section of this chapter discusses the epidemiologic and toxicological studies evaluated in Chapters 5 that provide information on potentially susceptible factors related to Pb-induced health effects. Highlighted studies include only those where the population was stratified into subgroups (e.g., males vs. females) 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. Additionally, the section on susceptibility and Pb body burden explores how susceptibility factors may be related to differential Pb exposures, where data are available. Numerous studies that focus on only one potentially susceptible population are described in previous chapters, but these studies are not discussed in detail in this chapter because they lack an adequate comparison group. For example, pregnancy is a potentially susceptible lifestage for mothers and fetuses, but because there are no comparison groups for stratified analyses, these studies are presented in Chapter 5 and are not included here. Included toxicological studies may categorize the study population by age, sex, diet, genetics, etc. or are those with animal models of disease.

Additionally, it is understood that some of the stratified variables may not be effect measure modifiers but instead may be mediators of Pb-related health effects. Factors that are mediators are on the causal pathway between Pb and health outcomes, while effect measure modifiers are factors that result in changes in the measured association between Pb 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 susceptibility factors. Some of the factors included in this chapter could be mediators and/or modifiers. These are noted in Table 6-3.

6.1. Susceptibility Factors and Lifestages Related to Lead Exposure and Dose

Elevated Pb biomarkers have been shown to be statistically related to several population characteristics, including age, gender, race and ethnicity, SES, and urbanization (U.S. EPA, 2006). In most cases, exposure, absorption, and biokinetics of Pb are all influenced to varying degrees by susceptibility factors. The relative importance of susceptibility factors on exposure, absorption, and biokinetics varies

on an individual basis and is difficult to quantify. The following section distinguishes studies demonstrating a relationship between each susceptibility factor and exposure status from those that are associated with increased biomarker levels without a clear attribution of the relative effects of those factors on exposure, absorption, and bioavailability.

6.1.1. Lifestage

6.1.1.1. Young Children

Typically, children have increased exposure to Pb compared with adults because children's behaviors and activities include increased hand-to-mouth contact, crawling, and poor hand-washing (U.S. EPA, 2006). Children can be susceptible to Pb exposure because outdoor play 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 2007-2008 NHANES data are presented in Table 6-1 by age and gender. Among children, highest blood Pb levels occurred in the 1-5 year age group, and within this subgroup, 1 year old children had the highest blood Pb levels (99th percentile: 16.9 µg/dL). 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). Jones et al. (2009) analyzed the NHANES dataset for the years 1988-2004 to study trends in blood Pb among different age groups over time (see Table 6-2). They observed greater percentages of children aged 1-2 year having blood Pb levels between 2.5 and 5 µg/L compared with 3-5 year old children. Similarly, the 1-2 year old group had larger percentages with blood Pb levels between 5 and 7.5 µg/dL compared with 3-5 year old children. These differences may be attributable to differences in exposure, age-dependent variability in absorption and biokinetics, or diet (milk/formula versus child diets). Yapici et al. (2006) studied the relationship between blood Pb level and age among a cohort of children younger than 73 months living in proximity to 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 6-1. Blood Pb levels broken down by age and gender, 2007-2008 NHANES

Age	Gender	Avg.	Std. Dev.	5%	25%	50%	75%	95%	99%
1-5 yr	total	2.03	2.01	0.69	1.08	1.54	2.34	4.50	10.56
	male	2.01	2.14	0.71	1.10	1.50	2.40	4.21	8.56
	female	2.05	1.85	0.66	1.02	1.60	2.28	4.65	10.70
1 yr	total	2.62	3.26	0.76	1.24	1.80	2.88	6.23	16.94

	male	2.70	3.98	0.76	1.29	1.80	2.75	5.59	19.82
	female	2.53	2.27	0.79	1.22	1.75	2.98	6.42	11.39
2 yr	total	2.09	1.52	0.76	1.28	1.77	2.40	4.34	8.40
	male	1.99	1.11	0.72	1.30	1.76	2.40	3.82	6.04
	female	2.20	1.84	0.82	1.27	1.77	2.35	4.52	10.75
3 yr	total	1.99	1.80	0.65	1.04	1.50	2.32	4.92	8.55
	male	1.99	1.55	0.68	1.11	1.50	2.35	5.09	8.46
	female	1.99	2.08	0.65	0.93	1.40	2.16	4.64	9.81
4 yr	total	1.75	1.31	0.69	1.00	1.40	1.90	4.03	6.31
	male	1.80	1.47	0.71	1.05	1.30	2.07	4.09	6.89
	female	1.66	1.03	0.64	0.98	1.41	1.89	3.78	5.31
5 yr	total	1.62	1.22	0.64	0.87	1.29	1.90	3.37	7.29
	male	1.58	0.97	0.72	0.95	1.29	1.88	3.38	4.96
	female	1.68	1.50	0.62	0.80	1.30	2.04	3.33	8.52
6-11 yr	total	1.27	0.87	0.49	0.75	1.06	1.50	2.84	4.80
	male	1.29	0.92	0.48	0.74	1.08	1.50	2.90	4.76
	female	1.25	0.83	0.50	0.78	1.03	1.49	2.80	4.80
12-19 yr	total	0.99	0.73	0.40	0.59	0.81	1.13	2.11	4.00
	male	1.13	0.82	0.44	0.69	0.94	1.30	2.41	4.11
	female	0.83	0.57	0.37	0.52	0.69	0.91	1.70	3.36
20-64 yr	total	1.76	1.57	0.53	0.91	1.40	2.10	4.20	7.42
	male	2.13	1.93	0.68	1.12	1.65	2.49	5.22	9.07
	female	1.41	1.01	0.47	0.78	1.13	1.73	3.13	5.10
65+ yr	total	2.31	1.64	0.80	1.30	1.90	2.70	5.22	8.54
	male	2.63	1.66	0.99	1.52	2.19	3.20	5.86	9.03
	female	1.98	1.56	0.75	1.17	1.62	2.40	4.14	6.95

Source: CDC (2009a).

Table 6-2. Percentage of children within six brackets of blood Pb levels, from NHANES 2003-2004

	n	Geometric mean	<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	2532	1.9	14.0	55.0	23.6	4.5	1.5	1.4
Gender								
Girl	1211	1.9	14.1	54.5	23.9	4.5	1.4	1.7
Boy	1321	1.9	14.0	55.5	23.2	4.6	1.5	1.3
Age								
1-2 yr	1231	2.1	10.6	51.0	27.9	6.7	1.4	2.4
3-5 yr	1301	1.7	16.2	57.6	20.7	3.1	1.5	0.9
Race/Ethnicity								
Non-Hispanic Black	755	2.8	4.0	42.5	36.2	9.4	4.6	3.4
Mexican American	812	1.9	10.9	61.0	22.1	3.4	1.3	1.2
Non-Hispanic White	731	1.7	17.6	57.1	19.7	3.6	0.8	1.2
PIR								
≤ 1.3	1302	2.4	6.7	49.3	32.5	6.9	2.8	1.8
>1.3	1070	1.5	19.9	60.4	16.0	2.3	0.6	0.8

Source: Jones et al. (2009)

6.1.1.2. Adults

Blood Pb levels tend to be higher in the elderly compared with the general adult population. Table 6-1 presents 2007-2008 NHANES data broken down by age group and shows that blood Pb levels were highest in the >65-years age group in comparison with adults aged 20-64 years. 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 both blood Pb ($p < 0.001$) and saliva Pb ($p < 0.001$). Average blood Pb levels among 14- to 24-year-old subjects was 2.6 μg/dL compared with 4.3 μg/dL among subjects aged 55 years or older; similarly, average saliva Pb levels among 14- to 24-years-old subjects was 2.5 μg/L compared with 3.6 μg/L 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 (see section 4.1) in conjunction with remobilization of stored Pb during bone loss (Section 4.2).

Theppeang et al. (2008) 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, Yale energy index, and 2 diet variables; $\beta = 0.57$, $p < 0.01$ in a model including age, gender, and an interaction term for gender and age, which was also statistically significant at $p = 0.03$). Theppeang et al. (2008) also noted that patella Pb concentrations increased with age, although the data quality for patella Pb was not as high, so 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.

Fetal and child Pb biomarkers have been demonstrated to relate to maternal Pb biomarkers; several older studies in the literature are presented in the 2006 AQCD (U.S. EPA, 2006). Kordas et al. (2010) observed that maternal hair Pb concentration was a statistically significant predictor of child hair Pb concentration ($\beta = 0.37 \pm 0.07$, $p < 0.01$). Miranda et al. (2010) observed that pregnant women ages 30-34 and 35-39 had statistically significant higher odds of having greater blood Pb levels than women in the 25- to 29-years-old 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 decay during pregnancy, as described in Section 4.2. Elevated blood Pb levels among mothers also present a potential exposure to their children in utero or through breast milk.

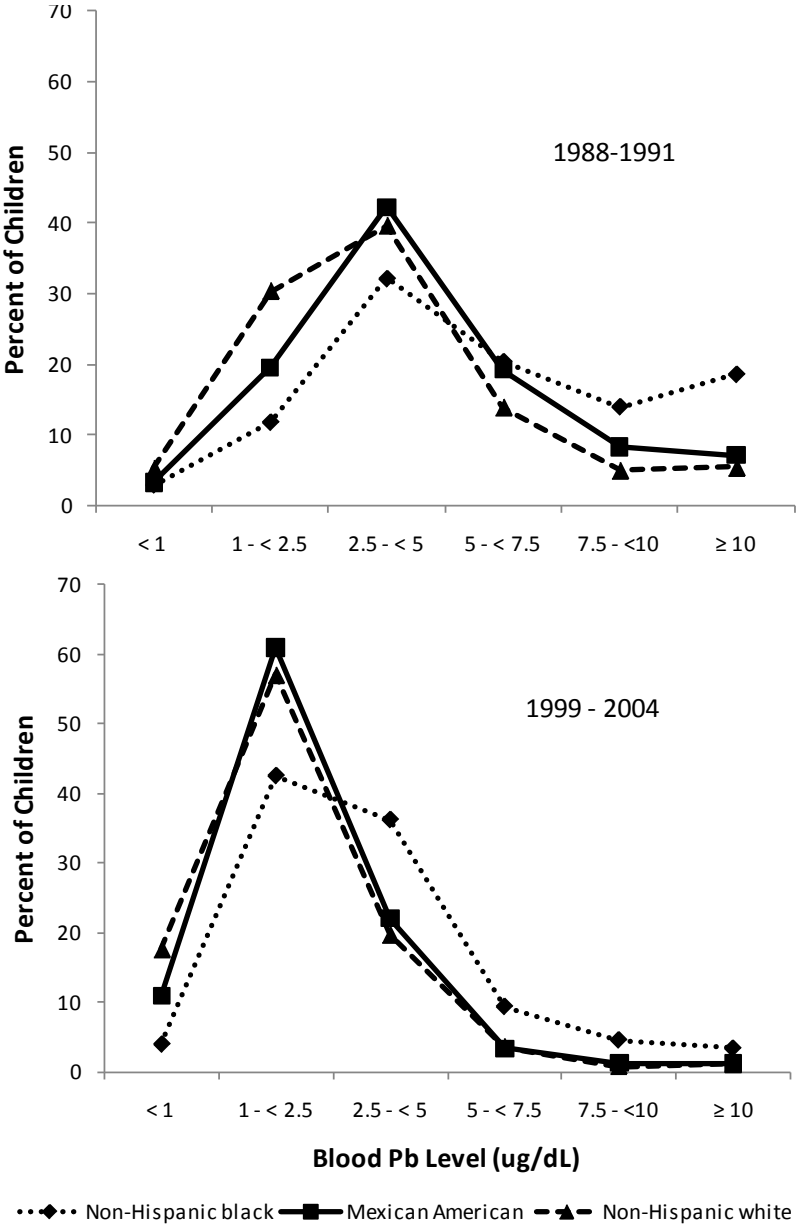
6.1.2. Sex

Several studies have suggested that sex influences levels of Pb biomarkers because differences in behavior between sexes may cause a differential increase in exposure. The 2007-2008 NHANES showed that overall, males have higher blood Pb levels (median: 1.50 µg/dL) than females (median: 1.14 µg/dL) (see Table 6-1). Among adults aged 20-64 years, median blood Pb levels among males was 46% higher than for females, and average levels were 51% higher for males compared with females. Among adults 65 years or older, median levels were 36% higher, and average levels were 33% higher. In their study of Pb burden among Baltimore adults aged 50-70 years, Theppeang et al. (2008) 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. (2008) noted no difference ($p = 0.12$) between men (18.0 µg/dL) and women (19.4 µg/dL).

Among U.S. children, the 2007-2008 NHANES data show that blood Pb levels are higher among girls than boys for the 1- to 5-years age group (Table 6-1). Blood Pb levels became slightly higher among boys for the 6- to 11-years age group, and levels were substantially higher among adolescent males in the 12- to 19-years age group. At the same time, blood Pb levels among both adolescent males and females were lower than blood Pb levels for the other age groups. The 2007-2008 NHANES data suggest that gender-based differences in blood Pb levels are not substantial until adolescence.

6.1.3. Race and Ethnicity

Higher blood Pb and bone Pb levels among African Americans has been well documented (U.S. EPA, 2006). Recent studies are consistent with those previous findings. For instance, Levin et al. (2008) and Jones et al. (2009) both analyzed NHANES survey data to examine trends in childhood blood Pb levels. Data from the Jones et al. (2009) study, using 2003-2004 NHANES data (CDC, 2011), are shown in Figure 6-1. They found that differences among racial and ethnic groups with regard to the percentage with blood Pb levels greater than 2.5 µg/dL have decreased since the period of 1976-1980 when NHANES II was conducted. The non-Hispanic black group still had higher percentages with blood Pb levels above 2.5 µg/dL compared with non-Hispanic whites and Mexican Americans, with the largest observable differences 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. Theppeang et al. (2008) also explored the effect of race and ethnicity on several Pb biomarkers in a study of older adults living in Baltimore. 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, $p = 0.69$). Differences between bone Pb levels in African American and Caucasian subjects could potentially be related to differential exposures in the home environment.

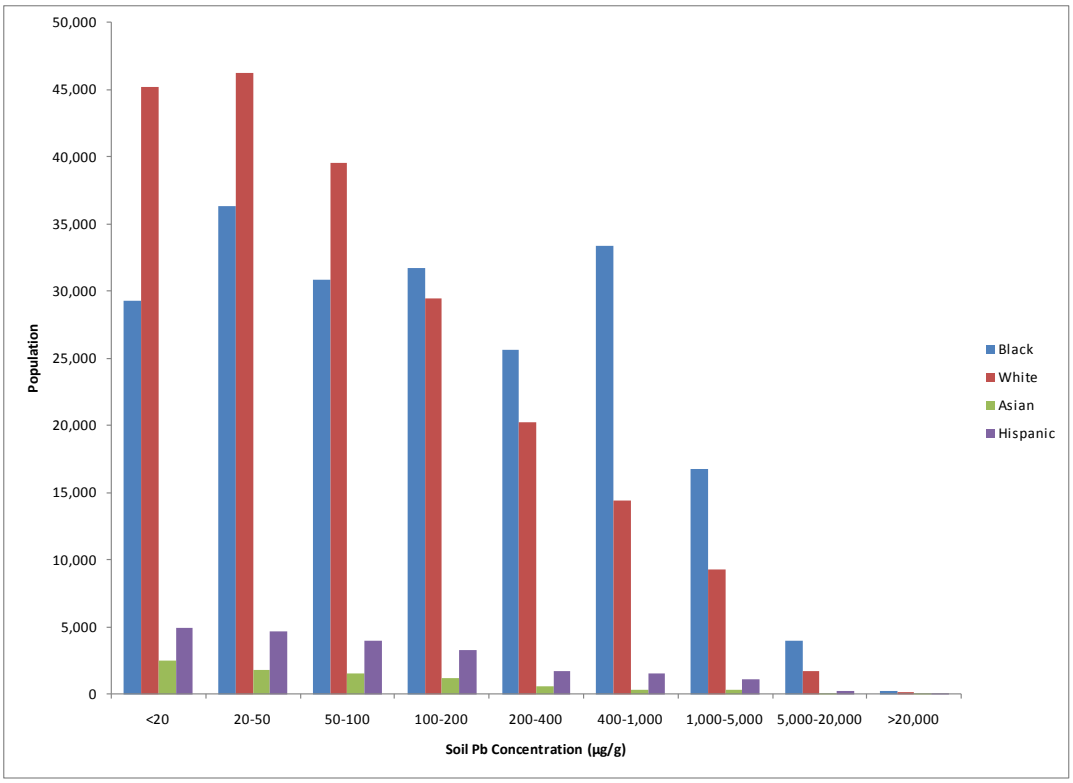


Data used with permission from the American Academy of Pediatrics, Jones et al. (2009)

Figure 6-1. 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).

Differences in exposure among ethnic and racial groups have also been noted. In a study of three parishes in the greater metropolitan New Orleans area, Campanella and Mielke (2008) found that, where soil Pb levels were less than 20 mg/kg, the population was 55% white, 36% black, 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 the population was 34% white, 62%

black, 1% Asian, and 4% Hispanic on Census blocks in which soil Pb levels were between 1,000 and 5,000 mg/kg (Figure 6-2). As described in Section 6.1.4, 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.



Source: Campanella and Mielke (2008).

Figure 6-2. Soil Pb concentration exposure among the population of three parishes within greater metropolitan New Orleans, by race and ethnicity.

6.1.4. Socioeconomic Status (SES)

Socioeconomic factors have sometimes been associated with Pb biomarkers, although these relationships have not always been consistent. Nriagu et al. (2006) performed a multiple regression of blood Pb and saliva Pb levels on various socioeconomic, demographic, and exposure variables among an adult population in Detroit. 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 gap between income levels with respect to blood Pb has been decreasing. For example, Levin et al. (2008) cite NHANES data analyzed in Pirkle et al. (1994) that the percentage of children aged 1-5 years with blood Pb levels above 10 µg/dL was 4.5% for the lowest income group compared with 0.7% for the highest group. For the 1999-2002 NHANES, there was 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%), although 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) (Levin et al., 2008). When adding data for 2003-2004 to the analysis (i.e., for 1999-2004), the gap between Medicaid enrolled and non-enrolled children widened for blood Pb levels exceeding 10 µg/dL (1.9% versus 1.1%), but the difference was still not statistically significant (p > 0.05). Median blood Pb levels with respect to Medicaid status did not change when adding the 2003-2004 data (R. L. Jones et al., 2009). Likewise, Jones et al. (2009) analyzed blood Pb levels with respect to poverty-income ratio (PIR). They found statistically significant differences in median blood Pb for PIR ≤ 1.3 compared with PIR >1.3. The percentage of 1- to 5-year0old children having blood Pb above 10 µg/dL was higher for PIR ≤ 1.3 (1.8 versus 0.8); however, this difference was not statistically significant. Additionally, Campanella and Mielke (2008) observed a linear increase in soil Pb content outside a home with respect to decreasing average household income, with soil Pb between 2.5 and 20 mg/kg associated with an average income of $40,000 per year, while soil Pb between 5,000 and 20,000 was associated with an average income of $24,000 per year.

6.1.5. Proximity to Lead Sources

Airborne and soil Pb concentrations are higher in some urbanized areas, as described in Sections 3.2, 3.3, 3.5 and 4.1, as a result of historical and contemporaneous Pb sources. High concentrations of ambient air Pb in PM tend to occur in the most urbanized areas and in close proximity to traffic in metropolitan areas (Laidlaw & Filippelli, 2008; Mielke et al., 2010; Weiss et al., 2006). Moreover, air Pb concentrations exhibit high spatial variability even at low concentrations (~0.01 µg/m^3) (Martuzevicius et al., 2004). These conditions present the potential for additional risk of Pb exposure in urban areas. Proximity to an industrial source likely contributes to higher Pb exposures, as described in the 2006 AQCD (U.S. EPA, 2006) 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 2007-2009 data presented in Section 3.5. Jones et al. (2010) found that neonates born near a Pb-contaminated urban industrial site had statistically significantly higher 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 current industrial Pb exposures 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, the Jones et al. (2010) study did not analyze covariation between exposure and maternal characteristics, so it cannot be determined if differences in characteristics among the groups with and without industrial exposures confounded these results.

6.2. Susceptibility Factors and Lifestages Related to Lead Induced Health Effects

The following section evaluates potential susceptibility factors examined as effect measure modifiers of various Pb-related health effects. Table 6-3 provides an overview of the factors examined and populations identified as susceptible to Pb-related health effects based on the recent evidence integrated across disciplines. Each characteristic is described in greater detail in the following sections.

Table 6-3. Summary of new evidence for characteristics examined as potential susceptibility factors and lifestages for lead-related health effects

Factor Evaluated	Susceptible Population
Lifestage	Children
Sex	Males[a] Females[a]
Genetics	ALAD[a], VDR[a]*, DRD4[a]*, GSTM1[a], TNF-alpha[a], eNOS[a], APOE[a], HFE[a]
Pre-existing Disease	Autism[ab], Atopy[ab], Hypertension[b]
Smoking	Smokers[a]
Race/Ethnicity	Non-Hispanic Blacks[a], Hispanics[a]
Socioeconomic Status (SES)	Low SES[a]
Nutrition	Iron deficiency
Stress	High stress
Cognitive Reserve	Low cognitive reserve[ab]
Other Metals	Cd[a], As[a], Mn[a]

[a]Additional evidence is needed to confirm whether the characteristic evaluated results in increased susceptibility to Pb-related health effects.
[b]Possible mediator

6.2.1. Lifestage

The previous Pb AQCD reported on susceptibility by age (U.S. EPA, 2006). The greatest ingestion of Pb is often at the same time as development in children. Older adults are more likely to have age-related degeneration of bones and organ systems and a possible redistribution of Pb. Therefore, increased susceptibility of Pb-related health effects is a concern for these populations. Below is information from epidemiologic and toxicological studies regarding studies of susceptibility for children and older adults.

6.2.1.1. Children

According to the 2000 Census, 28.6% of individuals living in the U.S. were under the age of 20, with 6.8% aged 0-4 years, 7.3% aged 5-9 years, 7.3% aged 10-14 years, and 7.2% aged 15-19 years (SSDAN, 2010a). It is recognized that Pb can cross the placenta to affect the developing nervous system of the fetus (Sections 4.2.2.4 and 5.3.2.1) and there is evidence of increased susceptibility to the neurocognitive effects of Pb exposure during several lifestages throughout childhood and into adolescence (for more detail, see Section 5.3.2.1). Epidemiologic studies have investigated susceptibility among infants compared to adults or infants to young children.

