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Integrated Science Assessment for Lead

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

Lead Project Team

Executive Direction

Dr. John Vandenberg (Director)—National Center for Environmental Assessment-RTP Division, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Debra Walsh (Deputy Director)—National Center for Environmental Assessment-RTP Division, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Mary Ross (Branch Chief)—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Doug Johns (Acting Branch Chief)—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Scientific Staff

Dr. Ellen Kirrane (Pb Team Leader)—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. James Brown—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Allen Davis—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Jean-Jacques Dubois—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Tara Greaver—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Erin Hines—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Dennis Kotchmar—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Meredith Lassiter—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Stephen McDow—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Qingyu Meng—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Elizabeth Oesterling Owens—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Molini Patel—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr Joseph P. Pinto—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Jennifer Richmond-Bryant—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Lindsay Stanek—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. David Svendsgaard—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Lisa Vinikoor-Imler—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Technical Support Staff

Mr. Kenneth J. Breito–Senior Environmental Employment Program, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Ellen Lorang—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. J. Sawyer Lucy–Student Services Authority, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Deborah Wales—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Richard N. Wilson–National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Barbara Wright—Senior Environmental Employment Program, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Authors, Contributors, and Reviewers

Authors

Dr. Ellen Kirrane (Pb Team Leader)—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Robyn Blain— Energy, Environment and Transportation, Environmental Science & Policy, ICF International, Lexington, MA

Dr. James Brown—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Allen Davis—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Gary Diamond—Syracuse Research Corporation, Akron, NY

Dr. Rodney Dietert—Cornell University College of Veterinary Medicine, Veterinary Medical Center, Ithaca, NY

Dr. Jean-Jacques Dubois—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Anne Fairbrother—Exponent, Inc., Bellevue, WA

Dr. Jay Gandy—Department of Environmental and Occupational Health, University of Arkansas for Medical Sciences, Little Rock, AR

Dr. Harvey Gonick—David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA

Dr. Margaret Graham—School of Geosciences, University of Edinburgh, Edinburgh, Scotland

Dr. Tara Greaver—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Erin Hines—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Dennis Kotchmar—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Meredith Lassiter—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Stephen McDow—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Qingyu Meng—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Bill Mendez—Energy, Environment and Transportation, Environmental Science & Policy, ICF International, Fairfax, VA

Dr. Howard Mielke—Center for Bioenvironmental Research, Tulane/Xavier Universities, New Orleans, LA

Ms. Chandrika Moudgal— Energy, Environment and Transportation, Environmental Science & Policy, ICF International, Dublin, CA

Dr. Elizabeth Oesterling Owens—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Katherine Palmquist—Exponent, Inc., Bellevue, WA

Dr. Molini Patel—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr Joseph P. Pinto—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Jennifer Richmond-Bryant—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Stephen Rothenberg—National Institute of Public Health, Cuernavaca, Morelos, Mexico

Dr. MaryJane Selgrade—Energy, Environment and Transportation, Environmental Science & Policy, ICF International, RTP, NC

Dr. Lindsay Stanek—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. David Svendsgaard—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Lisa Vinikoor-Imler—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Virginia Weaver—Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Dr. Marc Weisskopf—Department of Environmental Health and Department of Epidemiology, Harvard School of Public Health, Harvard University, Boston, Massachusetts

Dr. John Pierce Wise, Sr.—Maine Center for Toxicology and Environmental Health, Department of Applied Medical Sciences, Portland, ME

Dr. Rosalind Wright—Harvard Medical School and School of Public Health, Harvard University, Boston, MA

Dr. Robert Wright—Harvard Medical School and School of Public Health, Harvard University, Boston, MA

Contributors

Mr. Brian Adams—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Halil Cakir— Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Ye Cao—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Laura Datko—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Josh Drukenbrod—Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, RTP, NC

Mr. Mark Schmidt—Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Katie Shumake—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Kaylyn Siporin—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Lauren Tuttle—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Adrien Wilkie—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Brianna Young—Oak Ridge Institute for Science and Education, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Reviewers

Dr. Christal Bowman—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. David Buchwalter—Department of Toxicology, North Carolina State University, Raleigh, NC

Dr. Barbara Buckley—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Kevin Cavender—Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Rebecca C. Dzubow—Office of Children's Health Protection, U.S. Environmental Protection Agency, Washington, DC

Dr. David DeMarini—National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Pam Factor-Litvak—Department of Epidemiology, Mailman School of Public Health, New York, NY

Dr. Gabriel Filippelli—Department of Earth Sciences, Indiana University-Purdue University, Indianapolis, IN

Dr. Andrew Friedland—Environmental Studies Program, Darmouth College, Hanover, NH

Dr. Barbara Glenn—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Dr. Jeff Herrick—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Marion Hoyer—Office of Transportation and Air Quality, Office of Air and Radiation, U.S. Environmental Protection Agency, Ann Arbor, MI

Dr. Douglas Johns—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Thomas Luben—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Karen Martin—Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Connie Meacham—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Marie Lynn Miranda—Environmental Sciences and Policy, Nicholas School of the Environment, Duke University, Durham, NC

Dr. Deirdre Murphy—Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Paul Mushak—PB Associates, Durham NC

Dr. Kris Novak—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. David Orlin—Air and Radiation Law Office, Office of General Counsel, U.S. Environmental Protection Agency, Washington, DC

Dr. Meredith Pedde—Office of Transportation and Air Quality, Office of Air and Radiation, U.S. Environmental Protection Agency, Ann Arbor, MI

Dr. Pradeep Rajan—Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Joanne Rice—Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Mary Ross—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Joel Schwartz—Department of Environmental Health, Harvard School of Public Health, Boston, MA

Mr. Jason Sacks—National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Ginger Tennant—Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Jay Turner—Environmental and Chemical Engineering Department, Washington University, St. Louis, MO

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

Dr. Robert W. Vanderpool—National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Nosratola Vaziri—Division of Nephrology and Hypertension, School of Medicine, University of California, Irvine

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

Dr. Nasser Zawia—Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island

Clean Air Scientific Advisory Committee Lead NAAQS Review Panel

Chair of the Environmental Protection Agency's Clean Air Scientific Advisory Committee

Dr. Jonathan M. Samet*, Department of Preventive Medicine at the Keck School of Medicine, and Director of the Institute for Global Health at the University of Southern California, Los Angeles, CA

Chair of the Lead Review Panel

Dr. Christopher H. Frey*, North Carolina State University, Raleigh, NC

Lead Review Panel Members

Dr. George A. Allen*, Northeast States for Coordinated Air Use Management (NESCAUM), Boston, MA

Dr. Herbert Allen, University of Delaware, Newark, DE

Dr. Richard Canfield, Cornel University, Ithaca, NY

Dr. Deborah Cory-Slechta, University of Rochester, Rochester, NY

Dr. Cliff Davidson, Syracuse University, Syracuse, NY

Dr. Philip E. Goodrum, Environmental Resources Management (ERM), Dewitt, NY

Dr. Sean Hays, Summit Toxicology, Allenspark, CO

Dr. Philip Hopke, Clarkson University, Potsdam, NY

Dr. Susan Korrick, Harvard Medical School, Boston, MA

Dr. Michael Kosnett, University of Colorado Health Sciences Center, Denver, CO

Dr. Roman Lanno, Ohio State University, Columbus, OH

Dr. Richard L. Poirot, Vermont Agency of Natural Resources, Waterbury, VT

Dr. Joel Pounds, Battelle-Pacific Northwest National Laboratory, Richland, WA

Dr. Michael Rabinowitz, Harvard University, Newport, RI

Dr. William Stubblefield, Oregon State University, Corvallis, OR

Dr. Ian von Lindern, TerraGraphics Environmental Engineering, Inc., Moscow, ID

Dr. Gail Wasserman, Columbia University, New York, NY

Dr. Michael Weitzman, New York University School of Medicine, New York, NY

* Members of the statutory Clean Air Scientific Advisory Committee (CASAC) appointed by the EPA Administrator

Science Advisory Board Staff

Mr. Aaron Yeow, Designated Federal Officer, Office of Administration, Science Advisory Board Staff Office, U.S. Environmental Protection Agency, Washington, DC 20004. Phone: 202-564-2050, Email: yeow.aaron@epa.gov

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-Co	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;
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 underneath 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 ($NO + NO_2$)

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 a nominal aerodynamic diameter less than or equal to 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 a nominal aerodynamic diameter less than or equal to 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.
$PM_{10\text{-}2.5}$	In general terms, particulate matter with a nominal aerodynamic diameter less than or equal to 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 $PM_{10\text{-}2.5}$ concentration of $PM_{10\text{-}2.5}$ 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: U.S. Environmental Protection Agency

USF uptake slope factor

USGS U.S. Geological Survey

USL urban surface layer

UUDS urban dynamic driving schedule

UV ultraviolet radiation

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

PREAMBLE

Process of ISA Development

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

The fundamental process for developing an ISA includes:

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

An initial step in this process is publication of a call for information in the Federal Register that invites the public to provide information relevant to the assessment, such as new publications on health or welfare[1] effects of the pollutant, or from atmospheric and exposure sciences fields. EPA maintains an ongoing literature search process for identification of relevant scientific studies published since the last review of the NAAQS. Search strategies are designed for pollutants and scientific disciplines and iteratively modified to optimize identification of pertinent publications. Papers are identified for inclusion in several additional ways: specialized searches on specific topics; independent review of tables of contents for journals in which relevant papers may be published; independent identification of relevant literature by expert scientists; review of citations in previous assessments and identification by the public and CASAC during the external review process. References considered for inclusion in the ISA can be found at http://hero.epa.gov/; the references cited in the ISA include a hyperlink to each reference.

[1] Welfare effects as defined in Clean Air Act (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."

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

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

Selection of studies for inclusion in the ISA is based on the general scientific quality of the study, and consideration of the extent to which the study is informative and policy-relevant. Policy relevant and informative studies include those that provide a basis for or describe the relationship between the criteria pollutant and effects, including studies that offer innovation in method or design and studies that reduce uncertainty on critical issues, such as analyses of confounding or effect modification by copollutants or other variables, analyses of concentration-response or dose-response relationships, or analyses related to time between exposure and response. Emphasis is placed on studies that examine effects associated with pollutant concentrations relevant to current population and ecosystem exposures, and particularly those pertaining to concentrations currently found in ambient air. Other studies are included if they contain unique data, such as a previously unreported effect or MOA for an observed effect, or examine multiple concentrations to elucidate exposure-response relationships. In general, in assessing the scientific quality and relevance of health and welfare effects studies, the following considerations have been taken into account when selecting studies for inclusion in the ISA.

- Are the study populations, subjects, or animal models adequately selected, and are they sufficiently well defined to allow for meaningful comparisons between study or exposure groups?

- Are the statistical analyses appropriate, properly performed, and properly interpreted? Are likely covariates adequately controlled or taken into account in the study design and statistical analysis?
- Are the air quality data, exposure, or dose metrics of adequate quality and sufficiently representative of information regarding ambient conditions?
- Are the health, ecological or welfare effect measurements meaningful, valid and reliable?
- Do the analytical methods provide adequate sensitivity and precision to support conclusions?

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

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

Evaluation of controlled human exposure studies focuses on those that approximated expected human exposure conditions in terms of concentration and duration. Studies should include control exposures to filtered air, as appropriate. In the selection of controlled human exposure studies, emphasis is placed on studies that: (1) investigate potentially at-risk populations and lifestages such as people with asthma or cardiovascular diseases, children or older adults; (2) address issues such as concentration-response or time-course of responses; and (3) have sufficient statistical power to assess findings.

Review of the animal toxicological evidence focuses on studies that approximate expected human dose conditions, which vary depending on the dosimetry, toxicokinetics and biological sensitivity of the particular laboratory animal species or strains studied. Emphasis is placed on studies that: (1) investigate animal models of disease that can

provide information on populations potentially at increased risk of effects; (2) address issues such as concentration-response or time-course of responses; and (3) have sufficient statistical power to assess findings. Due to resource constraints on exposure duration and numbers of animals tested, animal studies typically utilize high-concentration exposures to acquire data relating to mechanisms and assure a measurable response. Emphasis is placed on studies using doses or concentrations generally within 1-2 orders of magnitude of current levels. Studies with higher concentration exposures or doses are considered to the extent that they provide useful information to inform our understanding of interspecies differences and potential differences between healthy and susceptible human populations. Results from in vitro studies may also be included if they provide mechanistic insight or further support for results demonstrated in vivo.

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

In developing an ISA, EPA reviews and summarizes the evidence from: studies of atmospheric sciences and exposure; the health effects evidence from toxicological, controlled human exposure and epidemiologic studies; and ecological and welfare effects evidence. In the process of developing the first draft ISA, EPA may convene a public workshop in which EPA and non-EPA experts review the scientific content of preliminary draft materials to ensure that the ISA is up to date and focused on the most policy-relevant findings, and to assist EPA with integration of evidence within and across disciplines.

EPA integrates the evidence from across scientific disciplines or study types and characterizes the weight of evidence for relationships between the pollutant and various outcomes. The integration of evidence on health, and ecological or welfare effects, involves collaboration between scientists from various disciplines. As an example, an evaluation of health effects evidence would include the integration of the results from epidemiologic, controlled human exposure, and toxicological studies, and application of the causal framework (described below) to draw conclusions. Using the causal framework described in the following section, EPA scientists consider aspects such as

strength, consistency, coherence, and biological plausibility of the evidence, and develop draft causality determinations on the nature of the relationships. Causality determinations often entail an iterative process of review and evaluation of the evidence. Two drafts of the ISA are typically released for review by the CASAC and the public, and comments received on the characterization of the science as well as the implementation of the causal framework are carefully considered in revising and completing the final ISA.

EPA Framework for Causal Determination

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

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

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

Evaluating Evidence for Inferring Causation

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

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

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

Publication bias is a source of uncertainty regarding the magnitude of health risk estimates. It is well understood that studies reporting non-null findings are more likely to

be published than reports of null findings, 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 (Bell et al., 2005).

Consideration of Evidence from Scientific Disciplines

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

Direct evidence of a relationship between pollutant exposures and human health effects comes from controlled human exposure studies. Controlled human exposure studies experimentally evaluate the health effects of administered exposures in human volunteers under highly controlled laboratory conditions. Also referred to as human clinical studies, these experiments allow investigators to expose subjects to known concentrations of air pollutants under carefully regulated environmental conditions and activity levels. In some instances, controlled human exposure studies can also be used to characterize concentration-response relationships at pollutant concentrations relevant to ambient conditions. Controlled human exposures are typically conducted using a randomized crossover design, with subjects exposed both to the pollutant and a clean air control. In this way, subjects serve as their own controls, effectively controlling for many potential confounders. However, controlled human exposure studies are limited by a number of factors, including small sample size and short exposure time. For example, exposure patterns relevant to understanding real-world exposures, especially long-term exposures, are generally not practical to replicate in a laboratory setting. In addition, although subjects do serve as their own controls, personal exposure to pollutants in the hours and days preceding the controlled exposures may vary significantly between and within individuals. Finally, controlled human exposure studies require investigators to adhere to stringent health criteria for subjects included in the study, and therefore the results often cannot be generalized to an entire population. Although some controlled human exposure studies have included health-compromised individuals such as those with respiratory or

cardiovascular disease, these individuals must also be relatively healthy and may not represent the most sensitive individuals in the population. In addition, the study design is limited to exposures and endpoints that are not expected to result in severe health outcomes. Thus, not observing an effect in controlled human exposure studies does not necessarily mean that a causal relationship does not exist. While controlled human exposure studies provide important information on the biological plausibility of associations observed in epidemiologic studies, observed effects in these studies may underestimate the response in certain populations.

Epidemiologic studies provide important information on the associations between health effects and exposure of human populations to ambient air pollution. In epidemiologic or observational studies of humans, the investigator generally does not control exposures or intervene with the study population. Broadly, observational studies can describe associations between exposures and effects. These studies fall into several categories: e.g., cross-sectional, prospective cohort, panel and time-series studies. "Natural experiments" offer the opportunity to investigate changes in health related to a change in exposure, such as closure of a pollution source.

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

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

Several statistical methods are available to detect and control for potential confounders, 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 likely causal pollutant from multipollutant regression models is made difficult by the possibility that one or more air pollutants may be acting as a surrogate for an unmeasured or poorly measured pollutant or for a particular mixture of pollutants. In addition, more than one pollutant may exert similar health effects, resulting in independently observed associations for multiple pollutants. The number and degree of diversity of covariates, as well as their relevance to the potential confounders, remain matters of scientific judgment. Despite these limitations, the use of multipollutant models is still the prevailing approach employed in most air pollution epidemiologic studies and provides some insight into the potential for confounding or interaction among pollutants.

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

Another important consideration in the evaluation of epidemiologic evidence is effect modification, which occurs when the effect differs between subgroups or strata; for example, effect estimates that vary by age group or potential risk factor. “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 and Greenland, 1998). When a risk factor is a confounder, it is the true cause of the association observed between the exposure and the outcome; when a risk factor is an effect modifier, it changes the magnitude of the association between the exposure and the outcome in stratified analyses. For example, the presence of a pre-existing disease or indicator of low socioeconomic status may be an effect modifier in causing increased risk of effects related to air pollution exposure. It is often possible to stratify the relationship between health outcome and exposure by one or more of these potential effect modifiers. For variables that modify the association, effect estimates in each stratum will be different from one another

and different from the overall estimate, indicating a different exposure-response relationship may exist in populations represented by these variables.

Another key consideration for ambient air pollution epidemiologic studies is exposure measurement error. There are several components that contribute to exposure measurement error in these 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.

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

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

For ecological effects assessment, both laboratory and field studies (including field experiments and observational studies) can provide useful data for causality determination. Because conditions can be controlled in laboratory studies, responses may be less variable and smaller differences easier to detect. However, the control conditions may limit the range of responses (e.g., animals may not be able to seek alternative food

sources), so they may not reflect responses that would occur in the natural environment. In addition, larger-scale processes are difficult to reproduce in the laboratory.

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

Intermediate between laboratory and field are studies that use environmental media collected from the field to examine response in the laboratory, and experiments that are performed in the natural environment while controlling for some environmental conditions (i.e., mesocosm studies). This type of study in manipulated natural environments can be considered a hybrid between a field experiment and laboratory study since some aspects are performed under controlled conditions but others are not. They make it possible to observe community and/or ecosystem dynamics, and provide strong evidence for causality when combined with findings of studies that have been made under more controlled conditions.

Application of Framework for Causal Determination

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

To aid judgment, various "aspects"[1] of causality have been discussed by many philosophers and scientists. The 1964 Surgeon General's report on tobacco smoking discussed criteria for the evaluation of epidemiologic studies, focusing on consistency, strength, specificity, temporal relationship, and coherence (HEW, 1964). Sir Austin Bradford Hill (1965) articulated aspects of causality in epidemiology and public health that have been widely used (Samet and Bodurow, 2008; IARC, 2006b; U.S. EPA, 2005c; CDC, 2004). These aspects (Hill, 1965) have been modified (Table I) for use in causal

[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.

determinations specific to health and welfare effects for pollutant exposures (U.S. EPA, 2009).[1] Although these aspects provide a framework for assessing the evidence, they do not lend themselves to being considered in terms of simple formulas or fixed rules of evidence leading to conclusions about causality (Hill, 1965). For example, one cannot simply count the number of studies reporting statistically significant results or statistically nonsignificant results and reach credible conclusions about the relative weight of the evidence and the likelihood of causality. Rather, these aspects 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 I cannot be used as a strict checklist, but rather to determine the weight of the evidence for inferring causality. In particular, not meeting one or more of the principles does not automatically preclude a determination of causality [see discussion in (CDC, 2004)].

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

Table I Aspects to aid in judging causality

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

Determination of Causality

In the ISA, EPA assesses the body of relevant literature, building upon evidence available during previous NAAQS reviews, to draw conclusions on the causal relationships between relevant pollutant exposures and health or environmental effects. ISAs use a

five-level hierarchy that classifies the weight of evidence for causation[1]. In developing this hierarchy, EPA has drawn on the work of previous evaluations, most prominently the IOM's *Improving the Presumptive Disability Decision-Making Process for Veterans* (Samet and Bodurow, 2008), EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005c), and the U.S. Surgeon General's smoking report (CDC, 2004), *The Health Consequences of Smoking: A Report of the Surgeion General*. 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 II.

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

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

Table II Weight of evidence for causal determination

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

	Health Effects	Ecological and Welfare Effects
Not likely to be a causal relationship	Evidence is suggestive of no causal relationship with relevant pollutant exposures. Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter and considering at-risk populations, are mutually consistent in not showing an effect at any level of exposure.	Several adequate studies, examining relationships with relevant exposures, are consistent in failing to show an effect at any level of exposure.

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

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

Quantitative Relationships: Effects on Human Populations

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

- What is the concentration-response, exposure-response, or dose-response relationship in the human population?
- What is the interrelationship between incidence and severity of effect?

- What exposure conditions (dose or exposure, duration and pattern) are important?
- What populations and lifestages appear to be differentially affected (i.e., more at risk of experiencing effects)?

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

An important consideration in characterizing the public health impacts associated with exposure to a pollutant is whether the concentration-response relationship is linear across the range of concentrations or if nonlinear relationships exist along any part of this range. Of particular interest is the shape of the concentration-response curve at and below the level of the current standards. Various sources of variability and uncertainty, such as low data density in the lower concentration range, possible influence of exposure measurement error, and variability between individuals in susceptibility to air pollution health effects, tend to smooth and "linearize" the concentration-response function, and thus can obscure the existence of a threshold or nonlinear relationship. Since individual thresholds vary from person to person due to individual differences such as genetic level susceptibility or preexisting disease conditions (and even can vary from one time to another for a given person), it can be difficult to demonstrate that a threshold exists in a population study. These sources of variability and uncertainty may explain why the available human data at ambient concentrations for some environmental pollutants (e.g., particulate matter [PM], ozone [O_3], lead [Pb], environmental tobacco smoke [ETS], radiation) do not exhibit thresholds for cancer or noncancer health effects, even though likely mechanisms include nonlinear processes for some key events. These attributes of human population dose-response relationships have been extensively discussed in the broader epidemiologic literature (Rothman and Greenland, 1998).

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

Quantitative Relationships: Effects on Ecosystems or Public Welfare

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

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

Evaluations of causality generally consider the probability of quantitative changes in ecological and welfare effects in response to exposure. A challenge to the quantification of exposure-response relationships for ecological effects is the great regional and local spatial variability, as well as temporal variability, in ecosystems. Thus, exposure-response relationships are often determined for a specific ecological system and scale, rather than at the national or even regional scale. Quantitative relationships therefore are available site by site and may differ greatly between ecosystems.