A study including multiple U.S. locations examined associations of blood Pb levels with various immune parameters among individuals living near Pb industries and matched controls (Sarasua et al., 2000). For several of the endpoints, the association in the youngest group (6-35 months) and the oldest group (16-75 years) were in different 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 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 susceptibility for Pb-associated inappropriate activation of humoral immune responses and perturbations in cell-to-cell interactions that underlie allergic, asthma, and inflammatory responses (for more information, see Sections 5.6.2.1 and 5.6.3).

A study among Lebanese children examined the association between blood Pb levels and transferrin saturation (TS) less than 12% and 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 note that it is

difficult to know whether the Pb levels were "a cause or a result of" IDA levels since previous studies linked iron deficiency with Pb toxicity.

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 susceptible to Pb exposure (Fullmer et al., 1985). Younger animals tend to attain a higher blood Pb level than older animals exposed to the same dose (mg/kg body weight/day) of Pb (Berrahal et al., 2011). Additionally, infants may be a susceptible population because Pb easily crosses the placental barrier and accumulates in fetal tissue during gestation (Pillai et al., 2009; Uzbekov et al., 2007; Y.-Y. Wang et al., 2009).

Overall evidence indicates young age as a potential susceptibility factor for Pb-related health effects. Both recent 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.

6.2.1.2. Older Adults

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, 2010a; U.S. Census Bureau, 2010). As of the 2000 Census, 7.2% of the U.S. population were ages 60-69, 5.8% were 70-79, and 3.3% were age 80 and older (SSDAN, 2010a).

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). Although the hazard ratios were greater for all-cause and cardiovascular mortality among those less than 60 years old, an increase in the hazard ratios was also observed among those 60 years of age and older and the interactions 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 of at least 10 µg/dL to blood Pb levels less than 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 comparing blood Pb levels of 5-9 µg/dL to blood Pb levels less than 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 comparing blood Pb levels of 10 µg/dL and greater to those less than 5 µg/dL were elevated among all age groups. Finally, the association

between blood Pb levels 10 µg/dL and greater 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 of 5-9 µg/dL to less than 5 µg/dL. The other age groups had similar point estimates but the associations were not statistically significant.

The NHANES III study cohort was also used to investigate the association between blood Pb and cognitive test scores (Krieg et al., 2009). The relationship was examined among adults aged 20-59 and 60 years and older, but no association was observed in either of the age groups. A study using the Normative Aging Study cohort reported an interaction between Pb and age (R. O. Wright, Tsaih, 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 a model of Pb 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 biological plausibility for increased susceptibility among older populations. Demineralization associated with aging may increase the pool of available Pb to the blood. Cory-Slechta (1990) administered various doses of Pb for a constant period to young animals, adults, or aged animals and found increased susceptibility to Pb in the aged animals due to increased exposure from elevated bone resorption.

Also the kidneys of older animals appear to be more susceptible to 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). Susceptibility 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 multiple species (mice and monkeys). These pathologies include the development of neurofibrillary tangles and increased amyloid precursor protein and its product beta-amyloid (Basha et al., 2005; Zawia & Basha, 2005). Some of these findings were seen in animals that no longer had elevated blood Pb levels.

Results for age-related modification of the association between Pb and mortality had mixed results and no difference by age was observed for the associations between Pb and other health effects. However, toxicological studies that inform on Pb-related health effects by age may be relevant in humans. Future studies will be instrumental in understanding older age as a susceptibility factor.

6.2.2. Sex

The distribution of males and females in the U.S. is similar. In 2000, 49.1% of the U.S. population was male and 50.9% were female. The distribution did vary by age with a greater prevalence of females ≥ 65 years old compared to males (SSDAN, 2010a). 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, 2006).

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 and sex for both prenatal and postnatal exposures; associations of prenatal and postnatal blood Pb decrements in memory, attention, and visuoconstruction were observed only among male adolescents. More recently, Wright et al. (2008) examined early life Pb exposure and criminal arrests in adulthood. The attributable risks were greater among males than females. Additionally, the association between childhood blood Pb levels and 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 inverse 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 Pb exposures at various ages throughout childhood and adolescence (Baghurst et al., 1992; McMichael et al., 1992; Tong et al., 2000). These studies observed Pb effects on cognition were stronger in girls throughout childhood and into early adolescence. A study in Poland also investigated the association between cord Pb levels and cognitive deficits and reported an inverse association for boys at 36 months but not for girls (Jedrychowski et al., 2009). No association was detected for boys or girls at 24 months.

An epidemiologic study examined the association between 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 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 among biomarkers. A study comprised mostly of females reported positive associations between 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 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 Pb levels and inflammatory markers (Songdej et

al., 2010). Although there was no pattern, a few of the associations were positive between 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). Additionally, blood Pb was inversely associated with the release of NO among males but not females.

Epidemiologic investigations of cancer have also examined the associations by sex. A study of the association between occupational exposure 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 inverse association was observed between ever exposure to Pb and meningiomas for females. 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 & Slesak, 2010).

A study of all-cause and cardiovascular mortality using the NHANES III cohort reported no modification of the association between 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 animals exposed to Pb. In a subsequent publication, Donald et al. (1987) showed that non-social behavior 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 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 males at all 3 doses of Pb exposure; memory retrieval was only impaired in low-dose females (Yang et al., 2003).

Sex-specific differences were also observed for gross motor skills; at the lowest Pb dose, balance and coordination were most affected among males (Leasure et al., 2008). In addition, obesity in adult offspring exposed to low dose Pb in utero was reported for males but not females (Leasure et al., 2008). Obesity was also found in male offspring exposed in utero to high doses of Pb that persisted to 5 weeks of age/end of the study, but among females, 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 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 also varied between males and females (Alghazal et al., 2008; Sobekova et al., 2009). 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).

Pb and stress are co-occurring factors that act in a sex-divergent manner to affect behavior, neurochemistry, and corticosterone. Pb and stress act synergistically to affect fixed interval operant behavior and corticosterone in female offspring. Virgolini et al. (2008) found that effects on the central nervous system by developmental Pb exposure are enhanced by combined maternal and offspring stress and females are most susceptible. Behavioral related outcomes after gestational and lactational Pb exposure with and without stress show sex-differences in exposed offspring (Virgolini, Rossi-George, Weston, et al., 2008). 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 males {McGivern, 1991, 49264}. Interestingly, another chemical that is known to cause a hypothalamic lesion in this area, monosodium glutamate, is associated with adult onset obesity; adult onset obesity is seen in the Pb literature.

Multiple associations between Pb and various health endpoints have been examined for effect measure modification by sex. Although not observed in all endpoints, some studies reported differences between the associations for males and females, especially in neurological studies. However, studies on cognition from the Cincinnati Lead Study cohort and a study in Poland reported males to be the susceptible population, whereas studies from Australia pointed to females as the susceptible population. A difference in sex is also supported by toxicological studies. Further research will confirm the presence or absence of sex-specific associations between Pb and various health outcomes.

6.2.3. Hormones

It is possible that hormone levels may affect susceptibility to Pb-related health effects. Among women, various hormone-related categories were examined for the relationship between blood Pb levels and follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels. A positive association was observed between Pb levels and FSH levels among women who were post-menopausal, who were pre-menopausal but not on birth control, menstruating, or pregnant, or who had both ovaries removed (Krieg, 2007). An inverse association was observed for women taking birth control pills. For Pb and LH, there

was a positive association among women who were post-menopausal or had both ovaries removed. No associations for either hormone were observed for women who were pregnant or menstruating.

Toxicological studies provide evidence that Pb affects hormones and support the possible susceptibility to Pb-related health effects by hormonal status. Delayed onset of puberty (Pine et al., 2006) as well as changes in the female reproductive tract have been reported in the literature after Pb exposure. Pb exposure can also alter estrous cyclicity (U.S. EPA, 2006), change cervical structure, and lead to ovarian dysfunction with altered membrane composition (Kolesarova et al., 2010; Nampoothiri et al., 2007; Nampoothiri & Gupta, 2006; U.S. EPA, 2006). Additionally, embryo development and implantation enzymes are aberrant with Pb exposure (Nandi et al., 2010). In female offspring, delays in F1 and F2 puberty onset, first estrous, and age of first parturition have been reported with in utero Pb exposure (Iavicoli et al., 2006).

6.2.4. Genetics

The 2006 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, 2006). The majority of discussion focused on aminolevulinate dehydratase (ALAD) and vitamin D receptor (VDR). These two genes, as well as additional genes examined in recent studies, are discussed below.

6.2.4.1. Aminolevulinate Dehydratase

The aminolevulinate dehydratase 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, 2006). ALAD is a polymorphic protein with three isoforms: ALAD 1-1, ALAD 1-2, and ALAD 2-2. Multiple studies have examined the association between ALAD2 polymorphisms and blood Pb levels (Y. Chen et al., 2008; Chia et al., 2007; Chia et al., 2006; Montenegro et al., 2006; Scinicariello et al., 2007; Scinicariello et al., 2010; Sobin et al., 2009; Zhao et al., 2007); ALAD polymorphisms may be biologically related to varying Pb levels. In addition, studies have examined whether ALAD variants alters associations between Pb and various health effects.

Associations between Pb and brain tumors observed in one study varied by ALAD genotype status (Rajaraman et al., 2006). Positive associations between Pb exposure 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.

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 (non-Hispanic white, non-Hispanic black, and Mexican-American) (Scinicariello et al., 2010).

Individuals with ALAD 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., 2003; Weaver et al., 2005). A study of Chinese battery plant storage workers reported workers with the ALAD2 allele demonstrated greater associations between Pb and renal injury (Gao et al., 2010). Another study of renal function among workers in Asia also reported greater associations between Pb and renal function by ALAD, especially at high levels of Pb (Chia et al., 2006).

Studies investigating the association between Pb levels and cognitive function have also examined modification by ALAD polymorphisms. In a study using a cohort from NHANES III, slight associations were observed in some cognitive tests for 20-59 year olds with CC and CG ALAD genotypes (Krieg et al., 2009). However, other studies reported no difference in the association between blood Pb and cognitive function by ALAD variant, although some difference was found when examining bone Pb levels and cognitive function (Rajan et al., 2008; Weisskopf et al., 2007; Weuve et al., 2006).

6.2.4.2. 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).

Three genetic variants or polymorphisms of the vitamin D receptor in humans have been characterized (BsmI, FokI, and ApaI) and have been reported to account for 75% of the differences in bone density in humans (Morrison et al., 1994; Morrison et al., 1992). The BsmI polymorphisms are denoted as bb (homozygote), BB (homozygote), and Bb (heterozygote). Bone measurements of Pb levels in exposed workers found that bone Pb was highest in individuals with the BB genotype, intermediate in the heterozygotes and lowest in the bb genotype group (Schwartz et al., 2000; Theppeang et al., 2004). People with the bb genotype or ff (FokI polymorphism) genotype have lower bone Pb than subjects with other genotypes. Subjects with the aa (ApaI polymorphism) or ff genotype have lower plasma Pb than subjects with other genotypes (Rezende et al., 2008). Plasma Pb is followed to look at bio-available Pb, instead of blood Pb, which largely reports Pb bound to the red blood cell. Thus, subjects with the haplotype combining a, b and f alleles for the aforementioned respective polymorphisms have lower

plasma Pb and bone Pb levels (Rezende et al., 2008). A study of pregnant women examining Pb in maternal and umbilical cord serum and blood found no association between VDR polymorphisms and these parameters. However there was lower Pb in women with the VDR haplotype combining the alleles f a and b, a group that is likely genetically less prone to Pb toxicity during pregnancy (Rezende et al., 2010).

6.2.4.3. 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.

6.2.4.4. Apolipoprotein E

Apolipoprotein E (APOE) is a transport protein for cholesterol and lipoproteins. The gene appears to regulate synapse formation (connections between neurons) and may be particularly critical in early childhood. A genetic variant, called the APOE4 allele is a haplotype between two exonic SNPs and is perhaps the most widely studied genetic variant with respect to increasing risk of neurologic disease. A study of occupationally-exposed adults observed Pb to be associated with greater decrements in tests such as digit symbol, pegboard assembly, and complex reaction time among adults with at least one APOE-ε4 allele (Stewart et al., 2002). Conversely, in a study of children in Mexico, children without the APOE-ε4 allele had a greater inverse association between umbilical cord Pb and Bayley's MDI than children with this allele, although the interaction term was not statistically significant (R. O. Wright, Hu, et al., 2003).

6.2.4.5. Hemochromatosis

The Hemochromatosis (HFE) gene encodes a protein believed to be involved in iron absorption. A difference was observed between the association of tibia Pb levels and cognitive function for men with and without HFE allele variants (F. T. Wang et al., 2007). No association between tibia Pb and cognitive function was present for men with HFE wildtype, but a decline in function associated with 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., 2010).

6.2.4.6. Other Genetic Polymorphisms

Some genetic polymorphisms have only one study examining whether they modify Pb-related health effects. These include dopamine 4 receptor (DRD4), glutathione S-transferase Mu 1 (GSTM1), tumor necrosis factor-alpha (TNF-alpha), 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 university students in 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 (J. H. Kim et al., 2007).

The relationship between blood Pb levels and inflammation was examined among individuals with TNF-alpha GG, GA, or AA alleles. An association was present for those with TNF-alpha GG but not for those with TNF-alpha GA or AA (J. H. Kim et al., 2007).

A study of blood Pb and plasma NOx reported no overall association but did report an inverse correlation among subjects with the eNOS TC+CC genotype (Barbosa et al., 2006). 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 Pb 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 glioblastma 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.

6.2.5. Pre-existing Diseases/Conditions

Studies have also been performed to examine whether certain morbidities make individuals more susceptible to Pb-related effects on health. Recent studies have explored relationships for autism, atopy, diabetes, and hypertension.

6.2.5.1. Autism

Rates of autism have increased in recent years. A study reported a prevalence rate in 2006 of 9.0 per 1,000 population (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, 2009b).

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 among both autistic and non-autistic children were associated with expression of the inflammation genes under study, however the associations observed were in opposite directions (positive association among autistic children and inverse among non-autistic children).

6.2.5.2. Atopy

Atopy, a type of allergic hypersensitivity, was evaluated as a susceptibility factor in a study of Pb and IgE (Annesi-Maesano et al., 2003). The study examined Pb levels (measured via hair) in infants and IgE and reported a positive correlation overall. However, in stratified analyses, this association remained only among infants of mothers without atopy. Among atopic mothers, the correlation was positive, although smaller, and was not statistically significant.

6.2.5.3. 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 susceptibility 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 was examined using the Normative Aging Study cohort (Tsaih et al., 2004). Tibia and blood Pb levels were positively associated with measures of renal function among diabetics 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 study evaluated whether the association between 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 did not differ for individuals with and without diabetes. However, results from the previous Pb AQCD found that individuals with diabetes are at "increased risk of Pb-associated declines in renal function" (U.S. EPA, 2006). 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 this potential susceptibility factor.

6.2.5.4. Hypertension

Hypertension affects approximately 24% of adults in the U.S. and the prevalence of hypertension increases with age (61% of individuals 75 years of age and older have hypertension) (Pleis et al., 2009).

The Normative Aging Study cohort mentioned above for 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 hypertensive but not normotensive individuals (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 Pb and heart rate variability was not observed among hypertensive or normotensive individuals, the estimates were different, with greater odds among hypertensive individuals (Pb positively related to low frequency power and the ratio of low frequency to high frequency power and inversely related to high frequency power).

A study using the NHANES III cohort reported a positive association between Pb and both all-cause and cardiovascular mortality for hypertensive and normotensive individuals 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 susceptibility to Pb-related effects on renal function (U.S. EPA, 2006). 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 hypertensive individuals, but the difference between hypertensive and normotensive adults is not observed for Pb-related mortality.

6.2.6. Smoking

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.

A study of Pb 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 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 non-smokers (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-alpha 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 non-smokers (Kiziler et al., 2007). The blood and seminal plasma Pb levels were higher for smokers of both groups. Additionally, the Pb levels were lowest among non-smoking fertile men and highest among smoking infertile men.

Prenatal smoking exposure was examined in a study of children's blood Pb levels and prevalence of attention-deficit/hyperactivity disorder (ADHD). An interaction was observed between Pb 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 mixed findings on whether smoking modifies the relationship between Pb and health effects. Future studies of Pb-related health effects and current, former, and prenatal smoking exposures among various health endpoints will aid in determining susceptibility by this factor.

6.2.7. Race/Ethnicity

Based on the 2000 Census, 69.1% of the U.S. population is comprised of Non-Hispanic Whites. Approximately 12.1% of people reported their race/ethnicity as Non-Hispanic Black and 12.6% reported being Hispanic (SSDAN, 2010b). Studies of multiple Pb-related health outcomes examined effect measure modification by race.

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 was reported. Another study using the NHANES III cohort reported on blood Pb levels and hypertension. While no association was observed between blood Pb and non-Hispanic Whites or Hispanics, a positive association was reported for non-Hispanic Blacks (Scinicariello et al., 2010). Another study using NHANES datasets examined the associations between blood Pb and hypertension (Muntner et al., 2005). Although none of the associations were statistically significant, increased odds were observed among non-Hispanic blacks and Mexican-Americans but not for non-Hispanic whites.

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 African Americans 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 African Americans and Mexican Americans had blood Pb levels greater than 5 µg/dL, respectively.

A study linking educational testing data for 4th grade students in North Carolina reported declines in reading and mathematics scores with increasing levels of Pb (Miranda et al., 2007). Although not quantitatively reported, a figure depicts the association stratified by race; the slopes appear to be similar for white and black children.

Blood Pb and asthma 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 suggest that there may be race-related susceptibility for some outcomes, although the overall understanding of potential effect measure modification by race is limited by the small number of studies. Additionally, these results may be cofounded by other factors, such as socioeconomic status.

6.2.8. Socioeconomic Status

Based on the 2000 Census data, 12.4% of Americans live in poverty (poverty threshold for family of 4 was $17,463) (SSDAN, 2010c). Ris et al. (2004) examined modification of the associations between early-life Pb exposure and Learning/IQ among adolescents in the Cincinnati Lead Study. In models examining the association between Pb and Learning/IQ, prenatal and 78-month Pb concentrations were associated with larger decrements in Learning/IQ in the lower two quintiles of socioeconomic status (SES) (measured based on family SES levels).

6.2.9. 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 the two categories of body mass index (BMI) (non-obese [<25 kg/m^2] and obese [≥ 25 kg/m^2]) but there was no difference between the categories. Using the Normative Aging Study, 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).

No modification by BMI/obesity was observed among recent epidemiologic studies. Future studies of Pb-related health effects and BMI will aid in determining susceptibility by this factor.

6.2.10. Alcohol Consumption

There are a limited number of studies examining alcohol as a susceptibility factor. 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). Pb and ethanol also decreased other endogenous renal antioxidants (glutathione and non-protein sulfhydryls) (Jurczuk et al., 2006).

6.2.11. Nutrition

Different components of diet may affect the association between Pb and health outcomes. Diets designed to limit or reduce caloric intake and induce weight loss have been associated with increased blood Pb levels in adult animals (Han et al., 1999). It is well established that diets sufficient in minerals such as calcium, iron, and zinc offer some protection from Pb exposure by preventing or competing with Pb for absorption in the GI tract. A recent 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 & Antonio-García, 2009). The previous Pb AQCD (U.S. EPA, 2006) reported limited data available to assess modification by nutritional status; however, potential modification by iron and calcium were noted. Recent epidemiologic and toxicological studies of specific mineral intakes/dietary components are detailed below.

6.2.11.1. Calcium

Using the Normative Aging Study, researchers examined the association between Pb and hypertension by calcium intake (Elmarsafawy et al., 2006). The associations between Pb (measured and modeled separately for blood, patella, and tibia) and hypertension did not differ based on dichotomized calcium intake (800 mg/day). However, toxicological studies have shown that dietary calcium deficiency induces increased Pb absorption and retention (Fullmer, 1992; Mykkanen & Wasserman, 1981; Six & Goyer, 1970). Also, low calcium levels in the body stimulate the production of vitamin D and increased synthesis of calcium-binding proteins to which Pb can bind (Richardt et al., 1986). Increased calcium intake reduces accumulation of Pb in bone and mobilization of Pb during pregnancy and lactation (Bogden et al., 1995).

6.2.11.2. Iron

The 2006 Pb AQCD included studies that indicated individuals with iron-deficiency and malnourishment had greater inverse associations between Pb and cognition/intellect (U.S. EPA, 2006). A recent epidemiologic study of pubertal development among girls observed inverse associations between blood Pb and inhibin B, but this association was modified by iron deficiency, with those girls with iron deficiency having a stronger inverse association between Pb and inhibin B than those who were iron sufficient (Gollenberg et al., 2010). Toxicological studies also report that iron deficient diets exacerbate or potentiate the effect of Pb. A study of pregnant rats given an iron deficient diet and exposed to Pb through drinking water over GD 6-14 had decreased litter size, 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 (Saxena et al., 1991; Singh et al., 1993). Thus, iron deficiency makes female rats of reproductive age more susceptible to Pb-dependent embryo and feto-toxicity (Singh et al., 1993).

6.2.11.3. Zinc

No epidemiologic studies have been performed to examine the effect of zinc on Pb-related health outcomes. Toxicological studies by Jamieson et al (2008; 2006) reported that a zinc deficient diet increases bone and renal Pb content (deposition in kidney tissue) and impairs skeletal growth and mineralization. A zinc-supplemented diet attenuated bone and renal Pb content.

6.2.11.4. Folate

A study by Kordas et al. (2009) examined Pb and birthsize among term births in Mexico City. The authors reported no interaction between Pb and folate levels.

6.2.11.5. Protein

No recent epidemiologic studies have evaluated protein intake as a susceptibility factor for Pb-related health effects. However, a toxicological study demonstrated that differences in maternal protein levels could affect the extent of Pb-induced immunotoxicity among offspring (S. Chen et al., 2004).

6.2.12. Stress

A study of bone Pb levels and hypertension reported modification of the association by perceived stress levels (Peters et al., 2007). Among individuals with greater stress levels, stronger associations of Pb levels on hypertension was present. Among the same study population, higher stress was also noted to affect the association between 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 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 (Cory-Slechta et al., 2008; Rossi-George et al., 2009; Virgolini, Rossi-George, Lisek, et al., 2008; Virgolini, Rossi-George, Weston, et al., 2008), including dysfunctional corticosterone responses (Rossi-George et al., 2009; Virgolini, Rossi-George, Weston, et al., 2008). Additionally, toxicological studies have demonstrated that immune stress also affects associations with Pb. Chicken with low Pb exposure in ovo and viral stressors had increased immune cell mobilization and trafficking dysfunction (Lee et al., 2002). Similarly, mice with neonatal Pb exposure and an immune challenge had a sickness behavior phenotype, likely driven by IL-6 production (Dyatlov & Lawrence, 2002).

Similar to studies of stress in animals, maternal self-esteem has also been shown to modify associations between Pb 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 observed of Pb on MDI and PDI scores; greater decreases in MDI and PDI associated with Pb levels were observed among mothers in the lower quartiles of self-esteem. The investigators indicated that high maternal self-esteem may serve as a buffer against stress by improving mother-child interactions and care giving practices but also may be a surrogate of biological stress responses in the child.

Although examined in a limited number of studies, recent epidemiologic studies observed modification of the association between Pb and health effects by stress-level. Susceptibility to Pb-related health effects by stress is supported by toxicological studies.

6.2.13. Cognitive Reserve

A study of Pb smelter workers reported that an inverse association between Pb levels and cognitive function was present among workers with low cognitive reserve but no association was present in workers with high cognitive reserve (Bleecker et al., 2007). Associations between Pb and motor functions existed among all workers regardless of cognitive reserve. No other recent epidemiologic studies were performed examining cognitive reserve as a susceptibility factor.

6.2.14. Other Metal Exposure

The 2006 Pb AQCD reported that the majority of studies examined other toxicants as confounders and not effect measure modifiers (U.S. EPA, 2006). Recent epidemiologic studies have, however, begun

to explore the possible interaction between Pb and other metals. These studies, as well as toxicological studies of these metals, are described below.

6.2.14.1. 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 have reported that the addition of Cd to Pb treatment of rats 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 (G. S. Wang & Fowler, 2008). In other studies, Cd synergistically exacerbated Pb-dependent renal mitochondrial dysfunction (L. Wang et al., 2009).

Overall, epidemiologic and toxicological studies have reported increased susceptibility to Pb-related health effects among those with high Cd levels as well.

6.2.14.2. Arsenic

In a study of immune function among children living at varying distances from a Pb smelter in Mexico, exposure to both metals were associated with greater decreases in NO and greater increases in superoxide anion (Pineda-Zavaleta et al., 2004). Recent toxicological studies that have examined the addition of arsenic (As) to Pb and Cd mixtures report increases in bioavailability of Pb (G. S. Wang & Fowler, 2008). Thus, there is biologic plausibility of increased susceptibility of Pb-related health effects when co-exposed to Pb and As.

6.2.14.3. Manganese

Among children in Korea taking part in a study of IQ, an interaction was reported between Pb and manganese (Mn) (Y. Kim et al., 2009). Compared to children with low blood Mn levels, those with high blood Mn levels had greater decreases in full scale IQ and verbal IQ associated with blood Pb levels. No effect modification was observed for the association between Pb levels and performance IQ.

6.2.15. Fluoride

Fl has been identified as a potential susceptibility factor in a toxicological study but has not yet been explored in epidemiologic studies. A recent toxicological study by Sawan et al. (2010) reported co-

exposure with Fl increased Pb deposition in calcified tissues. Future investigation among humans will be important for understanding whether fluoride present in water and other substances increases Pb deposition in humans and modifies the association between Pb and various health effects.

6.3. Summary

Section 6.1 of this chapter provides a review of the literature regarding factors influencing Pb exposure or biomarkers of Pb exposure. For most studies, relationships between factors and Pb biomarker levels were presented without attribution to exposure, diet, absorption, or biokinetic factors because the studies were not designed to make such conclusions. Where available, studies that shed light on the effect of susceptibility factors on exposure were included. The factors examined in Section 6.1 included age, gender, race and ethnicity, SES, and residential proximity to Pb sources.

In Section 6.2 of this chapter, epidemiologic and toxicological studies that contributed information on potential susceptibility factors for Pb-related health effects were evaluated. Overall, this review provided evidence that various factors may lead to increased susceptibility to Pb-related health effects (see Table 6-3 for evidence from current studies). Section 6.2 included most of the factors from Section 6.1, plus various genes, pre-existing diseases/conditions, smoking, BMI, nutrition, stress, cognitive reserve, and exposure to other metals.

Among children, the youngest age groups were observed to be most susceptible to having elevated Pb body burden, with blood Pb levels decreasing with increasing age of the children. Recent epidemiologic studies of infants/children detected susceptibility to Pb-related health effects, and this was supported by toxicological studies.

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 (see Section 4.2). Studies of older adults had inconsistent findings for effect 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 susceptibility to health effects.

Some studies suggest that males have higher blood Pb levels than females; this was supported by stratifying the total sample of NHANES subjects. Gender-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 mixed, although it appears that there are some differences in associations for males and females. This is also observed in toxicological studies. In addition, the associations among females may vary based on hormonal status. Future research will be useful in determining which Pb-related health effects are greater for males or females and whether hormones play a role in susceptibility.

Regarding race and ethnicity, recent data suggest that the difference in blood Pb levels between African American and White subjects is decreasing over time, but African Americans still tend to have higher Pb body burden and exposures. Similarly, the gap between socioeconomic groups with respect to Pb body burden appears to be diminishing, with Pb body burden being higher but not appreciably higher among lower income subjects. Studies of race as a susceptibility factor indicate that some modification of associations between Pb and health effects may be present. Compared to whites, non-white populations were observed to be more susceptible; 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. Although limited by the number of studies, lower SES individuals appear to represent a susceptible population. A study of Pb and IQ reported greater inverse associations among those in the lowest SES groups. Additionally, there is evidence associating proximity to areas with Pb sources, including urban areas with large industrial sources, with increased Pb body burden and risk of Pb exposure.

Various genes were examined as potentially modifying the associations between Pb and health effects. Epidemiologic and toxicological studies reported ALAD and VDR variants may be health-related susceptibility factors. Other genes examined that may also affect susceptibility to Pb-related health effects were MTHFR, DRD4, GSTM1, TNF-alpha, eNOS, APOE, and HFE.

Pre-existing diseases/conditions also have the potential to affect the association between Pb exposure and various health endpoints. Recent epidemiologic studies did not support modification of Pb and health endpoints by diabetes; however, past studies have found diabetics to be a susceptible population with regard to renal function. More research on this population will be important for determining which, if any, health effects related to Pb are different among diabetics. Hypertension was observed to be a susceptibility factor in both past and recent epidemiologic studies. Studies of Pb and both renal effects and heart rate variability demonstrated greater odds of the association among hypertensive individuals compared to those that are normotensive. Recent epidemiologic studies also examined autism and atopy as potential susceptibility factors. Future research will allow for a greater understanding of potential modification by these conditions, but current research has shown that autistic children and infants of mothers without atopy may have increased odds of Pb-induced health effects.

Recent epidemiologic studies examining smoking as a susceptibility factor reported mixed findings. It is possible that smoking modifies the effects of only some Pb-related health effects. Further studies of current, former, and prenatal smoking exposures related to Pb and health effects will provide additional information on susceptibility.

BMI, alcohol consumption, and nutritional factors were examined in recent epidemiologic and toxicological studies. Modification of associations between Pb and various health effects (mortality and heart rate variability) was not observed by 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 possibility of alcohol as a susceptibility factor. Among nutritional factors, those with

iron deficiencies were observed to be a susceptible population for Pb-related health effects in both epidemiologic and toxicological studies. Other nutritional factors, such as calcium, zinc, and protein, demonstrated the potential to modify associations between Pb and health effects in toxicological studies. Recent epidemiologic studies of these factors were either not performed or observed no modification. Folate was also examined in a recent epidemiologic study of birth size but no interaction was reported between Pb and folate. Further study of these and other nutritional factors will be useful in determining susceptibility among individuals with various nutritional levels/deficiencies.

Stress was also evaluated as a susceptibility factor and although there were a small number of recent epidemiologic studies, increased stress was observed to negatively impact the association between Pb and health endpoints. Toxicological studies supported this finding.

A recent 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. Future studies evaluating Pb-related health effects and cognitive reserve will provide more information on this possible susceptibility factor.

Finally, interactions between Pb and other metals were evaluated in recent epidemiologic and toxicological studies of health effects. High levels of other metals, such as Cd, As, and Mn, were observed to negatively affect the associations between Pb and various health endpoints.

Chapter 6. References

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Chapter 7. 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 four sections. The introduction (Section 7.1) presents the organizing principles of this chapter and several basic concepts of metal ecotoxicology and ecosystem services. Section 7.2 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, and critical loads for soils. A similar discussion of the effects of Pb on aquatic ecosystems is presented in Section 7.3, including water-only exposures and sediment related effects. Both the terrestrial and aquatic system sections conclude with a discussion of alterations in ecosystem service functions as a consequence of Pb deposition. Finally, an integrative synthesis of effects of Pb across biota and causal determinations for Pb in both terrestrial and aquatic systems are presented in Section 7.4. Areas not addressed here include literature related to Pb shot or pellets and studies that examine human health-related endpoints which are described in other chapters of this document.

7.1. 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 (Fairbrother et al., 2007). This section introduces critical concepts for understanding how Pb from atmospheric deposition may affect

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

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.

7.1.1. Ecosystem Scale, Function, and Structure

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. 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, 2008). 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.

Ecosystems are most often defined by their structure, and are based on the number and type of species present. 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 form populations, and populations of different species form communities. 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, effects on individuals must result in changes to their survival or reproductive output to have any effect on the population. 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, 2007). 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 & Latham, 1999); and (3) alterations in trophic structure.

Alternatively, ecosystems may be defined on a functional basis, 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 7.1.2) and critical loads (Section 7.1.3) serve as benchmarks or measures of the impacts of pollutants on ecosystems.

7.1.2. Ecosystem Services

Ecosystem structure and function may be translated into ecosystem services (Daily, 1997). Ecosystem services are the benefits people obtain from ecosystems (Millennium Ecosystem Assessment, 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 (Daily, 1997; Westman, 1977; WRI, 2000). 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 & Kennedy, 1997).

Particular concern has developed within the past decade regarding the consequences of decreasing biological diversity (Ayensu et al., 1999; Chapin et al., 1998; Hooper & Vitousek, 1997; Tilman, 2000; Wall, 1999). Human activities that decrease biodiversity also alter the complexity and stability of ecosystems and change ecological processes. In response, ecosystem structure, composition and function can be affected (Chapin et al., 1998; Daily & Ehrlich, 1999; Levlin, 1998; Peterson et al., 1998; Pimm, 1984; Tilman, 1996; Tilman & Downing, 1994; Wall, 1999). Biodiversity is an important consideration at all levels of biological organization, including species, individuals, 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 (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), 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.

7.1.3. Critical Loads as an Organizing Principle for Ecological Effects of Atmospheric Deposition

Critical loads were first defined for regulating emissions of sulfur and nitrogen oxides, but have since been applied to exposure of other pollutants, including metals (Adams & Chapman, 2007). 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 & Grennfelt, 1988). Because critical loads for Pb differ by ecosystem (aquatic-water; aquatic-sediment; terrestrial), differ by environmental chemistry properties that impact bioavailability, differ by species, and differ by endpoint of concern, they can be used as an organizing principle for linking atmospheric deposition with ecological impairment at multiple spatial scales. There are two aspects to consider: (1) the critical load at a steady state in the environment (i.e., how much input is required to balance the rate of output), and (2) the time required to reach the critical load (i.e., the lag time between onset of exposure and induction of measurable effects). This is particularly true for terrestrial ecosystems where changes in soil geochemistry, as a result of either changing land use or ecological succession, may significantly alter the amount of sequestration of Pb, thus changing its bioavailability and critical load if based on total metal. Ideally, therefore, critical loads for metals should be defined on the basis of bioavailable metal rather than total metal. This approach is being used in aquatic systems through the application of the biotic ligand model (BLM) (Di Toro et al., 2001; Di Toro et al., 2005), but is proving to be more difficult for modeling terrestrial systems.

For aquatic systems, a dynamic equilibrium exists between the surface water, the water column, and the sediment compartments (which must be defined when determining the critical load for each compartment). Although the sediment generally acts as a sink for pollutants in the water column, especially metals in particle form or as insoluble metal complexes, there may be some re-entrainment from the sediments into the water column. This sediment-water interface may change the solubility or bioavailability of the metal, thereby altering the critical load for the water column, particularly if expressed in the form of total metal. Note, however, that while sedimentation processes may change the time to steady state, they will not affect the ultimate critical load once steady state is achieved.

The following pieces 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.

As stated in the 2008 ISA for Oxides of Nitrogen and Sulfur-Ecological Criteria 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, 2008). 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.

In the short term, metal emissions generally have greater adverse effects on biota in aquatic systems than in terrestrial systems because metals are more readily immobilized in soils than in sediment. However, over the longer term, terrestrial systems may be more affected particularly by those metals with a long soil residence time, such as Pb. Thus, for a particular locale, either the terrestrial or the aquatic ecosystem at that site may have the lower critical load. Given the heterogeneity of ecosystems affected by 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.

7.1.4. Ecosystem Exposure, Lag Time and Re-entrainment of Historically Deposited Lead

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 the modifying factors listed in the previous section. However, there is frequently a lag time between when metals are emitted and when an effect is seen, particularly in terrestrial ecosystems and, to a lesser extent, in aquatic sediments; water exposures result in more immediate system responses. This is because the buffering capacity of soils and sediments permits Pb to become sequestered into organic matter, making it less available 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 from the time when the 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 is dependent 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:

[1] Time required to reduce the initial concentration by 50% if metal input is zero.

$$t_{1/2} = \frac{0.69 \times d \times 10000}{y \times TF + \frac{R}{\rho Kd}}$$

Equation 7-1

where:

d is the soil depth in meters (m)

y is the annual crop yield (tons/ha·yr)

TF is the ratio of the metal concentration in plant to that in soil

R is the net drainage loss out of the soil depth of concern (m^3/ha·yr)

ρ is the bulk density of soil ($kg_{(dry\ weight)}$/L)

Kd is the ratio of the metal concentration in soil to that in soil pore solution (L/kg)

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 7-1 is simplified to:

$$t_{1/2} = \frac{0.69 \times d \times 10000}{\frac{R}{\rho Kd}}$$

Equation 7-2

and becomes a function of soil depth, the amount of rainfall, soil density, and soil properties that affect *Kd*. Pb has a relatively long time to steady state compared to other metals, as shown in Table 7-1.

Table 7-1. 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)	Kd (L/Kg)	Time (yr)
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 *Kd* (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 23 cm, a rain infiltration rate of 3,000 m^3/ha·yr, and the assumption that background was zero at the start of loading.

Source: Smolders, Fairborother 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 half-life. Sediment $t_{1/2}$ is dependent upon the particulate and dissolved fractions and is calculated as for soils (Equation 7-2).

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 m^{-1}, or a resuspension rate (Λ), with units of sec^{-1} (Equation 7-3). 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 7-3

where:

R is the upward resuspension flux ($\mu g/m^2/sec$)

C is the soil (or dry sediment) Pb concentration ($\mu g/m^2$)

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 upper 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.

7.2. Terrestrial Ecosystem Effects

7.2.1. Introduction to Terrestrial Ecosystem Effects

Numerous studies of the effects of Pb on components of terrestrial systems were reviewed in the 2006 Pb AQCD. The literature on terrestrial ecosystem effects of Pb, published since the 2006 Pb AQCD, is considered with brief summaries from the AQCD where relevant. Section 7.2 is organized to consider uptake of Pb and effects at the species level, followed by community and ecosystem level effects. Soil biogeochemistry of Pb in terrestrial systems is reviewed in Section 7.2.2. Section 7.2.3. considers the bioavailability and uptake of Pb by plants, invertebrates, and wildlife in terrestrial systems. Biological effects of Pb on terrestrial ecosystem components including plants and lichen, invertebrates, and vertebrates (Section 7.2.4) are followed by data on exposure and response of terrestrial species (Section 7.2.5). Effects of Pb at the ecosystem scale are discussed in Section 7.2.6. Section 7.2 concludes with a

discussion of critical loads in terrestrial systems (Section 7.2.7), characterization of sensitivity and vulnerability of ecosystem components (Section 7.2.8), and effects on ecosystem services (Section 7.2.9).

7.2.2. Soil Biogeochemistry and Chemical Effects

According to data presented in the 2006 Pb AQCD, 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 small relative to 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.

7.2.2.1. pH and CEC

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 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 about one order of magnitude.

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 both (1) Fe and Mn oxide interactions; and (2) the formation of weak electrostatic bonds with charged soil surfaces. Similarly, the mobility of smelter-produced metals in forest soils was found to be greater than in adjacent agricultural lands (Douay et al., 2009). The higher solubility was caused by the decreased soil pH of the forest environments. Further, decreasing the soil pH via simulated acid rain events increased naturally occurring Pb bioavailability in field tests (X. Hu et al., 2009).

A sequential extraction procedure was employed by Ettler et al. (2005) to determine the relative bioavailability 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.

7.2.2.2. Organic Matter

Organic matter (OM) 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, OM, total calcium carbonate equivalent, and total free sesquioxides also exhibited higher total Pb concentration, indicating that less Pb had been removed 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 OM 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. (2006) 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-specific differences 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 atmosphere-deposited Pb in soil matrices was strongly influenced by soil type, indicating that certain soil types may retain Pb for longer periods of time. In humic forest soils, the highest Pb concentrations were measured in the humified bottom layer, whereas 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 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. Dayton et al. (2006) established significant negative correlations between log-transformed Pb content of lettuce plants (*Lactuca sativa*), soil organic content, and CEC, and similar negative relationships were also confirmed for soil pH and amorphous Fe and Al oxide content. 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).

7.2.2.3. Aging

Smolders et al. (2007) reviewed the effects of aging of Pb in soils on the toxicity of Pb to plants and soil invertebrates, with aging defined primarily as leaching following initial influx, but also as binding and complexation. In nearly half of the Pb soil studies reviewed, observed dose-response curves could not be established 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 soil 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 7.3.3), 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 individual simulated soil components freshly spiked with 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/L, whereas reproduction was always significantly affected at Pb concentrations of 0.7 mg/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 (*Plethodon cinereus*) exposed to Pb-amended soils (553, 1,700, 4,700, and 9,167 mg Pb/kg) exhibited lowered appetite and decreased white blood cell counts at the two highest concentrations, as compared to controls {B, 2010, 379076}. 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 for 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.

7.2.3. 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)." 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" (Fairbrother et al., 2007). Characteristics of the toxicant that affect bioavailability are: (1) chemical form or species; (2) particle size; (3) lability; and (4) source. New information on sources of Pb in terrestrial ecosystems, and their influence on subsequent bioavailability, was reviewed in Chapter 3, while new information on the influence of soil biogeochemistry on speciation and chemical lability was presented in Section 7.2.2. This section summarizes new literature on uptake and subsequent presence of Pb in tissues. Bioaccumulation factors (BAF's) (i.e., the ratio of Pb concentrations in tissues of terrestrial biota to concentrations in soil or in food items) reported in this section are summarized in Table 7-2. The 2006 Pb AQCD 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.

7.2.3.1. Plants

The 2006 Pb AQCD 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.

Leaf and Root Uptake

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 point source (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.

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.

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 determined to be from direct adsorption from the atmosphere. Further, Aznar et al. (2009) 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, which the authors attributed to "incidental processing" of atmospheric Pb. Foliar damage and growth reduction were also observed in the trees (Aznar, Richer-Lafleche, et al., 2009). They were significantly correlated with Pb concentration in the litter layer, where Pb comes from atmospheric deposition and closely reflects it. 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 adversely affecting trees (Aznar, Richer-Laflèchea, et al., 2009). 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). The advent of laser ablation inductively coupled plasma mass spectrometry has made measurement of Pb concentration in individual tree rings possible (Watmough, 1999; Witte et al., 2004). 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. (2008) 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 (L. 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 & 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 & 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-m 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 & Al-Khashman, 2007). However, decreases in tissue Pb concentration with increasing distance from point 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).

Translocation and Sequestration of Lead in Plants

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, 2006). 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, 2006).

The 2006 Pb AQCD 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. 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 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/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/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). 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).

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. (2006) 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 & Tsadilas, 2009).

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. (2009) 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 shoot concentration of *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 hieraciodides* (Pteridaceae) tissues collected from Jinping. These results indicate significant interspecies differences in Pb uptake, as well as potential soil-specific differences in Pb bioavailability. *S. vestita*, in particular, was determined 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 (Y. Li et al., 2009). 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.

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.

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 (G. Wang et al., 2006) and buckwheat has recently been shown to be an efficient translocator of Pb from soil to above-ground shoots (Tamura et al., 2005). However, there is broad variability in Pb uptake and translocation rates among plant species, and interspecies variability has been shown to interact with other factors such as soil type. Field studies carried out in the vicinity of Pb smelters (X. Hu et al., 2009) show that Pb may accumulate in shoot tissue through direct stomatal uptake rather than by soil-root-shoot translocation. 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).

7.2.3.2. Invertebrates

At the time of publication of the 2006 Pb AQCD, 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

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 & Richmond, 2010). While bioconcentration factors (BCF's) for Cd were observed to increase over the 64-day study period, the rate of Pb assimilation remained consistent over time and no evidence for a regulatory mechanism for Pb was observed. The authors observed that, for additional Pb to be retained, snails would have to grow additional soft tissue. *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, De Vaufleury, et al., 2006). There were no observed effects of Pb exposure or accumulation on survival or

growth in *C. asperses* or *H. aspersa*. In another study (Ebenso & 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. Although plant concentrations were low, they were correlated with elevated snail Pb tissue concentration. 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 µg Pb/g tissue). 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 aspera* snails to standardized (1999 European International Organization for Standardization methodology [ISO 11267:1999]) artificial-substrate soils 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 µg Pb/g dry weight versus concentration averaging 0.4 mg/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.

Earthworms

Soil characteristics that interact with bioavailability of Pb may include biogeochemistry associated with different soil horizons, source of Pb, and proportion of soil:leaf litter. These have been studied principally with various species of earthworms. 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 physical properties that were freshly spiked with Pb to give a standard concentration of 2,000 mg/kg dry weight. Both internal earthworm Pb concentration and mortality rates increased with decreasing pH and CEC although the apparent role of CEC may only have been due to its correlation with other soil characteristics. These data corroborate that Pb bioavailability and toxicity are increased in acidic soils and in soils with a low CEC (Section 7.2.2). 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 iron 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 reach depths of 6 inches). 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. Accumulation studies conducted with *Eisenia fetida* earthworms documented the difficulty of extrapolating accumulation kinetic constants from one soil type to another, and showed that many soil physiochemical properties, including pH, OM, and CEC, among others, work in conjunction to affect metal bioavailability (Nahmani et al., 2009). However, once taken up from the environment, more than half of the bioaccumulated Pb appears to be contained within earthworm tissue and cell membranes (Li et al., 2008).