Concepts in Evaluating Adversity of Health Effects

In evaluating health evidence, a number of factors can be considered in delineating between adverse and nonadverse health effects resulting from exposure to air pollution. Some health outcomes, such as hospitalization for respiratory or cardiovascular diseases, are clearly considered adverse. It is more difficult to determine the extent of change that constitutes adversity in more subtle health measures. These include a wide variety of responses, such as alterations in markers of inflammation or oxidative stress, changes in pulmonary function or heart rate variability, or alterations in neurocognitive function measures. The challenge is determining the magnitude of change in these measures when there is no clear point at which a change become adverse; for example, what percentage change in a lung function measure represents an adverse effect. What constitutes an adverse health effect may vary between populations. Some changes that may not be considered adverse in healthy individuals would be potentially adverse in more 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). An air pollution-induced shift in the population distribution of a given risk factor for a health outcome was viewed as adverse, even though it may not increase the risk of any one individual to an unacceptable level. For example, a population of asthmatics could have a distribution of lung function such that no identifiable individual has a level associated with significant impairment. Exposure to air pollution could shift the distribution such that no identifiable individual experiences clinically relevant effects. This shift toward decreased lung function, however, would be considered adverse because individuals within the population would have diminished reserve function and therefore would be at increased risk to further environmental insult. The committee also observed that elevations of biomarkers, such as cell number and types, cytokines and reactive oxygen species, may signal risk for ongoing injury and clinical effects or may simply indicate transient responses that can provide insights into mechanisms of injury, thus illustrating the lack of clear boundaries that separate adverse from nonadverse effects.

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

that range up to responses that are very clearly adverse, such as hospitalization or mortality.

Concepts in Evaluating Adversity of Ecological Effects

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

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

On a broader scale, ecosystem services may provide indicators for ecological impacts. Ecosystem services are the benefits that people obtain from ecosystems (UNEP, 2003). According to the *Millennium Ecosystem Assessment*, ecosystem services include:

"provisioning services such as food and water; regulating services such as regulation of floods, drought, land degradation, and disease; supporting services such as soil formation and nutrient cycling; and cultural services such as recreational, spiritual, religious and other nonmaterial benefits." For example, a more subtle ecological effect of pollution exposure may result in a clearly adverse impact on ecosystem services if it results in a population decline in a species that is recreationally or culturally important.

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PREFACE

Legislative Requirements for the NAAQS Review

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

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

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

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

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

Section 109(d)(1) requires that "not later than December 31, 1980, and at 5-year intervals thereafter, the Administrator shall complete a thorough review of the criteria published under section 108 and the national ambient air quality standards … and shall make such revisions in such criteria and standards and promulgate such new standards as may be appropriate … ." Section 109(d)(2) requires that an independent scientific review committee "shall complete a review of the criteria … and the national primary and secondary ambient air quality standards … and shall recommend to the Administrator any new … standards and revisions of existing criteria and standards as may be appropriate

… .” Since the early 1980's, this independent review function has been performed by the Clean Air Scientific Advisory Committee (CASAC).

History of the NAAQS for Pb

On October 5, 1978, EPA initially promulgated primary and secondary NAAQS for Pb under Section 109 of the Act (43 FR 46246). Both primary and secondary standards were set at a level of 1.5 micrograms per cubic meter ($\mu g/m^3$), measured as Pb in total suspended particles (Pb-TSP), not to be exceeded by the maximum arithmetic mean concentration averaged over a calendar quarter. These standards were based on the 1977 Air Quality Criteria for Lead (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 Air Quality Criteria for Lead (U.S. EPA, 1986a), the associated Addendum (U.S. EPA, 1986c) and the 1990 Supplement (U.S. EPA, 1990a). As part of the review, the Agency designed and performed human exposure and health risk analyses (U.S. EPA, 1989), the results of which were presented in a 1990 Staff Paper (U.S. EPA, 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 developed during the review and the significantly changed circumstances since Pb was listed in 1976, the Agency did not propose any revisions to the 1978 Pb NAAQS. In a parallel effort, the Agency developed the broad, multi-program, multimedia, integrated U.S. Strategy for Reducing Lead Exposure (U.S. EPA, 1991). As part of implementing this strategy, the Agency focused efforts primarily on regulatory and remedial clean-up actions aimed at reducing Pb exposures from a variety of non-air sources judged to pose more extensive public health risks to U.S. populations, as well as on actions to reduce Pb emissions to air, such as bringing more areas into compliance with the existing Pb NAAQS (U.S. EPA, 1991).

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

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

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

The November 2008 notice described EPA's decision to revise the primary and secondary NAAQS for Pb from a level of 1.5 $\mu g/m^3$ to a level of 0.15 $\mu g/m^3$. EPA's decision on the level for the primary standard was based on the much-expanded health effects evidence on neurocognitive effects of Pb in children. The level of 0.15 $\mu g/m^3$ was established to protect against air Pb-related health effects, including 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

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).

estimates. The averaging time was revised to a rolling three-month period with a maximum (not-to-be-exceeded) form, evaluated over a three-year period. As compared to the previous averaging time of calendar quarter, this revision was considered to be more scientifically appropriate and more health protective. The rolling average gives equal weight to all three-month periods, and the new calculation method gives equal weight to each month within each three-month period. Further, the rolling average yields 12 three-month averages each year to be compared to the NAAQS versus four averages in each year for the block calendar quarters pertaining to the previous standard. The indicator of Pb-TSP was retained, reflecting the evidence that Pb particles of all sizes pose health risks. The secondary standard was revised to be identical in all respects to the revised primary standards.[1]

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.2 One aspect of the new data handling requirements is the allowance for the use of Pb-PM_{10} monitoring for Pb NAAQS attainment purposes in certain limited circumstances at non-source-oriented sites. The monitoring network requirements resulted in a substantial number of new monitors being required as of January 2010, Subsequent to the 2008 rulemaking, additional revisions were made to the monitoring network requirements, which required additional monitors as of December 2011; the complete current requirements are described in Section 3.4.

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

As part of the science assessment phase of the current review, EPA held a workshop in December 2010 (75 FR 69078) to discuss, with invited scientific experts, preliminary

1 The 2008 NAAQS for Pb are specified at 40 CFR 50.16.
2 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).

draft materials prepared during the ongoing development of the Pb ISA. The first external review draft ISA for Lead was released on May 6, 2011 (U.S. EPA 2011). The CASAC Pb Review Panel met at a public meeting on July 20, 2011 to review the draft ISA (76 FR 36120). Subsequently, on December 9, 2011, the CASAC panel provided a consensus letter for their review to the Administrator of the EPA (U.S. EPA 2011). The current document, the second external review draft *ISA for Lead*, will be discussed at a public meeting of the CASAC Pb Review Panel, and timely public comments received will be provided to the CASAC panel. A Federal Register notice will inform the public of the exact date and time of that CASAC meeting.

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CHAPTER 1 EXECUTIVE SUMMARY

1.1 Introduction

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

EPA has developed a process for evaluating the scientific evidence and drawing conclusions and causal judgments regarding air pollution-related health and environmental effects. The ISA development process includes literature search strategies, criteria for selecting and evaluating studies, approaches for evaluating weight of the evidence, and a framework for making causality determinations. The ISA uses a five-level hierarchy that classifies the weight of evidence for causation:

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

The process and causality framework are described in more detail in the Preamble to the ISA. Considerations that are specific to the causal determinations drawn for the health and ecological effects of Pb are described in Section 2.1 of the document.

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

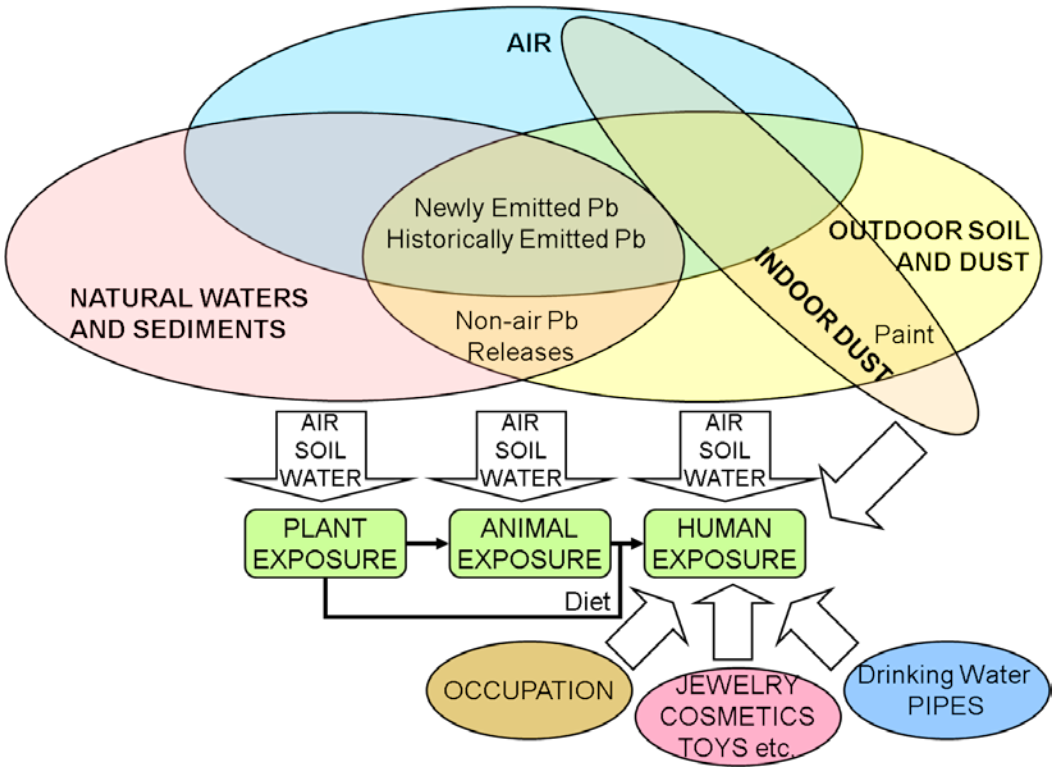
Emissions of Pb to ambient air have declined by more than two orders of magnitude over the period 1970 to 2008 following the ban on alkyl-Pb additives for on-road gasoline and tightened industrial standards. Emissions were estimated to be 970 tons in 2008, with more than half emitted from piston-engine aircraft. 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. During the same period that saw this dramatic decrease in Pb emissions, ambient air Pb concentrations have also declined. The median annual concentrations in 2010 (0.03 $\mu g/m^3$) were approximately thirty times lower than they were in 1980. The sharpest drop in Pb concentration occurred during 1980-1990 and concentrations continued to decline through 2010. Concentrations near Pb sources as well as concentrations away from Pb sources have shown a sharp decline (Section 2.2.2).

The indicator for the Pb NAAQS is Pb in total suspended particles (Pb-TSP). The Pb-TSP indicator was retained in 2008 in recognition of the role of all PM sizes in ambient air Pb exposures. The Federal Reference Method (FRM) Pb-TSP sampler's size selective performance is known to be affected by wind speed and direction, and collection efficiency has been demonstrated to decline with particle size. The size distribution of Pb-bearing particulate matter (PM) varies substantially depending on the nature of Pb sources and proximity of the monitors to the Pb sources. Coarse Pb-bearing PM deposits to a great extent near its source, contributing to local soil Pb concentrations, while fine Pb-bearing PM (e.g. aircraft emissions) can be transported long distances, potentially contributing Pb to remote areas. Depending on local conditions, deposited particles may be resuspended and redeposited multiple times before further transport becomes unlikely. In regulatory Pb monitoring, ambient Pb sampled using the FRM for Pb-PM_{10} is allowed in certain instances where the expected Pb concentration does not approach the NAAQS and no sources of ultracoarse Pb are nearby.

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 the 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. 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). 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.

Human exposure to Pb involves multiple pathways (Figure 1-1) and is difficult to assess because Pb has multiple sources in the environment. Air-related pathways of Pb exposure are the focus of this assessment. In addition to inhalation of Pb from ambient air, air-related Pb exposure pathways include inhalation and ingestion of Pb from indoor dust and/or outdoor soil that originated from recent or historic ambient air. Non-air-related exposures to humans include occupational exposures, hand-to-mouth contact with Pb-containing consumer goods, hand-to-mouth contact with dust or chips of peeling Pb-containing paint, or ingestion of Pb in drinking water conveyed through Pb pipes. Soil can act as a reservoir for deposited Pb emissions and exposure to soil contaminated with deposited Pb can occur through resuspended PM as well as shoe tracking and hand-to-mouth contact, which is the main pathway of childhood exposure to Pb. Infiltration of Pb dust into indoor environments has been suggested, 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 measurements of Pb concentration are typically higher than are indoor or outdoor ambient Pb concentrations.



Note: The Venn diagram is used to illustrate the passage of Pb through multiple environmental media compartments through which exposure can occur.

Figure 1-1 Conceptual model of multimedia Pb exposure.

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. The burden of Pb in the body may be viewed as that divided between a dominant slow compartment (bone) and smaller fast compartment(s) (soft tissues). Pb uptake and elimination in soft tissues is much faster than it is in bone. Pb accumulates in bone regions undergoing the most active calcification at the time of exposure. Pb in bone becomes distributed in trabecular (e.g. patella) and the more dense cortical bone (e.g. tibia).

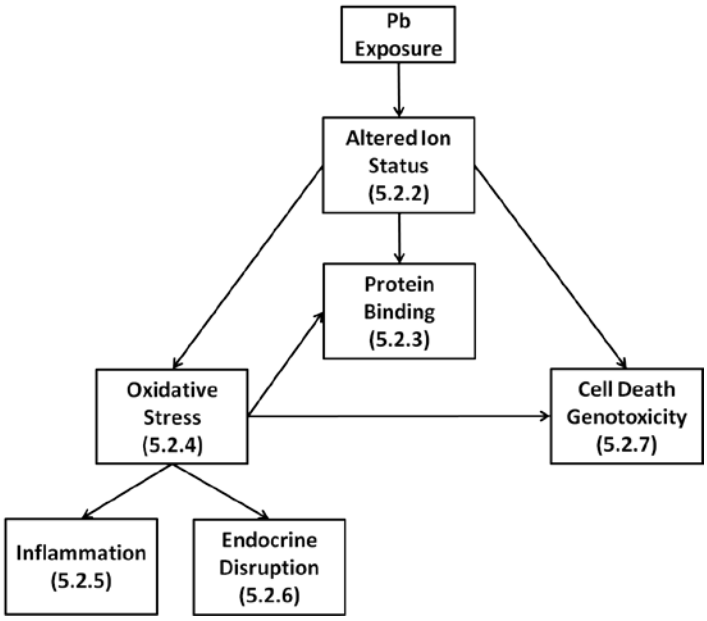
Blood Pb is the most common metric of Pb dose or exposure used in epidemiologic studies of Pb health effects. Overall, blood Pb levels have been decreasing among U.S. children and adults over the past twenty years. The median blood Pb level for the entire U.S. population is 1.2 µg/dL with a 95th percentile blood Pb level of 3.7 µg/dL based on the 2007-2008 NHANES (National Health and Nutrition Examination Survey) data. Among children aged 1-5 years, the median and 95th percentiles are slightly higher at 1.4 µg/dL and 4.1 µg/dL, respectively. Other common metrics of Pb dose or exposure used in

epidemiologic studies are Pb in bone (a metric of cumulative exposure) and Pb cord blood (a metric of prenatal exposure).

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 in an individual depending on the duration and intensity of the exposure, age, and various other physiological stressors that may affect bone remodeling (e.g., nutritional status, pregnancy, menopause, extended bed rest, hyperparathyroidism) beyond that which normally and continuously occurs. In children, largely due to faster exchange of Pb to and from bone, blood Pb is both an index of recent exposure and potentially an index of body burden. In adults and children whose current exposure to Pb has effectively ceased or greatly decreased, a slow decline in blood Pb concentrations mainly reflects the gradual release of Pb from bone. Generally, bone Pb is an index of cumulative exposure and body burden. Pb is sequestered in two bone compartments; with Pb in trabecular bone exchanging more rapidly with the blood, than Pb in cortical bone. Therefore, Pb in cortical bone a better marker of cumulative exposure and Pb in trabecular bone more likely to be correlated with blood Pb, in adults and children. There is evidence for maternal-to-fetal transfer of Pb in humans. Transplacental transfer of Pb may be facilitated by an increase in the plasma/blood Pb concentration ratio during pregnancy. Maternal-to-fetal transfer of Pb appears to be related partly to the mobilization of Pb from the maternal skeleton.

1.3 Integrative Overview of Health and Ecological Effects

There is substantial overlap between the ecological and health endpoints related to Pb exposure in that they can be mediated through multiple, interconnected modes of action (Table 2-5). The principal cellular/subcellular effects contributing to modes of action for human health and ecological endpoints are altered ion status, protein binding, oxidative stress, inflammation, endocrine disruption and cell death/genotoxicity (Figure 1-2). The commonalities with regard to the endpoints and modes of action allowed the integration of the evidence across disciplines (Table 2-4). The mechanisms of Pb toxicity are likely conserved from invertebrates to vertebrates, including humans in some organ systems. See Section 2.8.1 for additional details.



Note: The subsections where these MOAs are discussed are indicated in parentheses.

Figure 1-2 Schematic representation of the relationships between the various MOAs by which Pb exerts its effects.

1.3.1 Health Effects of Lead

Evidence from epidemiologic and toxicological studies was considered in combination with the evidence from other disciplines such as exposure sciences and toxicokinetics in determining the causal relationships for the health outcomes discussed in this assessment. The evidence related to the specific endpoints comprising the weight of the evidence for the organ system-specific causal determination are summarized in Table 1-1 below. Table 1-1 also specifies the sections sections in Chapter 2 and Chapter 5 where detailed information can be found.

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

Outcome[a]	Causality Determination
Nervous System Effects (Section 2.6.1)	Causal Relationship
Cardiovascular Effects (Section 2.6.2)	Causal Relationship
Renal Effects (Section 2.6.3)	Causal Relationship
Immune System Effects (Section 2.6.4)	Causal Relationship
Effects on Heme Synthesis and Red Blood Cell Function (Section 2.6.5)	Causal Relationship
Reproductive and Developmental Effects (Section 2.6.6)	Causal Relationship
Cancer (Section 2.6.7)	Likely Causal Relationship

[a]Based upon the framework described in the Preamble, a determination of causality was made for a broad outcome category (e.g., nervous system effects) by evaluating the coherence of evidence across disciplines and across a spectrum of related endpoints. However, the evidence on which the causal judgment is based, including the strength of evidence for the individual endpoints within the major outcome category, is characterized within the discussion. Causal determinations were made within approximately 1-2 orders of magnitude of current levels.

Nervous System Effects

Recent epidemiologic and toxicological studies substantiated the strong body of evidence presented in the 2006 Pb AQCD that Pb exposure is associated with nervous system effects. The weight of evidence clearly supports associations of increases in blood Pb levels with decrements in cognitive function in children, i.e., full-scale IQ and various measures of learning and memory. Several studies find that there is a larger incremental effect of Pb on cognition at lower blood Pb levels. Studies have not provided evidence of a threshold for Pb-related impaired cognition. Epidemiologic and toxicological evidence also clearly demonstrates Pb-associated increases in behavioral problems, in particular, inattention and impulsivity in children. In epidemiologic studies, associations with cognitive function and behavior were observed after adjustment for a range of potential confounding variables, but most commonly, parental IQ, parental education, and other SES-related variables. In children, the weight of evidence supports cognitive function decrements and behavioral problems in association with concurrent blood Pb levels. Associations also are observed with prenatal, early childhood, and childhood average blood Pb levels, thus uncertainty remains regarding the lifestage of exposure within childhood that is associated with the greatest risk. The weight of toxicological evidence demonstrates neurodevelopmental effects with prenatal and early postnatal Pb exposures that can have effects persisting to adulthood. The biological plausibility for epidemiologic and toxicological findings for effects on cognitive function and behavior is provided by evidence characterizing underlying mechanisms, including Pb-induced

changes in neurogenesis, synaptogenesis and synaptic pruning, long term potentiation, and neurotransmitter function. Based most heavily on cognitive function decrements and inattention in children, the collective body of evidence integrated across epidemiologic and toxicological studies is sufficient to conclude that there is a causal relationship between Pb exposures and nervous system effects (Section 2.6.1).

Cardiovascular Effects

The 2006 Pb AQCD concluded that there was a relationship between blood Pb and bone Pb and cardiovascular effects in adults, in particular increased blood pressure (BP) and increased incidence of hypertension. Building on this strong body of evidence, recent epidemiologic and toxicological studies substantiated the evidence that long-term Pb exposure is associated with cardiovascular effects in adults with the largest body of evidence demonstrating associations of Pb with increased BP and hypertension. The epidemiologic evidence is strengthened by several recent prospective studies that find associations between biomarkers of Pb and BP and hypertension and by effect estimates that are observed after adjustment for multiple potential confounding factors. However, there is uncertainty regarding the level, timing, frequency, and duration of Pb exposure contributing to the observed associations since the populations studied were likely to have higher past than recent Pb exposure. Animal toxicological studies provide mechanistic evidence to support the biological plausibility of Pb-induced hypertension, including Pb-induced oxidative stress, activation of renin-angiotensin aldosterone system (RAAS), altered sympathetic activity, and vasomodulator imbalance. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of other cardiovascular endpoints examined is sufficient to conclude that there is a causal relationship between Pb exposures and cardiovascular health effects (Section 2.6.2).

Renal Effects

New epidemiologic and toxicological studies evaluated in the current review support or expand upon the strong body of older evidence on the effect of Pb on the renal system presented in the 2006 Pb AQCD. The weight of epidemiologic evidence consistently demonstrates a relationship between higher blood Pb level and kidney dysfunction (e.g., lower creatinine clearance, higher serum creatinine, and lower glomerular filtration rate [GFR]) in nonoccupationally-exposed adults. Associations between Pb biomarker levels and renal function are observed after adjustment for multiple potential confounding factors such as age, sex, comorbid cardiovascular conditions, body mass index, smoking, and alcohol use; however, there is uncertainty regarding the level, timing, frequency, and

duration of Pb exposure contributing to the observed associations, since the populations studied were likely to have higher past exposure than recent exposure. Coherence for epidemiologic findings is provided by observations from animal models that Pb exposure for longer than 6 months decreases GFR and increases serum creatinine. The weight of evidence from animal studies indicates that Pb induces histopathological changes, including tubular atrophy and sclerosis. By demonstrating Pb-induced renal oxidative stress, inflammation, mitochondrial dysfunction, apoptosis, and glomerular hypertrophy, toxicological studies provide biological plausibility for the associations observed in epidemiologic studies between blood Pb levels and kidney dysfunction. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of renal outcomes is sufficient to conclude that there is a causal relationship between Pb exposures and renal health effects (Section 2.6.3).

Immune System Effects

As described in the 2006 Pb AQCD, rather than producing overt cytotoxicity or pathology, Pb exposure was found to be associated with alterations in several subclinical parameters related to cellular and humoral immunity (Figure 5-42). The principal findings are Pb-induced increased production of Th2 cytokines, suppressed production of Th1 cytokines, increased inflammation, and elevated IgE, with the weight of evidence provided by toxicological studies. Collectively, these findings are coherent with the observed effects of Pb exposure on decreasing responses to antigens (e.g., DTH, bacterial resistance) in animals. Both toxicological studies in animals and epidemiologic studies in children provide evidence for Pb-associated increases in IgE. The toxicological and epidemiologic findings for the Pb-induced effects on Th2 cytokines, IgE, and inflammation provide biological plausibility for the associations observed for blood Pb levels with asthma and allergic conditions in children. Associations with asthma and allergy were observed after considering potential confounding by several factors, including, SES and allergen exposure. The blood Pb levels and Pb exposure lifestage, magnitude, frequency, and duration associated with immune effects are not well characterized in children or adults. Epidemiologic studies of children and adults primarily examined concurrent blood Pb levels. The consistency and coherence of findings across the continuum of related immune parameters that demonstrate a stimulation of Th2 responses in toxicological studies combined with the supporting epidemiologic evidence in children are sufficient to conclude that there is a causal relationship between Pb exposures and immune system effects (Section 2.6.4).

Heme Synthesis and Red Blood Cell Function

Recent epidemiologic and toxicological studies support findings from the 2006 Pb AQCD which concluded that Pb exposure was associated with effects on developing red blood cells (RBCs) as well as heme synthesis in children and occupationally exposed adults. The principal findings for RBC survival and function from recent studies are the consistent Pb-induced alterations in several inter-connected hematological parameters such as hemoglobin (Hb), hematocrit (Hct), and mean corpuscular volume (MCV) observed across multiple studies. The effects on heme synthesis occurred through the inhibition of multiple key enzymes, most notably aminolevulinic acid dehydratase (ALAD), the enzyme that catalyzes the second, rate-limiting step in heme biosynthesis. For heme synthesis, the largest body of evidence indicates decreased ALAD activity in association with Pb exposure or blood Pb levels in occupationally-exposed adults or in children (3-12 years old), respectively. Evidence for oxidative stress, an important mode of action by which Pb may exert effects, primarily comes from studies of occupationally-exposed adults and of children. the level, frequency, timing and duration of exposure associated with altered RBC survival and function, heme synthesis, or the state of oxidative stress in RBCs is uncertain in both adults and in children. The consistency of findings in epidemiologic studies investigating effects in occupationally-exposed adults and children, and the coherence of findings in the toxicological literature and coherence across the disciplines is sufficient to conclude that there is a causal relationship exists between Pb exposures and effects on heme synthesis and RBC function (Section 2.6.5).

Reproductive and Developmental Effects

Supporting conclusions from the 2006 Pb AQCD, recent toxicological and epidemiologic literature provides strong evidence that Pb exposure is associated with effects on reproduction and development, and expands evidence for additional endpoints. The weight of the evidence supports the association of Pb exposure with delayed onset of puberty in both males and females; and detrimental effects on sperm and semen quality in occupationally-exposed males and in laboratory animals. In cross-sectional epidemiologic studies of girls (ages 6-18 years) consistent associations with delayed pubertal development (measured by age at menarche, pubic hair development, and breast development) were observed. In boys (ages 8-15 years), fewer studies were conducted but associations with delayed puberty were observed in most. There is uncertainty with regard to the exposure frequency, timing, duration and level that contributed to these observed association in studies of adolescents. The collective body of evidence integrated across epidemiologic and toxicological studies (which examined Pb-induced detrimental effects on sperm and on delayed onset of puberty), is sufficient to conclude that there is a

causal relationship between Pb exposures and reproductive and developmental effects (Section 2.6.6).

Cancer

The previous epidemiologic studies included in the 2006 Pb AQCD provided very limited evidence suggestive of Pb exposure associations with cancers of the lung and stomach and genotoxic effects in humans. The conclusions of the 2006 Pb AQCD were consistent with those of International Agency for Research on Cancer (IARC) and the National Toxicology Program. The animal toxicological literature continues to provide the strongest evidence for an association between cancer and Pb exposure, with some supporting evidence provided by the epidemiologic literature. Evidence from toxicological studies demonstrates a relationship between Pb and cancer, genotoxicity/clastogenicity or epigenetic modification. Carcinogenicity in previous animal toxicology studies with Pb exposure has been reported in the kidneys, testes, brain, adrenals, prostate, pituitary, and mammary gland, albeit at high doses of Pb. Epidemiologic studies of cancer incidence and mortality reported inconsistent results. Recent occupational studies of Pb exposure and lung cancer reported no associations. The majority of epidemiologic studies of brain cancer had null results overall, but positive associations between Pb exposure and brain cancer were observed among individuals with certain genotypes (e.g. ALAD2). The collective body of evidence integrated across toxicological and epidemiologic studies is sufficient to conclude that there is a likely causal relationship between Pb exposure and cancer (Section 2.6.7).

1.3.2 Ecological Effects of Lead

The 2006 Pb AQCD and previous assessments found that the most commonly observed effects in terrestrial organisms included decreased survival, reproduction and growth as well as development and behavior. In aquatic invertebrates, effects of Pb exposures included inhibition of ALAD, reduced reproduction, growth and survival. Evidence of toxicity to fish (e.g. heme formation, alterations in brain receptors, effects on blood chemistry and hormonal systems, and decreases in some enzyme activities) was also observed. The effects of Pb can be observed in endpoints common to both terrestrial and aquatic biota. These effects can be observed at multiple levels of biological organization, starting at the cellular and subcellular level, then at the whole organism level, and finally at the community and ecosystem level. Effects of Pb on physiological stress, blood, and neurobehavior may increase susceptibility to other stressors and impact fitness of individual organisms. Effects on development, reproduction, growth and survival may

lead to effects on communities and ecosystems. The relationship of exposure and responses is difficult to quantitatively characterize because of the influence of multiple environmental variables on both Pb bioavailability and toxicity, and the substantial species and lifestage differences in Pb sensitivity.

A brief discussion of the conclusions regarding Pb effects on physiological stress, hematology, neurobehavior, development, reproduction, growth, survival and community and ecosystem level effects is provided below and summarized in Table 1-2. Causal determinations were based on consideration and integration of information on biogeochemistry, bioavailability, biological effects, and exposure-response relationships of Pb in terrestrial and aquatic environments. A detailed discussion for all relevant welfare effects is provided in Chapters 2 and 7.

Table 1-2 Summary of Pb causal determinations for plants, invertebrates and vertebrates

Effect[a]	Terrestrial	Aquatic[b]
Physiological Stress-All organisms (Section 2.7.3.1)	Causal	Causal
Hematological Effects-Invertebrates (Section 2.7.3.2)	Inadequate	Causal
Hematological Effects-Vertebrates (Section 2.7.3.2)	Causal	Causal
Neurobehavioral Effects-Invertebrates and Vertebrates (Section 2.7.3.3)	Likely Causal	Likely Causal
Developmental and Reproductive Effects-Plants (Section 2.7.3.4)	Inadequate	Inadequate
Developmental and Reproductive Effects-Invertebrates and Vertebrates (Section 2.7.3.4)	Causal	Causal
Growth-Plants (Section 2.7.3.5)	Causal	Causal
Growth-Invertebrates (Section 2.7.3.5)	Inadequate	Causal
Growth-Vertebrates (Section 2.7.3.5)	Inadequate	Inadequate
Survival-Plants (Section 2.7.3.6)	Inadequate	Inadequate
Survival- Invertebrates and Vertebrates (Section 2.7.3.6)	Causal	Causal
Community and Ecosystem Level Effects (Section 2.7.3.7)	Likely Causal	Likely Causal

[a]Effects observed at or near ambient levels of Pb were emphasized, and studies generally within 1-2 orders of magnitude above current conditions were considered in the body of evidence for terrestrial and aquatic ecosystems.

[b]Causal determinations for aquatic biota are based primarily on evidence from freshwater organisms.

Effects on physiological stress

Upregulation of antioxidant enzymes and increased lipid peroxidation are considered to be reliable biomarkers of stress. Alterations in these biomarkers are associated with Pb exposure in plants, invertebrates and vertebrates, and they may be indicative of increased susceptibility to other stressors, and of reduction in individual fitness. Markers of oxidative damage and antioxidant activity have been observed in a wide range of species

in terrestrial and aquatic environments when Pb is present, and following laboratory exposures. Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and physiological stress in terrestrial and aquatic plants, invertebrates and vertebrates (Section 2.7.3.1).

Hematological Effects

Changes in hematological variables including ALAD activity, blood cell counts and serum profiles are associated with Pb exposure in both aquatic and terrestrial animal taxa. Evidence from both field and laboratory studies suggest that ALAD is an indicator for Pb exposure across a wide range of biota, and it is commonly recognized as such. Studies conducted across the last two decades have indicated that hematological responses are associated with Pb in the environment. Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and hematological effects in aquatic invertebrates and vertebrates. Evidence is inadequate to establish a causal relationship between Pb exposures and hematological effects in terrestrial invertebrates (Section 2.7.3.2).

Neurobehavioral Effects

Historical and recent evidence from Pb-exposed animals indicates that Pb affects behaviors such as food consumption, avoidance and escape from predators, behavioral thermoregulation, and prey capture. Alterations to these behaviors may decrease the overall fitness of the organism. Evidence from laboratory studies has shown effects of Pb on nervous system endpoints in both aquatic and terrestrial animal taxa. Overall, the evidence from terrestrial and aquatic systems is sufficient to conclude that there is a likely causal relationship between Pb exposures and neurobehavioral effects in terrestrial and aquatic invertebrates and vertebrates (Section 2.7.3.3).

Effects on Development and Reproduction

Various endpoints measured in multiple taxa of terrestrial and aquatic organisms have documented effects of Pb on development, fecundity and hormone homeostasis. In plants, few studies have addressed reproductive effects of Pb exposure. Decreased reproduction at the organismal level can result in declining abundance and/or extirpation of populations, decreased taxa richness, and decreased relative or absolute abundance at the community level. Among the animal species tested, aquatic invertebrates were the most sensitive to Pb with respect to reproduction. Effects of Pb on reproduction in invertebrates and vertebrates indicate that Pb is 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. The evidence is inadequate to conclude that there is a causal relationship between Pb exposures and reproductive effects in plants (Section 2.7.3.4).

Effects on Growth

Effects on growth observed at the species level may translate into effects at the ecosystem level. Exposure to Pb has been shown to have effects on growth in plants and in some species of invertebrates and vertebrates. Evidence for effects of Pb on growth is strongest in plants, although they are typically observed in laboratory studies with high exposure concentrations or in field studies near point sources. Evidence for Pb effects on growth in invertebrates has been observed most extensively in freshwater aquatic taxa, with inhibition in sensitive species occurring near the current range of Pb in surface waters. In general, juvenile organisms are more sensitive than are adults. There are limited data on growth effects in vertebrates. Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and growth effects in aquatic and terrestrial plants and aquatic invertebrates. Evidence is inadequate to to conclude that there is a causal relationship between Pb exposures and growth effects in terrestrial invertebrates and in terrestrial and aquatic vertebrates (Section 2.7.3.5).

Effects on Survival

Decreased survival of individuals within a population can have ecosystem-level impacts. Pb is generally not toxic to aquatic or terrestrial plants at concentrations found in the environment away from point sources, probably due to the fact that plants often sequester large amounts of Pb in roots, with little translocation to other parts of the plant. Aquatic invertebrates are generally more sensitive to Pb exposure than other taxa, with survival adversely impacted in a few species at concentrations occurring near point sources, or at concentrations near common ambient levels. Terrestrial invertebrates typically tolerate higher concentrations of Pb. Limited studies with vertebrates indicated adverse effects of Pb on survival at concentrations typically higher than ambient Pb in the environment, although juvenile organisms appear to be more sensitive than adults. The evidence is inadequate to conclude that there is a causal relationship between Pb exposure and survival in terrestrial and aquatic plants. Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and survival in terrestrial and aquatic invertebrates and vertebrates (Section 2.7.3.6).

Community and Ecosystem Effects

At higher levels of ecological organization, exposure to Pb may alter community and ecosystem structure and function. Decreases in abundance, reduced species diversity and shifts in community composition have been observed following Pb exposure in laboratory and field experiments, but considerable uncertainties exist in generalizing to natural ecosystem level effects. Assessment of Pb-specific exposure-response relationships is difficult at the whole ecosystem level because potential confounders such as other metals, physico-chemical variables, or various stressors cannot be controlled. These factors make it difficult to quantify relationships between ambient concentrations of Pb and ecosystem responses. The cumulative evidence that has been reported for effects of Pb at the highest levels of ecological organization, and for species-level endpoints with direct relevance to population and ecosystem level effects (i.e., development and reproduction, growth, survival) is sufficient to conclude that there is a likely causal relationship between Pb exposures and the alteration of species richness, species composition and biodiversity in terrestrial and aquatic ecosystems (Section 2.7.3.7).

1.4 Policy Relevant Considerations

Public Health Significance

The concept of population risk is relevant to the interpretation of findings for the continuously-distributed subclinical health endpoints frequently studied in association with Pb biomarkers in the assessment of their public health significance. A seemingly small increase in the mean of a continuously distributed health index may push the most susceptible group in the population above a critical cut point on the continuum of disease development, such that their condition meets the clinical definition of a disease. Moreover, small changes at the population level could translate into large numbers of clinical events if a large population is affected. Pb-associated changes in subclinical indices of disease may also increase an individual's risk for health effects that are of greater clinical consequence, thus of greater public health concern. For example, a downward shift in the mean IQ value can also reduce the proportion of the population achieving very high IQ scores. Additional small increases in blood pressure or decreases in renal function that are associated with Pb biomarkers, may shift the population mean resulting in a larger proportion of the population that is diagnosed with hypertension or chronic kidney disease, respectively.

Air Lead-to-Blood Lead Relationships

A limited number of epidemiological studies evaluated relationships between air Pb and blood Pb (Section 2.9.2). Much of the pertinent earlier literature described in the 1986 Pb AQCD and more recent studies provide data from which estimates of the blood Pb-air Pb slope can be derived for children. The range of estimates from these studies is 2 to 9 µg/dL per µg/m^3. The differences in the estimates across studies may reflect model selection by the investigators (e.g., some models predict an increase in the blood Pb-air Pb slope with decreasing air Pb concentration while other models predict a constant blood Pb-air Pb slope across all air Pb concentrations) as well as inclusion parameters (e.g. soil Pb) that may account for some of the variation in blood Pb that is attributable to air Pb. Other factors that may explain the variation in the derived blood Pb-air Pb slope include differences in the populations examined and Pb sources (e.g. leaded gasoline or smelter).

Ecological Effects and Corresponding Lead Concentrations

There is limited evidence to relate ambient air concentrations of Pb to levels of deposition onto terrestrial and aquatic ecosystems and to subsequent movement of atmospherically-deposited Pb through environmental compartments (e.g., soil, sediment, water, and biota) (Section 2.9.3). Therefore, the connection between air concentration and ecosystem exposure continues to be poorly characterized for Pb and the contribution of atmospheric Pb to specific sites is not clear. Furthermore, the level at which Pb elicits a specific effect is difficult to establish in terrestrial and aquatic systems, due to the influence of other environmental variables on both Pb bioavailability and toxicity, and also to substantial species differences in Pb susceptibility. Current evidence indicates that Pb is bioaccumulated in biota; however, the sources of Pb in biota have only been identified in a few studies, and the relative contribution of Pb from all sources is usually not known. In addition, 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.

Concentration-Response Relationships for Health Effects

With each successive assessment to-date, the epidemiologic and toxicological study findings show that progressively lower blood Pb levels or Pb exposures are associated with cognitive deficits and behavioral impairments (Section 2.9.4). Compelling evidence for a steeper slope for the relationship between blood Pb level and children's IQ at lower blood Pb levels was presented in the 2006 Pb AQCD based on the international pooled analysis of seven prospective cohort studies. A subsequent reanalysis of these data focusing on the shape of the concentration-response function and several recent studies

support these findings. The majority of the epidemiologic evidence from stratified analyses comparing the lower and the higher ends of the blood Pb distributions indicates larger negative slopes at lower blood Pb levels (Figure 2-2). Several lines of toxicological evidence support the possibility of lower and higher Pb exposures acting through differential activation of mechanisms underlying cognition. The shapes of concentration-response relationships were examined in a more limited number epidemiologic studies of other effects and findings were mixed (Section 2.9.4).

Pb Exposure and Nervous System Effects in Children

Young children, do not have lengthy exposure histories and consequently the interpretation of associations with blood Pb levels for this age group may be less complicated compared to older age groups. Several lines of evidence inform the interpretation of study findings as they relate to aspects of exposure that can be attributed to the cognitive and behavioral effects of Pb observed in young children (Section 2.9.5).

Epidemiologic studies find associations of cognitive function and behavior with prenatal, early-childhood, lifetime average, and concurrent blood Pb levels as well as with childhood tooth Pb levels. However, the weight of epidemiologic evidence supports associations of concurrent blood Pb level. Studies of children up to three years of age that found associations with concurrent blood Pb levels also tended to find associations with prenatal cord or maternal blood Pb levels. Thus, both postnatal child and maternal Pb exposures may contribute to lower cognitive function in young children. Exposures that are reflected by concurrent blood Pb measured when children are older, by cumulative blood Pb levels or by tooth Pb levels have also been demonstrated to be associated with neurodevelopmental deficits throughout school-age and into adolescence. These findings are consistent with the understanding that the nervous system continues to develop throughout childhood.

Potentially At-Risk Populations

The NAAQS are intended to provide an adequate margin of safety for both the population as a whole and those groups with unique factors that make them potentially at increased risk for health effects in response to ambient air pollutant exposure. The most well-substantiated at-risk population for the effects of Pb exposure is children. Among children, the youngest age groups were observed to be most at risk of elevated blood Pb levels, with levels decreasing with increasing age of the children. Recent epidemiologic studies of infants/children detected increased risk of Pb-related health effects, and this was supported by toxicological studies. Synaptic pruning, which is active throughout early childhood (ages 1-4 years), may underlie the elevated risk of neurodevelopmental

effects in young children. Evidence is less consistent for other factors that may increase Pb-related risk including sex, genetics, pre-existing disease (e.g., hypertension), race/ethnicity, SES, nutrition, stress and co-exposure to other metals (Table 2-9).

1.5 Summary

Overall, the evidence evaluated for the current review expands upon findings of the 2006 Pb AQCD, which concluded that there was a strong body of evidence substantiating the health effects of Pb and strong evidence of effects on some ecological endpoints. Table 2-10 of Chapter 2 summarizes the main conclusions from the 2006 Pb AQCD and compares them to the findings from this assessment, including causality determinations, regarding the health and ecological effects of Pb.

Nervous system effects, specifically cognition and behavior, in children are the effects that are best substantiated as occurring at the lowest blood lead concentrations. Some new epidemiologic and toxicological findings indicate that some effects (e.g., behavioral impairments) are observed in populations of children with lower mean blood lead levels than in previous assessments (e.g. <2 μg/dL). Causal relationships were also determined between Pb exposure and several adult outcomes including renal and cardiovascular effects, for which the evidence strongly suggests that cumulative exposure plays a role. As lead exposures were generally higher in the past than they are today, uncertainties exist, regarding the relative importance of recent versus past exposure in the development of the lead-related health effects in the adult populations studied.

With regard to the ecological effects of Pb at or near concentrations currently present in the environment, there is new evidence to support previous findings of effects on growth and on reproduction and development in aquatic invertebrates as well as effects on survival in both invertebrate and vertebrate aquatic biota. These effects are observed at or near ambient levels of Pb in only a few species with greater toxicity generally associated with early lifestages. Hematological and stress related responses were also associated with elevated Pb levels in polluted areas in some terrestrial and aquatic species. In both aquatic and terrestrial systems, uptake of Pb into flora and fauna and subsequent effects on reproduction, growth and survival at the species level are likely to lead to effects at the population, community and ecosystem level of biological organization.

CHAPTER 2 INTEGRATIVE SUMMARY

2.1 ISA Development and Scope

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

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

EPA has developed a process for evaluating the scientific evidence; and drawing conclusions and causal judgments regarding air pollution-related health and environmental effects. The ISA development process includes literature search strategies, criteria for selecting and evaluating studies, approaches for evaluating weight of the evidence, and a framework for making causality determinations. The process and causality framework are described in more detail in the Preamble to the ISA. This section provides a brief overview of the process for development of this ISA.

EPA initiated the current review of the NAAQS in April 2010 with a call for information from the public (75 FR 20843). Literature searches were conducted routinely to identify studies published since the last review, focusing on studies published from 2006 (close of previous scientific assessment) through September 2011. References that were considered for inclusion or cited in this ISA can be found at http://hero.epa.gov/lead.

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

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

In drawing judgments regarding causality for the criteria air pollutants, evidence of health effects in the range of relevant pollutant exposures or doses is considered. With regard to the causal determinations drawn for human health effects of Pb, population-based epidemiology studies were emphasized over occupational studies. Recent occupational studies were considered insofar as they added to the evidence base for an outcome for which sufficient numbers of population-based studies were unavailable or, to the extent that they addressed a topic area that was of particular relevance to the NAAQS review (e.g., longitudinal studies designed to examine recent versus historical Pb exposure). Evidence from toxicological studies of effects observed in experimental animals at doses that were relevant to, or somewhat above, those currently experienced by the U.S. general population were emphasized. The extent to which studies of higher concentrations were considered varied by major outcome category, but generally studies with blood Pb levels within one order of magnitude above the upper end of the distribution of U.S. blood Pb levels were considered (i.e., toxicological studies reporting blood Pb levels less than

about 100 µg/dL).[1] The majority of these studies reported blood Pb levels below approximately 30-40 µg/dL, however. Studies with higher blood Pb levels were considered to the extent that they provided useful information to inform modes of action or mechanisms or kinetics. For toxicological studies where blood Pb levels were not measured, judgments regarding how to distinguish high from the more relevant low doses were made considering the range of doses across the available body of evidence and emphasizing studies at the lower end of the range.

Relevant concentrations for drawing causality judgments for ecological effects of Pb were determined considering the range of Pb concentrations in the environment and the available evidence for concentrations at which effects were observed in biota. Effects observed at or near ambient levels of Pb were emphasized and studies generally within one to two orders of magnitude above current conditions were considered in the body of evidence for terrestrial and aquatic ecosystems. Studies at higher concentrations were used to the extent that they informed modes of action and illustrated the wide range of sensitivity to Pb across taxa. For aquatic biota, generally, the number of studies available on freshwater organisms is greater than on saltwater organisms, covering more taxa as well as more endpoints. The causal determinations for aquatic biota are, therefore, based primarily on evidence from freshwater species. When available, data from saltwater ecosystems are discussed separately under the appropriate causal endpoint. For most of the endpoints under consideration, evidence is inadequate to establish causality in saltwater species. When sufficient evidence is available for marine organisms, data on concentrations at which effects are observed are presented in Section 7.4.2.

The causal statements for terrestrial and aquatic effects are arranged according to ecologically meaningful levels of biological organization (organism, population, community, ecosystem). As recognized in EPA's Framework for Ecological Risk Assessment (U.S. EPA, 1992), and in the adverse outcome pathway (AOP) framework (Ankley et al., 2010) endpoints that are measured at one level of ecological organization may be related to an endpoint at a higher level. The AOP conceptual framework was proposed to link mechanistic data from initiating events at the molecular level through a series of higher order biological responses to survival and to developmental and reproductive endpoints that can be used in ecological risk assessment, i.e., at the population level and higher. Fecundity, growth, and survival are organism-level attributes that lead to population-level (e.g., abundance, production, extirpation), community-level (taxa richness, relative abundance) and ecosystem-level responses (Ankley et al., 2010;

1 Studies considered were generally within an order of magnitude above 10 µg/dL (i.e., 100 µg/dL), which was used to indicate the upper end of the blood Pb distribution in the U.S. population. Currently approximately 1.4% of U.S. children overall have blood Pb levels that exceed 10 µg/dL (Jones et al., 2009a); however, the proportion of individuals with blood Pb levels that exceed this concentration varies depending on factors including age and/or residence near a source of Pb exposure (Section 4.1).