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). From this, the authors concluded that only a minority of earthworm-absorbed Pb would be toxicologically available to cause adverse effects in the earthworms or in their predators. Earthworm activity can alter Pb bioavailability and subsequent uptake by earthworms themselves and other organisms. The presence of earthworms may increase soil pH though the secretion of cutaneous mucus, and worm activity is generally associated with increased bioavailability of Pb. Sizmur and Hodson (2009) speculated that earthworms affect Pb mobility by modifying the availability of cations or anions. 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.

Arthropods

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, 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.

7.2.3.3. Terrestrial Vertebrates

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 coramandelicus*) following oral dosing of 0.5 mg/kg, 1.25 mg/kg or 2.5 mg/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 & 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).

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 cerussite and manganese-Pb oxides and poor absorption of 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).

Interestingly, dietary Ca deficiency (0.45 mg Ca 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/L Pb (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-deficient diet. However, it is not known whether this level of dietary Ca deficiency is common in wild populations of birds.

7.2.3.4. Food Web

In addition to the individual 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 particulates 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-zinc mine was limited along a soil-plant-insect-chicken food chain (Zhuang et al., 2009). In soils averaging 991 mg Pb/kg, Rumex K-1 plants sequestered an average of 1.6 mg/kg wet weight Pb 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, an insectivorous 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*), elmleaf 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 concentration 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/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 periodically returns to background level with each shedding. Granular Pb is unavailable. Trophic attenuation is thus likely.

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.

Table 7-2. Soil-to-tissue bioaccumulation factors for various terrestrial plant, invertebrate, and vertebrate species

Species	Soil Concentration	Tissue Type	Tissue Concentration	Bioaccumulation Factor	Reference
Vegetable	379 mg/kg	Root	47.3 mg/kg	0.12	X. Hu & Ding (2009)
Vegetable	379 mg/kg	Shoot	36.2 mg/kg	0.1	X. Hu & Ding (2009)
P. angulata	4020 mg/kg	Root	527.1 mg/kg	0.13	Cui et al. (2007)
P. angulata	4020 mg/kg	Shoot	331.1 mg/kg	0.08	Cui et al. (2007)
A. theophrasti	4020 mg/kg	Root	38.7 mg/kg	0.01	Cui et al. (2007)
A. theophrasti	4020 mg/kg	Shoot	61.4 mg/kg	0.02	Cui et al. (2007)

Species	Soil Concentration	Tissue Type	Tissue Concentration	Bioaccumulation Factor	Reference
S. vestita	2409 mg/kg	Shoot	3142 mg/kg	1.3	Y. Li et al. (2009)
S. vestita	2409 mg/kg	Root	7457 mg/kg	3.1	Y. Li et al. (2009)
S. grevilloides	5330 mg/kg	Shoot	69 mg/kg	0.01	Y. Li et al. (2009)
D. carota	40.1 mg/kg	Root	12.2 mg/kg	0.3	Murray et al. (2009)
A. fistulosum	3560 mg/kg	Whole plant	23.7 mg/kg	0.007	Cho et al. (2009)
C. baccatum	0.6 mg/kg	Fruit	0.86 mg/kg	1.4	Antonious & Kochhar (2009)
A. achatina	1200 mg/kg	Whole body	468.5 mg/kg	0.39	Ebenson & Ologhobo (2009b)
A. achatina	200 mg/kg	Whole body	91.4 mg/kg	0.46	Ebenson & Ologhobo (2009b)
A. achatina	20 mg/kg	Whole body	12.2 mg/kg	0.61	Ebenson & Ologhobo (2009b)
H. aspersa	39 mg/kg	Viscera	1.1 mg/kg	0.03	De Vaufleury et al. (2006)
H. aspersa	52 mg/kg	Viscera	1.2 mg/kg	0.02	De Vaufleury et al. (2006)
L. terrestris	710 mg/kg	Whole body	20.6 mg/kg	0.03	Darling & Thomas (2005)
O. asellus	245 mg/kg	Whole body	43 mg/kg	0.18	Gál et al. (2008)
T. talpoides	777 mg/kg	Liver	1.63 mg/kg	0.002	Reynolds et al. (2006)
T. talpoides	777 mg/kg	Whole body	3.85 mg/kg	0.005	Reynolds et al. (2006)
L. limosa	336 mg/kg	Egg	0.17 mg/kg	0.0005	Roodbergen et al. (2008)
L. limosa	336 mg/kg	Feather	2.79 mg/kg	0.008	Roodbergen et al. (2008)

7.2.4. Biological Effects

Various adverse 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. In this section, the observed biological effects caused by exposure to atmosphere-derived Pb will be discussed, while the results of dose-response experimentation will be addressed in Section 7.2.5. 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.

7.2.4.1. 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 & Lorenc-Plucinska, 2008).

Effects on Photosystem and Chlorophyll

The effect of Pb exposure on the structure and function of plant photosystem II was studied in giant duckweed, *Spirodela polyrrhiza* (Ling & Hong, 2009). Although this is an aquatic plant, photosystem II is present in all plants, and effects on photosystem II observed in any plant species are likely to occur in all of them. 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. (2008) 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 $PbCl_2$ for 48 hours prior to germination, and following 42 days of growth, plants were sprayed with $PbCl_2$ 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. Liu et al. (2010) 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 exposures of 0.1 and 0.5 mM Pb in hydroponic solution for 50 days, concentration of chlorophyll *a* and *b* was decreased in radish (*R. sativus*) (Kumar & 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 & Pignata, 2007). *Usnae amblyoclada* lichen was exposed to aqueous Pb solutions of 0.5, 1, 5, and 10 mM Pb nitrate; 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. As compared to other metals, Pb caused less physiological damage, which the authors attributed to the metal's high affinity for binding to and sequestration within cell walls.

The effect of Pb exposure on chlorophyll content of the moss and liverwort species *Thuidium delicatulum, T. sparsifolium*, and *Ptychanthus striatus* was investigated following simulated atmospheric exposures of 10^{-10} to 10^{-2} M Pb (Shakya et al., 2008). Both chlorophyll *a* and total chlorophyll content of the mosses (*T. delicatulum* and *T. sparsifolium*) decreased with increasing Pb exposure, but the effect was not statistically significant. For the liverwort, Pb exposure resulted in significant 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 10 mM Pb, while lower exposures slightly elevated chlorophyll content.

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 have been demonstrated in broad bean (*Vicia faba*) (C.-R. Wang et al., 2010; C.-R. Wang, Wang, Tian, Yu, et al., 2008; C. Wang, Y. Tian, et al., 2010) and tomato (*Lycopersicon esculentum*) (C.-R. Wang, Wang, Tian, Xue, et al., 2008), where they were accompanied by proportional increases in superoxide dismutase (SOD), glutathione, guaiacol peroxidase, as well as lipid peroxidation, and decreases in catalase. Reddy et al. (2005) found that horsegram (*Macrotyloma uniflorum*) and bengalgram (*Cicer arietinum*) plants exposed to Pb solutions of 200, 500, and 800 mg Pb/kg exhibited increased antioxidant activity: at exposures of 800 mg Pb/kg, 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 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. 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, 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., 2010) 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.

Growth

There is evidence of effects of Pb on higher growth processes as well. Both growth and carotenoid and chlorophyll content of *Brassica juncea* (mustard) plants were negatively affected by Pb exposure (John et al., 2009). Pb treatments of 1,500 µM (as Pb acetate solution) decreased root lengths and stem heights by 50% after 60 days. Exposure to 600 µM Pb and greater decreased carotenoid content, while chlorophyll *a* was decreased at Pb exposures of 450 µM and higher. However, no effects were seen in growth or chlorophyll production of maize (*Zea mays*) following growth in smelter ash–spiked soils containing 1,466 mg Pb/kg (and 18.6 mg Cd/kg) (Komarek et al., 2009). Pb concentrations of 7,331 mg/kg (98.0 mg/kg Cd) were required to elicit chlorosis and the expected decreased in growth.

Chinese cabbage (*Brassica pekinensis*) exposed to Pb-containing soils (0, 4, and 8 mM/kg dry weight) 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 concentration of 0.2, 0.4, and 0.8 mg/kg dry weight total Pb and collected leaf samples for 11 days. At exposures of 0.4 and 0.8 mg/kg, leaf nitrite content was decreased by 29% and 20%, while nitrate content was affected only at the highest Pb exposure (70% of control levels). 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.

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 & 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 nM of Pb nitrate for 6 days and showed decreased genetic template stability (as quantified by random amplified polymorphic DNA profiles) and decreased photosynthetic pigments.

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 (Kumar & Tripathi, 2008) Plants 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 and iron and decreased sulfur concentration in radish tops.

Interestingly, as in zebra finch (Section 7.2.3.3), Ca was found to counteract the toxic 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/L in the presence of Ca concentration of 1.2 mM and higher (Antosiewicz, 2005).

7.2.4.2. 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. 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 & Wang, 2009). The authors determined that exposures as low as 2.5 µM Pb nitrate 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. Younger *C. elegans* larvae were more likely to exhibit neurobehavioral toxicity symptoms in response to Pb exposure (2.5 µM) (Xing, Guo, et al., 2009). 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, Rui, et al., 2009). *C. elegans* nematodes exposed to Pb concentration as low as 2.5 µM for 24 hours also exhibited significantly altered behavior characterized by decreased head thrashes and body bends. Exposures of 50 µM Pb and greater decreased the number of nematode forward turns (D. Y. Wang & Xing, 2008). Chemotaxis towards NaCl, cAMP, and biotin was also decreased in *C. elegans* nematodes exposed to Pb concentration greater than 2.5 µM (Xing, Du, et al., 2009). 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 (D. Y. Wang & Xing, 2008).

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 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 to 2.5, 75, and 200 µM Pb nitrate exhibited significant indications of multi-generational toxicity (D. Y. Wang & Peng, 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.

E. andrei earthworms exposed to 21 different soils, each containing 2,000 mg/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/kg) also decreased the protein content of *E. fetida* earthworms during a 7-day exposure (M. Li et al., 2009). 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), indicating that Pb exerted a detrimental effect 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.

As in plants, induction of antioxidant activity is affected by exposure to Pb in invertebrates. The induction of antioxidant activity was correlated to standard toxicity measurements in *Theba pisana* snails (Radwan et al., 2010). Topical application of Pb solutions (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. 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. Further, decreased food consumption, growth, and shell thickness were observed in juvenile *A. achatina* snails exposed to Pb-contaminated (concentration greater than 134 mg/kg) diet for 12 weeks (Ebenso & 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/kg, a concentration-dependent relationship was established for growth, with significant reduction observed at 12-week exposures to 20 mg Pb/kg (Ebenso & Ologhobo, 2009b). However, consumption of field-collected Pb-polluted *U. dioica* leaves containing 3 mg/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.

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 had no significant adverse effect on *Sinella curviseta* springtail survival or reproduction (Xu et al., 2009).

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 & 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."

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 were demonstrated to decrease with increasing Pb exposure. Conversely, in a study by Zheng and Li (2009), vitellogenin mRNA was detected at higher levels in males exposed to 12 and 25 mg Pb/kg, although vitellogenin levels were not affected. Similarly, the sperm morphology of the Asian earthworm (*Pheretima guillelmi*) was found to be altered significantly following 2-week exposure to soils containing 1,000, 1,400, 1,800, and 2,500 mg Pb/kg (Zheng & 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).

7.2.4.3. Terrestrial Vertebrates

According to the 2006 Pb AQCD, 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.

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 intraperitoneal 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 concentration 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 adverse biological effects can be difficult. Pb tissue concentration in field-collected urban 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 breast feathers, washed tail feathers, and blood, respectively (Scheifler, Coeurdassier, et al., 2006). Although these levels were significantly higher than those measured in rural blackbirds, elevated Pb tissue concentration was not significantly correlated to any index of body condition. On the other hand, Hargreaves et al. (2010) showed that Pb tissue concentration of female arctic shorebirds was negatively correlated with reproductive success. Maternal blood Pb levels were negatively associated with hatching success in black bellied plovers (*Pluvialis squatarola*) and ruddy turnstones (*Arenaria interpres*), and with nest duration in all species tested. There was no significant correlation between adult whole-blood or feather Pb concentration and Pb levels in produced eggs.

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 and 1.7 higher after mining operations in the mining region than in reference areas, respectively. 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.

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 91 µg/L (± 51), which was higher than blood Pb levels from the mining area (44 ± 34 µg/L). 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 intraperitoneal 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.

Again, dietary or other health deficiencies unrelated to Pb exposure are likely to exacerbate the effects of Pb. Ca-deficient female zebra finches (*T. guttata*) had a suppressed secondary humoral immune response following 28-day exposures to 20 mg/L Pb in drinking water (Snoeijs et al., 2005). This response, however, was not observed in birds fed sufficient Ca. 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 adversely affect several mammalian species. Young adult rats reared on a diet containing 1,500 mg/kg Pb acetate for 50 days demonstrated less plasticity in learning than non-exposed rats (McGlothan et al., 2008), indicating that Pb exposure caused significantly altered 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, lutenizing 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-zinc 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.

Pb-treated oocytes of buffalo (*Bubalus bubalis*) assessed in vitro at concentrations ranging from 0.5 to 1,000 µg/dL in one-day cultures indicated a significant decline in viability of oocytes at 100 µg/dL (Nandi et al., 2010). 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 250 µg/dL and 100% oocyte death at 3,200 µg/dL. Similarly, 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., 2009).

7.2.5. Exposure and Response of Terrestrial Species

Given that exposure to Pb may adversely affect organisms at the individual, population, or community level, determining the rate and concentration at which these effects occur is essential in predicting the overall risk to terrestrial organisms. However, data from controlled studies using a single compound are scarce relative to field studies, which in turn often investigate effects of multiple metal contaminants and afford too little control on interacting variables to be of use in establishing a general dose-response function. This section updates available information derived since the 2006 Pb AQCD, summarizing several dose-response studies with soil invertebrates. No new exposure-response information was available for plants, birds, or mammals.

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., 2009). 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/kg and 1,000 mg/kg Pb, respectively. 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 soil, 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 (2009) 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.

The individual and population-level responses of the springtail *Paronychiurus kimi* to Pb were determined by Son et al. (2007) using artificial soils, following the 1999 European 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/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 concentration 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.

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 7.2.2, 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 of 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 biotic ligand model 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).

7.2.6. Community and Ecosystem Effects

According to the 2006 Pb AQCD, natural terrestrial ecosystems near significant Pb point 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, 2006). 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 & 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. R.Y. Yang et al. (2008) 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 & 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. 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 (C. B. 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 (R. Y. 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 a better estimate of ecological impacts than microbial diversity or abundance measurements. When the microbial properties of metal-contaminated urban soils were compared to those of rural soils, significant differences were detected in basal community respiration rates and microbial abundance (Y. Yang et al., 2006). 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. This suggests that anthropogenic Pb contamination may adversely affect soil microbial communities, and alter their ecological function. Most studies of metal-induced changes in microbial communities have been conducted using mixtures of metals. 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. Total phospholipid fatty acid content was determined to negatively correlate with increasing Pb exposure, indicating alteration of the microbial community. (S. Khan et al., 2010), found that following a 2-week exposure to three levels of Pb (150, 300, and 500 mg Pb/kg), both acid phosphatase and urease levels (measures of soil microbial activity) decreased significantly, although they had recovered by the ninth week. In addition, the number of culturable bacteria was also decreased, but only at the highest exposure concentration tested.

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. Basal respiration was adversely affected in two soil types tested at the highest Pb treatment (32 mM), and in a third at the two highest Pb treatments (8 and 32 mM). 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 exposure negatively affected the prey capture ability of certain fungal species. The densities of traps constructed by nematophagous fungi decreased in soils treated with 0.15 mM Pb chloride (Mo et al., 2008). Nematophagous fungi are important predators of soil-dwelling nematodes, collecting their prey with sticky nets, branches, and rings. This suppression caused a subsequent reduction in fungal nematode capturing capacity, and could result in increased nematode abundance.

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 from the contributions of copper and zinc that were added with the Pb.

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) (Q. Hu et al., 2007). 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. 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. Thus, it was concluded that even long-term exposure to minimally bioavailable Pb can alter the structure of soil decomposer communities, which could in turn decrease decomposition rates.

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 625 or 2,500 µg 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 adversely 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 protect the host plant from Pb uptake and fungal viability seems to be protected by the 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. However, it is well known from historical data that Pb can have a widespread and dramatic effect on populations of waterfowl exposed to spent shot (Beyer et al., 1996) and may be negatively affecting loons in the northeastern U. S. through ingestion of Pb fishing sinkers (Scheuhammer & Norris, 1996). Studies at shooting ranges and downwind of smelters previously reported in the 2006 Pb AQCD demonstrate effects of dispersed Pb on terrestrial soil and plant communities with resulting decreases in secondary consumer vertebrate species.

7.2.7. Critical Loads in Terrestrial Systems

The critical load is the environmental concentration predicted or estimated from available literature data, above which adverse effects to organisms are likely to occur. It is based on the relative toxicity of the compound to species or ecosystem processes of concern, and an estimate of residence time in the soil environment. The concept of critical load is discussed in more detail in Section 7.1.3 of this chapter and in Section 7.3 of the 2006 Pb AQCD (U.S. EPA, 2006).

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, the Pennines, and southern Wales), 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 et al. (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 & Groenenberg, 2009). Similarly, although Pb soil concentration 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).

In some cases, risk assessment for Pb predicts risks to terrestrial animals at environmental concentrations that fall below natural background levels, and uncertainties associated with standard toxicity testing can become magnified through the risk assessment process, degrading the reliability of

estimates of hazardous environmental concentrations. Smolders et al. (2009) concluded from their comparison of field soils, spiked soils, and artificially aged spiked soils that corrections for aging and interacting soil properties in spiked soils will make predicted-no-effect concentrations rise above background. Buekers et al. (2009) proposed the use of a Tissue Residue Approach as a risk estimation method for terrestrial vertebrates that does not predict risks at background levels, and has smaller uncertainty. This approach eliminates the need for quantitative estimation of food intake or Pb species bioavailability. Blood Pb no-adverse-effect concentration (NAEC) and lowest adverse effects concentration (LAEC) derived from 25 literature 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. For mammals, the hazardous concentration for 5% of species (HC5) values obtained from the species sensitivity distributions ranged from 11 to 18 µg Pb/dL blood; HC5 values for birds ranged from 65 to 71 µg/dL. The authors proposed the use of 18 and 71 µg/dL as critical threshold values for mammals and birds, respectively, which are below the lowest NAEC for both data sets used, and are above typical background Pb values.

Short of conducting expensive in vivo toxicity studies, it is difficult to determine environmental Pb toxicity given the variation of physiochemical and soil properties that alter bioavailability and toxicity. 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.

7.2.8. 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, 2006). 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, 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.

7.2.9. 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 7.2.2), 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.

7.2.9.1. 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. Similarly, 28-day EC_{50} values derived for *F. candida* collembola (springtails) were between 2,060 and 3,210 mg/kg in Pb-spiked soils (Lock et al., 2006), while the springtail species *S. curviseta* exhibited no response to a 28-day exposure to 3,200 mg/kg Pb-spiked soil (Xu et al., 2009). 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, Guo, et al., 2009), and also demonstrated more pronounced neural degeneration than older larvae and adults (Xing, Rui, et al., 2009).

7.2.9.2. 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 describes how Ca, 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 adverse behavioral effects in conjunction with a nutrient-deficient diet. As previously reported in the 2006 Pb AQCD, Ca 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 determined that, for plants, Ca 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 channel pores, in the presence of low Ca 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 (Simkiss et al. (1982), as cited in Jordaens et al. (2006)). Hence, in environments with disproportionately high Pb versus Ca concentration, accumulation of Pb may be accelerated, as in plants. Ca deficiency in birds was demonstrated to stimulate the production of Ca-binding proteins in the intestinal tract, which extract more Ca from available diet; however, this response also enhances the uptake and accumulation of Pb from diet and drinking water (Fullmer et al. (1997), as cited in Dauwe et al. (2006)).

7.2.9.3. 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, the proportionality of toxicity to Pb content disappeared following experimental aging of freshly spiked soils through leaching. The leaching-aging factor for Pb was determined to be 4.2, and represented the ratio of ED_{10} values derived in aged soils to freshly spiked soils (factors greater than one indicate decreased toxicity in aged field soils relative to laboratory spiked soils). Consequently, the sensitivity of terrestrial vertebrates to environmental Pb exposures will be heavily dependent on the relative rate of aging and site-specific bioavailability.

7.2.9.4. 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, beach, 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, that 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.

7.2.10. Ecosystem Services

There are no publications at this time that specifically focus on the ecosystem services affected by Pb in terrestrial systems. The evidence reviewed in this ISA illustrates that Pb can cause ecological effects in each of the four main categories of ecosystem services (Section 7.1.2) 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. 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 & 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 cadmium and Pb concentrations (Migula & 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 copper (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 & 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 on 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 & Hodson, 2009), all of which may directly affect soil fertility.

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.

7.2.11. Summary of Effects in Terrestrial Systems

This summary of the effects of Pb on terrestrial ecosystems covers information from the publication of the 2006 Pb AQCD to present. Refer to Section 7.4: Causality determinations for Pb in Terrestrial and Aquatic Systems for a synthesis of all evidence dating back to the 1977 AQCD considered to determine causality.

7.2.11.1. Biogeochemistry and Chemical Effects

The amount of Pb dissolved in 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 both solubility and 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.

7.2.11.2. Bioavailability and Uptake

Plants

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. Multiple new studies showed that in trees, this proportion 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. 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. Studies with trees found that soil Pb is generally translocated from the roots.

Invertebrates

Since the 2006 Pb AQCD, various species of terrestrial snails have been found to accumulate Pb from both diet and soil. New 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 may result from layer differences in interacting factors such as pH and CEC. 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 point sources.

Vertebrates

There were few new studies of Pb bioavailability and uptake in birds 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/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 point sources, and none was able to identify the origin of the Pb. 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.

Food web

New 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.

7.2.11.3. Biological Effects

Plants

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, reduced growth was observed in some experiments, as well as genotoxicity, decreased germination, and pollen sterility.

Invertebrates

Recently published studies have shown neuronal damage in nematodes exposed to low concentrations of Pb (2.5 μM), 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, 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 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. Evidence suggested that aging and pH are important modifiers.