Suter et al., 2005). In the case of Pb, physiological stress, hematological effects and neurobehavioral alterations may decrease the overall fitness of an organism, even though their connection to effects at higher levels of biological organization may not have been characterized. Furthermore, the effects of Pb on ecosystems necessarily begin with some initial effects at the molecular level of specific organisms within the ecosystem (U.S. EPA, 1986b). There are many different molecular and cellular level effects, and toxicity of Pb in ecosystems may be attained through multiple modes of action.

The ISA considers evidence of health effects for both short- and long-term pollutant exposures. Biomarkers are typically used in epidemiologic studies of the health effects associated with Pb as an index of exposure or dose. Consequently, the timing, frequency, level, and duration of Pb exposure(s) associated with the observed health effects are uncertain. Evidence from animal toxicological studies is also drawn upon to understand and interpret Pb exposures in epidemiologic studies. Animal toxicological studies that are relied upon to inform our understanding of the exposures needed to induce health effects in humans include chronic exposure studies (i.e., over 10% of the lifespan of the animal), long-term exposure studies (e.g., greater than 4 weeks in rodents) as well as acute or short-term exposure studies (e.g., less than 4 weeks in rodents). In this ISA, short-term human exposures are generally defined to include exposures of months (e.g., < one year) while long-term human exposures include those greater than one year in duration. In addition, information including the age of the population studied, study period and study location can be used to aid in the interpretation of findings from epidemiologic studies because Pb exposures have declined over time and exposures vary depending on proximity to Pb sources.

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

This chapter summarizes and integrates the newly available scientific evidence that best informs consideration of the policy-relevant questions that frame this assessment. The organization of this chapter generally follows the organization of the document as a whole, with several additional sections including: (Section 2.1) a discussion of the assessment development and scope; (Section 2.8) an integration of the evidence across the disciplines of health and ecology; (Section 2.9) a discussion of policy-relevant considerations; and, (Section 2.10) an overall summary. This ISA itself is composed of six chapters including this integrative summary. Chapter 3 highlights key concepts or issues relevant to understanding the sources, ambient concentrations, and fate and transport of Pb in the environment. 0 summarizes key concepts and recent findings on Pb exposures, toxicokinetics, and biomarkers reflecting Pb exposure and body burden. 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 nervous system, cardiovascular, renal, immunological, reproductive and developmental, and cancer outcomes. Chapter 6 summarizes the evidence on potentially susceptible populations. Chapter 7 evaluates ecological effects evidence that is relevant to the review of the secondary NAAQS for Pb.

2.2 Ambient Lead: Source to Concentration

2.2.1 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, 2006b), 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 1995 and then by an additional 76% from 1995

to 2008, at which time emissions were estimated at 970 tons per year. As was the case at the time of the last Pb NAAQS review, data from the 2008 National Emissions Inventory (NEI) (U.S. EPA, 2011a) illustrate that piston-engine aircraft emissions comprise the largest share (56%) of total atmospheric Pb emissions; the 2008 NEI estimated that 550 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. Global atmospheric Pb deposition peaked in the 1970s, followed by a decline. See Section 3.2 for additional information.

The atmosphere is the main environmental transport pathway for Pb. On a local scale atmospheric Pb is primarily associated with coarse particulate matter (PM), and on a global scale atmospheric Pb is primarily associated with fine PM. 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. Pb associated with coarse PM deposits to a great extent near local industrial sources, contributing to soil Pb concentrations in those locations, while fine Pb-bearing PM can be transported long distances, contributing Pb to remote areas. Depending on local conditions, once deposited particles may be resuspended and redeposited before reaching a site where further transport is unlikely, especially for dry deposition. See Section 3.3 for additional information.

Environmental distribution of Pb occurs mainly through the atmosphere, from which it is deposited into surface waters and soil. Surface waters act as an important reservoir, with Pb lifetimes in the water column largely controlled by deposition and resuspension of Pb in sediments. Substantial amounts of Pb from vehicle wear and building materials can also be transported by runoff waters to surface waters and sediments without becoming airborne. Pb containing sediment particles can be remobilized into the water column, and sediment concentrations tend to follow those in overlying waters. See Section 3.3 for additional information.

2.2.2 Monitoring and Concentrations of Ambient Air Lead

The indicator for the Pb NAAQS is Pb in total suspended particles (Pb-TSP). The Pb-TSP indicator was retained in 2008 in recognition of the role of all PM sizes in ambient air Pb exposures. The Federal Reference Method (FRM) for Pb-TSP specifies that ambient air is drawn through a high-volume TSP sampler onto a glass fiber filter. The Pb-TSP sampler's size selective performance is known to be affected by wind speed and direction, and collection efficiency has been demonstrated to decline with particle

size with an uncertain upper size limit (Wedding et al., 1977). In recognition of the steep spatial gradients associated with sources of ultra-coarse particles, ambient Pb sampled using the FRM for Pb-PM_{10} is allowed in certain instances where the expected Pb concentration does not approach the NAAQS and no sources of ultra-coarse Pb are nearby. A new 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. The Pb-PM_{10} FRM is free of wind direction bias. Several FEMs have also been approved. The Pb-TSP FRM is based on flame atomic absorption spectroscopy (AAS), while the Pb-PM_{10} FRM is based on x-ray fluorescense spectroscopy (XRF). Inductively-coupled plasma mass spectrometry (ICPMS) is under consideration as a new FRM for Pb-TSP. See Section 3.4.1 for additional information.

Pb monitoring requirements have experienced several changes since publication of the 2006 Pb AQCD (U.S. EPA, 2006b). The current Pb monitoring network design requirements include two types of FRM monitoring sites: source-oriented and non-source-oriented. Source-oriented FRM Pb-TSP monitoring sites are required near sources of air Pb emissions which are expected to or have been shown to contribute to ambient air Pb concentrations in excess of the NAAQS. At a minimum, monitoring is required near sources that emit 0.50 tons/year or more of Pb unless it can be determined that the source will not contribute to an ambient concentration exceeding 50% of the Pb NAAQS. With the December 2010 completion of action on regulatory requirement of Pb monitoring, one-year of Pb-TSP FRM monitoring is also required near 15 specific airports to gather additional information on the likelihood of NAAQS exceedances near airports due to combustion of leaded aviation gasoline. Non-source-oriented monitoring is also required at national core multipollutant monitoring network (NCore) sites in Core Based Statistical Areas (CBSA) with a population of at least 500,000. In addition to FRM monitoring, Pb is also routinely measured in smaller particle fractions in the chemical speciation network (CSN), interagency monitoring of protected visual environment (IMPROVE), and the national air toxics trends station (NATTS) networks. While monitoring in multiple networks provides extensive geographic coverage, measurements between networks are not directly comparable in all cases because there are differences in the methods, including the different particle size ranges sampled in the different networks. Depending on monitoring network, Pb is monitored in TSP, PM_{10}, or $PM_{2.5}$. For the purpose of analyzing data for the ISA, monitors reporting to the AQS were considered 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, 2008a) or the 2008 NEI (U.S. EPA, 2011a). See Section 3.4 for additional information.

Ambient air Pb concentrations have declined drastically over the period 1980-2010. The median annual concentrations have dropped by 97% from 0.87 $\mu g/m^3$ in 1980 to 0.03 $\mu g/m^3$ in 2010. While the sharpest drop in Pb concentration occurred during 1980-1990, a declining trend was observed between 1990 and 2010. There was an 84% reduction in the median annual source-oriented Pb concentration and a 85% reduction in the median annual non-source-oriented Pb concentration for 1990-2010. Recent estimates for the natural contribution to background Pb are ~0.3 to 1 ng/m^3. These estimates exceed estimates of natural background presented in the 1986 Pb AQCD by a factor of 2 to 20. The more recent estimate still indicates that background airborne Pb concentrations are well below current ambient concentrations. See Section 3.5 for additional information.

AQS data for source-oriented and non-source-oriented FRM monitoring were analyzed for 2008-2010. For source-oriented monitors, the three-month rolling average was above the level of the NAAQS in twenty counties across the U.S. The three-month rolling average was never above the level of the NAAQS for any of the non-source-oriented FRM monitors. 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. See Section 3.5 for additional information.

Size distribution of Pb-bearing PM varied substantially 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 related to differences in sources across land use types; for example, ultra-coarse Pb-PM has been observed near industrial sites. 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. Moderate associations with Pb concentrations were observed for Br, Cu, and K. Such correlations may suggest some common sources affecting the concentrations of various pollutants. See Section 3.5 for additional information.

2.2.3 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, as well as surface sediments and soils in some locations, have also been observed (U.S. EPA, 2006b). In contrast, Pb is retained in soils and sediments, where it 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.

Information on ambient Pb concentrations in non-air media and biota is reported in Section 3.6, and concentrations considered in the interpretation of the ecological evidence are tabulated in Table 2-1. As noted in the preamble, the ecological causal determinations focus on studies where effects of Pb exposure are observed at or near ambient levels of Pb and studies generally within the range of one to two orders of magnitude above current or ambient conditions were also considered in the body of evidence.

Table 2-1 Ambient Pb Concentrations in Non-Air Media and Biota Considered for Ecological Assessment

Media	Pb Concentration	Years Data Obtained	References
Soil	National Average: 18.9 mg Pb/kg Range of state averages: 5-38.6 mg Pb/Kg	1961-1976	U.S. EPA (2007d, 2006b, 2003b)
Sediment[1]	Median: 28 mg Pb/kg (dry weight)	1996-2001	Mahler et al. (2006), U.S. EPA (2006b)
Fresh Surface Water (Dissolved Pb)[a]	Median: 0.5 µg Pb/L; Max: 30 µg Pb/L Range: 0.0003-0.075 µg Pb/L	2001-2003 2002-2007	U.S. EPA (2006b) Field and Sherrell (2003), Landers et al. (2008)
Saltwater[b]	Range: 0.01 – 27 µg Pb/L Range: 0.005-0.04 µg Pb/L	Dates not available	Sadiq (1992) Leland and Kuwabara (1985)
Vegetation	Lichens: 0.3-5 mg Pb/kg Grasses: 31% (percent of soil Pb in grass)	2002-2007 1980s-2000s	Landers et al. (2008) Vandenhove et al. (2009)
Vertebrate	Fish: Geometric Mean: 0.54 mg Pb/kg (dry weight) Maximum: 23 mg Pb/kg (dry weight)	2001-2003	U.S. EPA (2006b)
	Fish: 0.0033-0.97 mg Pb/kg Moose: 0.021-0.23 mg Pb/kg	2002-2007	Landers et al. (2008)

[a]Based on synthesis of NAWQA data reported in 2006 Pb AQCD (U.S. EPA, 2006b)
[b]Data from a combination of brackish and marine saltwater samples. In general, Pb in seawater is higher in coastal areas and estuaries since these locations are closer to sources of Pb contamination and loading from terrestrial systems.

2.3 Exposure to Ambient Lead

Exposure data considered in this assessment build upon the conclusions of the 2006 Pb AQCD (2006b), which found air Pb concentrations in the U.S. and associated biomarkers of exposure to have decreased substantially following the ban on Pb in on-road gasoline as well as an earlier ban on Pb in house-hold paints. Pb exposure is difficult to assess because Pb has multiple sources in the environment and passes through various media. The atmosphere is the main environmental transport pathway for Pb, and, on a global scale, atmospheric Pb is primarily associated with fine particulate matter, which can deposit to soil and water. In addition to primary emission of particle-bearing or gaseous Pb to the atmosphere, Pb can be suspended to the air from soil or dust. Air-related pathways of Pb exposure are the focus of this assessment. In addition to inhalation of Pb from ambient air, air-related Pb exposure pathways include inhalation and ingestion of Pb from indoor dust and/or outdoor soil that originated from recent or historic ambient air (e.g., air Pb that has penetrated into the residence either via the air or tracking of soil). Non-air-related exposures include occupational exposures, hand-to-mouth contact with t with dust or chips of peeling Pb-containing paint, or ingestion of Pb in drinking water conveyed through Pb pipes. Several study results indicate that exposure to Pb-containing

paint and home age (often a surrogate for the presence of Pb paint) are important residential factors that increase risk of elevated blood Pb (Sections 2.9.6.7 and 6.2.6). Most Pb biomarker studies do not indicate species or isotopic signature, and so non-air exposures are reviewed in this section because they can also contribute to Pb body burden. See Section 4.1 for additional information on exposure to ambient Pb.

A number of monitoring and modeling techniques have been employed for ambient Pb exposure assessment. Environmental Pb concentration data can be collected from ambient air Pb monitors, soil Pb samples, dust Pb samples, and dietary Pb samples to estimate human exposure. Exposure estimation error depends in part on the collection efficiency of these methods; collection efficiency for ambient air Pb FRM samplers is described in Section 3.4. Additionally, high spatial variability of the Pb concentrations in various media also can contribute to exposure error, as described in the 2009 PM ISA (U.S. EPA, 2009). Models, such as the Integrated Exposure Uptake Biokinetic (IEUBK) model, simulate human exposure to Pb from multiple sources and through various routes including inhalation, ingestion, and dermal exposure. IEUBK model inputs include soil Pb concentration, air Pb concentration, dietary Pb intake including drinking water, Pb dust ingestion, human activity, and biokinetic factors. Measurements and/or assumptions can be utilized when formulating the model inputs; error in measurements and assumptions thus have the potential to propagate through the exposure models.

Section 4.1 presents data illustrating potential exposure pathways. Soil can act as a reservoir for deposited Pb emissions and exposure to soil contaminated with deposited Pb can occur through resuspended PM as well as shoe tracking and hand-to-mouth contact, which is the main pathway of childhood exposure to Pb. Recent data by Yamamoto et al. (2006) have shown that the size distribution of particles collected on children's hands have a mode around 40 μm with the upper tail of the distribution extending to 200-300 μm. Infiltration of Pb dust into indoor environments has been suggested, 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 measurements of Pb concentration are typically higher than indoor or outdoor ambient Pb concentrations. These findings regarding personal exposure to Pb dust may be related to the personal cloud effect.

2.4 Toxicokinetics

The majority of Pb in the body is found in bone (roughly 90% in adults, 70% in children); only about 1% of Pb is found in the blood. Pb in blood is primarily (~99%) bound to red blood cells (RBCs). It has been suggested that the small fraction of Pb in plasma (<1%)

may be the more biologically labile and toxicologically active fraction of the circulating Pb. The relationship between Pb in blood and plasma is pseudo-linear at relatively low daily Pb intakes (i.e., <10 µg/day–kg) and at blood Pb concentrations <25 µg/dL, and becomes curvilinear at higher blood Pb concentrations due to saturable binding to RBC proteins. As blood Pb level increases and the higher affinity binding sites for Pb in RBCs become saturated, a larger fraction of the blood Pb is available in plasma to distribute to brain and other Pb-responsive tissues. See Section 4.2 for additional details.

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

The exchange of Pb from plasma to the bone surface is a relatively rapid process. Pb in bone becomes distributed in trabecular and the more dense cortical bone. The proportion of cortical to trabecular bone in the human body varies by age, but on average is about 80% cortical to 20% trabecular. Of the bone types, trabecular bone is more reflective of recent exposures than is cortical bone due to the slow turnover rate and lower blood perfusion of cortical bone. Some Pb diffuses to deeper bone regions where it is relatively inert, particularly in adults. These bone compartments are much more labile in infants and children than in adults as reflected by half-times for movement of Pb from bone into the plasma (e.g., cortical half-time = 0.23 years at birth, 3.7 years at 15 years of age, and 23 years in adults; trabecular half-time = 0.23 years at birth, 2.0 years at 15 years of age, and 3.8 years in adults) (Leggett, 1993). See Section 4.2 for additional details.

Evidence for maternal-to-fetal transfer of Pb in humans is derived from cord blood to maternal blood Pb ratios. Group mean ratios range from about 0.7 to 1.0 at the time of delivery for mean maternal blood Pb levels ranging from 1.7 to 8.6 µg/dL. Transplacental transfer of Pb may be facilitated by an increase in the plasma/blood Pb concentration ratio during pregnancy. Maternal-to-fetal transfer of Pb appears to be related partly to the mobilization of Pb from the maternal skeleton. See Section 4.2 for additional details.

The dominant elimination phase of Pb kinetics in the blood, exhibited shortly after a change in exposure occurs, has a half-life of ~20-30 days. An abrupt change in Pb uptake

gives rise to a relatively rapid change in blood Pb, to a new quasi-steady state, achieved in ~75-100 days (i.e., 3-4 times the blood elimination half-life). A slower phase of Pb clearance from the blood may become evident with longer observation periods following a decrease in exposure due to the gradual redistribution of Pb among bone and other compartments. See Section 4.3 for additional details.

2.5 Lead Biomarkers

Overall, trends in blood Pb levels have been decreasing among U.S. children and adults over the past 20 years (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.

Blood Pb is dependent on both the recent exposure history of the individual, as well as the long-term exposure history that determines body burden and Pb in bone. The contribution of bone Pb to blood Pb changes depending on the duration and intensity of the exposure, age, and various other physiological stressors that may affect bone remodeling (e.g., nutritional status, pregnancy, menopause, extended bed rest, hyperparathyroidism) beyond that which normally and continuously occurs. In children, largely due to faster exchange of Pb to and from bone, blood Pb is both an index of recent exposure and potentially an index of body burden. In adults and children, where exposure to Pb has effectively ceased or greatly decreased, a slow decline in blood Pb concentrations over the period of years is most likely due to the gradual release of Pb from bone. Bone Pb is an index of cumulative exposure and body burden. Even bone compartments should be recognized as reflective of differing exposure periods with Pb in trabecular bone exchanging more rapidly than Pb in cortical bone with the blood. This difference in the compartments making Pb in cortical bone a better marker of cumulative exposure and Pb in trabecular bone more likely to be correlated with blood Pb, even in adults. See Section 4.3 for additional details.

Sampling frequency is an important consideration when evaluating blood Pb and bone Pb levels in epidemiologic studies, particularly when the exposure is not well characterized. It is difficult to determine what blood Pb is reflecting in cross-sectional studies that sample blood Pb once, whether recent exposure or movement of Pb from bone into blood from historical exposures. In contrast, cross-sectional studies of bone Pb and longitudinal samples of blood Pb concentrations over time provide more of an index of cumulative exposure and are more reflective of average Pb body burdens over time. The degree to

which repeated sampling will reflect the actual long-term time-weighted average blood Pb concentration depends on the sampling frequency in relation to variability in exposure. High variability in Pb exposures can produce episodic (or periodic) oscillations in blood Pb concentration that may not be captured with low sampling frequencies. Furthermore, similar blood Pb concentrations in two individuals (or populations), regardless of their age, do not necessarily translate to similar body burdens or similar exposure histories.

The concentration of Pb in urine follows blood Pb concentration, in that it mainly reflects the exposure history of the previous few months and therefore, is likely a relatively poor index of Pb body burden. There is added complexity with Pb in urine because concentration is also dependent upon urine flow rate, which requires timed urine samples that is often not feasible in epidemiologic studies. Other biomarkers have been utilized to a lesser extent (e.g., Pb in teeth). See Section 4.3.

2.6 Health Effects

This section evaluates the evidence from toxicological and epidemiologic studies that examined the health effects associated with exposure to Pb and integrates that evidence across these disciplines. The results from the health studies are also considered in combination with the evidence from other disciplines (e.g., toxicokinetics) for the causal determinations (Section 2.1) 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 2-2). A more detailed discussion of the underlying evidence used to formulate each causal determination can be found in Chapter 5 of this document.

Table 2-2 Summary of causal determinations[a] between exposure to Pb and health outcomes

Outcome	Causality Determination[a]
Nervous System Effects (Section 5.3.13)	Causal Relationship
Cardiovascular Effects (Section 5.4.7)	Causal Relationship
Renal Effects (Section 5.5.6)	Causal Relationship
Immune System Effects (Section 5.6.8)	Causal Relationship
Effects on Heme Synthesis and Red Blood Cell Function (Section 5.7.7)	Causal Relationship
Reproductive and Developmental Effects (Section 5.8.10)	Causal Relationship
Cancer (Section 5.10.6)	Likely Causal Relationship

[a]Based upon the framework described in the Preamble, a determination of causality was made for a broad outcome category (i.e., nervous system effects) by evaluating the coherence of evidence across disciplines and across a spectrum of related endpoints. However, the evidence on which the causal judgment is based, including the strength of evidence for the individual endpoints within the major outcome category, is characterized within the discussion. Causal determinations were made within approximately 1-2 orders of magnitude of current levels.

2.6.1 Nervous System Effects

Recent epidemiologic and toxicological studies substantiated the strong body of evidence presented in the 2006 Pb AQCD that Pb exposure is associated with nervous system effects. The weight of epidemiologic and toxicological evidence clearly supports associations of higher blood Pb levels with decrements in cognitive function in children, i.e., full-scale IQ and various measures of learning and memory. In epidemiologic studies, these associations were substantiated in children ages 1 to 11 years and in populations with mean blood Pb levels between 2 and 7 µg/dL. Observation of a supralinear concentration-response relationship and associations with mean (or quantile) blood Pb levels < 2 µg/dL do not provide evidence for a threshold for effects of Pb exposure on the developing nervous systems of children. Epidemiologic and toxicological evidence clearly demonstrates Pb-associated increases in behavioral problems, in particular, inattention and impulsivity (prior symptoms of ADHD). Associations are substantiated in children ages 1 to 12 years with mean concurrent blood Pb levels of 2 to 5 µg/dL. In epidemiologic studies, associations with cognitive function and behavior were observed after adjustment for a range of potential confounding variables, but most commonly, parental IQ, parental education, and other SES-related variables.

In animals, the weight of evidence demonstrates effects on cognition and behavior with prenatal and early postnatal Pb exposures that resulted in blood Pb levels of 10 to 40 µg/dL. In children, the weight of evidence supports cognitive function decrements and behavioral problems in association with concurrent blood Pb levels. Associations also are observed with prenatal, early childhood, and childhood average blood Pb levels, thus uncertainty remains regarding the lifestage of exposure within childhood that is associated with the greatest risk. The uncertainty regarding the frequency, timing duration and level of exposure contributing to the observed associations is greater in studies of older children compared to younger children. The weight of toxicological evidence demonstrates neurodevelopmental effects on cognition and behavior with prenatal and early postnatal Pb exposures that can have effects persisting to adulthood. The biological plausibility for epidemiologic and toxicological findings for effects on cognitive function and behavior is provided by evidence characterizing underlying mechanisms, including Pb-induced changes in neurogenesis, synaptogenesis and synaptic pruning, long term potentiation, and neurotransmitter function. Based most heavily on cognitive function decrements and inattention in children, the collective body of evidence integrated across epidemiologic and toxicological studies is sufficient to conclude that there is a causal relationship between Pb exposures and nervous system effects (Section 5.3.13).