Vertebrates

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. 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. Effects of dietary exposure were studied in several mammalian species, and cognitive, endocrine, immunological, and growth effects were observed.

7.2.11.4. Exposure Response

Evidence reviewed in previous sections demonstrates clearly that increased exposure to Pb is generally associated with increases in observed effects in terrestrial ecosystems. It also demonstrates that many factors, including species and various soil physiochemical properties, interact strongly with 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, a few studies of exposure-response have been conducted with earthworms, and results have been inconsistent.

7.2.11.5. Community and Ecosystem Effects

New evidence of effects of Pb at the community and ecosystem scale 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 scale 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 (Akerblom 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.

7.2.11.6. Critical Loads, Sensitivity and Vulnerability

Exploratory studies of critical load approaches for risk assessment for Pb have been recently conducted in the U. K., the Netherlands, and Italy. Their authors suggested that the main limitations of critical loads approaches in those countries were gaps and uncertainty in both ecotoxicological and Pb deposition data. The most visible indication of the need for improvement was that critical load values were often below background values. Smolders (2009) suggested that correcting for aging and other interacting factors would likely raise predicted-no-effect concentrations, and others proposed basing risk management on tissue residue in organisms, or creating extraction methods that more closely mimic uptake and accumulation.

Recent studies have addressed differences in sensitivity explicitly, and clearly demonstrated high variability between related species, as well as within larger taxonomic groupings. 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). Protective effects of dietary Ca have been found in plants, birds, and invertebrates.

7.3. Aquatic Ecosystem Effects

7.3.1. Introduction to Aquatic Ecosystem Effects

This section of the Pb ISA reviews the new literature since the 2006 Pb AQCD (U.S. EPA, 2006) on the effects of Pb on aquatic systems. The focus is on the effects of Pb, with particular focus on ambient level, to aquatic organisms including algae, aquatic plants, invertebrates, vertebrates, and other biota with

an aquatic life stage (e.g., amphibians). The current mean and range of Pb concentrations in surface waters (mean 0.66 µg Pb/L, range 0.04 to 30 µg Pb/L), sediments (mean 120 µg Pb/g dry weight, range 0.5 to 12,000 µg Pb/g dry weight) and fish tissues (mean 1.03 µg Pb/g dry weight, range 0.08 to 23 µg Pb/g dry weight [whole body]) in the U.S. based on a synthesis of National Water Quality Assessment (NAWQA) data was reported in the previous 2006 Pb AQCD (U.S. EPA, 2006). 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 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 (AWQC) for Pb 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 currently expressed as a criteria maximum concentration (CMC) for acute toxicity and criterion continuous concentration (CCC) for chronic toxicity (U.S. EPA, 2010). 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. In saltwater, these values are 210 µg Pb/L CMC and 8.1 µg Pb/L CCC, respectively.

A draft document intended to update the AWQC for Pb (Great Lakes Environmental Center, 2008) was recently prepared for the EPA. This draft included significantly revised values for both acute and chronic endpoints that were also based on a hardness equation; the revised chronic AWQC in particular is much higher than in previous AWQC due to a substantial reduction in the acute to chronic ratio (ACR). Recent studies have suggested that the ACRs used in the existing criteria documents were too high, possibly because of the age and size of fish used in those studies (Mebane et al., 2008).

The 2006 Pb AQCD summarized two approaches for establishing sediment criteria for Pb based on either bulk sediment or equilibrium partitioning (Section 7.2.1, Table 7-2 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, 2005). 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 acid-volatile sulfides (AVS) since metals that bind to AVS (such as Pb) should not be toxic in sediments where AVS occurs in greater qualities than SEM.

Since the publication of 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 Section 7.3.3. 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 (J. A. 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, new information since the 2006 Pb AQCD on Pb in aquatic ecosystems will be presented. Throughout the sections, brief summaries of conclusions from the 2006 Pb AQCD are included where appropriate. Section 7.3 is organized to consider uptake of Pb and effects at the species level, followed by community and ecosystem level effects. Section 7.3.2 considers the biogeochemistry and chemical effects of Pb in aquatic systems. New research on the bioavailability and uptake of Pb into aquatic organisms including plants, invertebrates and vertebrates is presented next (Section 7.3.3). Effects of Pb on the physiology of aquatic fauna and biota (Section 7.3.4) are followed with data on exposure and response of aquatic organisms (Section 7.3.5). Ecosystem-scale responses are reviewed in Section 7.3.6 followed by a brief consideration of critical loads in aquatic systems (Section 7.3.7), characterization of sensitivity and vulnerability of ecosystem components (Section 7.3.8) and the effects of Pb on ecosystem services associated with aquatic environments (Section 7.3.9).

7.3.2. Biogeochemistry and Chemical Effects

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 and discussed in detail in Section 3.3. 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 concentration, pH, alkalinity, total suspended solids, and dissolved organic carbon (DOC), including humic acids. In sediments, Pb is further influenced by the presence of sulfides and iron and manganese 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 [$Pb(OH)_2$], while in alkaline waters, common forms of Pb include Pb carbonates [$Pb(CO_3)$] and hydroxides [$Pb(OH)_2$]. 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.

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 calcium and magnesium ions) of the water in a specific aquatic system. Models such as the BLM (Paquin et al., 2002) 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 While the BLM is not yet used in setting regulatory criteria for Pb in the U.S., its application in risk analysis has become widely recognized (Fairbrother et al., 2007). Sediment quality criteria have yet to be adopted by EPA, although an equilibrium partitioning procedure is now available to predict which sediments have metal concentrations that are not toxic to aquatic organisms (U.S. EPA, 2005). 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 (2006). 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 iron, SHM predicted Pb speciation predominantly characterized by both iron 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 (both in the Coeur D'Alene River Basin in Idaho), indicating that such models may not be able to accurately describe metal speciation under all environmental conditions (Balistrieri & Blank, 2008).

Quantification of different sediment metal-binding phases, including sulfide, organic C, Fe, and Mn phases, is important to fully understand the bioaccessible fraction of Pb and the toxicity to benthic organisms (Simpson & 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).

7.3.2.1. Other Metals

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 concentrations (0.25 µM) 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 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 (2.5 mM). Ca 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).

7.3.2.2. 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. 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.

7.3.2.3. 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., 2011).

7.3.2.4. Dissolved Organic Matter (DOM)

Uptake of Pb by water-column organisms is affected by the concentration of DOM (Mager et al., 2010). 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 & 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, *C. Kesslerii,* 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 marine invertebrate *Paracentrotus lividus* embryo-larval bioassay (Sanchez-Marin, Santos-Echeandia, et al., 2010). 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, Slaveykova, et al., 2010).

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, Slaveykova, et al., 2010). 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).

As little as 1 µmol of humic acid introduced into surface waters was sufficient to reduce Pb uptake by perennial ryegrass, *Lolium perenne*, grown in nutrient solution. This resulted from a decrease in the concentration of the free Pb fraction by several orders of magnitude following complexation with the OM. Pb content on the root surface was reduced to 8 µmol/g from 20 µmol/g following humic acid addition, and relative Pb absorption (absorption in the presence of humic acid divided by absorption in the absence of humic acid) was determined to be approximately 0.2 (Kalis et al., 2006). Conversely, 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 *Chlorella 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 & 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-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, the attempted field verification of freshwater bioaccessibility models was conducted at sites with distinct point-sources of Pb contamination, and only one model (SHM) was found to adequately predict Pb bioaccessibility.

7.3.3. Bioavailability in Aquatic 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" (Fairbrother et al., 2007). 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. 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, zinc regulated transporter/iron-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.

According to the 2006 Pb AQCD, 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 adverse effects (Luoma & 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 7.3.2. 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, recent information on bioavailability and uptake in algae, plants, invertebrates and vertebrates from marine and freshwater systems are reviewed with summary material from the 2006 Pb AQCD where appropriate. 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. This is followed by a discussion of bioavailability in algae, plants, invertebrates and vertebrates. 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 3.3) 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.

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 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. 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 susceptible 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.

Similarly, 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 & 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 can 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 & Wilkinson, 2005). Despite this, the authors noted that, in most cases, the BLM could predict acute metals toxicity with a reasonable degree of accuracy.

Veltman et al. (2010) proposed an integration of BLM and bioaccumulation models in order to more accurately predict metal uptake by fish and invertebrates. Although Pb was not the specific focus of the paper, calculated metal absorption efficiencies for marine fish species from both BLM and bioaccumulation models were determined to be highly comparable for Ag, Cd, Cu, and Zn. Authors also noted that affinity constants for Ca, 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.

7.3.3.1. Plants and Algae

Aquatic macrophytes and algae can accumulate Pb from either the water column or sediments, based on their specific microhabitats. For instance, rooted macrophytes may be more likely to accumulate Pb from sediment sources, while floating macrophytes or algae will take up Pb suspended or dissolved in the water column. However, significant species-dependent differences in bioaccumulation rates, as well as concentrations of sequestered metals within different parts of the plants (shoots versus roots), have also been observed and some authors have concluded that the plant species is a more important determinant of Pb uptake than is habitat type. Uptake and translocation studies of Pb in plants and algae reviewed in the 2006 Pb AQCD indicated that all 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 2006 Pb AQCD and provide additional evidence for species-dependent differences in responses to Pb in water and sediments.

The microalgae *Spirulina platensis* was demonstrated to accumulate Pb from the water column, with 2.7, 6.9, 19, 45 and 145 µg Pb/mg accumulated at aqueous Pb concentrations of 5, 10, 30, 50, and 100 µg Pb/L, 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 (known as "biodilution"). An aquatic moss, *Fontinalis antipyretica*, accumulated up to an average of 3 µmol Pb/g dry weight over a 7-day exposure to 100 µmol Pb, despite saturation of intracellular Pb concentrations after 5 days of exposure (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 (*Z. Z. Chen et al., 2010*)*. 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.

Pb bioaccumulation studies conducted with five species of marine algae, (*Tetraselmis chuii*, *Rhodomonas salina*, Chaetoceros sp., *Isochrysis galbana* and *Nannochloropsis gaditana*) demonstrated that bioaccumulation rates varied with species. *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 1.4 and 6,348 µg Pb/L (Debelius et al., 2009).

When exposed to water concentrations of up to 100 µmol Pb, 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 (S. Mishra et al., 2006). 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 100 µmol

Pb) 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, 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 BCF's 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 wetlands 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 µg/g dry weight), while *Potamogeton malainus* tissue contained the least amount of Pb, 2.4 µg/g 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 & 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 *Marsilia 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 & Mushrifah, 2009).

Lemna sp., a rooted 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 5 and 10 mg Pb/L, plant biomass was found to contain an average of 2.9 and 6.6 mg Pb, respectively, versus 0.2 and 0.3 mg in sediment (Hurd & Sternberg, 2008).

Young *Typha latifolia*, another rooted macrophyte, were grown in 5 and 7.5 mg/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 environmental Pb exposure (Alonso-Castro et al., 2009).

Common reeds (*Phragmites australis*) grown in metal-impacted aquatic environments in Sicily, Italy, preferentially accumulated Pb in root and rhizome tissues (Bonanno & Lo Giudice, 2010). Environmental 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 & Lo Giudice, 2010). The roots of two salt marsh species, *Sacorconia fructicosa* 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 sediments in vegetated zones indicating that Pb uptake by plants reflects the input in sediments. 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 & 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 & Kempers, 2007). A similar significant correlation was established between reed sweet grass

root Pb concentration and sediment Pb concentrations, yielding BCFs ranging from 0.5 to 1.5, with an average BCF of 0.9 (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 (H. J. Yang et al., 2008). The authors proposed that this diminished transfer ability explained the relatively low BCF's for Pb uptake in these two species, when compared with those of other metals (Table 7-3).

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 measured 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/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.

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).

In contrast, 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.

Reported values for BCF's in aquatic plants from the 2006 Pb AQCD range from 840 to 20,000 (, Table AX7-2.3.1U.S. EPA, 2006). Duckweed (*Lemna minor*) had BCF values ranging from 840 to 3,560 depending on the method of measurement. Additional BCF's established for aquatic plants since the 2006 Pb AQCD are summarized in Table 7-3 and include data on field-collected plants as well as BCF's obtained from laboratory exposures.

Table 7-3. Bioconcentration factors for Pb in aquatic plants

Species	BCF	Test conditions	Reference
Typha latifolia	649	10 days, Pb nitrate-spiked water	Alonso-Castro et al. (2009)
Spirulina platensis	1500	10 days, Pb nitrate-spiked water	Arunakumara et al. (2008)
Ceratophyllum demersum	0.2	Field-collected plants	Bi et al. (2007)
Spirogyra communis	0.2	Field-collected plants	Bi et al. (2007)
Phragmites australis	6.4	Field-collected plants	Bonanno and Lo Giudice (2010)
Taxodium distichum	0.02	Field-collected tissue	Devall et al. (2006)
Hydrilla verticillata	26	Field-collected plants	Dwivedi et al. (2008)
Eleocharis acicularis	0.8	11 mo, field-collected sediment	Ha et al. (2009)
Lemna sp.	0.01	7 days, ash water extract	Horvat et al. (2007)
Mangrove species	0.8	Field-collected tissue	MacFarlane et al. (2007)
Glyceria aquatica	0.9	Field-collected tissue (roots)	Skorbiowicz (2006)
Phragmites australis	0.7	Field-collected plants	Yang et al. (2008)
Ludwigia prostrata	1.5	Field-collected plants	Yang et al. (2008)

7.3.3.2. Invertebrates

Uptake and subsequent bioaccumulation of Pb in marine and 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 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, life stage, age, and metabolism can alter the accumulation rate. In this section, new information on Pb uptake from 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.

The 2006 Pb AQCD 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., 2008). 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. Additionally, Metian et al. (2009) showed that king scallop (*Pecten maximus*) exhibited low bioaccumulation efficiency of Pb from spiked sediment.

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 (J. M. 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 (0.05 µmol Pb in the water column) by the freshwater cladoceran *D. magna* directly from water and through food, the green algae *Pseudokirchneriella subcapitata*. (Komjarova & 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 300 µmol Pb/kg dry weight, and was similar to the amount absorbed by algae (Komjarova & Blust, 2009a).

Stable isotope analysis was to used measure uptake and elimination simultaneously in netspinning caddisfly larvae (Hydropsyche sp.) exposed to aqueous Pb concentrations of 0.2 to 0.6 µg Pb/L (Evans et al., 2006). The measured uptake constant for Pb in this study was 7.8 g/dry weight·day and the elimination rate constant of 0.15 d^{-1} for Pb-exposed larvae was similar in both presence and absence of the metal in the water. Caddisflies accumulated significant amounts of the metal over 18 days of

exposure. Measured tissue concentrations ranged from approximately 15 to 35 µg Pb/g. Hydropsychid Pb BCF's ranged from 41 to 65, and averaged 54, indicating a relatively high accumulation rate 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). In larvae of the mosquito, *Culex quinquefasciatus*, exposed to 100 µg Pb/L for seven days the BCF was 62 (Kitvatanachai et al., 2005).

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). Metian et al. (2009) investigated the uptake and bioaccumulation of ^{210}Pb in *Chlamys varia* (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 *C. varia* than *P. maximus* and soft tissue distribution patterns differed between the species. *C. varia* accumulated Pb preferentially in the digestive gland (50%) while in *P. maximus*, 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 with *P. maximus* showed low bioaccumulation efficiency of Pb from sediment.

With the exception of the above-mentioned study with scallops (Metian et al., 2009) recent reports on Pb distribution generally supports 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 5,000 µg Pb/L for 21 days resulted in accumulation at the highest concentration in gill, followed by exoskeleton < mid-gut gland < muscle < hemolymph (Morris et al., 2005). The gills were the main sites of Pb accumulation in *Pinctada fucata* (pearl oyster) 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). Body burden analysis following 96 hour exposure to 50, 100 and 500 µg Pb/L 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).

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 & 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, Pb was also mainly bound to organelles, i.e., 66% of the total metal burden (Bustamante & 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).

Since the publication of the 2006 Pb AQCD, additional factors have been considered that may affect Pb uptake in aquatic organisms. Pb tissue concentrations fluctuated seasonally in mussels (*Mytilus galloprovincialis*) harvested near Istanbul, Turkey (Ozden, 2008). Tissue Pb concentrations were lowest during the summer months (average of 0.9 mg Pb/kg), followed by spring, autumn and winter (1.3, 1.4, and 1.6 mg Pb/kg, respectively). The authors speculated that the slight seasonal differences indicate that bioavailability of the metal may be related to seasonal changes in surface water or sediment chemistry. Additionally, alterations in growth over the year, as well as different rates of Ca uptake may have impacted Pb bioaccumulation rates. 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).

Reported BAF values for Pb in aquatic invertebrates from the 2006 Pb AQCD ranged from 499 to 3,670 [See Table AX7-2.3.2 (U.S. EPA, 2006)]. Since the publication of 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 & Fisher, 1997) (Table 7-4). 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). Mean Pb levels in both predatory and grazing zooplanktonic species in El Niagra reservoir, (in Aguascalientes, Mexico) were used to calculate BAF values (Rubio-Franchini et al., 2008) to assess biomagnification of Pb. The BAF of the predatory rotifer *Asplanchna brigthwellii* (BAF 49,300) was up to four times higher than the grazing cladocerans *Daphnia similis* (BAF 9,022) and *Moina micrura* (BAF 8,046). Limpet (Patella sp.) from the Lebanese Coast had Pb BAF values ranging from 2,500 to 6,000 and in the same field study mussel (*Brachidontes variabilis*) Pb BAF values ranged from 7,500-8,000 (Nakhle et al., 2006).

Table 7-4. Bioaccumulation factors for Pb in aquatic invertebrates

Species	BAF	Test conditions	Reference
*Hydropsyche sp. (*caddisfly larvae*)*	54	18 days, Pb laboratory exposure	Evans et al. (2006)
Culex Quinquefasciatus (mosquito)	62	7 days, Pb laboratory exposure	Kitvatanachai et al. (2005)
Daphnia similis (zooplankton)	9,022	Field-collected	Rubio-Franchini et al. (2008)
Moina micrura (zooplankton)	8,046	Field-collected	Rubio-Franchini et al. (2008)
Asplanchna brigthwellii (rotifer)	49,344	Field-collected	Rubio-Franchini et al. (2008)
Patella sp. (limpet)	6,000	Field-collected	Nakhle et al.(2006)
Brachidontes variabilis (mussel)	8,000	Field-collected	Nakhle et al.(2006)

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). A mean log of 4.3 of the metal concentration in mussels (ng Pb/kg wet flesh weight)/metal concentration in water (ng Pb/L) was determined for Pb for *M. galloprovincialis* in this study 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.

7.3.3.3. 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. 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 vertebrates, 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). New information since the 2006 Pb AQCD on uptake of Pb by fish from water 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. New data on uptake studies in marine fishes are

presented followed by new evidence for additional Pb detoxification mechanisms in fish. Finally, new data on uptake and tissue distribution of Pb in amphibians and aquatic mammals are presented.

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, accumulation rates of Pb are influenced by both environmental factors, such as water pH, DOC, and Ca 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).

Since the publication of the 2006 Pb AQCD, multiple studies on uptake of Pb from water by fathead minnow 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 µg Pb/g), followed by whole fish and carcass (whole fish minus gill, liver, muscle and intestine) Pb concentrations (approximately 70 to 80 µg Pb/g). 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 200 nmol Pb/g tissue, although this number was decreased from initial body burdens of greater than 500 nmol Pb/g 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 157 nM Pb in base water for 150 days resulted in fathead minnow whole body concentrations of approximately 150 nmol Pb/g tissue, 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: 177 nM Pb in hard water (Ca^{2+} 500 µM) or 187 nM Pb in humic

acid supplemented water (4 mg/L). While the addition of humic acid significantly reduced Pb bioaccumulation in minnows (to approximately 50 nmol Pb/g on a whole body basis), Ca sulfate did not alter uptake. Despite the fact that Ca-mediated Pb toxicity occurred in larval fathead minnow, there was no concurrent effect on whole body Pb accumulation.

Uptake studies in other teleosts of Pb from freshwater have generally followed the pattern of 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 10 µM, 57.4 +26.1 µg/g dry weight at 20 µM) and liver (14.3 µg/g dry weight at 10 µM and 10.2 µg/g dry weight at 20 µM) during a 14-day exposure (Atli & Canli, 2008). In rainbow trout exposed to 100 µg Pb/L 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). Sloman et al. (2005) investigated the uptake of Pb in dominant-subordinate pairings of rainbow trout exposed to 46 µg/L or 325 µg/L 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 µg Pb/g dry weight in gill tissue and Pb concentrations in liver tissue reached 14 µg Pb/g 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 from media containing 0.025 µmol Pb was significantly increased by neutral pH (versus a pH of 6 or 8) and by Ca concentrations of 0.5 mmol; uptake rate of Pb was increased from 10 L/kg·h to 35 L/kg·h by increasing pH from 6 to 7, and from 20 L/kg·h to 35 L/kg·h by increasing Ca concentration from 0.1 mmol to 0.5 mmol (Komjarova & Blust, 2009c). This study also 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.

The Eurasian silver crucian carp (*Carrasius auratus*) collected from a pond containing an average of 1,600 mg Pb/kg in the sediments exhibited increased Pb body burdens ranging from 12 to 68 mg Pb/kg dry weight (Khozhina & 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 of 0.03 mg 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 µg Pb/g tissue and 1.3 and 1.0 µg Pb/g 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 µg Pb/g) than did *L. rohita* (0.5 and 0.4 µg Pb/g).

The studies reviewed above generally support the conclusions of the 2006 Pb AQCD 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 aqueous 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 publication of the 2006 Pb AQCD, several studies have focused on dietary uptake of Pb in teleosts. Alves et al. (2006) administered a diet of three concentrations of Pb (7, 77 and 520 µg Pb/g 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 µg Pb/g tissue wet weight) and this accumulation decreased to 2.2 µg Pb/g 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 µg Pb g wet weight in the high dose), however, Pb was elevated in blood cells (1.5 µg Pb g 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 (100, 400, and 800 µg Pb/g dry weight) accumulated the greatest concentration of Pb in the intestine, followed by the stomach and then the liver (Dai, Du, et al., 2009). 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 50 or 500 µg Pb/g, accumulated Pb preferentially in anterior intestine (Alves & Wood, 2006). Pb accumulation in the gut was followed by bone, kidney, liver, spleen, gill, carcass, brain and white muscle (Alves & 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 & 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 µg Pb/g wet weight. Fish skin accumulated the greatest Pb concentrations (0.02 to 0.05 µg Pb/g wet weight), followed by kidney, gills, liver, and muscle. Pb accumulation in muscles (5 ng Pb/g) 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 internally 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 & 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). The Western Airborne Contaminants Assessment Project 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. Results from this study are considered in in Chapter 3 of this ISA.