New evidence demonstrates associations of blood Pb level with Attention Deficit Hyperactivity Disorder (ADHD) in children (8-17 years old) and this evidence is consistent with findings demonstrating the effect of Pb on inattention and impulsivity in children. Several new epidemiologic studies indicate associations between higher concurrent blood Pb level and higher prevalence or incidence of ADHD diagnosis and its contributing diagnostic indices, whereas previous evidence was inconsistent. The biological plausibility for associations with ADHD is strongly supported by the large epidemiologic and toxicological evidence base demonstrating Pb-associated increases in inattention and impulsivity, both of which are primary symptoms of ADHD.

The evidence for Pb-associated effects on other nervous system endpoints was also evaluated. Compared to the body of evidence on behavior and inattention, a smaller but equally consistent evidence base indicated associations of concurrent and early childhood blood Pb levels with social misconduct in children and delinquent behaviors in adolescents and young adults (Table 5-11). Associations of blood Pb levels with ADHD, misconduct, and delinquency were observed in populations of children with a wide range of blood Pb levels, 1 to 11 µg/dL, all similar in the strength of evidence. While the different behavioral indices are examined, Pb exposure also was found to affect behavior (decreased ability to escape predators or capture prey) in aquatic and terrestrial species (Section 7.2 and Section 7.3).

A few new toxicological studies augmented the evidence for Pb-related effects to the visual system by demonstrating retinal changes in male rodent offspring in association with lower blood Pb levels (<15 µg/dL) than previously examined (20 to > 100 µg/dL) (Section 5.3.4.3). A small body of epidemiologic evidence together with a large historical base of toxicological evidence indicated associations of Pb biomarkers or exposure with impaired auditory function. Associations were found in children ranging from 4 to 19 years in age and with mean concurrent blood Pb levels of 7-12 µg/dL (Section 5.3.4.1). While mood and emotional state have been examined less frequently compared with inattention and misconduct, several studies found associations of biomarkers of cumulative Pb exposure (i.e., tooth or childhood average blood Pb) and concurrent blood Pb levels with parental or teacher reports of withdrawn behavior or depression in children with mean blood Pb levels 8-28 µg/dL (Section 5.3.3.3). These findings in children are supported by a small body of toxicological studies in which prenatal plus lactational Pb exposure resulted in depression-like behavior in rodent models. Studies also reported associations of early childhood average and concurrent blood Pb levels with lower fine and gross motor function in children ages 3 to 17 years (Section 5.3.5). A common observation across studies was finding that biomarkers of Pb exposure were associated with decrements in multiple neurodevelopmental outcomes, including cognitive function, externalizing behaviors, internalizing behaviors, and motor function, within the same population. These findings in combination with previously discussed cognitive and behavioral effects indicate that Pb exposure affects a broad spectrum of neurodevelopmental effects in children.

In adults, the frequency, duration, timing and level of Pb exposure implicated in nervous system effects remain uncertain. Among occupationally-exposed adults, a spectrum of nervous system effects is associated with concurrent blood Pb level (≥ 14 µg/dL), which reflects both current and cumulative exposure. However, in adults without occupational exposure, cognitive performance is more strongly associated with tibia Pb levels than blood Pb levels, which indicates an effect of long-term, cumulative Pb exposures. Based on a smaller body of epidemiologic studies, blood and bone Pb levels were associated with essential tremor and PD, respectively, in adults (Section 5.3.7.1). However, in these case-control studies, it is difficult to establish temporality between Pb exposure and disease. Support for epidemiologic findings for PD is provided by toxicological evidence for Pb-induced decreased dopaminergic cell activity in the substantia nigra, which contributes to the primary symptoms of Parkinson’s disease. Whereas evidence for association with Alzheimer's disease in adults is weak, developmental Pb exposures of monkeys (early postnatal, PND 1-400) and rats (lactational) has been shown to induce formation of amyloid plaques, pathology that underlies Alzheimer's disease (Section 5.3.7.2). A small body of studies of behavior in adults examined and found

associations of blood and tibia Pb levels with depression and anxiety symptoms (Section 5.3.3.6).

2.6.2 Cardiovascular Effects

The 2006 Pb AQCD concluded that there was a relationship between higher blood Pb and bone Pb and cardiovascular effects in adults, in particular increased blood pressure (BP) and increased incidence of hypertension (U.S. EPA, 2006b). Building on this strong body of evidence, recent epidemiologic and toxicological studies substantiated the evidence that long-term Pb exposure is associated with cardiovascular effects in adults with the largest body of evidence demonstrating associations of Pb with increased BP and hypertension. The epidemiologic evidence is strengthened by several recent prospective studies that find associations between biomarkers of Pb and BP and hypertension and by effect estimates that are observed after adjustment for multiple potential confounding factors. The weight of epidemiologic evidence supported associations in adults with mean concurrent blood Pb levels less than 5 µg/dL. As these outcomes in epidemiologic studies were most often observed in adults with likely higher past than current Pb exposures, uncertainty exists as to the Pb exposure level, timing, frequency, and duration contributing to the observed associations. Recent epidemiologic studies found that bone Pb level, a metric of cumulative exposure, is strongly related to hypertension risk in adults with mean bone Pb levels greater than 20 µg/g. However, uncertainties also exist as to the specific Pb exposure conditions that contributed to the associations. The weight of animal evidence also demonstrates an increase in BP after long-term (i.e., greater than 4 weeks in rodents) exposure to Pb. Whereas the majority of studies examined and found increases in BP in animals with mean blood Pb levels greater than 10 µg/dL (Table 5-18), a recent study found elevated BP in animals with a mean blood Pb level of 2 µg/dL (Tsao et al. 2000). Also, animal toxicological studies provided mechanistic evidence to support the biological plausibility of Pb-induced hypertension, including Pb-induced oxidative stress, activation of RAAS (renin-angiotensin-aldosterone system), altered sympathetic activity, and vasomodulator imbalance. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of other cardiovascular endpoints examined is sufficient to conclude that there is a causal relationship between Pb exposures and cardiovascular health effects (Section 5.4.7).

Studies in the 2006 Pb AQCD (U.S. EPA, 2006b) also found associations between Pb biomarkers of exposure and other cardiovascular diseases such as IHD, cerebrovascular disease, peripheral vascular disease, and cardiovascular disease related mortality; however, the available evidence was limited. Recent epidemiologic and toxicological studies continue to provide evidence in adults for blood Pb-associated increased

atherosclerosis, thrombosis, IHD, PAD, arrhythmia, and cardiac contractility in populations with mean blood Pb levels >2.5 µg/dL (Table 5-19). Further, animal toxicological evidence continued to build on the evidence supporting the mechanisms leading to these cardiovascular system responses, as well as Pb-induced changes in BP and hypertension. 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 consistent associations observed between higher blood and bone Pb levels and greater cardiovascular effects.

Recent epidemiologic studies of adults also investigated the interaction of Pb biomarkers with genetic variants in associations with cardiovascular effects. Evidence was presented for a larger blood Pb-associated increase in BP in carriers of the ALAD2 allele, which is associated with greater binding affinity for Pb in the bloodstream (Figure 5-26). Additionally, bone Pb concentration was associated with larger increases in PP, which is a good predictor of cardiovascular morbidity and mortality and an indicator of arterial stiffness, among adults with the HFE H63D and/or C282Y variant and there was evidence of HFE and transferrin gene variants, related to iron metabolism, impacting the associations of bone Pb levels with cardiovascular effects, evaluated by QT interval changes (Figure 5-26). The evidence for genetic factors to potentially increase the risk of Pb related health effects was limited (Table 6-4). New evidence extended the potential continuum of Pb-related cardiovascular effects by demonstrating associations of blood Pb and bone Pb with both cardiovascular and all-cause mortality with follow-up periods ranging between 8 and 12 years. The associations of Pb with cardiovascular morbidity observed in both epidemiologic and toxicological studies support recent epidemiologic findings of increased Pb-associated cardiovascular mortality. Since the populations enrolled in the epidemiologic studies were largely composed of adults with likely higher past than current Pb exposures, uncertainty exists as to the Pb exposure level, timing, frequency, and duration contributing to the observed associations.

2.6.3 Renal Effects

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 over follow-up of 4 to 15 years in association with higher baseline blood and bone Pb levels (U.S. EPA, 2006b). Uncertainty remained on the contribution of past Pb exposures to associations observed in adults, the renal effects of Pb in children, and the implication of hyperfiltration. Because blood Pb level in nonoccupationally-exposed adults reflects

both recent and past Pb exposures, the magnitude, timing, frequency, and duration of Pb exposure contributing to the observed associations remains uncertain.

New epidemiologic and toxicological studies evaluated in the current review support or expand upon the strong body of evidence presented in the 2006 Pb AQCD (U.S. EPA, 2006b). The weight of epidemiologic evidence demonstrates consistently a relationship between higher blood Pb level and kidney dysfunction (e.g., lower creatinine clearance, higher serum creatinine, and lower GFR [glomerular filtration rate]) in nonoccupationally-exposed adults with mean concurrent or baseline blood Pb levels of 2-10 µg/dL. A few analyses find higher blood Pb levels to be associated with a greater longitudinal decrease in kidney function over time (4-15 years), suggesting that past Pb exposures may contribute to ongoing renal effects. The epidemiologic evidence is strengthened by associations between Pb biomarker levels and renal function that are observed after adjustment for multiple potential confounding factors such as age, sex, comorbid cardiovascular conditions, BMI (body mass index), smoking, and alcohol use. Coherence for epidemiologic findings is provided by observations in animal models that Pb exposure for greater than 6 months decreases GFR and increases serum creatinine. The weight of evidence in animal studies indicates that Pb induces histopathological changes, including tubular atrophy and sclerosis. Overall, reduced renal function and increased kidney damage in animals are observed with chronic Pb (> 4 weeks) exposure that results in blood Pb levels > 20 µg/dL. By demonstrating Pb-induced renal oxidative stress, inflammation, mitochondrial dysfunction, apoptosis, and glomerular hypertrophy, toxicological studies provide biological plausibility for the associations observed in epidemiologic studies between blood Pb levels and kidney dysfunction. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of renal outcomes is sufficient to conclude that there is a causal relationship between Pb exposures and renal health effects (Section 5.5.6).

In addition, data on the effects of Pb on the kidney in children were reported in a recent NHANES analysis of adolescents, ages 12-20 years, which observed an association between higher concurrent blood Pb (mean: 1.5 µg/dL) and lower cystatin C-based eGFR (Section 5.5.6). These findings are consistent with results from a rodent model study 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. This recent epidemiologic study along with several previous studies that included children with higher Pb exposures (due to residence near sources, Pb poisoning, or parental occupational exposure) provide evidence that renal function in children may be affected by Pb exposure. The NHANES adolescents, however, likely had higher Pb exposures earlier in childhood, thus, the magnitude, timing, frequency, and duration of Pb exposure contributing to the observed

association is uncertain. Research on the association of Pb with kidney function in the occupational setting is less consistent than that in environmentally exposed populations (Section 5.5.2.25.5.2.2). 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 reflects limitations of the study design.

2.6.4 Immune System Effects

The 2006 Pb AQCD presented consistent evidence for immune system effects associated with Pb exposure (U.S. EPA, 2006b). Rather than producing overt cytotoxicity or pathology, Pb exposure was found to be associated with alterations in several subclinical parameters related to cellular and humoral immunity (Figure 5-42). Recent toxicological and epidemiologic studies support the strong body of evidence presented in the 2006 Pb AQCD that Pb exposure may be associated with a broad spectrum of changes in both cell-mediated and humoral immunity that cumulatively promote a Th2 phenotype and hyperinflammatory state (U.S. EPA, 2006b). The principal findings are Pb-induced increased production of Th2 cytokines, suppressed production of Th1 cytokines, increased inflammation, and elevated IgE, with the weight of evidence provided by toxicological studies. Collectively, these findings are coherent with the observed effects of Pb exposure on decreasing responses to antigens (e.g., DTH, bacterial resistance) in animals. Both toxicological studies in animals and epidemiologic studies in children provide evidence for Pb-associated increases in IgE. The toxicological and epidemiologic findings for Th2 cytokines, IgE, and inflammation provide biological plausibility for the associations observed for blood Pb levels with asthma and allergic conditions in children. Associations with asthma and allergy were observed after considering potential confounding by several factors, including, SES and allergen exposure. Animal studies found a range of immune effects with prenatal exposure in juvenile animals and long-term postnatal (> 4 weeks) Pb exposures in adult animals. The blood Pb levels and Pb exposure lifestage, magnitude, frequency, and duration associated with immune effects are not well characterized in children or adults. Epidemiologic studies of children and adults primarily examined concurrent blood Pb levels. Little information was provided on concentration-response functions. In epidemiologic studies, higher IgE and higher asthma prevalence were examined and found in children with blood Pb levels > 10 µg/dL. The consistency and coherence of findings across the continuum of related immune parameters that demonstrate a stimulation of Th2 responses in toxicological studies combined with the supporting epidemiologic evidence in children are sufficient to conclude that there is a causal relationship between Pb exposures and immune system effects (Section 5.6.8).

The toxicological evidence for Pb-induced production of Th2 cytokines provides biological plausibility for the evidence linking Pb exposure with elevated IgE levels (Section 5.6.8). 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. However, epidemiologic studies did not find blood Pb level to be associated consistently with B cell abundance. In addition to T cell responses, a prominent effect of Pb exposure, as demonstrated in an extensive historical toxicological evidence base, was the induction of 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). Consistent with these observations, a previous epidemiologic study examined and found greater release of ROS and lower release of NO from macrophages, primarily in children with concurrent blood Pb levels 10.31-47.49 µg/dL (Pineda-Zavaleta et al., 2004). Misregulated inflammation represents one of the major modes of action for Pb-induced immune effects. Toxicological studies provide evidence for the modulation of inflammatory cell function, production of pro-inflammatory cytokines and metabolites, enhanced inflammatory chemical messengers, and pro-inflammatory signaling cascades. In addition to the associations reported with IL-4, epidemiologic evidence for Pb effects on inflammation is limited to a few recent studies in nonoccupationally-exposed adults in which concurrent blood Pb level was associated with other indicators of inflammation such as CRP and IL-6 (Section 5.6.5.1). The studies commonly adjusted for potential confounding by age, sex, BMI, and smoking status. However, because only concurrent blood Pb levels in adults were examined, there is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposures that contributed to the observed associations.

The effects of Pb exposure on macrophages also suggest a role for the immune system in mediating Pb-associated effects in multiple other physiological systems. A small body of new toxicological studies indicated Pb-induced changes in specialized macrophages in nonlymphoid tissue such as alveolar macrophages, testicular macrophages, and brain microglia (Section 5.6.4.5); however, these studies primarily used the i.p. route to administer Pb. Thus, the relevancy of observations to those expected from typical routes of human exposure is not clear.

In the large body of studies of adults (mostly males) with occupational Pb exposures (Section 5.6.2.5), the most consistent findings were decreased neutrophil functionality in workers with mean blood Pb levels 21-71 µg/dL. Recent epidemiologic studies provided new evidence in adults without occupational Pb exposures; however, each examined a different immune endpoint, for example, IgE, eNO, IL-6. These endpoints were associated with concurrent blood Pb levels in populations of adults with mean blood Pb

levels of 1.9 µg/dL to 7 µg/dL; however, there is uncertainty regarding the contributions of current Pb exposures and cumulative Pb stores in bone. The small body of epidemiologic studies of nonoccupationally-exposed adults examined different endpoints and found associations with concurrent blood Pb levels, which are influenced by current Pb exposures as well as cumulative Pb stores in bone (Section 5.6).

2.6.5 Heme Synthesis and Red Blood Cell Function

The 2006 Pb AQCD reported that Pb affects developing red blood cells (RBCs) in children and occupationally exposed adults as noted by anemia observed with 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 Pb-induced changes on iron uptake or inhibition of enzymes in the heme synthetic pathway may be responsible. Pb was also observed to exert effects on heme synthesis through the inhibition of multiple key enzymes, most notably ALAD (aminolevulinic acid dehydratase), the enzyme that catalyzes the second, rate-limiting step in heme biosynthesis (Figure 5-45 presents a schematic representation of the heme biosynthetic pathway). Decreased RBC ALAD activity was concluded to be the most sensitive measure of human Pb exposure, in that measurement of ALAD activity is correlated with blood Pb levels. Oxidative stress was identified as an important potential mechanism of action by which Pb exposure induced effects on RBCs (U.S. EPA, 2006b).

Recent epidemiologic and toxicological studies support findings from the 2006 Pb AQCD. The principal finding regarding RBC survival and function are consistent Pb-induced alterations in several inter-connected hematological parameters such as Hb (hemoglobin), Hct (hematocrit), and MCV (mean corpuscular volume) across multiple studies, with the weight of evidence provided by epidemiological studies in occupationally-exposed adult populations and children. In occupationally-exposed adults, these findings are most substantiated in populations with current blood Pb levels > 20 µg/dL, although effects on hematological parameters were observed in some occupationally-exposed populations at concurrent blood Pb levels in the range of 5-7 µg/dL. The weight of evidence in adult rodents exposed long-term to Pb (i.e., ≥ 4 weeks) is coherent with epidemiologic studies regarding decrements in hematological parameters at blood Pb levels as low as 6.6-7.1 µg/dL in rats and mice. Regarding alterations in heme synthesis, the largest body of evidence again is provided by decreased ALAD activity in association with Pb exposure or blood Pb levels in occupationally-exposed adults or in children (3-12 years old), respectively. In the occupationally-exposed adult populations, the observation of decreased ALAD activity was most often observed in populations with concurrent blood Pb levels >15 µg/dL.

Animal toxicological studies also provide to the weight of evidence regarding altered ALAD activity, with effects seen in adult animals exposed for 3-4 weeks with blood Pb levels as low as 6.5 µg/dL. The weight of evidence for oxidative stress (i.e., increased lipid peroxidation or alterations in antioxidant enzyme levels) primarily comes from epidemiological studies in occupationally-exposed adults and children. The majority of evidence for increased oxidative stress in Pb-exposed adults comes from occupational cohorts with concurrent blood Pb levels >15 µg/dL. In children, concurrent blood Pb levels of 7-22 µg/dL were associated with measures of oxidative stress. The frequency, timing and duration of exposure necessary to alter RBC survival and function, heme synthesis, or the state of oxidative stress in RBCs is uncertain in both adults and children. The consistency of findings in epidemiologic studies investigating effects in occupationally-exposed adults and in children, and the coherence of findings in the toxicological literature and coherence across the disciplines, is sufficient to conclude that there is a causal relationship between Pb exposures and heme synthesis and RBC function (Section 5.7.7).

2.6.6 Reproductive and Developmental Effects

Recent toxicological and epidemiologic literature provides strong evidence that Pb exposure is associated with effects on reproduction and development supporting conclusions of the 2006 Pb AQCD and expanding evidence for additional endpoints. The weight of the evidence supports the association of Pb exposure with delayed onset of puberty in both males and females and detrimental effects on sperm and semen quality in occupationally-exposed males and in laboratory animals. In cross-sectional epidemiologic studies of girls (ages 6-18 years) with mean and/or median concurrent blood Pb levels less than 5 µg/dL consistent associations with delayed pubertal development (measured by age at menarche, pubic hair development, and breast development) were observed. Toxicological studies of rodents indicate that prenatal and lactational exposures to Pb can cause a delay in the onset of female puberty at blood Pb levels as low as 8 µg/dL. Recent studies show that pubertal onset is one of the more sensitive markers of Pb exposure with effects observed after maternal exposures leading to blood Pb levels in the pup of 3.5-13 µg/dL. In boys (ages 8-15 years), fewer studies were conducted but associations were observed in most. Male animal toxicology studies have reported delayed sexual maturity as measured with prostate weight, among other outcomes, seeing significant decrements at blood Pb levels of 34 µg/dL. There is uncertainty with regard to the exposure frequency, timing, duration and level that contributed to these observed association in these studies of adolescents. Multiple studies were performed in areas, which were contaminated with other chemicals as well as Pb.

Additionally, Pb exposure has been shown to have detrimental effects on sperm. These were observed in epidemiologic studies at population mean blood Pb levels of 30 µg/dL and greater among men occupationally exposed (mean blood Pb levels in study controls around 10 µg/dL) and in animal toxicological studies with rabbits exposed to subcutaneous Pb 3 times per week for 15 weeks with blood Pb levels of 20 µg/dL. The collective body of evidence integrated across epidemiologic and toxicological studies with a focus on the strong relationship observed with detrimental effects on sperm and delayed pubertal onset is sufficient to conclude that there is a causal relationship between Pb exposures and reproductive and developmental effects (Section 5.8.10).

The evidence on hormonal influences and fecundity are less consistent (Section 5.8.1, Section 5.8.2). 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. 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.

Consistent conclusions in the 2006 Pb ISA recent studies of pregnancy outcomes were reported mixed results. Inconsistent evidence of a relationship with Pb was available for preterm birth and little evidence was available to study the associations with spontaneous abortions (Section 5.8.6, Section 5.8.3). The 2006 Pb AQCD included a few studies that reported potential associations between Pb and neural tube defects, but the recent epidemiologic studies found no association. Some associations were observed between Pb and low birth weight (Section 5.8.7) 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 (maternal blood Pb or umbilical cord and placenta Pb were the biomarkers most commonly used in studies of low birth weight). Effects of Pb exposure during early development on toxicological studies included reduction in litter size, implantation, birth weight and postnatal growth (Section 5.8.4). Findings from epidemiologic studies of postnatal growth are inconsistent (Section 5.8.8). 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 (Section 5.8.9). Negative developmental outcomes were also noted including effects on the eyes and teeth (Section 5.8.9).

2.6.7 Cancer

The previous epidemiologic studies included in the 2006 Pb AQCD (U.S. EPA, 2006b) "provide[d] only very limited evidence suggestive of Pb exposure associations with carcinogenic or genotoxic effects in humans" and the studies were summarized as follows:

> "The epidemiologic data …suggest a relationship between Pb exposure and cancers of the lung and the stomach… Studies of genotoxicity consistently link Pb-exposed populations with DNA damage and micronuclei formation, although less consistently with chromosomal aberrations."

The International Agency for Research on Cancer (IARC) classified inorganic Pb compounds as probable human carcinogens (Group 2A of IARC classifications) based on stronger evidence in animal studies than human studies, and organic Pb compounds as not classifiable (Group 3 of IARC classifications) (IARC, 2006a; Rousseau et al., 2005). Additionally, the National Toxicology Program has listed Pb and Pb compounds as "reasonably anticipated to be human carcinogens" (NTP, 2004). The typical cancer bioassays used by IARC or NTP as evidence of Pb-induced carcinogenicity used rodents that were continuously exposed to Pb-acetate in chow or drinking water for 18 months to two years in duration. These two year cancer bioassays and the doses administered are typical of cancer bioassays used with other chemicals.