Marine 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 gills and intestine (W. X. Wang & Rainbow, 2008). Pb was significantly accumulated in gill, liver, plasma, kidney, rectal gland, intestine, skin, muscle of a marine shark species, spotted dogfish (*Scyliorhinus canicula*) exposed to 2,072 µg Pb/L for one week (De Boeck et al., 2010). 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).

The 2006 Pb AQCD considered detoxification mechanisms in fish including mucus production and Pb removal by scales through chelation with keratin. Since the 2006 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 & 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.

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 diffuse, non-quantified sources of Pb (e.g., in sediments or in dietary sources) may be contributing to Pb uptake by marine fish.

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.

Reported BCF's in fish species from the 2006 Pb AQCD were 42 for brook trout (*Salvelinus fontinalis*) and 45 for bluegill (*Lepomis macrochirus*). Since the 2006 Pb AQCD, additional BAF's have been established in water-only exposures, from dietary exposures and from field-collected fish (Table 7-5).

Table 7-5. Bioaccumulation factors for Pb in fish

Species	BAF	Test conditions	Reference
Clarias gariepinus	800	56 days, Pb nitrate	Kudirat (2008)
Ctenopharyngodon idella	44	Field-collected	Malik et al. (2010)
Pimephales promelas	100 to 100,000	30 days, Pb nitrate	Grosell et al. (2006a)
Carassius auratus	0.04	Field-collected	Khozhina and Sherriff (2008)
Danio rerio	1.4	63 days, dietary exposure	Boyle et al. (2010)

Amphibians

Since the 2006 Pb AQCD, there are a few new studies that consider uptake of Pb in amphibians. In a chronic 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 (T. H. Chen et al., 2006). Dose-dependent bioaccumulation of Pb was observed in the livers of tadpoles of the African clawed frog (*Xenopus laevis*) exposed to concentrations ranging from 0.001 to 30 mg Pb/L (2.91 to 114.5 Pb/g 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 spenocephala*) 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.

Mammals

Studies that consider uptake of Pb in aquatic mammals are limited. Kannan et al. (2006) in a comparison of trace element concentrations in livers of free-ranging sea otters (*Enhydra lutris nereis*) found dead along the California coast, detected Pb in all individuals sampled (N=80) in a range of 0.019 to 1.06 µg Pb/g. The otters were classified by cause of death (infectious causes, non-infectious causes, those that died in an 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.

7.3.3.4. Food Web

At the time of the publication of the 2006 Pb AQCD, trophic transfer of Pb through aquatic food chains was considered to be negligible (U.S. EPA, 2006). 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 bioconcentrated but not biomagnified (Eisler, 2000; U.S. EPA, 2006). New literature since the 2006 Pb AQCD provides evidence of the potential for Pb to be transferred in aquatic food webs while other studies indicate Pb is decreased with increasing trophic level. This section incorporates recent literature on transfer of Pb through 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 Niagra reservoir, Aguascalientes, Mexico, an ecosystem that lacks fishes (Rubio-Franchini et al., 2008). Pb was measured in sediment, water, and zooplankton samples of this freshwater system. BCF's were calculated for predatory and grazing zooplanktonic species. The BCF of the rotifer *A. brigthwellii* (BCF 49,300) was up to four times higher than the grazing cladocerans *D. similis* (BCF 9,022) and *M. micrura* (BCF 8,046). According to the authors, since *M. micrura* are prey for *A. brigthwellii* this may explain the biomagnifications of Pb observed in the predatory rotifer and provides evidence that Pb biomagnifies at intermediate trophic levels. 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 & Páez-Osuna, 2008). The highest rate of transference of Pb as measured in muscle tissue occurred between the prey species white shrimp (*Litopenaeus vannimei*) and mullet (*Mugil cephalus*) to pelican (*Pelecanus occidentalis*).

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 *Neris 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, U.S. Generally, Pb concentrations decreased with increasing trophic level: detritus contained 20 to 60 µg Pb/g dry weight, while periphyton and algae contained 1 to 30 µg Pb/g dry weight; invertebrates and fish collected from the same areas exhibited Pb tissue concentrations of 0.1 to 8 µg Pb/g dry weight (J. M. 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 (J. M. Besser et al., 2007).

Other studies have traced Pb in aquatic food webs and have found no evidence of biomagnification of Pb with increasing trophic level. Pb exposure at the base of the food web did not biomagnify in a simplified-four level marine food chain from *Tetraselmis suecica* (phytoplankton) to *Artemia franciscana* (crustacean, brine shrimp) then *L. vannamei* (crustacean white shrimp) and finally to *Haemulon scudderi* (fish, grunt) (Soto-Jiménez et al.). 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). No biomagnification of Pb was detected from mesozooplankton to macrozooplankton in Bahia Blanca estuary, Argentina (Fernández Severini et al.). In a Brazilian coastal lagoon food chain, Pb was significantly higher in invertebrates than in fishes (Pereira et al., 2010). Watanabe et al. (2008) also observed decreasing Pb concentrations through a stream macroinvertebrate food web in Japan from producers to primary and secondary consumers.

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 melanastomus*) 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.

7.3.4. Biological Effects

This section focuses on the studies of biological effects of Pb on aquatic biota including algae, aquatic plants, invertebrates, fish and other biota with an aquatic lifestage (e.g., amphibians) published since the 2006 Pb AQCD. Waterborne Pb is highly toxic to aquatic organisms with toxicity varying depending upon the species and lifestage tested, duration of exposure, the form of Pb tested, and water quality characteristics. The 2006 Pb AQCD noted that the physiological effects of Pb in aquatic organisms can occur at the biochemical, cellular, and tissue levels of organization and include inhibition of heme formation, adverse effects to blood chemistry, and decreases in enzyme levels. Functional growth responses resulting from Pb exposure include changes in growth patterns, gill binding affinities, and absorption rates. 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. Since this document focuses on atmospheric sources of Pb to ecosystem receptors, areas of research not addressed here include literature related to exposure to Pb from shot or pellets. Biological effects of Pb on algae and plants are considered below, followed by information on effects on aquatic invertebrates and vertebrates.

7.3.4.1. Plants and Algae

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. Pb exposure in microalgae species has been linked to several adverse effects, including disruption of

thylakoid structure and inhibition of growth in both *Scenedesmus quadricauda* and *Anabeana flos-aquae* (Arunakumara & Zhang, 2008). Arunakumara et al. (2008) determined the effect of aqueous Pb on the algal species *S. platensis* using solutions of Pb-nitrate. While low Pb exposures (5 µg Pb/mL) stimulated 10-day algal growth, growth was inhibited at higher concentrations of 10, 30, 50, and 100 µg Pb/mL 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 µg Pb/mL. The effect of Pb exposure on the structure and function of plant photosystem II was studied in giant duckweed, *S. polyrrhiza* (Ling & Hong, 2009). 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.

An increase in levels of antioxidant enzymes is commonly observed in aquatic plant, algae, and moss species exposed to Pb. An aquatic moss, *F. antipyretica*, exhibited increased SOD and ascorbate levels following a 2-day exposure to Pb-chloride solutions of concentrations of 1, 10, 100, and 1000 µmol. 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 exposures of 1, 10, and 100 mg 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, 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).

Toxicity and oxidative stress were also observed in coontail (*C. demersum*) rooted aquatic macrophytes following 7-day exposures to aqueous Pb (1 to 100 µmol), 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 (S. Mishra et al., 2006).

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 to Pb (50 to 300 mg Pb/L); total phenols also increased with increasing Pb exposure. Authors noted that this species exhibited similar responses under extreme temperatures, drought, and disease (Sobrino et al., 2010). According to Odjegba and Fasidi (2006), exposure to 0.3 mmol of Pb for 21 days was sufficient to induce a gradual reduction of both chlorophyll and protein content in the macrophyte *Eichhornia crassipes*. Decreased proteins were theorized to be related to inefficient protein formation following disruption of nitrogen metabolism after Pb exposure (Odjegba & Fasidi, 2006). Foliar proline (which is thought to act as an antioxidant) concentrations were found to increase in a concentration-dependent manner as Pb concentrations increase from 0.1 to 5.0 mmol.

Following 72-hour aqueous exposure to 41 µmol Pb-nitrate, phytochelatin and glutathione concentrations in the algae *Scenedesmus vacuolatus* were significantly increased over that of non-exposed algal cultures {F, 2006, 358857}. 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.

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 10 mmol, while chlorophyll *a* content was decreased at concentrations greater than 1 mmol Pb (Piotrowska et al., 2010).

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 Pb exposures of 20 mg Pb/L (Deng et al., 2009). Further, while both Zn and Fe exposures exerted some selective pressure on plants, the authors did not observe the same with Pb, leading them to theorize that concentrations of bioavailable Pb were not present in high enough quantities to have such an effect. However, while Lemna sp. aquatic plants were determined to effectively sequester aqueous Pb, the plant growth rate was not significantly different from zero following exposures of 5 and 10 mg Pb/L, while exposure to 15 mg Pb/L was associated with notable plant mortality (Hurd & Sternberg, 2008). In fact, Paczkowska et al. (2007) observed that low Pb exposures (0.1 to 1.0 mmol 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)

7.3.4.2. Invertebrates

Effects of Pb on aquatic invertebrates recognized in the 2006 Pb AQCD include adverse impacts on reproduction, growth, survival and metabolism. 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. As observed in terrestrial invertebrates, the antioxidant system, survival, growth and reproduction are affected by Pb in aquatic organisms. In aquatic invertebrates, Pb has also been shown to affect stress responses and osmoregulation. New evidence that supports previous findings of Pb on reproduction and growth in invertebrates are reviewed here as well as limited studies on behavioral effects.

Recent literature strengthens the evidence indicating that Pb affects enzymes and antioxidant activity in aquatic invertebrates. Increased SOD activity was observed in mantles of pearl oyster but decreased with time although always remaining higher than in the control animals during 72-hour exposures to 0.5 µM Pb (Jing et al., 2007). In contrast, activity of Se-dependent glutathione peroxidase was not changed 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* (Y. Zhang et al., 2010). In contrast, Einsporn et al. (2009) observed no change in catalase activity in the digestive gland and gill of blue mussel *M. edulis* following exposures to 2,500 µg Pb/L as Pb nitrate for 10 days and measured 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 concentrations of Pb, fluctuations in SOD activity were observed over the length of the exposure (Vlahogianni & 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 (Y. Zhang et al., 2010). In black mussel exposed for 10 days to sublethal concentrations of Pb, MDA levels were increased in mantle and gill (Vlahogianni & Valavanidis, 2007).

Aminolevulinic acid dehydratase (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. 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 from 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 *Crassotrea angulata*), however, the authors of this study indicated the need to consider species-dependent responses to Pb (Company et al., 2011). Pb content varied significantly among species and was related to habitat (sediment versus substrate) and feeding behavior. In red fingered marsh crab, *Parasesarma erythodactyla*, 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).

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. Induction of heat shock proteins in zebra mussel exposed to 500 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 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. Activity of enzymes associated with the immune defense system in the mantle of pearl oyster were measured at 0, 24, 48 and 72 hour exposure to 104 µg Pb/L (Jing et al., 2007). Activity of AcPase, a lysosomal marker enzyme, was detected at 24 hours and decreased at subsequent time points. Phenoloxidase activity was depressed compared with controls and remained significantly lower than control at 72 hours of exposure to Pb.

The effect of Pb on the osmoregulatory response has been studied after the 2006 Pb AQCD. The combined effects 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. 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.

Additional evidence of reproductive and developmental effects of Pb on aquatic invertebrates is available since the 2006 Pb AQCD. Sublethal concentrations of Pb negatively affected the total number of eggs, hatching success and embryonic survival of the freshwater snail *B. glabrata* exposed to 50, 100, or 500 µg Pb/L (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.

Formation of tentacles and eyes was significantly impaired in embryos of the freshwater ramshorn snail *Marisa cornuarietis* at 15,000 µg Pb/L (Sawasdee & Köhler, 2010). 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. 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 (Garcia-Garcia et al., 2007).

In larvae of the mosquito, *C. quinquefasciatus*, exposed to 50 µg Pb/L , 100 µg Pb/L or 200 µg Pb/L, 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/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.

Since the publication of the 2006 Pb AQCD, limited studies on marine invertebrates have indicated adverse effects of Pb on reproduction in saltwater environments. In a long term (approximately 60 days) sediment bioassay with the marine amphipod *Elasmopus laevi*, onset to reproduction was significantly delayed at 118 µg Pb/g compared to controls. In the higher concentrations, start of offspring production was delayed further; 4 days in 234 µg Pb/g and 8 days in 424 µg Pb/g (Ringenary et al., 2007). Fecundity was also reduced with increasing Pb concentration in sediment. 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 100 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 50 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).

The protective barrier against Pb toxicity formed by the egg structure in some invertebrates (e.g., Daphnia) 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 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, however concentrations associated with the eggshell increased throughout the 48 day exposure. These results are consistent with cuttlefish eggs collected from the field in which Pb was only detected in the eggshell and indicate the protective barrier provided by cuttlefish egg to Pb toxicity (Miramand et al., 2006).

As noted in the 2006 Pb AQCD, Pb exposure negatively affects the growth of aquatic invertebrates. Some studies reviewed in the previous document suggested that juveniles do not discriminate between the uptake of essential and non-essential metals (Arai et al., 2002). In new literature, the freshwater pulomonate 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 & Brix, 2009; Grosell et al., 2006b). In *L. stagnalis* exposed to 18.9µg/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 (Grosell & 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 susceptibility of this species.Wang et al., {, 2009, 533439} 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/L (A. S. Sobrino-Figueroa et al., 2007). Rate of growth of the deposit feeding polychaete Capitella sp. decreased significantly with increasing concentrations of Pb associated with sediment (Horng et al., 2009).

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. Additionally, the carapace of hard shelled crabs have lower concentrations of Pb than the exuvium of the soft shell crabs, leading the authors to speculate that some of the metal might be excreted during the molting process. 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.

Behavioral responses of aquatic invertebrates to Pb reviewed in the 2006 Pb AQCD included avoidance. A limited number of new studies have considered additional behavioral endpoints. Valve closing speed was used as a measure of physiological alterations due to Pb exposure in the Catarina scallop (A. Sobrino-Figueroa & 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 Pb (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. 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.

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. In a 10-day Pb-spiked sediment exposure (1,000 mg Pb/kg), 100% of individuals of the Australian estuarine bivalve *Tellina deltoidalis* survived (King et al., 2010). In the deposit feeding polychaete Capitella sp., exposure to varying concentrations of Pb associated with sediment up to 0.41 µmol/g had no effect on survival (Horng et al., 2009). In freshwater habitats, odonates are highly tolerant of Pb with no significant differences in survival time of dragonfly larvae (*Pachydiplax longipennis* and *Erythemis simplicicollis*) exposed to concentrations as high as 185 mg Pb/L Pb (185,000 µg Pb/L) (Tollett et al., 2009). Other species are more susceptible to Pb in the environment and these responses are reviewed in Section 7.3.5.

7.3.4.3. Vertebrates

Biological effects of Pb on fish that have been studied since the 2006 Pb AQCD report are reviewed here, and limited new evidence of Pb effects on amphibians, and marine mammals are considered. As noted in the 2006 Pb AQCD, commonly observed effects of Pb on fish included inhibition of heme formation, alterations in brain receptors in fish, adverse effects on blood chemistry, and decreases in some enzyme activities. (U.S. EPA, 2006). 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. More recent experimental data presented here expand and support these observations. As in terrestrial vertebrates, Pb has been shown to affect antioxidant and enzymatic activity in aquatic vertebrates and new evidence of this since the 2006 Pb

AQCD is reviewed in this section. This section also presents the limited new 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 and growth effects of Pb on fish is discussed along with limited new information on behavioral effects of Pb. Finally, limited new information since the 2006 Pb AQCD on physiological effects of Pb on amphibians and marine mammals is presented.

Fish

In environmental assessments of metal-impacted habitats, ALAD is a recognized biomarker of Pb exposure (U.S. EPA, 2006). 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., 2005; Schmitt et al., 2007). 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). Costa et al. (2007) observed inhibition of ALAD in hepatocytes of the neotropical traira (*Hoplias malabaricus*) following dietary dosing of 21 µg Pb/g 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), $CaSO_4$ (37µg Pb/L) or (39 µg Pb/L) humic acid-supplemented water 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).

Pb was shown to inhibit hepatic cytochrome P450 in carp (*Cyprinus carpio*), silver carp (*Hypothalmichtys molitrix)* and wels catfish (*Silurus glanis*) in a concentration-dependent manner from 0-4.0 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/L) while carp EROD activity was inhibited at 0.76 µg Pb/L. Interaction of Pb with cytochrome P450 was verified by spectral changes using Fourier Transform Infrared (FTIR) spectroscopy. Liver damage to African catfish exposed to 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.

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 (*Carassius auratus gibelio*) injected with 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 & 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. In fathead minnow, three genes, glucose-6-phosphate dehydrogenase, glutathione-S-transferase and ferritin were upregulated, in microarray analysis, during 30 day exposures to Pb in base water (33µg Pb/L), or (37µg Pb/L [hard]-water supplemented with 500 µM Ca^{2+}) or (39 µg Pb/L [DOC]-water supplemented with 4 mg/L humic acid). However, no changes in whole body ion concentrations were observed (Mager et al., 2008). In the freshwater fish Nile tilapia, liver catalase, liver alkaline phosphatase, sodium and potassium-ATPase (NA, K-ATPase) and muscle Ca-ATPase activities were quantified in various tissues following a 14 day exposure to 5, 10, and 20 µM concentrations of Pb nitrate (Atli & Canli, 2007). Liver catalase activity significantly increased in the 5 and 20 µM concentrations while liver alkaline phosphatase activity was significantly increased only at the 20 µM concentration. No significant change in alkaline phosphatase activity was observed in intestine or serum. Ca-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. In another study with *O. niloticus*, Pb had no effect on glutathione measured in liver, gill, intestine, muscle and blood and liver metallothionein levels following a 14 day exposure to 5, 10, and 20 µM concentrations of Pb nitrate (Atli & 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 50 to 500 µg Pb/g 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 µg Pb/g indicated that alanine transaminase, aspirate transaminase, and lactate dehydrogenase activities significantly decreased in kidney in a concentration-dependent manner (Dai, Fu, et al., 2009) 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, Du, et al., 2009). 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.

Data on the physiological effects of Pb on marine elasmobranchs are limited. De Boeck et al. (2010) exposed the spotted dogfish to 2,072 µg Pb/L for one week and measured metallothionein induction, and the electrolytes Na, K, Ca and Cl. No effects were observed in Pb-exposed fish in any of the physiological parameters measured in this study, however Pb was measured in all organs (De Boeck et al., 2010).

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 1,000 µg Pb/L 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 5 and 10µM 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 (*Hoplias malabaricus*) muscle cholinesterase was significantly inhibited after 14 dietary doses of 21 µg Pb/g wet weight (Rabitto et al., 2005). Histopathological observations of brains of African catfish exposed to 500 µg Pb/L or 1,000 µg Pb/L Pb for 4 weeks included perivascular edema, focal areas of malacia, and diffuse areas of neuronal degeneration (Adeyemo, 2008b).

Adverse effects of Pb on blood chemistry of fish were noted in the 2006 Pb AQCD and limited new literature since the last Pb review has considered effects on blood. In the African catfish, packed cell volume decreased with increasing 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.

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 homeostasis as previously characterized in the 2006 Pb AQCD (Rogers & Wood, 2004; Rogers & 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 µg Pb/g dry weight) to rainbow trout for 21 days, and measured physiological parameters including Na^+ and Ca^+ influx rate from water. Dietary Pb had no effect on brachial Na^+ and Ca^+ rates except on day 8 where Na^+ influx rates were significantly elevated. These studies suggest that Pb is intermediate between purely Ca antagonists such as Zn and Cd and disruptors of Na and Cl balance such as Ag and Cu. This finding has implications for BLM modeling since it suggests that both Ca and Na need to be considered as protective cations for Pb toxicity. Indeed, protection from Pb toxicity by both Na and Ca have been documented in freshwater fish (Komjarova & Blust, 2009b).

Long-term exposures of Pb can adversely impact gill structure and function. Histopathological observations of gill tissue in the catfish (*C. gariepinus*) following an 8-week aqueous exposure to 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 2.5, 12.5 and 25 mg Pb/L 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).

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 reabsorption of Ca^+, 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 1.2 mg 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^+ 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).

Limited new studies on reproductive effects of Pb in fish from oocyte formation to spawning are available. Decreased oocyte diameter and density in the toadfish (*Tetractenos glaber*) were associated with elevated levels of Pb in the gonad (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. The effects of metals on embryonic stage of fish development in *Cyprinus 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. 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 g Pb/kg·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 (120 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.

Reproductive effects of Pb have also been observed at the cellular level, including alterations in gonadal tissue and hormone secretions that are associated with Pb-exposure. 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 Pb-nitrate (0, 001, 0.1, 1, 3, and 10 µg Pb/mL). 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 disruption of steroid production and altered hormone secretion patterns observed at the low concentrations of Pb in this study are suggestive of the potential for impacts to fish reproduction (Chaube et al., 2010).

Reduction of growth was noted as an adverse effect of Pb on fish in the 2006 Pb AQCD. No new evidence of growth effects in fish have been reported with the exception of Grosell et al. (2006a). 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). No effects on growth rates were observed in rainbow trout administered a diet containing three concentrations of Pb (7, 77 and 520 µg Pb/g dry weight) for 21 days (Alves et al., 2006) or in Nile tilapia fed diets with 100, 400, or 800 µg/g Pb dry weight for 60 days (Dai, Du, et al., 2009). Growth and survival were not adversely affected in juvenile rainbow trout, fathead minnow and channel catfish (*Ictalurus punctatus*) fed a live diet of *L. variegatus* contaminated with Pb (850-1,000 µg Pb/L·g dry mass for 30 days. (Erickson et al., 2010). 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. (2011) did not observe growth impairment in fathead minnows at environmentally relevant concentrations of Pb.

Since the publication of the 2006 Pb AQCD, several studies integrating behavioral and physiological measures of Pb toxicity have been conducted on fish. The ornate wrasse (*Thalassoma pavo*) was exposed 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. No changes in feeding activity were noted between non-treated and treated fish.