The animal toxicological literature continues to provide the strongest evidence for Pb exposure and cancer with some supporting evidence provided by the epidemiologic literature. Evidence from toxicological studies demonstrates an association between Pb and cancer, genotoxicity/clastogenicity or epigenetic modification. Carcinogenicity in historical animal toxicology studies with Pb exposure has been reported in the kidneys, testes, brain, adrenals, prostate, pituitary, and mammary gland, albeit at high doses of Pb. Epidemiologic studies of cancer incidence and mortality reported inconsistent results; one large epidemiologic study demonstrated an association between blood Pb and increased cancer mortality, but the other studies reported weak or no associations. In the 2006 Pb AQCD, Pb exposure was found to be associated with stomach cancer, but there was only one recent study on stomach cancer and Pb exposure, which reported mixed findings. Similarly, some studies in the 2006 Pb AQCD reported associations between Pb exposure and lung cancer (U.S. EPA, 2006b). More recent occupational studies of Pb exposure and lung cancer reported no associations. The majority of epidemiologic studies of brain cancer had null results overall, but positive associations between Pb exposure and brain

cancer were observed among individuals with certain genotypes (e.g., ALAD2). In toxicological studies, chromosomal aberrations after Pb exposure are most often reported with Pb chromate exposure, which is likely due to toxicity of the chromate moiety. Mechanistic understanding of Pb and its effect on cancer and genotoxicity is expanding through toxicological work focusing on antioxidants and other proteins that sequester Pb or reduce its bioavailability. The collective body of evidence integrated across toxicological and epidemiologic studies is sufficient to conclude that there is a likely causal relationship between Pb exposure and cancer (Section 5.10.6).

2.7 Ecological Effects of Lead

This section evaluates the evidence from studies of ecological effects associated with exposure to Pb. Causal determinations are developed for the ecological outcomes discussed in this assessment, in combination with evidence from other disciplines (e.g., fate and transport) where relevant. Pb effects on terrestrial and aquatic systems (Section 2.7.1 and Section 2.7.2) are summarized from Chapter 7 in which the effects on terrestrial and aquatic ecosystems are presented separately. These sections are followed by a summary of the evidence for the causal determinations (Section 2.7.3) and consideration of atmospheric deposition of Pb as related to ecological effects (Section 2.9.3). The ecological causal determinations are integrated across endpoints (physiological stress, hematological effects, neurobehavioral effects, development and reproduction, growth, survival) common to both terrestrial and aquatic biota (Table 2-4). Where the causal determination varies substantially between types of organisms (typically between plants and other organisms or between aquatic and terrestrial biota), the divergence is noted.

2.7.1 Summary of Effects on Terrestrial Ecosystems

Historically, Pb poisoning is one of the earliest recognized toxicoses of terrestrial biota, occurring primarily through ingestion of spent shot by birds (Section 7.4.2.3). At the time of the 1977 Pb AQCD few studies of Pb exposure and effects in wild animals other than birds were available. A limited number of rodent trapping studies and observations from grazing animals near smelters provided evidence for differences in Pb sensitivity among species and these findings were further supported in the 1986 and 2006 Pb AQCDs (U.S. EPA, 2006b, 1986b, 1977). ALAD was recognized as a sensitive indicator of Pb exposure in rats and waterfowl in the 1977 Pb AQCD and now is regarded as a biomarker of exposure in many terrestrial organisms. According to the 2006 Pb AQCD and supported by evidence from previous Pb AQCDs, commonly observed effects of Pb on

terrestrial organisms include decreased survival, reproduction, and growth, as well as effects on development and behavior. Furthermore, the toxicity of Pb to terrestrial vertebrates and invertebrates varies with bioavailability of the metal, species and lifestage, duration of exposure, form of Pb, and soil characteristics.

In plants, Pb effects have also been studied for several decades. At the time of the 1977 Pb AQCD, it was understood that Pb uptake in plants was influenced by plant species and by the available Pb pool in the soils (U.S. EPA, 1977). Furthermore, most of the Pb taken up by plants from soil, remains in the roots and that distribution to other portions of the plant is variable among species (U.S. EPA, 1977). Plant growth was recognized as an endpoint of Pb toxicity in plants in the 1977 Pb AQCD and additional effects of Pb on growth processes were reported in subsequent Pb AQCDs (U.S. EPA, 2006b, 1986b, 1977). A study reviewed in the 1977 Pb AQCD provided evidence for Pb effects on forest-nutrient cycling and shifts in arthropod community composition in the vicinity of a smelting complex. A number of ecosystem-level effects, including decreased species diversity, changes in floral and faunal community composition, and decreasing vigor of terrestrial vegetation have subsequently been reported near Pb-point sources (U.S. EPA, 2006b, 1986b, 1977; Watson et al., 1976).

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) cation exchange capacity (CEC); and (6) aging. Since 2006, further details have been contributed to the understanding of the role of pH, CEC, organic matter, and aging. Smolders et al. (2009) demonstrated that the two most important determinants of both Pb solubility and toxicity in soils are pH and CEC. However, they had previously shown that experimental aging, primarily in the form of initial leaching following addition of Pb, decreases soluble metal fraction by approximately one order of magnitude (Smolders et al., 2009). Since 2006, organic matter has been confirmed as an important influence on Pb sequestration, leading to longer-term retention in soils with higher organic matter content, and also creating the potential for later release of deposited Pb. Aging, both under natural conditions and simulated through leaching, was shown to substantially decrease bioavailability to plants, microbes, and vertebrates.

There is evidence over several decades of research previously reviewed in Pb AQCDs and in recent studies reviewed in this ISA that Pb accumulates in terrestrial plants,

invertebrates and vertebrates. Studies with herbaceous plant species growing at various distances from smelters added to the existing strong evidence that atmospherically transported Pb is taken up by 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 the proportion of atmospheric Pb up directly from the atmosphere, and suggested it amounts to 50% of the Pb contained in Scots Pine in Sweden (Klaminder et al., 2005). 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 generally is translocated to other parts.

Since the 2006 Pb AQCD, various species of terrestrial snails have been found to accumulate Pb from both diet and soil (U.S. EPA, 2006b). 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 between species of earthworms that burrow in different soil layers. The rate of accumulation in each of these species may result from differences in interacting factors such as pH and CEC between layers. 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 are a few new studies of Pb bioavailability and uptake in birds since the 2006 Pb AQCD (U.S. EPA, 2006b). 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. Evidence of oxidative stress in response to Pb exposure has also been observed in plants. Reactive oxygen species were found to increase in broad bean and tomato plants exposed to increasing concentrations of soil Pb, and a concomitant increase in superoxide dismutase, glutathione, peroxidases, and lipid peroxidation, as well as decreases in catalase were observed in the same plants. Monocot, dicot, and bryophytic taxa grown in Pb-contaminated soil or in experimentally spiked soil all responded to increasing exposure with increased antioxidant activity. In addition, reduced growth was observed in some experiments, as well as genotoxicity, decreased germination, and pollen sterility.

In terrestrial invertebrates, evidence for Pb effects has included neurological and reproductive endpoints. Recently published studies have shown neuronal damage in nematodes exposed to 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 malformations. Within each study, increasing concentration of Pb in the exposure medium generally resulted in increased effects, but the relationship between concentration and effects varied between studies, even when the same medium, e.g., soil, was used. Evidence suggested that aging and pH are important modifiers.

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.

New evidence reviewed in Section 7.2.3 and Section 7.2.4 demonstrates that exposure to Pb is generally associated with negative effects in terrestrial ecosystems. It also demonstrates that many factors, including species and various soil physiochemical properties, interact strongly with Pb concentration to modify those effects. In these ecosystems, where soil is generally the main component of the exposure route, Pb aging is a particularly important factor, and one that may be difficult to reproduce experimentally. Without quantitative characterization of those interactions, characterizations of exposure-response relationships would likely not be transferable outside of experimental settings. Since the 2006 Pb AQCD, a few studies of exposure-response have been conducted with earthworms, and results have been inconsistent (U.S. EPA, 2006b).

New evidence of effects of Pb at the community and ecosystem levels of biological organization include several studies of the ameliorative effects of mycorrhizal fungi on plant growth in the presence of Pb, 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-level 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.

2.7.2 Summary of Effects on Aquatic Ecosystems

Pb effects on aquatic biota were previously assessed in the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD (U.S. EPA, 2006b, 1986a, 1977). Evidence of toxicity of Pb and other metals to freshwater organisms goes back to early observations of contamination of natural areas by Pb mining leading to extirpation of fish from streams (U.S. EPA, 1977). Observed responses of fish to Pb reported in the 1986 Pb AQCD and the 2006 Pb AQCD include inhibition of heme formation, alterations in brain receptors, effects on blood chemistry and hormonal systems, and decreases in some enzyme activities (U.S. EPA, 2006b, 1986a). In the 1986 Pb AQCD and 2006 Pb AQCD,

additional evidence for Pb toxicity was available for aquatic invertebrates. Inhibition of ALAD, and reduced reproduction, growth, and survival were reported. Few studies of Pb toxicity to saltwater organisms are reported in previous Pb AQCDs. The toxicity of Pb to saltwater and freshwater organisms varies with bioavailability of the metal, species and lifestage, duration of exposure, form of Pb, and water quality parameters (U.S. EPA, 2006b, 1986a). As reviewed in the 2006 Pb AQCD, the biotic ligand model (BLM) constitutes an attractive means of quantifying factors that affect bioavailability, such as pH, dissolved organic carbon (DOC), and alkalinity (U.S. EPA, 2006b).

The toxicity of Pb to aquatic algae and plants has been recognized in earlier agency reviews of this metal. In the 1977 Pb AQCD, differences in sensitivity to Pb among different species of algae were reported and concentrations of Pb varied among genera and within a genus, an observation subsequently found across aquatic taxa (U.S. EPA, 1977). At the time of the 1977 Pb AQCD, the information available on effects of Pb on aquatic plants was limited. For plants in general, Pb was recognized to affect photosynthesis, mitosis, and growth, but at concentrations higher than typically found in the environment (U.S. EPA, 1977). Findings from field studies of aquatic communities in the vicinity of Pb point sources include changes in species composition and species richness, predator/prey interactions, nutrient cycling and energy flow; however, Pb is often found coexisting with other metals and other stressors, which risk confounding the observed effects.

Effects of Pb observed in aquatic organisms are tied to terrestrial systems via watershed processes (Section 3.3). Atmospherically-derived Pb can enter aquatic systems through runoff from terrestrial systems or via direct deposition over a water surface. Once 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.

Recent evidence supports the 2006 Pb AQCD conclusion that processes such as Pb adsorption, complexation, and chelation 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; which include ingestion of contaminated food and water, uptake from sediment pore waters, or incidental ingestion of sediment (U.S. EPA, 2006b).

There are considerable differences between species in both the amount of Pb taken up from the environment and in the amount retained in the organism. 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 accumulated 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.

Recent evidence also supports the 2006 conclusions that the gill is a major site of Pb uptake in fish, and that there are species differences in the rate of Pb accumulation and distribution of Pb within the organism 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 consumers at higher trophic levels, indicating that Pb was bioconcentrated but not biomagnified. Some studies published since the 2006 Pb AQCD support the potential for transfer of Pb in aquatic food webs, while other studies indicate that Pb concentration decreases with increasing trophic level (U.S. EPA, 2006b).

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. 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. 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 and marine amphipods. As reviewed below, growth effects are observed at lower concentrations in some aquatic invertebrates since the 2006 Pb AQCD, especially in juvenile organisms. 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 aquatic vertebrates. Since the 2006 Pb AQCD, possible molecular targets for Pb neurotoxicity have been identified in fish and additional mechanisms of Pb toxicity have been elucidated in the fish gill and the fish renal system. In the 2006 Pb AQCD, amphibians were considered to be relatively tolerant to Pb. Observed responses to Pb exposure included decreased enzyme activity (e.g., ALAD reduction) and changes in behavior. Since the 2006 Pb AQCD, studies conducted at concentrations approaching environmental levels of Pb have indicated sublethal effects on tadpole endpoints including growth, deformity, and swimming ability. In fish, several recent studies on behavioral effects of Pb indicate decreased prey capture rate, slower swim speed and decline in startle response and visual contrast with Pb exposure.

Concentration-response data from plants, invertebrates and vertebrates are consistent with findings in previous AQCDs of species differences in sensitivity to Pb in aquatic systems (Section 7.3.17.4). In this ISA, as in 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 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 and Brix, 2009; Grosell et al., 2006a). 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 (Wang et al., 2010e). In a series of 48 hour acute toxicity tests using a variety of natural waters across North America, LC_{50} values ranged from 29 to 180 µg Pb/L tests with the cladoceran *Ceriodaphnia dubia* (Esbaugh et al., 2011).

In 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 near point sources (mining, effluent) 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-level effects, specifically in aquatic macrophyte communities and sediment-associated communities. Different species may exhibit different responses to Pb-impacted ecosystems dependent not only upon other environmental factors (e.g., temperature, pH), but also on the species sensitivity, lifestage, or seasonally-affected physiological state. Aquatic ecosystems with low pH and low DOM are likely to be the most sensitive to the effects of atmospherically-deposited Pb.

2.7.3 Determinations of Causality for Effects on Ecosystems

Table 2-3 Summary of Pb causal determinations for plants, invertebrates and vertebrates

Effect	Terrestrial	Aquatic[a]
Physiological Stress-All organisms	Causal	Causal
Hematological Effects-Invertebrates	Inadequate	Causal
Hematological Effects-Vertebrates	Causal	Causal
Neurobehavioral Effects-Invertebrates and Vertebrates	Likely Causal	Likely Causal
Developmental and Reproductive Effects-Plants	Inadequate	Inadequate
Developmental and Reproductive Effects-Invertebrates and Vertebrates	Causal	Causal
Growth-Plants	Causal	Causal
Growth-Invertebrates	Inadequate	Causal
Growth-Vertebrates	Inadequate	Inadequate
Survival-Plants	Inadequate	Inadequate
Survival- Invertebrates and Vertebrates	Causal	Causal
Community and Ecosystem Level Effects	Likely Causal	Likely Causal

[a]Causal determinations for aquatic biota are based primarily on evidence from freshwater organisms.

2.7.3.1 Effects on Physiological Stress

Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and physiological stress in terrestrial and aquatic plants, invertebrates and vertebrates (Sections 7.4.1.1 and 7.4.2.1).

Endpoints associated with physiological stress received no consideration prior to the 2006 Pb AQCD and this ISA. Studies published since the 2006 Pb AQCD support previous associations of Pb exposure with physiological stress. New evidence includes upregulation of antioxidant enzymes, production of reactive oxygen species and increased lipid peroxidation associated with Pb exposure in additional species of terrestrial and aquatic plants, invertebrates and vertebrates that support, and expand upon findings in the previous Pb AQCD (U.S. EPA, 2006b).

In this ISA and the 2006 Pb AQCD, there is strong evidence of upregulation of antioxidant enzymes and increased lipid peroxidation associated with Pb exposure in many species of plants, invertebrates and vertebrates. In plants, increases of antioxidant

enzymes with Pb exposure occur in algae, aquatic mosses, floating and rooted aquatic macrophytes, and terrestrial species. Most observations of antioxidant responses in plants typically occur at concentrations of Pb higher than found in the environment. However, in a few terrestrial plant species, increases of antioxidant enzymes occur at concentrations approaching the average Pb concentrations in U.S. soils and limited transplantation studies with aquatic plants indicate elevated antioxidant enzyme activity associated with Pb levels measured in sediments at polluted sites. There is considerable evidence for antioxidant activity in invertebrates, including gastropods, mussels, and crustaceans, in response to Pb exposure. Some recent evidence for invertebrate antioxidant responses in aquatic species indicates effects at Pb concentrations associated with polluted sites. Markers of oxidative damage are also observed in fish, amphibians and mammals, both in the laboratory and in exposed natural environments. Across all biota, there are differences in the induction of antioxidant enzymes that appear to be species-dependent.

Additional stress responses observed in terrestrial and 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 following 96-hour exposures 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. The oxidative stress responses associated with Pb exposure are consistent in terrestrial biota and in aquatic organisms. Furthermore, these responses are also observed in experimental animal studies, and in humans.

2.7.3.2 Hematological Effects

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 aquatic invertebrates and terrestrial and aquatic vertebrates (Section 7.4.1.2, Section 7.4.2.2). The evidence is inadequate to conclude that there is a causal relationship between Pb exposures and hematological effects in terrestrial invertebrates.

Recent studies add support to the strong body of evidence presented in previous Pb AQCDs that Pb exposure is associated with hematological responses in aquatic invertebrates and terrestrial and aquatic vertebrates. In environmental assessments of metal-impacted habitats, ALAD is a recognized biomarker of Pb exposure (U.S. EPA, 2006b). 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 (U.S. EPA, 1986a). Further evidence from the 2006 Pb AQCD and this review suggests this enzyme is an indicator for Pb exposure across a wide range of taxa. Since the 2006 Pb AQCD, evidence of Pb effects on ALAD activity has been found in additional species of amphibians and fish, and has been identified in bacteria. New field studies of ALAD activity include observations in songbirds and owls near historical mining areas. In addition to consideration of ALAD activity, there is new evidence for deceased white blood cell counts in amphibians affected by Pb exposure. The consistency and coherence of these findings of effects on ALAD activity are also supported by some evidence of Pb-induced alterations of blood chemistry in fish reported in the 2006 Pb AQCD (U.S. EPA, 2006b). This evidence is strongly coherent with observations from human epidemiologic and animal toxicology studies where a causal relationship was identified between exposure to Pb and hematological effects in humans and laboratory animals (Section 2.6.5 and Section 5.7).

2.7.3.3 Neurobehavioral Effects

Overall, the evidence from terrestrial and aquatic systems is sufficient to conclude that there is a likely causal relationship between Pb exposures and neurobehavioral effects in terrestrial and aquatic invertebrates and vertebrates (Section 7.4.1.3, Section 7.4.2.3). Evidence from laboratory studies reviewed in Chapter 7 and previous Pb AQCDs have shown adverse effects of Pb on neurological endpoints in both aquatic and terrestrial animal taxa. Studies that consider mode-of-action and molecular targets of Pb toxicity in biota are now available for a few species. New studies have continued adding to the evidence from both invertebrate and vertebrate studies that Pb adversely affects behaviors such as food consumption, avoidance and escape from predators, behavioral thermoregulation, and prey capture. These changes are likely to decrease the overall fitness of the organism. New evidence in this ISA includes reports of behavioral responses across a larger variety of organisms including reptiles and fish larvae born from Pb-exposed adults, while some impairments in feeding and escaping behaviors were reported for the first time. More evidence has become available for marine organisms.

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 (*Caenorhabditis elegans*) (Du and Wang, 2009).

Decreased food consumption of Pb-contaminated diet has been demonstrated in some invertebrates (snails) and vertebrates (lizards, pigs, fish). Behavioral effects in grunt fish *Haemulon scudderi*, occupying the top level of a simulated marine food chain included lethargy and decreased food intake in a 42-day feeding study (Soto-Jiménez et al., 2011a). These fish were fed white shrimp exposed to Pb via brine shrimp that were initially fed microalgae cultured at 20 µg Pb/L. In the same study, surfacing, reduction of motility, and erratic swimming were observed in the white shrimp after 30 days of exposure to Pb via diet. Pb may also decrease the ability of an organism to capture prey or escape predation. For example, Pb exposure has been demonstrated to adversely affect prey capture ability of certain fungal and fish species, and the motility of nematodes was adversely affected in Pb-contaminated soils (Wang and Xing, 2008). Prey capture ability was decreased in 10 day old fathead minnows born from adult fish exposed to 120 µg Pb/L for 300 days, then subsequently tested in a 21-day breeding assay (Mager et al., 2010). In a laboratory study, Pb-exposed gull chicks exhibited abnormal behaviors such as decreased walking, erratic behavioral thermoregulation and food begging that could make them more vulnerable in the wild (Burger and Gochfeld, 2005). The chicks were exposed to Pb via injection to produce feather Pb concentration approximately equivalent to those observed in wild gulls. Lizards exposed to Pb through diet in the laboratory exhibited abnormal coloration and posturing behaviors. Other behavioral effects affected by Pb exposure include increased hyperactivity in fish and hypoxia-like behavior in frogs.

These findings show strong coherence with findings from studies in laboratory animals described in Section 2.6.1 and Section 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).

2.7.3.4 Effects on Development and Reproduction

Evidence in this review and the previous Pb AQCDs from invertebrate and vertebrate studies indicate that Pb is affecting reproductive performance in multiple species. Various

endpoints have been measured in multiple taxa of terrestrial and aquatic organisms to assess the effect of Pb on development, fecundity and hormone homeostasis, and they have demonstrated the presence of adverse effects. Reproductive effects are important when considering effects of Pb because impaired fecundity at the organismal level can result in a decline in abundance and/or extirpation of populations, decreased taxa richness, and decreased relative or absolute abundance at the community level (Suter et al., 2005; U.S. EPA, 2003a). The evidence is sufficient to conclude that there is a causal relationship between Pb exposures and developmental and reproductive effects in terrestrial and aquatic invertebrates and vertebrates (Section 7.4.1.4, Section 7.4.2.4). The evidence is inadequate to conclude that there is a causal relationship between Pb exposures and developmental and reproductive effects in plants (Section 7.4.2.4).

In terrestrial invertebrates, new developmental and reproductive endpoints shown to be affected by Pb include hatching success in collembolans, increased development time in fruit flies and aphids, and disrupted hormone homeostasis in moths; however, these studies have been conducted at Pb concentrations that exceed Pb soil concentrations found in most U.S. locations. In terrestrial vertebrates, new evidence for decreased sperm count and quality in deer at a location contaminated by mining, and for decreased testis weight in lizards, support previous associations between Pb exposure and reproductive and developmental effects. Recent studies in freshwater rotifers and cladocerans provide additional evidence for reproductive and developmental effects of Pb at concentrations at or near ambient Pb levels in some aquatic species. New evidence in frogs and fish continue to support developmental and reproductive effects of Pb in aquatic vertebrates reported in earlier Pb AQCDs. Few studies are available that specifically address reproductive effects of Pb exposure in plants.

In terrestrial invertebrates, Pb can alter developmental timing, hatching success, sperm morphology, and hormone homeostasis. In fruit flies, Pb exposure increased time to pupation and decreased pre-adult development. Sperm morphology was altered in earthworms exposed to Pb-contaminated soils. Pb may also disrupt hormonal homeostasis in invertebrates as studies with moths have suggested. Evidence of multi-generational toxicity of Pb is also present in terrestrial invertebrates, specifically springtails, mosquitoes, carabid beetles, and nematodes where decreased fecundity in progeny of Pb-exposed individuals was observed. However, effects have only been studied in a small number of species and at concentrations that typically exceed Pb levels in U.S. soils.

For aquatic invertebrates, reproductive effects were reported to begin at 19 µg Pb/L for the freshwater snail *Lymnea palustris* and 27 µg Pb/L for Daphnia sp. as reported in the 1986 Pb AQCD (U.S. EPA, 1986b). Several new studies of snails, clams, and rotifers

support previous findings of adverse impacts to embryonic development. Reproductive effects have also been observed in multi-generational studies with aquatic invertebrates. Larval settlement rate and rate of population increase was decreased in rotifers and marine amphipods. Rotifers have a reduced fertilization rate associated with Pb exposure that appears to be due to decreased viability of sperm and eggs.

In aquatic vertebrates there is evidence for reproductive and developmental effects of Pb. Pb-exposure in tadpoles has been demonstrated to delay metamorphosis, decrease larval size, produce subtle skeletal malformations, and to result in slower swimming speed. Previous Pb AQCDs have reported developmental effects in fish, specifically spinal deformities in larvae at a concentration of 120 µg Pb/L. In the 2006 Pb AQCD, decreased spermatocyte development in rainbow trout was observed at 10 µg Pb/L and in fathead minnow testicular damage occurred at 500 µg Pb/L. In 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 observed impacts of Pb on reproductive endpoints in fish 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 Pb exposure and observed adverse reproductive effects. In mammals, few studies in the field have addressed Pb specifically: most available data in wild or grazing animals are from near smelters, where animals are co-exposed to other metals. Evidence obtained using mammals in the context of human health research demonstrates adverse effects of Pb on sperm, and on onset of puberty in males and females (Chapter 5), which is coherent with the partial evidence from mammals in the wild. Other reproductive endpoints including spontaneous abortions, pre-term birth, embryo development, placental development, low birth weight, subfecundity, hormonal changes, and teratology were also affected, but less consistently. 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 in humans and laboratory animals.

Many studies of effects on reproductive and developmental endpoints in terrestrial invertebrates and vertebrates have been conducted with soil Pb concentrations exceeding those found in most U.S. locations. Studies in this ISA include exposure-response experiments showing exposure-dependent responses with exposure increasing from background level to levels greater than near point sources. For some aquatic species,

recent evidence supports previous findings of reproductive and developmental effects of Pb and differential lifestage response at near ambient concentrations of Pb.

2.7.3.5 Effects on Growth

Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and effects on growth in aquatic and terrestrial plants and aquatic invertebrates (Section 7.4.1.5, Section 7.4.2.5). Evidence is inadequate to establish a causal relationship between Pb exposures and effects on growth in terrestrial invertebrates and in terrestrial and aquatic vertebrates (Section 7.4.2.5).

Alterations in the growth of an organism can impact population, community and ecosystem level variables. Evidence for effects of Pb on growth is strongest in aquatic and terrestrial plants. In invertebrates, evidence for effects of Pb on growth has been observed most extensively in aquatic taxa, with inhibition in sensitive species occurring near the current range of Pb concentrations in surface waters. Vertebrates, particularly terrestrial, have been the object of a comparatively much smaller number of studies of the effects of Pb on growth. Growth effects observed in both invertebrates and vertebrates, however, underscore the importance of lifestage to overall Pb susceptibility. In general, juvenile organisms are more sensitive than adults. Evidence for growth effects of Pb in freshwater and terrestrial plant species is primarily supported by earlier Pb AQCDs. In aquatic invertebrates, the weight of the evidence continues to support growth effects of Pb with several new studies reporting effects at ≤ 10 µg Pb/L, specifically in snails and mussels. Also, growth effects in frogs are reported at lower concentrations in this ISA than in earlier reviews.

There is evidence over several decades of research that Pb inhibits photosynthesis and respiration in plants, both of which reduce growth (U.S. EPA, 2006b, 1977). Many laboratory and greenhouse toxicity studies have reported effects on plants. These effects are typically observed in laboratory studies with high exposure concentrations or in field studies near point sources. 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. Most primary producers experience EC_{50} values for growth in the range of 1,000 to 100,000 µg Pb/L with minimal inhibition of growth observed as low as 100 µg Pb/L (U.S. EPA, 2006b).

Growth effects of Pb on aquatic invertebrates are reviewed in the draft Ambient Aquatic Life Water Quality Criteria for Pb (U.S. EPA, 2008b) and the 2006 Pb AQCD. In the

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

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

2.7.3.6 Effects on Survival

The evidence is sufficient to conclude that there is **a** causal relationship between Pb exposures and survival in terrestrial and aquatic invertebrates and vertebrates (Section 7.4.1.6, Section 7.4.2.6). The evidence is inadequate to conclude that there is a causal relationship between Pb exposure and survival in terrestrial and aquatic plants (Section 7.4.1.6, Section 7.4.2.6). In terrestrial vertebrates and invertebrates, evidence for Pb effects on survival is primarily supported by previous Pb AQCDs with no new studies reporting effects on survival at lower concentrations. For aquatic invertebrates recent studies support previous associations between Pb exposure and mortality at concentrations near the range of Pb measured in U.S. surface waters in cladocerans, amphipods and rotifers. In aquatic vertebrates, there is new evidence for effects in fish at <100 µg Pb/L. Pb is generally not phytotoxic to aquatic or terrestrial 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.

The relationship between Pb exposure and decreased survival rate has been well demonstrated in terrestrial and aquatic species, as presented in Section 7.2.5, Section 7.3.6, and Section 7.3.17.4 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. LC_{50} values are usually much higher than Pb concentrations near point-sources, although physiological dysfunction that adversely impacts the fitness of an organism often occurs well below concentrations that result in mortality.

Freshwater aquatic invertebrates are generally more sensitive to Pb exposure than other taxa, with survival adversely impacted in a few species at concentrations occurring near point-sources, or at concentrations near ambient levels. These impacted species may include endangered species or candidates for the endangered species list, such as the freshwater mussel *Lampsilis rafinesqueana* (Neosho mucket), The EC_{50} for foot movement (a measure of viability) for newly transformed juveniles of this species was 188 µg Pb/L. Freshwater biota that exhibit sensitivity to Pb in the range of Pb concentrations measured in U.S. waters [median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L (U.S. EPA, 2006b)], include some species of gastropods, amphipods, cladocerans, and rotifers although the toxicity of Pb is highly dependent upon water quality variables such as DOC, hardness, and pH. Other aquatic invertebrates such as odonates may be tolerant of Pb concentrations that greatly exceed environmental levels.

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

Data on mortality of saltwater species associated with exposure to Pb is limited; however, in general, marine organisms are less sensitive to this metal than freshwater organisms and the highest toxicity is observed in juveniles. In one study, effects of Pb on survival at environmentally relevant concentrations of Pb in diet have been demonstrated though a simulated marine food chain in which the primary producer, the microalgae *T. suecica*, was exposed to 20 µg Pb/L and subsequently fed to brine shrimp *Artemia franciscana*, (mean Pb content 12 to 15 µg Pb/g) which were consumed by white-leg shrimp

Litopenaeus vannamei, itself consumed by grunt fish *H. scudderi* representing the top of the marine food chain (Soto-Jiménez et al., 2011a). Survival of brine shrimp was 25 to 35 % lower than the control and both white shrimp and grunt fish had significantly higher mortalities than controls.

In 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, 2005b). The NOAELs for survival ranged from 3.5 to 3,200 mg Pb/kg • day.

In aquatic vertebrates there is considerable historic information on Pb toxicity to freshwater fish. New studies support findings in previous AQCDs of Pb effects on fish survival and indicate effects at lower concentrations in testing with juvenile lifestages. In a series of 96-hour acute toxicity tests with fathead minnow conducted in a variety of natural waters across North America, LC_{50} values ranged from 41 to 3,598 µg Pb/L and no Pb toxicity occurred in three highly alkaline waters (Esbaugh et al., 2011). Thirty day LC_{50} values for larval fathead minnows ranged from 39 to 1,903 µg Pb/L in varying concentrations of DOC, $CaSO_4$ and pH (Grosell et al., 2006a). In a recent study of rainbow trout fry at 2-4 weeks post-swim up, the 96-hour LC_{50} was 120 µg Pb/L at a hardness of 29 mg/L as $CaCO_3$, a value much lower than in testing with older fish (Mebane et al., 2008).

2.7.3.7 Community and Ecosystem Effects

More evidence for Pb toxicity to terrestrial and aquatic biota has been reported from single-species assays in laboratory studies than from whole ecosystem studies. The evidence is strong for effects of Pb on growth, reproduction and survival in very diverse species, but considerable uncertainties exist in generalizing effects observed under particular, small-scale conditions, up to the ecosystem level of biological organization. At the ecosystem level, the presence of multiple stressors, variability in field conditions, and differences in bioavailability of Pb make it difficult to measure the magnitude of effects, and to quantify relationships between ambient concentrations of Pb and ecosystem response. However, the cumulative evidence that has been reported for Pb effects at higher levels of ecological organization and for endpoints in single species with direct relevance to population and ecosystem level effects (i.e., development and reproduction, growth, survival) is sufficient to conclude that there is a likely causal relationship between Pb exposures and the alteration of species richness, species composition and biodiversity in terrestrial and aquatic ecosystems (Section 7.4.1.7, Section 7.4.2.7).

Ecosystem-level studies are complicated by the confounding of Pb exposure with other factors such as the presence of other 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. In laboratory studies and simulated ecosystems, where it is possible to isolate the effect of Pb, this metal has been shown to alter competitive behavior of species, predator-prey interactions and contaminant avoidance. At higher levels of ecological organization, these effects may change species abundance and community structure. Uptake of Pb into aquatic and terrestrial organisms and its effects on survival, growth, physiological stress, blood, neurobehavior, and developmental and reproductive endpoints at the organism level are expected to have ecosystem-level consequences. Where evidence of effects is observed at the ecosystem level of organization, evidence from lower levels brings consistency and plausibility for causality.

Most direct evidence of community and ecosystem level effects is from near point sources where Pb concentrations are higher than typically observed in the environment. For terrestrial systems, evidence of impacts on natural ecosystems near smelters, mines, and other industrial sources of Pb has been assembled in previous decades. Those impacts include decreases in species diversity and changes in floral and faunal community composition. For aquatic systems, the literature focuses on evaluating ecological stress from Pb originating from urban and mining effluents rather than atmospheric deposition. In 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. 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).These dynamics are likely to change species abundance and community structure at higher levels of ecological organization.

Recent studies continue to demonstrate associations between Pb exposures and effects at higher levels of biological organization that were shown in field and microcosm studies in previous Pb AQCDs. New studies on plant and soil microbial communities and sediment-associated and aquatic plant communities increase the total number of types of ecological associations impacted by Pb.

In terrestrial ecosystems, most studies show decreases in microorganism abundance, diversity, and function with increasing soil Pb concentration. Specifically, shifts in nematode communities, bacterial species, and fungal diversity have been observed. Furthermore, presence of arbuscular mycorrhizal fungi may protect plants growing in Pb-contaminated soils. Increased plant diversity ameliorated effects of Pb contamination on a microbial community.

In aquatic ecosystems, Pb effects reviewed in the 2006 Pb AQCD included reduced species abundance, richness and diversity, decreased primary productivity, and altered predator-prey interactions. Since the 2006 Pb AQCD, there is further evidence for effects of Pb in sediment-associated communities. Exposure to three levels of sediment Pb contamination 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 and Nabelkova, 2005). Although surface water Pb concentrations in monitored streams were determined to be very low, concentrations of the metal in sediment were high enough to pose a risk to the benthic community (e.g., 34-101 mg Pb/kg). These risks were observed to be linked to benthic invertebrate functional feeding group, with collector-gatherer species exhibiting larger body burdens of heavy metals than benthic predators and collector-filterers.

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

2.8 Integration of Health and Ecological Effects

The health and ecological effects considered for causal determination are summarized in the Table 2-4. The health outcomes were nervous system, cardiovascular, renal, immune, effects on heme synthesis and RBC function, reproductive effects, and cancer. The ecological endpoints considered for causal determination were: physiological stress, hematological effects, neurobehavioral effects, developmental and reproductive effects, growth, survival, and community and ecosystem level effects. The evidence relating to specific ecological endpoints is also integrated across aquatic and terrestrial habitats. Further, the substantial overlap between the ecological and health endpoints considered in the causal determinations allowed the integration of the evidence across these disciplines.

Table 2-4 Summary of Causal Determinations[a] for Health and Ecological Effects

Outcome/Effect	Human Health Causal Determination	Ecological Receptors Causal Determination
Neurological Effects[b]	Causal Relationship	Likely Causal Relationship: Invertebrates and Vertebrates
Cardiovascular Effects	Causal Relationship	N/A[e]
Renal Effects	Causal Relationship	N/A[e]
Immune System Effects	Causal Relationship	N/A[e]
Heme Synthesis and RBC Function[c]	Causal Relationship	Causal Relationship: Terrestrial Vertebrates, Aquatic Invertebrates and Vertebrates
Reproductive Effects and Birth Outcome[d]	Causal Relationship	Causal Relationship: Invertebrates and Vertebrates Inadequate to Infer Causal Relationship: Plants
Cancer	Likely to be a causal relationship	N/A[e]
Mortality	N/A[e] (The strongest evidence of Pb-induced mortality in humans was observed for cardiovascular disease related mortality and this evidence was considered in determining the causal relationship between Pb exposure and cardiovascular effects.)	Causal Relationship: Invertebrates and Vertebrates Inadequate to Infer Causal Relationship: Plants
Growth	N/A[e] (There is evidence from toxicological and epidemiologic studies of Pb effects on fetal and postnatal growth, which was considered in determining the causal association between Pb exposure and reproductive and developmental effects.)	Causal Relationship: Plants and Aquatic Invertebrates Inadequate to Infer Causal Relationship: Vertebrates and Terrestrial Invertebrates
Physiological Stress	N/A[e] (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[e]	Likely Causal Relationship

[a]Based upon the framework described in the Preamble, a determination of causality for health effects was made for a broad outcome category (i.e., nervous system effects) by evaluating the coherence of evidence across disciplines and across a spectrum of related endpoints. However, the evidence on which the causal judgment is based, including the strength of evidence for the individual endpoints within the major outcome category, is characterized within the discussion. Causal determinations were made within approximately 1-2 orders of magnitude of current levels.

[b]In ecological receptors, the causal determination was developed considering neurobehavioral effects that can be observed in toxicological studies of animal models and studies of ecological effects in vertebrates and invertebrates. The human epidemiologic evidence evaluated included a wider range of health endpoints such as cognition.

[c]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.

[d] 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, was considered.

[e]N/A, not applicable, i.e., for health effects no causal determination for this specific endpoint, considered within the causal determination for the larger outcome category; for ecological effects there was no comparable endpoint.

2.8.1 Modes of Action Relevant to Downstream Health and Ecological Effects

The diverse health and ecological effects of Pb are mediated through multiple, interconnected modes of action. This section summarizes the principal cellular/subcellular effects contributing to modes of action for human health endpoints associated with Pb exposure and the concentrations at which those effects are observed. Then, effects of Pb observed in aquatic and terrestrial species (Section 2.7) are evaluated along with evidence from epidemiological and laboratory animal studies to determine the extent to which common modes of action can be inferred from the observed effects. The rationale for this approach is that the mechanism of Pb toxicity is likely conserved from invertebrates to vertebrates to humans in some organ systems.

Each of the modes of action discussed in Section 5.2 has the potential to contribute to the development of a number of Pb-induced health effects (Table 2-5). Evidence for the majority of these modes of action is observed at low blood Pb levels in humans and laboratory animals, between 2 and 5 µg/dL, and at doses as low as the picomolar range in animals and cells. The concentrations eliciting the modes of action (reported in Table 2-5) are drawn from the available data and do not imply that these modes of action are not acting at lower exposure levels or that these doses represent the threshold of the effect. Also, the data in presented this table does not inform regarding the exposure frequency and duration required to elicit a particular MOA.

Table 2-5 MOAs, their related health effects, and information on concentrations eliciting the MOAs

Mode of Action [Related Health Effects (ISA Section)]	Concentrations or doses (Conditions)[a]	
	Blood Pb	Dose
Altered Ion Status [All Health Effects of Pb]	3.5 µg/dL (mean in cord blood; association with cord blood Ca^{2+}-ATPase pump activity) Huel et al. (2008)	0.00005 µM free Pb^{2+} (In vitro; 30 minutes; calmodulin activation assay) Kern et al. (2000)
Protein Binding [Renal (5.5), Heme Synthesis and RBC Function (5.7)]	17.0 µg/dL (concurrent mean in adult workers with wildtype metallothionein expression; increased BP susceptibility) Chen et al. (2010a)	50 µM Pb glutamate (In vitro; 24 hours; increased nuclear protein in neurological cell) Klann and Shelton (1989)
Oxidative Stress [All Heath Effects of Pb]	5.4 µg/dL (concurrent mean in adult male workers; decreased CAT activity in blood) Conterato et al. (In Press)	0.1 µM Pb-acetate (In vitro; 48 hours; decreased cellular GSH in neuroblastoma cells) Chetty et al. (2005)
Inflammation [Nervous System (5.3), Cardiovascular (5.4), Renal (5.5), Immune (5.6), Respiratory (5.6.4), Hepatic (5.9.1)]	2.5 µg/dL (concurrent minimum in adult males; increased serum TNF-α and blood WBC count) Kim et al. (2007)	0.01 µM Pb-acetate (In vitro; 48 hours; increased cellular PGE_2 in neuroblastoma cells) Chetty et al. (2005)
Endocrine Disruption [Reproductive and Developmental Effects (5.8), Endocrine System (5.9.3), Bone and Teeth (5.9.4)]	1.7 µg/dL (concurrent minimum in women with both ovaries removed; increased serum FSH) Krieg (2007)	10 µM Pb nitrate (In vitro; 30 minutes; displaced GHRH binding to rat pituitary receptors) Lau et al. (1991)
Cell Death/Genotoxicity [Cancer (5.10), Reproductive and Developmental Effects (5.8), Bone and Teeth (5.9.4)]	3.3 µg/dL (concurrent median in adult women; increased rate of HPRT mutation frequency) Van et al. (2004)	0.03 µM Pb-acetate (In vitro; 18 hours; increased formation of micronuclei) Bonacker et al. (2005)

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

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

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 can interfere with endogenous cation homeostasis, necessary as a cell signal carrier mediating normal cellular functions. Pb is able to displace metal ions, such as Zn, Mg, and Ca^{2+}, from proteins due to the flexible coordination numbers and multiple ligand binding ability of Pb, leading to abnormal conformational changes to proteins and altered protein function. Disruption of ion transport leading to increased intracellular Ca^{2+} levels is due in part to the alteration of the activity of transport channels and proteins, such as Na^{+}-K^{+} ATPase and voltage-sensitive Ca^{2+} channels. Pb can interfere with these proteins through direct competition between Pb and the native metals present in the protein metal binding domain or through disruption of proteins important in 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. Evidence for these effects in Pb-exposed experimental animals and cell cultures has been linked to altered neurobehavioral endpoints and other neurotoxicity. Neurobehavioral changes that may decrease the overall fitness of the organism have also been observed in aquatic and terrestrial invertebrate and vertebrate studies. There is evidence in tadpoles and fish to suggest Pb may alter neurotransmitter concentrations, possibly resulting in some of these neurobehavioral changes.

Altered cellular ion status following Pb exposure can result in the inhibition of heme synthesis. Pb exposure is commonly associated with altered hematological responses in aquatic and terrestrial invertebrates, experimental animals, and human subjects. The proteins 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 in experimental animals. A number of unique Pb binding proteins have been detected, constituting the observed inclusion bodies. The major Pb binding protein in blood is ALAD with carriers of the ALAD-2 allele potentially exhibiting higher Pb binding affinity. Inhibition of ALAD activity is a widely recognized response to Pb in environments where Pb is present and is considered to be biomarker of Pb exposure in both terrestrial and aquatic biota. Additionally, metallothionein is an important protein in the formation of inclusion bodies and mitigation of the toxic effects of Pb. Protein binding of Pb is a recognized mechanism of Pb detoxification in some terrestrial and aquatic biota. For example, plants can sequester Pb through binding with phytochelatin and some fish have the ability to store accumulated Pb in heat-stable proteins.

A second major mode of action of Pb is the development of oxidative stress, due in many instances to the antagonism of normal metal ion functions. Disturbances of the normal redox state of tissues can cause toxic effects and is involved in the majority of health and ecological outcomes observed after Pb exposure. The origin of oxidative stress produced after Pb exposure is likely a multi-pathway process. Studies in humans and experimental animals provide evidence to conclude that oxidative stress results from oxidation of δ-ALA, NAD(P)H oxidase activation, membrane and lipid peroxidation, and antioxidant enzyme depletion. Evidence of increased lipid peroxidation associated with Pb exposure exists for many species of plants, invertebrates, and vertebrates. Enhanced lipid peroxidation can also result from Pb potentiation of Fe^{2+} initiated lipid peroxidation and alteration of membrane composition after Pb exposure. Increased Pb-induced ROS will also sequester and inactivate biologically active $^{\bullet}NO$, 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 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 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.

2.9 Policy Relevant Considerations

2.9.1 Public Health Significance

The weight of the epidemiologic and toxicological evidence clearly supports the causal relationship of Pb with decrements in cognitive function and behavior in young children (Section 5.3.2 and Section 5.3.13). Associations of blood Pb level with higher IgE, asthma and allergy (Section 5.6.8) and delays in the pubertal development in children (Section 5.8.10), as well as associations of blood and bone Pb with increased blood pressure, hypertension (Section 5.4.7), and kidney function (Section 5.5.6) in adults are also well-substantiated in the evidence base. These endpoints were among those comprising the weight of the evidence for determining causal relationships for the organ system effects.

The rationale for establishing the public health significance of the various health endpoints associated with Pb exposure is multifaceted. The 2006 Pb AQCD concluded that neurological effects in children and cardiovascular effects in adults were among the effects best substantiated as occurring at blood Pb levels as low as 5-10μg/dL (or possibly lower), and that these categories of effects were clearly of the greatest public health concern. Evidence of newly demonstrated immune and renal system effects in the general population was also noted to be of potential public health concern during the previous review (U.S. EPA, 2006b). No safe level of Pb exposure has been identified in current or previous assessments and any threshold for Pb neurotoxicity would have to exist at distinctly lower levels than those associated with the lowest blood Pb concentrations examined in the epidemiologic studies included in this assessment. Recent epidemiologic studies of children continue to find associations with several health endpoints in populations with lower mean blood Pb levels than previously reported (Chapter 5, Table 2-9).

The concept of population risk is relevant to the interpretation of findings for the continuously-distributed subclinical health endpoints frequently studied in association with Pb biomarkers in the assessment of their public health significance. The World Health Organization definition of “health” is “the state of complete physical, mental and social well-being and not merely the absence of disease or infirmity” (WHO, 1948). By

this definition, even decrements in health status that are not severe enough to result in the assignment of a clinical diagnosis might be undesirable if they reflect a decrement in the well-being of an individual. Further, deficits in subtle indices of health or well-being may not be observable except in aggregate, at the population level, so the critical distinction between population and individual risk is essential for interpreting the public health significance of study findings. The American Thoracic Society (ATS) discusses concepts related to understanding the shift in a population distribution of a health endpoint (ATS, 2000). As shown in Figure 2-1, a seemingly small increase in the mean of a continuously distributed health index may push the most susceptible group in the population above a critical cut point on the continuum of disease development, such that their condition meets the clinical definition of a disease. Moreover, small changes at the population level could translate into large numbers of clinical events if a large population is affected.