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, 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 momoamines. 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 either 35 or 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.

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 7.2.2.3 and 7.2.4.3. 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, 2006). Since the 2006 Pb AQCD, studies conducted at environmentally relevant concentrations of Pb 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.

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 (T. H. Chen et al., 2006). 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, 2002). 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 2,360 mg Pb/kg (8,121 µg Pb/L sediment pore water) treatment. Conversely, no effects were observed on organogenesis in *X. laevis* embryos exposed to a range of Pb concentrations from 8,600 to 220,500 µg Pb/L using the Frog Embryo Teratogenesis Assay (Gungordu et al., 2010).

Endpoints of oxidative damage were measured in testes of the black-spotted frog (*Rana nigromaculata*) treated with 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 adsorption for 30 days (M. Z. Wang & 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 genotoxic potential of Pb to larvae of the toad (*X. laevis*) was assessed by determining the number of micronucleated erythrocytes per thousand (MNE) following a 12 day exposure (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* (Y. M. Zhang et al., 2007). 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.

In a study with 4-day-old *X. laevis* tadpoles exposed to a range of concentrations of Pb from 25,500 to 137,000 µg Pb/L for 24 hours, acetylcholinesterase was significantly inhibited in all treatments (Gungordu et al., 2010). The authors suggest that the 35-60% inhibition of acetylcholinesterase is indicative of a neurotoxic effect. In the same study, glutathione-s-transferase activity significantly increased in a concentration-dependent manner. Alanine aminotransferase and aspartate aminotransferase activities were decreased, however, the degree of inhibition did not reflect Pb concentration. The concentrations used in this study were selected based on the LC_{50} of Frog Embryo Teratogenesis Assay results with *X. laevis* and are much higher than ambient levels of Pb.

Birds

In addition to effects on amphibians, 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 100 µg Pb/L for 30 minutes on day 0 of development did not increase malformations or mortality of embryos (Kertész & 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 (Kertesz et al., 2006). The second study was conducted to emulate environmental levels of Pb following a dam failure in Hungary.

Mammals

Although Pb continues to be detected in tissues of marine mammals in U.S. coastal waters (Bryan et al., 2007; Kannan et al., 2006; Stavros et al., 2007) few studies exist that consider biological effects associated with Pb-exposure. Pb effects on immune parameters 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 concentrations of 1, 10, 20 and 50 mg Pb/L. Proliferative response of bottlenose dolphin leukocytes was significantly reduced at 50 mg Pb/L, albeit by only 10% in comparison to the control.

7.3.5. Exposure and Response of Aquatic Species

To support the development of air quality criteria standards that are protective of aquatic ecosystems, threshold levels for Pb effects on aquatic populations must be evaluated. The Annex of the 2006 Pb AQCD (U.S. EPA, 2006) summarized data on exposure-response functions for freshwater and marine invertebrates (Table AX7-2.4.1) and freshwater and marine 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).

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. Additionally, the aquatic freshwater microalgae *Scenedesmus obliquus* was significantly more susceptible 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 4,000 and 24,500 µg Pb/L were derived (Atici et al., 2008). Experiments with the blue-green algae *Spirulina platensis* produced a LC_{50} value of 75.3 µg Pb/mL (95% CI: 58.5, 97.0)(Arunakumara et al., 2008).

In the 2006 Pb AQCD, adverse 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 publication of the 2006 Pb AQCD, recent studies have identified the freshwater snail *L. stagnalis* as a species that is extremely sensitive to Pb exposure (Grosell & Brix, 2009; Grosell et al., 2006b). Growth of juvenile *L. stagnalis* was inhibited at an EC_{20} of < 4 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 (Sawasdee & Köhler, 2010).

Additional studies on Pb effects in aquatic invertebrates published since the 2006 Pb AQCD have indicated differences in susceptibility 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} for adults was 3,000 µg Pb/L and 1,520 µg Pb/L for juveniles. Ten-day exposures of adults in seawater resulted in an LC_{50} of 1,270 µg Pb/L, an NOEC of 190 µg Pb/L and a LOEC of 390 µg Pb/L. In comparison, the LC_{50}, NOEC, and LOEC value for the adults exposed in sediment was 3,560 µg Pb/L. Juvenile sediment tests results were LC_{50} 1,980, NOEC 580 and LOEC 1,020 µg Pb/L.

In the freshwater mussel, *Lampsilis 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 (N. Wang et al., 2010). 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 (N. Wang et al., 2010). 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. In contrast, 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 (Vlahogianni & Valavanidis, 2007).

The acute toxicity of Pb to first-instar *C. riparius* larvae was tested in soft water, with hardness of 8 mg/L as $CaCO_3$. (Bechard 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). 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).

Cladocerans are commonly tested aquatic organisms, with data from three species: *D. magna*, *D. pulex* and *Cerodaphnia dubia*, representing approximately 70% of available metal toxicological literature on this group (L. C. Wong et al., 2009). Since the publication of the 2006 Pb AQCD, additional studies have generated acute toxicity values for other cladocerans. 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} 7,000 µg Pb/L) indicate differences in susceptibility to Pb in these freshwater species from Mexico (Garcia-Garcia et al., 2006). 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).

Exposure-response assays on other freshwater species have been conducted since the 2006 Pb AQCD. 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). For rotifer *Brachionus patulus* neonates, the 24-hour LC_{50} was 6,150 µg Pb/L (Garcia-Garcia et al., 2007). In a 48-hour toxicity test with the rotifer

Brachionus calyciflorus, an NOEC (194 µg Pb/L), a LOEC (284 µg Pb/L), and an EC_{20} of 125 µg Pb/L was established for this species (Grosell et al., 2006b).

Since the publication of 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, 2009, 533439}. In comparison, juvenile Catarina scallop (*A. ventricosus*) had a LC_{50} of 830 µg Pb/L in a 96-hour exposure (A. S. Sobrino-Figueroa et al., 2007). 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 to Pb, suggesting this species is not as sensitive to Pb as other marine invertebrate larvae (Gorski & Nugegoda, 2006).

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*. The EC_{50} for developmental defects in this species was 1,150 µg Pb/L with a NOEL of 250 µg Pb/L.

There have been only a few new exposure-response studies in amphibians since the 2006 Pb AQCD. Southern leopard frog tadpoles exposed to Pb in sediment (45 to 7,580 mg/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/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). A 96-hour LC_{50} of 96,100 µg Pb/L was determined for *X. laevis* embryos exposed to a range of Pb concentrations from 8,600 to 220,500 µg Pb/L using the Frog Embryo Teratogenesis Assay (Gungordu et al., 2010).

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, 2006). 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 freshwater and marine fish. At the time of the 2006 Pb AQCD, there was a lack of exposure-response data

in marine fish. No new exposure-response studies in marine fish have been conducted since the previous Pb review.

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. The 30-day LC_{50} for low hardness (19 mg $CaSO_4$/L) in basic test 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).

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. (2011) 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., 2011).

In the 2006 Pb AQCD, fish size was recognized as an important variable in determining the adverse effects of Pb. Acute (96-hour) and chronic (60-day) early-lifestage test exposures were conducted with rainbow trout to develop 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/L, maximum acceptable toxicant concentration=36 µg/L, EC_{10}=26 µg/L, EC_{20}=34 µg/L, and LC_{50}=55 µg/L. Results from the 62-day test, with fish length as the endpoint, were NOEC=8 µg/L, MATC=12 µg/L, EC_{10}=7µg/L, EC_{20}=102 µg/L and LC_{50}=120 µg/L. In acute tests run concurrently with the chronic tests, 96-hour LC_{50} values were 120 and 150 µg/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.

7.3.6. Community and Ecosystem Effects

As discussed in the 2006 Pb AQCD, exposure to Pb is likely to have adverse impacts in aquatic environments via effects at several levels of ecological organization (individuals, populations, communities, or ecosystems). These adverse 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 individual 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, Pb exposure has been demonstrated to alter predator-prey interactions, as well as feeding and avoidance behaviors. 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 type of habitat 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 & 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. A total of 11 species of macrophytes were collected. 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 (V. K. Mishra et al., 2008). The site with the highest Pb concentrations (103 to 118 µg Pb/L) had the lowest number of resident macrophyte species; these included *E. crassipes*, *L. minor*, *Azolla pinnata* and *Spirodela 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 (V. K. Mishra et al., 2008).

Exposure to three levels of sediment Pb contamination (322, 1,225, and 1,465 µg Pb/g dry weight) had variable effects on different species within an aquatic 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.

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.

In 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.

Rhea et al. (2006) examined the effects of multiple heavy metals in the Boulder River, MT, U.S., 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 & 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 ecosystems dynamics.

Invertebrate functional feeding group may also affect invertebrate Pb body burdens in those systems where Pb bioconcentration occurs. The predaceous zooplanktonic rotifer, *A. brigthwellii* collected from a Pb-impacted reservoir in Mexico, contained 384 ng Pb/mg and exhibited a water-to-tissue BCF of 49,344. The authors theorized that Pb biomagification 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 & 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. 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 (Larsson et al., 2007).

A risk assessment conducted in southern Florida freshwater canals determined that the 90th percentile of bulk Pb sediment concentrations in systems was 105 mg Pb/kg, which was predicted to result in a sediment pore water concentration of 2.6 µg Pb/L. This estimated pore water concentration was contrasted with acute 10th percentile toxic concentrations derived from a series of species sensitivity distributions: 8.7 µg Pb/L for arthropods, 223 µg Pb/L for fish species, and 116 µg Pb/L for all species (Rand & Schuler, 2009). Although the predicted sediment pore water Pb concentration was below the derived acute toxicity hazardous concentration for 10% of species (HC10) values, it was considered possible that chronic exposure to such concentrations could impact some arthropod populations. A chronic species sensitivity distribution constructed with Pb NOEC values for all aquatic species produced

a chronic 10th percentile Pb NOEC value of 1.6 µg/L, a further indication that some aquatic species (likely arthropod species) may be impacted by the Pb contamination in southern Florida canals (Rand & Schuler, 2009).

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. fructicosa* 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.

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 & 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.

7.3.7. Critical Loads in Aquatic Systems

Since the publication of the 2006 Pb AQCD there is no new significant information on critical loads of Pb in aquatic systems. Refer to Section 7.3.6 of the 2006 Pb AQCD for a discussion of critical loads of Pb in aquatic systems.

7.3.8. Characterization of Sensitivity and Vulnerability

Data from the literature indicate that exposure to Pb may affect survival, reproduction, growth, metabolism, and development in a wide range of 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 may also alter Pb toxicity. The 2006 Pb AQCD 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 life-stage as other important determinants of differential sensitivity to Pb.

7.3.8.1. 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 & Anbazhagan, 2009).

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 biota 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. 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 high molecular weight (>10 kDaltons) cationic Pb species 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.

7.3.8.2. 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.

7.3.8.3. 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 (M. A. Q. Khan et al., 2006). 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 & Taner, 2009). Decreased pH was also demonstrated to increase the uptake of environmental Pb in aquatic plants (Uysal & Taner, 2009; C. Wang, X. Yan, et al., 2010). 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.

7.3.8.4. Life Stage

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. Bechard 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}s 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 (Bechard et al., 2008). Similarly, Wang et al. (2010) 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 (N. Wang et al., 2010).

Pb exposures also differentially affected life-stages of the marine polychaete *H. elegans*. Pb water concentrations of 100 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 off-set (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 50 µg Pb/L and greater (Gopalakrishnan et al., 2008).

7.3.8.5. Species Sensitivity

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 Hydropysche 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). Data from 20 years of monitoring of contaminant levels in filter-feeding mussels Mytilus sp. and oysters *Crassotrea virginica* 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).

Species-specific Ca requirements have also been shown to affect the vulnerability of aquatic organisms to Pb. The snail, *L. stagnalis*, exhibits an unusually high Ca demand due to $CaCO_3$ formation

required for shell production and growth, and exposure to Pb prevents the uptake of needed Ca, leading to toxicity. Consequently, aquatic species that require high assimilation rates of environmental Ca for homeostasis are likely to be more sensitive to Pb contamination (Grosell & Brix, 2009). Grosell and colleagues also noted that reduced snail growth following chronic Pb exposure was likely a result of reduced Ca 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 (Bergey & Weis, 2007; Mohapatra et al., 2009; Soto-Jiménez et al.; Tollett et al., 2009). Crab species *U. pugnax* (Bergey & Weis, 2007) and *Scylla serrata* (Mohapatra et al., 2009), white shrimp *L. vannamei* (Soto-Jiménez et al.) as well as Libellulidae dragonfly nymphs (Tollett et al., 2009) have been shown to preferentially sequester Pb in exoskeleton tissue, where it is later shed along with the hardened exterior 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 & Wallace, 2010). Fish with such abilities are more likely to thrive in Pb-contaminated environments than other species. 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 & Gopal, 2009).

Tolerance to prolonged Pb exposure may develop in aquatic invertebrates and fish. Multi-generational exposure to low levels of Pb appears to confer some degree of metal tolerance in invertebrates such as *Chironomus plumosus* larvae; consequently, previous population Pb exposures may decrease species' susceptibility to Pb contamination (Vedamanikam & Shazilli, 2008). 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) 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 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 (L. C. Wong et al., 2009).

7.3.8.6. Ecosystem Vulnerability

Relative vulnerability of different aquatic 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 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 susceptible 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.

7.3.9. Ecosystem Services

There are limited publications at this time that address Pb impacts to ecosystem services associated with aquatic systems and most of the available literature is for estuaries, salt marsh and freshwater wetlands rather than lakes and streams. The evidence reviewed in the ISA illustrates that Pb can affect the ecological effects in each of the four main categories of ecosystem services (Section 7.1.2) 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

A few recent studies consider the impact of Pb and other heavy metals on ecosystem services provided by estuaries (Smith et al., 2009) and salt marsh {Bromberg Gedan, 2009, 672706}. These systems are natural sinks for metals and other contaminants. They provide habitat and breeding areas for both terrestrial and marine wildlife and are locations for bird watching. In a Monte-Carlo 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 may pose an ecologically relevant risk to dunlin, *Calidris alpina* (Smith et al., 2009). However, the authors noted that a major source of uncertainty in this study was the NOAEL values for Pb. Pb can be toxic to salt marsh plant species and decaying plant detritus may result in resuspension of Pb into the aquatic food chain {Bromberg Gedan, 2009, 672706}.

Ecological services provided by freshwater wetlands are similar to those provided by estuaries and are sinks for atmospheric Pb as well as Pb from terrestrial runoff (Landre et al., 2010; Watmough & Dillon, 2007). Several studies have addressed the response of natural wetlands to Pb (Gambrell, 1994; Odum, 2000). Recent reviews of pollution control by constructed wetlands (Mander & Mitsch, 2009), removal of metals by constructed wetlands (Marchand et al., 2010) and phytoremediation of metals by wetland plants (Rai, 2008) indicate that these systems can remove Pb from the aquatic environment. 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. 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. The conclusions of a recent assessment report are that the highest concentrations of Pb are found in mussels and oysters near urban and industrial centers and the only region that exceeded the Food and Drug Administration action level for Pb in clams, oysters and mussels of 1.7 mg Pb/kg wet weight was Lake Michigan, where maximum concentrations were 1.9 mg Pb/kg wet weight in the years 2004-2005 (Kimbrough et al., 2008). 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 an ecosystem service that 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). Other aquatic organisms have been

considered for their role in remediation of Pb in the environment. 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.

7.3.10. Summary of Aquatic Effects

This summary of the effects of Pb on aquatic ecosystems covers information from the publication of the 2006 Pb AQCD to present. Refer to Section 7.4: Causality determinations for Pb in Terrestrial and Aquatic Systems for a synthesis of all evidence dating back to the 1977 AQCD considered to determine causality.

7.3.10.1. Biogeochemistry and Chemical Effects

Once the atmospherically-derived Pb enters surface waters its fate and bioavailability are influenced by Ca concentration, pH, alkalinity, and total suspended solids, and DOC, including humic acids. Once in sediments, Pb bioavailability may be influenced by the presence of sulfides and Fe and Mn oxides, physical disturbance, the presence of other metals, biofilm and organisms. In many, but not all aquatic organisms, Pb dissolved in the water can be the primary exposure route to gills or other biotic ligands. 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. As recognized in the 2006 Pb AQCD and further supported in this review, chronic exposures to Pb may also include dietary uptake, and there is an increasing body of evidence showing that differences in uptake and elimination of Pb vary with species. 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.

7.3.10.2. Bioavailability

There is evidence over several decades of research previously reviewed in Pb AQCDs and in recent studies reviewed in this ISA that Pb bioaccumulates in plants, invertebrates and vertebrates in aquatic systems, just as it does in terrestrial systems. According to the 2006 Pb AQCD, and further supported in this review, Pb adsorption, complexation, chelation, etc., are processes that alter bioavailability to aquatic biota. Given the low solubility of Pb 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.

As reviewed by Wang and Rainbow (2008) and supported by additional studies reviewed in this ISA, 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 toxicity of Pb to

aquatic organisms are closely linked to the environmental fate of the metal under variable environmental conditions (Section 3.3) as they are highly dependent upon the proportion of free metal ions in the water column.

Recent studies on bioavailability of Pb in aquatic plants and algae support the findings of previous Pb AQCDs that all 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. Trees that inhabit semi-aquatic environments have also been shown to absorb Pb from contaminated sediments.

In the case of invertebrates, Pb can be bioaccumulated from multiple sources, including the water column, sediment, and dietary exposures, and factors such as amount of bioavailable Pb, lifestage, age, and metabolism can alter the accumulation rate. Additional studies have considered the relative importance of water versus dietary uptake of Pb in aquatic invertebrates. Use of stable isotopes has enabled simultaneous measurement of uptake and elimination in several aquatic species 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.

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*), scallop and cuttlefish and was found to be bound principally to organelles (Einsporn et al., 2009; Einsporn & Koehler, 2008). Since the 2006 Pb AQCD, BCF's have been measured for several species in field studies and these BCF's tend to be higher than calculated BCF's from laboratory exposures (Table 7-4).

Tissue accumulation of Pb via gill and dietary uptake has been further characterized in aquatic vertebrates and stable isotope techniques have been applied to further elucidate bioaccumulation of Pb in this ISA. The conclusions of the 2006 Pb AQCD that the gill is a major site of Pb uptake in fish and that there are species differences in the of Pb accumulation and distribution of Pb within the organism are supported in this review. In general, the accumulation of Pb in fish tissues is observed to be gill>kidney>liver. The anterior intestine has been newly identified as a site of uptake of Pb through dietary exposure studies (Alves et al., 2006). Additional detoxification strategies for Pb have been elucidated since the 2006 Pb AQCD. 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 (Goto & Wallace, 2010).

There are few new studies on Pb uptake by amphibians and mammals. 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 higher trophic-level consumers indicating that Pb was bioconcentrated but not biomagnified (Eisler, 2000; U.S. EPA, 2006). Some studies published since the 2006 Pb AQCD 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).

7.3.10.3. Biological Effects

Evidence in this review further supports the findings of the previous Pb AQCDs that waterborne Pb is highly toxic to aquatic organisms, with toxicity varying with species and lifestage, duration of exposure, form of Pb, and water quality characteristics.

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. Effects on plants supported by additional evidence in this review and evidence from previous reviews include oxidative damage, decreased photosynthesis and reduced growth. The mechanism of Pb toxicity in plants is likely mediated by damage to photosystem II through alteration of chlorophyll structure. Elevated levels of antioxidant enzymes are commonly observed in aquatic plant, algae, and moss species exposed to Pb.

As observed in terrestrial invertebrates, upregulation of antioxidant enzymes is a common biomarker of Pb exposure in aquatic invertebrates. Since the 2006 Pb AQCD, there is additional evidence for Pb effects on antioxidant enzymes, lipid peroxidation, stress response and osmoregulation. Studies of reproductive and developmental effects of Pb in aquatic invertebrates in this review provide further support for findings in the 2006 Pb AQCD. These new studies include reproductive endpoints for rotifers and freshwater snails as well as multigenerational effects of Pb in mosquito larvae. Growth effects are observed at lower concentrations in some aquatic invertebrates since the 2006 Pb AQCD, including juveniles of the freshwater snail *L. stagnalis* where growth is affected at <4 µg Pb/L (Grosell et al., 2006b). Behavioral effects of Pb in aquatic invertebrates reviewed in this ISA include decreased valve closing speed in scallops and slower feeding rate in blackworms.

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, behavioral, growth effects and effects on blood parameters have become available since the 2006 Pb AQCD. The mitogen-activated protein kinases, ERK1/2 and $p38^{MAPK}$ were identified for the first time as possible molecular targets for Pb neurotoxicity in a teleost (Leal et al., 2006). Pb toxicity at the fish gill primarily involves disruption of

Ca homeostasis as previously characterized in the 2006 Pb AQCD (Rogers & Wood, 2004; Rogers & 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.

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 of the 2006 Pb AQCD (U.S. EPA, 2006). Since the 2006 Pb AQCD, studies conducted at concentrations approaching environmental levels of Pb have indicated sublethal effects on tadpole endpoints including growth, deformity, and swimming ability.

7.3.10.4. Exposure and Response

Concentration-response data on plants, invertebrates and vertebrates is consistent with findings in previous reviews of species differences in sensitivity to Pb in aquatic systems. Growth in plants continues to be an endpoint adversely affected by Pb exposure. The lowest EC_{50} for growth observed in marine microalgae and freshwater microalgae was in the range of 100 µg/L.

In the 2006 Pb AQCD, concentrations at which effects were observed in aquatic invertebrates ranged from 5 to 8,000 µg Pb/L. Several studies in this review have provided evidence of effects at lower concentrations. Among the most sensitive species, growth of juvenile freshwater snails *L. stagnalis* was inhibited at an EC_{20} of <4 µg Pb/L. (Grosell & Brix, 2009; Grosell et al., 2006b). A chronic value of 10 µg Pb/L obtained in 28-day exposures of 2-month-old *L. siliquoidea* juveniles was the lowest genus mean chronic value ever reported for Pb (N. Wang et al., 2010).

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. However, adverse effects in fish observed in recent studies fall within the range of concentrations observed in the previous AQCD.

7.3.10.5. Community and Ecosystem Effects

Since the publication of the 2006 Pb AQCD, additional evidence for community and ecosystem level effects of Pb have been observed primarily in microcosm studies or field studies with other metals present. One ecological effect reported in previous Pb AQCDs is a shift in community composition in Pb-impacted habitats towards more Pb-tolerant species. New studies in this ISA provide evidence in additional habitats for community composition shifts associated with Pb. Alteration of aquatic plant community composition was demonstrated in the presence of elevated surface water Pb concentrations at three lake sites impacted by mining effluents. Lakes with the highest levels of Pb had the lowest number

of aquatic plant species when compared to sites with lower Pb concentrations. In an aquatic macrophyte community, both plant species and type of habitat were determined to be factors affecting the rate of Pb accumulation from contaminated sediments. While the rooted macrophyte *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 & Osmolovskaya, 2006). This would suggest that certain types of aquatic plants, such as rooted and submerged species, may be most susceptible to atmospherically-deposited Pb, resulting in shifts in plant community composition as a result of Pb pollution.

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.

Sensitive species may become locally extinct from habitats where Pb toxicity is greater. 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. A series of species sensitivity distributions constructed by Brix et al. (2005) 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.

7.3.10.6. Critical Loads, Sensitivity and Vulnerability

Since the 2006 Pb AQCD there is no new significant information on critical loads of Pb in aquatic systems. Recent studies have identified seasonally-affected physiological changes and life-stage as important determinants of differential sensitivity to Pb in aquatic organisms. These factors are in addition to species differences in metabolism, sequestration, and elimination rates, diet, lifestage, genetics, age, and body size that were considered in the 2006 Pb AQCD. Although evidence is available to support Pb impacts to supporting, provisioning, regulating and cultural ecosystem services, there is insufficient data available to adequately quantify these adverse effects.

7.4. Causality Determinations for Lead in Terrestrial and Aquatic Systems

This section presents key conclusions regarding causality determinations for welfare effects of Pb (Table 7-6). Evidence considered in establishing causality was drawn from the 1977 (U.S. EPA, 1977), 1986 (U.S. EPA, 1986) and 2006 Pb AQCD (U.S. EPA, 2006) for Pb where appropriate as well as the current ISA. EPA's framework for causality described in Chapter 1 was applied and the causal determinations are highlighted. In this ISA, effects determined to be causal at the species level contribute to the body of evidence for causal effects at the community and ecosystem scale. Some of the effects of Pb observed in terrestrial and aquatic organisms are also considered in the chapters of the ISA that evaluate evidence for human health effects associated with Pb exposure.

Table 7-6. Summary of Pb causal determinations for plants, invertebrates and vertebrates

Effect	Causality Determination
Bioaccumulation as it Affects Ecosystem Services-All organisms	Causal
Mortality-Plants	Inadequate
Mortality- Invertebrates and Vertebrates	Causal
Growth-Plants	Causal
Growth-Invertebrates	Causal
Growth-Vertebrates	Suggestive
Physiological Stress-All organisms	Causal
Hematological Effects-Invertebrates and Vertebrates	Causal
Development and Reproduction-Invertebrates and Vertebrates	Causal
Development and Reproduction-Plants	Inadequate
Neurobehavior-Invertebrates and Vertebrates	Causal
Community and Ecosystem Level Effects	Causal

7.4.1. Bioaccumulation of Lead in Terrestrial and Aquatic Biota as it Affects Ecosystem Services

Pb deposited on the surface of, or taken up by organisms has the potential to alter the services provided by terrestrial and aquatic biota to humans. Ecosystem services are the benefits people obtain from ecosystems. They include supporting, provisioning, regulating and cultural services that are vital for the functioning of the biosphere and provide the basis for the delivery of tangible benefits to human society. There is compelling evidence over several decades of research that Pb bioaccumulates in plants, invertebrates and vertebrates in terrestrial and aquatic systems. Generally, there are considerable differences between species in the amount of Pb taken up from the environment and in the amounts of Pb

retained in the organism. In order for Pb to reach a biological receptor, the metal must first cross the membranes of organisms to the target organ or site of storage. This process varies between plants, invertebrates and vertebrates, and furthermore, uptake and sequestration are at times similar in unrelated species, while substantially different between related ones. Uptake of Pb from environmental media is dependent upon the bioaccessibility of Pb (reviewed in Chapter 3) which is influenced by many factors including, but not limited to, temperature, pH, presence of humic acid and dissolved organic matter, presence of other metals, and speciation of Pb.

Terrestrial plants accumulate Pb via direct stomatal uptake into foliage and incorporation of atmospherically-deposited Pb from soil into root tissue, followed by variable translocation to other tissues. Near smelters and other point-sources of Pb, leaf uptake may in some cases be greater than root uptake, otherwise root uptake is predominant. Translocation of soil Pb to shoots and leaves is limited in most species as plants sequester a large portion of Pb in root tissue. There are considerable species-dependent differences in rate of uptake and translocation of Pb to other parts of the plant. Uptake and sequestration of Pb primarily in roots of terrestrial plants is also observed in wetland species and algae. Rooted aquatic plants take up Pb primary from sediments while floating aquatic plants take up Pb from water.

Uptake of atmospherically deposited Pb from soil is the primary exposure route in terrestrial plants and invertebrates. Bioaccessibility of Pb to soil-dwelling organisms is influenced by soil factors including soil type, amount of OM, pH, and CEC and there are considerable differences in uptake among species. Species exhibit different accumulation efficiencies and compartmentalize sequestered Pb differently. There is limited evidence of contributions to total Pb body burden via dietary exposures in primary and secondary consumers.

Aquatic organisms can uptake and bioaccumulate Pb from the water column, sediments or via dietary exposure. However, as in terrestrial organisms, uptake and subsequent bioaccumulation of Pb in aquatic plants, invertebrates and vertebrates varies greatly between species and across taxa. Invertebrates may also sequester Pb in the exoskeleton, which is subsequently shed. Pb in aquatic invertebrates is primarily sequestered in the gill and hepatopancreas. Uptake of Pb in fish is well characterized and occurs primarily via direct uptake of dissolved Pb from the water column through gill surfaces and by ingestion of Pb-contaminated diets. Pb in these organisms is primarily sequestered in the gill. In dietary exposures in fish, Pb also targets the anterior intestine indicating the importance of water-only versus dietary uptake exposures.

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. 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). 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. Overall, based on the consistency of findings across taxa, the evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and bioaccumulation of Pb that affects ecosystem services associated with terrestrial and aquatic biota.**

7.4.2. Mortality

The relationship between Pb exposure and mortality has been well demonstrated in terrestrial and aquatic species as presented in Sections 7.2.5 and 7.3.5 of this ISA and in the 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. However, the LC_{50} is usually much higher than current environmental levels of Pb, even though physiological dysfunction that adversely impacts the fitness of an organism often occurs well below concentrations that result in mortality.

Pb is generally not phytotoxic to plants at concentrations found in the environment away from point-sources, 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.

Invertebrates are generally more sensitive to Pb exposure than other taxa, with survival adversely impacted in a few species at concentrations occurring near point-sources or at concentrations that approach ambient levels. These impacted species may include candidate or endangered species. The freshwater mussel *Lampsilis 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. (N. Wang et al., 2010). However, tolerance to Pb varies substantially among invertebrate species. As reviewed in the 2006 Pb AQCD, the LC_{50} values for soil nematodes vary from 10-1,550 mg Pb/kg dry weight dependent upon soil OM content and soil pH (U.S. EPA, 2006). 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. Toxicity of Pb to aquatic invertebrates is highly dependent on water quality parameters such as pH, DOC and Ca^{2+}. For example, 48 hour LC_{50} values for *C. dubia* range from 280 to >2,700 µg Pb/L when tested at varying pH levels (U.S. EPA, 2006). Other invertebrates such as odonates may be tolerant of Pb concentrations that greatly exceed environmental levels. Some invertebrates are able to detoxify Pb such as through sequestration of Pb in the exoskeleton which is subsequently molted.

Early experiments from Pb mining areas indicated local extinction of fish from streams. Mortality in fish is dependent on the sensitivity of the species tested and on water quality parameters. Higher

toxicity tends to occur in acidic waters where more free-Pb ion is available for uptake. The interaction between water quality parameters and Pb toxicity may result in a range of concentrations that cause equivalent toxicity. For example, 96-hr LC_{50} values in fathead minnow range from 810->5,400 µg Pb/L in varying pH and hardness (U.S. EPA, 2006). Increased mortality is also a function of age of the fish. Thirty day LC_{50} values for larval fathead minnows ranged from 39 to 1,903 µg Pb/L in varying concentrations of DOC, $CaSO_4$ and pH (Grosell et al., 2006b). 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 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-day) as reviewed for the development of Eco-SSL's {U.S. EPA, 2005 #19390}. Mortality associated with Pb exposure in vertebrates is supported by the consistently positive associations between Pb exposure and mortality observed in human epidemiologic studies (Section 5.3.1).

The evidence is **inadequate to conclude that there is a causal relationship between Pb exposure and mortality in plants.**

The evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and mortality in sensitive terrestrial and aquatic animal taxa.**

7.4.3. Growth Effects

Evidence for Pb effects on growth is strongest in plants with limited information on invertebrates and vertebrates. There is evidence over several decades of research that Pb inhibits photosynthesis and respiration in plants all of which reduce the growth of the plant (U.S. EPA, 1977, 1986, 2006). Many laboratory toxicity studies report effects on plants; however, there are few field studies. Specifically, Pb has been shown to affect photosystem II with the hypothesized mechanism being that Pb may replace either Mg or Ca in chlorophyll, altering the pigment structure and decreasing the efficiency of visible light absorption in exposed plants. Decreases in chlorophyll *a* and *b* content have been observed in various algal and plant species. The lowest 72-hour EC_{50} for growth inhibition reported for algae was 105 µg Pb/L in Chaetoceros sp. Most primary producers experience EC_{50} values for growth in the range of 1,000 to 100,000 µg Pb/L (U.S. EPA, 2006).

In previous AQCDs, growth effects of Pb have been reported in fish (growth inhibition), birds (changes in juvenile weight gain), and frogs (delayed metamorphosis, smaller larvae). Growth effects observed in invertebrates and vertebrates underscore the importance of lifestage to overall Pb susceptibility. In general, juvenile organisms are more sensitive than adults. Several studies since the 2006 Pb AQCD have demonstrated adverse effects of Pb on growth at lower concentrations than in previous literature. Among the animal taxa tested, aquatic invertebrates were the most sensitive to the effect of Pb,

with adverse effects being reported as low as 4 µg Pb/L. Growth of juvenile freshwater snails *L. stagnalis* was inhibited at EC_{20} <4 µg Pb/L (Grosell & Brix, 2009; Grosell et al., 2006b). In the freshwater mussel, fatmucket (*L. siliquoidea*) juveniles were the most sensitive lifestage (N. Wang et al., 2010). 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. Evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and growth effects in plants and invertebrates.** Evidence is **suggestive of a causal relationship between Pb exposures and growth effects in vertebrates.**

7.4.4. Physiological Stress

In this review and in previous Pb AQCDs there is consistent and coherent evidence that Pb induces oxidative stress in plants, invertebrates, and vertebrates. This is consistent with evidence in humans and experimental animal studies for oxidative stress development, due in many instances to the antagonism of normal metal ion functions (Section 5.2.4). This oxidative stress is characterized by increased reactive oxygen species and membrane and lipid peroxidation that can promote tissue damage, cytotoxicity, and dysfunction. Increased reactive oxygen species are often followed by a compensatory and protective upregulation in antioxidant enzymes, such that this observation is indicative of oxidative stress conditions. Additionally, continuous reactive oxygen species production may overwhelm this defensive process leading to further oxidative stress and injury.

Building on this strong body of evidence presented previously, recent studies provide consistent and coherent 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. There is considerable evidence for antioxidant activity in invertebrates, including gastropods, mussels, and crustaceans, in response to Pb exposure. Markers of oxidative damage are also observed in fish and amphibians. Across all biota, there are differences in the induction of antioxidant enzymes that appear to be species-dependent.

Additional stress responses to Pb exposure observed in terrestrial and aquatic invertebrates including elevated heat shock proteins, osmotic stress, lowered metabolism and decreased glycogen levels have been reported since the 2006 Pb AQCD. Heat shock protein induction by Pb exposure has been observed in zebra mussels and mites. 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). Crayfish exhibit a Pb-induced hypometabolism under conditions of environmental hypoxia in the presence of the metal (Morris et al., 2005). Tissue volume regulation is adversely affected in freshwater crabs (Amado et al., 2006). Glycogen levels in the freshwater snail *B. glabrata* were significantly

decreased at near environmentally relevant concentrations of Pb (50 µg/L and higher) (Ansaldo et al., 2006).

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. Evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and physiological stress in plants, invertebrates and vertebrates.**

7.4.5. Hematological Effects

Hematological responses are commonly reported effects of Pb exposure in invertebrates and vertebrates in both aquatic and terrestrial systems. In environmental assessments of metal-impacted habitats, ALAD is a recognized biomarker of Pb exposure (U.S. EPA, 2006). ALAD activity is negatively correlated with total Pb concentration in bivalves. Lower ALAD activity has been correlated with elevated blood Pb levels in fish and mammals as well. In the 1986 AQCD, decreases in red blood cell ALAD activity following Pb exposure were well documented in birds and mammals. 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 fish, suggest this enzyme is an indicator for Pb exposure across a wide range of taxa. Limited evidence of Pb effects on other blood parameters including altered serum profiles and changes in white blood cell counts in fish and amphibians support the finding of the hematological system as a target for Pb in natural systems. This evidence is strongly coherent with observations from human epidemiologic and animal toxicology studies (Section 5.7). There is consistent toxicological and epidemiologic 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. Taken together, the overall weight of epidemiologic and toxicological evidence is sufficient to conclude that a causal relationship exists between exposure to Pb and hematological effects in humans and laboratory animals (Section 5.7). Based on observations in both terrestrial and aquatic 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 invertebrates and vertebrates.**

7.4.6. Developmental and Reproductive Effects

Evaluation of the literature on Pb effects in aquatic and terrestrial species indicates that exposure to Pb is associated with reproductive effects. Various endpoints have been measured in aquatic and terrestrial organisms to assess the effect of Pb on development, fecundity and hormone homeostasis. Evidence in

this review and the previous Pb AQCDs from invertebrate and vertebrate studies indicate that Pb is adversely affecting reproductive performance in multiple species. However, there are typically only limited studies available from different taxa.

In plants, few studies are available that specifically address reproductive effects of Pb exposure. One study with grass pea showed Pb exposure increased pollen sterility.

Several studies with invertebrates provide evidence for adverse impacts to embryonic development, specifically in snails, clams and rotifers. In addition to affecting the embryo, Pb can alter developmental timing, 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.

Reproductive effects have also been observed in multi-generational studies. Larval settlement rate, and rate of population increase was adversely impacted in rotifers. Rotifers have decreased fertilization rate associated with Pb exposure that appeared to be due to decreased viability of sperm and eggs. 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.

In 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 subtle skeletal malformations. Previous Pb AQCDs have reported developmental effects in fish, specifically spinal deformities in larvae. 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 fish, there is new evidence of Pb in this ISA on effects on steroid profiles and additional reproductive parameters. Reproduction in fathead minnows was affected in breeding exposures following 300 day chronic toxicity testing. However, reproductive performance was unaffected in zebrafish exposed to Pb-contaminated prey. Additional reproductive parameters in fish observed to be impacted by Pb include decreased oocyte diameter and density in toadfish associated with elevated Pb levels in gonad.

In terrestrial vertebrates, evidence from Chapter 7 of this ISA and in previous Pb AQCDs indicates an association between observed adverse reproductive effects and Pb exposure. Reproductive effects observed in birds near areas of Pb-contamination or where Pb tissue concentration has been correlated with effects include declines in clutch size, number of young hatched, number of young fledged, decreased fertility, and decreased eggshell thickness. Decreased testis weight was observed in lizards. In mammals, few studies in the field have addressed Pb specifically, due to most available data in wild or grazing animals being from near smelters, where animals are co-exposed to other metals.

In Chapter 5 evidence from mammals demonstrates a consistency of adverse effects of Pb on sperm and the onset of puberty in males and females 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. New toxicological data support trans-generational effects, a finding that is also an area of emerging interest in ecology. The evidence presented in Section 5.8 is sufficient to conclude that there is a causal relationship between Pb exposure and reproductive effects.

Adverse effects of Pb on reproduction in invertebrates and vertebrates indicate that Pb is likely adversely affecting fecundity of Pb-exposed organisms in both aquatic and terrestrial habitats, and therefore the evidence is sufficient to conclude that there is a **causal relationship between Pb exposures and reproductive effects in terrestrial and aquatic invertebrates and vertebrates.** In plants, the evidence is **inadequate to conclude a causal relationship between Pb exposures and plant reproductive effects.**

7.4.7. Neurobehavioral Effects

Evidence from laboratory studies and limited data from field studies reviewed in Chapter 7 have shown adverse effects of Pb on neurological endpoints in both aquatic and terrestrial animal taxa. These include changes in behaviors that may decrease the overall fitness of the organism. There is also evidence from both invertebrate and vertebrate studies that Pb adversely affects behaviors that may decrease the ability of an organism to escape predators or capture prey.

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). Additionally, there is new evidence for neurotoxic action of Pb in invertebrates with exposure to Pb observed to cause changes in the morphology of GABA motor neurons in nematodes (*C. elegans*) (Du & Wang, 2009).

Decreased food consumption of Pb-contaminated diet has been demonstrated in some invertebrates (snails) and vertebrates (lizards, pigs). 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. In limited studies available on snails, tadpoles, scallops, hatchling turtles and fish there is evidence that Pb may affect the ability to escape or avoid predation. The motility of nematodes was adversely affected in Pb-contaminated soils (D. Y. Wang & Xing, 2008). 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 & Gochfeld, 2005). Lizards exposed to Pb through diet in the laboratory exhibited abnormal coloration and posturing behaviors. Other behavioral effects affected by Pb exposure include increased hyperactivity in fish and hypoxia-like behavior in frogs.

These findings are coherent with findings from studies in laboratory animals as described in Section 5.3 that show that Pb induces changes in learning, memory, attention and motor skills. Pb induced new behaviors including hyperactivity and mood disorders. Within the sensory organs, the visual and auditory systems are affected by Pb exposure. Changes in structure and function of neurons and supporting cells in the brain are detailed including effects on the blood brain barrier. Mechanisms including the displacement of physiological cations, oxidative stress and changes in neurotransmitters and receptors are detailed. Based on evidence from several cohort and cross-sectional studies in diverse populations, the overall weight of the available evidence provides clear and consistent evidence of association between blood Pb concentrations and decrements in neurodevelopmental outcomes in young children (Section 5.1). In addition to the consistency of findings in children, the evidence is strengthened by the coherence of findings with toxicological studies and by coherence of association of blood Pb with a spectrum of related endpoints including IQ, verbal and reading skills, motor coordination, mood and attention problems, and behavioral problems. The evidence presented in the health chapter is sufficient to conclude that there is a causal relationship between Pb exposure and neurobehavioral effects (Section 5.3). These data from laboratory toxicology studies, especially neurobehavioral findings and structural changes are highly coherent with data from ecological studies. Overall, the evidence from aquatic and terrestrial systems is sufficient to conclude that there is a **causal relationship between Pb exposures and neurobehavioral effects in invertebrates and vertebrates.**

7.4.8. Other Physiological Effects

In addition to the above mentioned physiological effects of Pb on organisms in terrestrial and aquatic systems for which there is sufficient evidence to infer causality, there are a few recent studies that can be linked to effects observed in humans for which there is insufficient evidence across taxa. Pb exposure has been demonstrated to result in changes to DNA structure and chromosomal alterations in plants, gastropods, mussels and fish. DNA damage, chromosomal damage and aberrations, and micronucleus formation are also observed in humans and laboratory animals exposed to Pb (Section 5.10). Additional new evidence in this review indicates that Pb can interfere with renal function in fish, specifically with ionoregulation of Na and Cl and reabsorption Ca^{2+}, Mg^{2+} glucose and water (Patel et al., 2006). In humans and laboratory animals, Pb is a recognized nephrotoxicant and is considered to be causal of kidney damage (Section 5.5).

7.4.9. Community and Ecosystem Level Effects

Uptake of Pb into aquatic and terrestrial organisms and subsequent effects on survival, reproduction, growth, behavior and other physiological variables at the species scale are likely to result in effects at the population, community and ecosystem scale. The effects may include alteration of predator-

prey dynamics, species richness, species composition, and biodiversity. There are few field studies available that directly consider effects of Pb on these measures of ecosystem health. Ecosystem-level studies are complicated by the confounding of Pb exposure with other factors such as trace metals and acidic deposition. In natural systems, Pb is often found co-existing with other stressors, and observed effects may be due to cumulative toxicity.

Most direct evidence of community and ecosystem level effects is from near point sources. For terrestrial systems there are several decades of research on impacts to natural ecosystems near smelters, mines, and other industrial sources of Pb where Pb levels are elevated. Those impacts include decreases in species diversity and changes in floral and faunal community composition. For aquatic systems, the literature focuses on evaluating ecological stress from Pb originating from urban and mining effluents rather than atmospheric deposition. 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. Effects of Pb on mortality, growth, physiological stress, blood, neurobehavior and developmental and reproductive endpoints at the individual level are expected to have ecosystem-level consequences, and thus provide consistency and plausibility for causality in ecosystem-level effects.

Avoidance response to Pb exposure varies widely in different species and this could affect community composition. For example, frogs and toads lack avoidance response while snails and fish avoid higher concentrations of Pb (U.S. EPA, 2006). New evidence since the 2006 Pb AQCD indicates that some species of worms avoid Pb-contaminated soils (Langdon et al., 2005)

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 was shown to ameliorate effects of Pb contamination on a microbial community.

In aquatic ecosystems there are numerous field studies on reductions of species abundance, richness or diversity particularly in benthic macroinvertebrate communities coexisting with other metals. 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 & Antonio-Garcia, 1988)

Since the last Pb AQCD, there is further evidence for effects of Pb in sediment-associated communities. Exposure to three levels of sediment Pb contamination (322, 1,225, and 1,465 µg Pb/g dry

weight) in a microcosm experiment significantly reduced nematode diversity and resulted in profound restructuring of the community structure (Mahmoudi et al., 2007). Sediment-bound Pb contamination appears to differentially affect members of the benthic invertebrate community, potentially altering ecosystems dynamics in small urban streams (Kominkova & 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 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.

In a new 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) (V. K. Mishra et al., 2008). This shift toward more Pb-tolerant species is also observed in terrestrial plant communities near smelter sites (U.S. EPA, 1986, 2006). 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.

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 in natural systems. However, the evidence for Pb effects at higher levels of ecological organization is sufficient to conclude that there is a **causal relationship between Pb exposures and the alteration of species richness, species composition and biodiversity in terrestrial and aquatic ecosystems.**

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