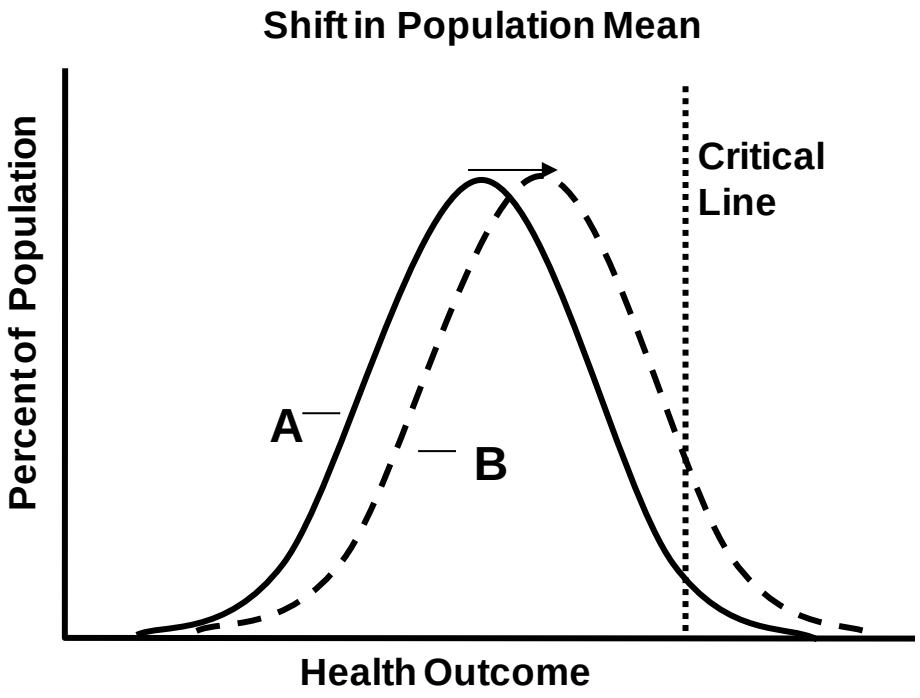


Figure 2-1 The effect of a small shift in population mean on the proportion of individuals in the population diagnosed with clinical disease (i.e., the proportion to the right of the "Critical Line.")

As shown in Table 2-6, the size of the Pb-associated effects observed in epidemiologic studies may be small relative to the effect sizes that are considered clinically relevant. Still, these small shifts in population means are often significant from a public health perspective. For example, small changes in IQ or inattention are often significant at the

population level, especially in the two tails of the outcome distribution. Further, a small shift in the population mean may result in a substantial increase in the number of individuals functioning in the low range of the IQ distribution who may fall into the IQ range associated with increase risk of educational, vocational, and social failure after experiencing a Pb-associated decrement in IQ. In cohorts of children where Pb-associated decrements in cognition and attention were observed, lower academic performance, antisocial behavior, or delinquent behavior assessed in adolescence or in early adulthood were also observed in association with blood Pb levels. Studies also find that higher blood Pb level is associated with measures of inattention and hyperactivity as well as with ADHD or other diagnostic indices used in a clinical setting (Section 5.3.3.1). A downward shift in the mean IQ value can also reduce the proportion of the population achieving very high IQ scores. It is shown that interventions that shift the population mean, in a beneficial direction, by an amount that is without clinical consequence for an individual produce substantial decreases in the proportion of individuals with clinically significant disorders. This evidence, which informs the public health significance of the evidence on the nervous system effects of Pb, is summarized in detail in Section 5.3.12.

It is also important to note that the change in a population mean observed in an epidemiologic study may be small compared to its standard error of measurement for the test. As noted in the 2006 Pb AQCD, statistics that pertain to individual-level data should not be used to draw inferences about group-level data. Measurement error affects the likelihood of detecting an association and is not relevant to the size of the association that is detected. If a study is large enough it will have adequate statistical power to detect small changes. Bias may be introduced if the measurement error of the outcome is highly correlated with the exposure and there is no evidence indicating that individuals with higher blood Pb levels test systematically lower than their true IQ.

Pb-associated changes in other subclinical indices also increase an individual's risk for health effects that are of greater clinical consequence, thus of greater public health concern. Small increases in blood pressure or decreases in GFR that are associated with Pb biomarkers, may shift the population mean resulting in a larger proportion of the population that is diagnosed with hypertension or CKD, respectively. Results from the Framingham Heart Study show that higher levels of blood pressure, even within the nonhypertensive range, impose increased rates of cardiovascular disease (Kannel, 2000a, b). A continuous graded increase in cardiovascular risk is observed as blood pressure increases, with no evidence of a threshold value. Most events arise not in the most severe cases, but mainly in those with high normal blood pressure (i.e., mild hypertension). Kannel (2000a) emphasized that systolic blood pressure exerts a strong influence on more serious cardiovascular events, as it is the primary cause of hypertension and its adverse cardiovascular sequelae. In addition to the small increases in blood pressure associated

with Pb, Pb-associated effects on cardiovascular morbidity outcomes such as ischemic heart disease (Section 5.4.3.5) and mortality (Section 5.4.5) have been observed. The high correlation between blood pressure and clinical cardiovascular outcomes combined with the high prevalence of cardiovascular disease in the U.S. adult population translate into a large increase in the prevalence of conditions in the population. In addition to the small changes in markers of kidney function, which are potentially significant from a public health perspective, Navas-Acien (2009) and Munter et al. (2003) both report increased risk of clinically significant CKD and albuminuria in association with Pb among NHANES III participants. 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 heart 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.

Table 2-6 Illustrative examples contrasting the effect sizes observed in epidemiologic studies to effect sizes considered significant in a clinical setting.[a]

Organ System	Endpoint and Findings from Epidemiologic Studies	Clinical Significance Context
Nervous	IQ: 1 µg/dL increase in blood Pb is typically associated with small decrement in IQ (e.g., < 3 point decrement in FSIQ score)	IQ below 70 is considered a disability by the Social Security Disability Insurance Administrationb
Cardiovascular	Systolic Blood Pressure (SBP): Doubling of blood Pb is associated with an approximate 1 mmHg increase in blood pressure (Nawrot et al., 2002)	Hypertension is defined as systolic over diastolic blood pressure that is greater than 140/90. Pre-hypertension is blood pressure that is 120 to 139/80 to 89. Hypertension is a risk factor for other cardiovascular diseases and mortality.
Renal[a]	Glomerular Filtration Rate (GFR): Increased blood Pb level is associated with reductions in GFR (e.g., Akesson et al. (2005) reports a decrease of -2.0 mL/min (95% CI: -3.2, -0.9) per 1 µg/dL increase in blood Pb)	GFR <60mL/min/1.73m2 for ≥ 3 months indicates Chronic Kidney Disease (CKD). Estimated GFR (eGFR) can be calculated using several different equations. An equation using serum cystatin C rather than serum creatinine may be a more sensitive marker of early kidney damage.
	Serum Creatinine: Increased blood Pb is associated with increased serum creatinine (e.g., (Kim et al., 1996) observed an increase 0.030 mg/g (95% CI: 0.011, 0.049) among those with peak blood Pb levels < 10 µg/dL).	Creatinine ≥ 30 mg/g creatinine indicates albuminuria.
	Creatinine Clearance: [Increased blood Pb is associated with decreased creatinine clearance (e.g., Akesson et al. (2005) reports a -1.8 mL/min (95% CI: -3.0, 0.7) per 1 µg/dL increase in blood Pb)	Normal values range from 97 to 137 mL/min (males) and 88 to 128 mL/min (females). Abnormal results indicate poor kidney function.

[a]Effect sizes based on general population studies

[b]Information on Adult Mental Disorders can be found at: http://www.socialsecurity.gov/disability/professionals/bluebook/12.00-MentalDisorders-Adult.htm

Rather than producing overt cytotoxicity or pathology, blood Pb level is associated with alterations in several subclinical parameters related to cellular and humoral immunity (Figure 5-42). Increases in IgE may contribute to more consequential health conditions including allergy and asthma and the small changes observed in epidemiologic studies may result in a population shift and increased prevalence of these conditions. Several studies found associations between blood Pb levels and asthma and allergy in children (Section 5.6.4.2). Immune changes may also be associated with autoimmune diseases later in life as well as reduced capacity to combat certain viral infections and cancer. Although the public health significance of a delay in onset of puberty is less clear, there is some limited evidence that delay in puberty is associated with other health outcomes (Gilsanz et al., 2011; Naves et al., 2005).

There is a new body of literature suggesting there may be relationship between maternal bone Pb, cord Pb and/or concurrent blood Pb and cardiovascular effects in children including increased blood pressure, Total Peripheral Resistance (TPR) responses to acute stress tasks, and acute stress-induced autonomic and cardiovascular dysregulation (Section 5.4.4). This evidence, however, is difficult to interpret with respect to the risk

for health effects because cardiovascular and renal effects are associated with long-term exposures to Pb and there is uncertainty regarding the frequency, timing, duration and level of exposure associated with the effects. There is some evidence that the pathogenesis of CVD begins in childhood (Kapuku et al., 2006). Although compensatory mechanisms may be more active in children compared to adults, these early cardiovascular effects may persist and worsen if Pb exposure persists over the long-term. A recent study that strengthens the evidence of an association between blood Pb and altered renal function in children is included in this review. This NHANES analysis in adolescents found that association between higher blood Pb and lower cystatin C-based eGFR (Section 5.5.6). This association is reported in a population with mean blood Pb levels less than 5 μg/dL; however, those enrolled in the study were likely to have higher past than recent Pb exposure and the pattern and level of their exposure was not assessed in the study.

In addition to the long-term effects of Pb, early life exposure to Pb (postnatally) may permanently alter normal development of the CNS to increase risk of pathology in adults (Section 5.3.2.2). For example connections between developmental exposure to Pb with early life programming and the resulting inflammation-associated DNA damage with neurodegeneration have been demonstrated in rats. Additionally, there is animal toxicological evidence for a Pb exposure contribution to Alzheimer's disease pathology through the generation of neuronal plaques is in early life (Section 5.3.7.2). Early life Pb exposure is also associated with impaired auditory function (Section 5.3.4.3).

2.9.2 Air Lead-to-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 1986 Pb AQCD was drawn from a meta-analysis by Brunekreef (1984). Based on the studies available at that time that considered multiple air-related Pb exposure pathways in the aggregate, the 1986 Pb AQCD concluded that "the blood Pb versus air Pb slope β is much smaller at high blood and air levels." This is to say that the slope β was much smaller for occupational exposures where high blood Pb levels (>40 μg/dL) and high air Pb levels (much greater than 10 $\mu g/m^3$) prevailed relative to lower environmental exposures which showed lower blood Pb and air Pb concentrations (<30 μg/dL and <3 $\mu g/m^3$). For those environmental exposures, it was concluded that the relationship between blood Pb and air Pb "…for direct inhalation appears to be approximately linear in the range of normal ambient exposures (0.1-2.0 $\mu g/m^3$)" (pp 1–98 of the 1986 Pb AQCD). In addition to the meta-analysis of Brunekreef (1984), more recent studies have provided data from which estimates of the blood Pb-air Pb slope can be derived for children (Table 2-7, Table

4-11). The range of estimates from these studies is 2-9 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). Most studies have described the blood Pb-air Pb relationship as either log-log (Schnaas et al., 2004; Hayes et al., 1994; Brunekreef, 1984), which predicts an increase in the blood Pb-air Pb slope with decreasing air Pb concentration or linear (Hilts, 2003; Tripathi et al., 2001; Schwartz and Pitcher, 1989), which predicts a constant blood Pb-air Pb slope across all air Pb concentrations. These differences may simply reflect model selection by the investigators; alternative models are not reported in these studies.

The blood Pb-air Pb slope may also be affected in some studies by the inclusion of parameters (e.g., soil Pb) that may account for some of the variance in blood Pb attributable to air Pb. Other factors that likely contribute to the derived blood Pb-air Pb slope include differences in the populations examined and Pb sources, which varied among individual studies. See Section 4.5.1 for a detailed discussion of studies that inform air Pb-to blood-Pb relationships.

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

Reference	Study Methods	Model Description	Blood Pb–Air Pb Slope[a]
Children Populations			
Brunekreef et al. (1984)	**Location:** Various countries **Years:** 1974-1983 **Subjects:** Children (varying age ranges, n>190,000) **Analysis:** Meta analysis of 18 studies	**Model:** Log-Log **Blood Pb:** 5-41 µg/dL (mean range for studies) **Air Pb:** 0.1-24 µg/m^3 (mean range for studies)	**All children:** 4.6 (1.5)[c] **Children <20 µg/dL:** 4.8 (0.54)[d]
Hayes et al. (1994)	**Location:** Chicago, IL **Years:** 1974-1988 **Subjects:** 0.5-6 yr (n = 9,604) **Analysis:** Regression of quarterly median blood Pb and quarterly mean air Pb	**Model:** Log-Log **Blood Pb:** 12-30 µg/dL (annual median range) **Air Pb:** 0.05-1.2 µg/m^3 (annual mean range)	8.2 (0.62)[e]
Hilts et al. (2003)	**Location:** Trail, BC **Years:** 1989-2001 **Subjects:** 0.5-6 yr (Estimated n = 220-460, based on 292-536 blood Pb measurements/yr with 75-85% participation). **Analysis:** Regression of blood Pb screening and community air Pb following upgrading of a local smelter	**Model:** Linear Blood Pb: 4.7-11.5 µg/dL (annual geometric mean range) **Air Pb:** 0.03-1.1 µg/m^3 (annual geometric mean range)	6.5 (0.48)[f]
Ranft et al. (2008)	**Location:** Germany **Years:** 1983-2000 **Subjects:** 6-11 yr (n = 843) **Analysis:** Pooled regression 5 cross-sectional studies	**Model:** Multivariate 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 (0.1)[g]
Schnaas et al. (2004)	**Location:** Mexico City **Years:** 1987-2002 **Subjects:** 0.5-10 yr (n = 321) **Analysis:** Regression of lifetime blood Pb from longitudinal blood Pb measurements and annual average air Pb data	**Model:** Log-Log **Blood Pb**: 5-12 µg/dL (annual GM range) **Air Pb**: 0.07-2.8 µg/m^3 (annual mean range in yr of birth)	2.2 (0.4)[h]
Schwartz and Pitcher (1989), U.S. EPA (1986a)	**Location:** Chicago, IL **Years:** 1976-1980 **Subjects**: Black children, 0-5 yr (n = 5,476) **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)[b]	8.6 (0.75)[i]
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 (0.45)[j]

Reference	Study Methods	Model Description	Blood Pb–Air Pb Slope[a]
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)	0.01 (58)[k]
Mixed Child-Adult Populations			
Schwartz and Pitcher (1989), U.S. EPA (1986a)	**Location:** U.S. **Years:** 1976-1980 **Subjects:** 0.5-74 yr (n = 9,987) **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 (0.75)[l]

[a]Slope is predicted change in blood Pb (µg/dL per µg/m3) evaluated at ± 0.01 µg/m3 from central estimate of air Pb for the study (shown in parentheses)
[b]Based on data for U.S. (1986 Pb AQCD).
[c]ln(PbB) = ln(PbA) × 0.3485 + 2.853
[d]ln(PbB) = ln(PbA) × 0.2159 + 2.620
[e]ln(PbB) = ln(PbA) × 0.24 +3.17
[f]PbB = PbA × 6.5
[g]PbB =1.5 × EXP(0.9361 × (PbA-0.1)/0.44), where 1.5 µg/dL is the background PbB, and 0.1 µg/m^3 is the median PbA for the study; model also adjusted for soil Pb concentration, which may reduce estimated slope
[h]ln(PbB) = Ln(PbA) × 0.213 +1.615 for the 1987 cohort, see text in Section 4.5.1 for more study details.
[i]PbB = PbA × 8.6
[j]PbB =Pb A × 3.6
[k]ln(PbB) = ln(PbA) × 0.05 +2.12
[l]PbB = PbA × 9.63

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

2.9.3 Ecological Effects and Corresponding Lead Concentrations

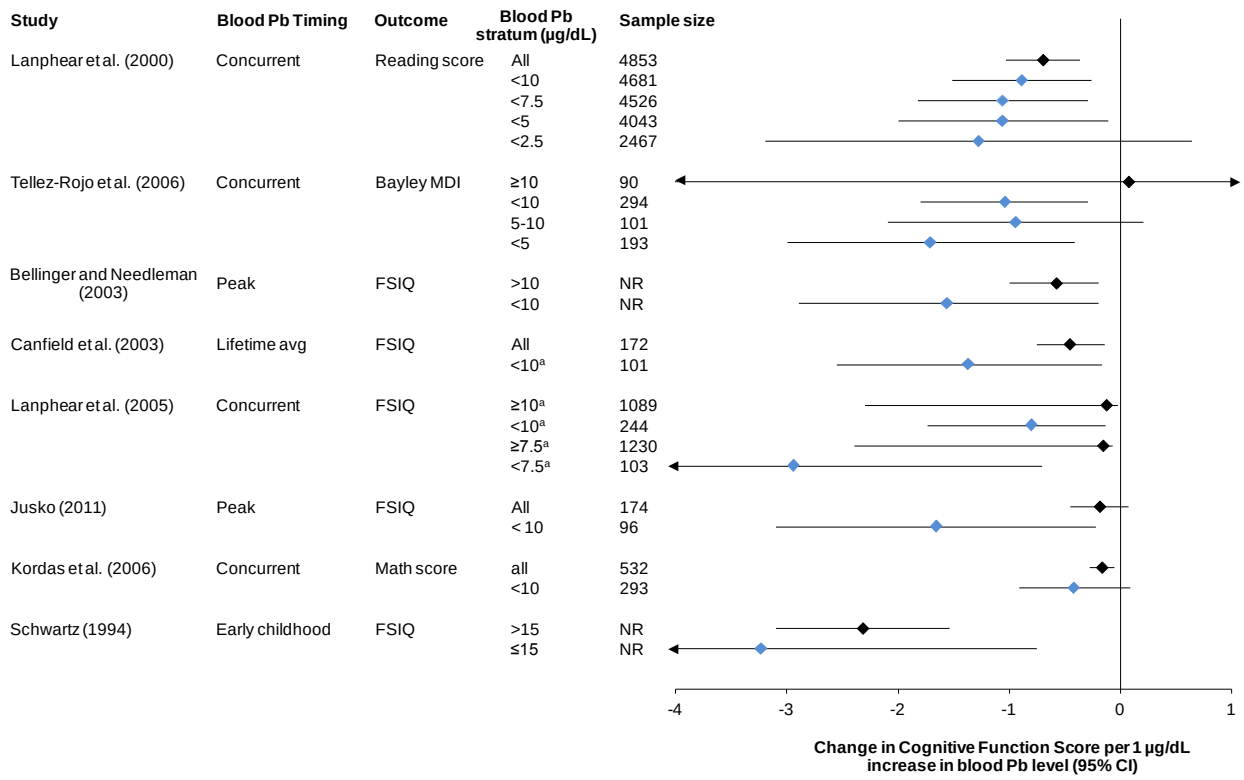
There is limited evidence to relate ambient air concentrations of Pb to levels of deposition onto terrestrial and aquatic ecosystems and to subsequent movement of atmospherically-deposited Pb through environmental compartments (e.g., soil, sediment, water, biota). The proportion of observed effects of 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. Therefore, the connection between air concentration and ecosystem exposure continues to be poorly characterized for Pb, and the contribution of atmospheric Pb to specific sites is not clear.

Furthermore, the level at which Pb elicits a specific effect is difficult to establish in terrestrial and aquatic systems, due to the influence of other environmental variables on both Pb bioavailability and toxicity, and also to substantial species differences in Pb susceptibility. Current evidence indicates that Pb is bioaccumulated in biota; however,

the sources of Pb in biota have only been identified in a few studies, and the relative contribution of Pb from all sources is usually not known. There are large differences in species sensitivity to Pb, and many environmental variables (e.g., pH, organic matter) determine the bioavailability and toxicity of Pb.

2.9.4 Concentration-Response Functions for Health Effects

With each successive assessment to-date, the epidemiologic and toxicological study findings show that progressively lower blood Pb levels or Pb exposures are associated with cognitive deficits and behavioral impairments (Section 5.3.9). C-R functions have been examined across different populations and the interpretation of these functions depends on several factors including lifestage and Pb biomarker used for the analysis. Compelling evidence for a steeper slope for the relationship between blood Pb level and children's IQ at lower blood Pb levels was presented in the 2006 Pb AQCD 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 and Rothenberg, 2005), and several individual studies noted in 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 negative slopes at lower blood Pb levels.



Note: Studies are presented in order of increasing mean blood Pb level.
[a]Strata refer to peak blood Pb level measured in child at any point during follow up.
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. Blue symbols represent effect estimates in lower blood Pb strata.

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

Relatively few studies examined the shape of the concentration-response relationship between Pb in blood or bone and effects in adults. There is uncertainty regarding the frequency, duration, timing and level of exposure contributing to the blood Pb or bone Pb levels in the adult population studied. Some of the populations examined (e.g., NHANES, NAS) are likely to have higher past than recent Pb exposure. Other populations (e.g., worker populations) studied have ongoing exposure to Pb. As described elsewhere in the document (Section 4.3, Section 5.3, Section 5.4, Section 5.5), the interpretation of the study findings depends on the exposure history and the choice of the biomarker in the context of what is known about that exposure history.

In NHANES analyses describing the C-R function for blood Pb level and neurocognitive effects in adults, only log-linear models were used to fit the data (Krieg et al., 2010; Krieg and Butler, 2009; Krieg et al., 2009). With regard to C-R relationships for cumulative exposure metrics, nonlinearity in the BMS and NAS cohorts of

community-dwelling older adults was examined with the use of quadratic terms, penalized splines, or visual inspection of bivariate plots (Bandeen-Roche et al., 2009; Weisskopf et al., 2007a; Shih et al., 2006). There was some evidence for nonlinearity for some but not all cognitive tests or subjects in the NAS cohort (Section 5.3.10). In analyses of the BMS cohort, observations of a statistically nonsignificant quadratic term (Shih et al., 2006) or spline (Bandeen-Roche et al., 2009) for tibia Pb indicated that a linear model adequately fit the relationship between tibia Pb level and various tests of cognitive performance.

Concentration-response relationships were examined in several epidemiologic studies of blood pressure and mortality (Section 5.4.5). With regard to the concentration-response relationship, meta-analysis of human studies found that each doubling of blood Pb level (between 1 and >40 μg/dL measured concurrently in most studies) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP (Nawrot et al., 2002). However, there is uncertainty in the concentration-response relationship to cardiovascular endpoints at lower blood Pb levels since most studies model a linear relationship. Weaver et al. (2010) provided the results of further analysis of this Korean worker cohort, with a focus on determining the functional form of the concentration-response relationships. The coefficient indicated that every doubling of blood Pb level was associated with a systolic BP increase of 1.76 mmHg. The J test, a statistical test for determining which, if either, of two functional forms of the same variable, provides superior fit to data in non-nested models. Davidson and MacKinnon (1981) returned a p-value of 0.013 in favor of the natural log blood Pb level, over the linear blood Pb level specification. This analysis indicates that 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. 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.

Studies investigating both all-cause and cardiovascular mortality report both linear and non-linear relationships (Section 5.4.5). 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. There is uncertainty regarding the frequency, duration, timing and magnitude of exposure contributing to the blood Pb levels among the NHANES population studied; individuals in this population are likely to have higher past than recent exposures. Weisskopf et al. (2009) examined the concentration-response

relationships of patella and tibia Pb with mortality. Concentration-response relationships were approximately linear for patella Pb on the log HR scale for all CVD, but appear nonlinear for IHD ($p < 0.10$). The peak HR is shown around 60 µg/g, beyond which the HR tends to decrease. It is important to note the wide confidence limits, which increase uncertainty at the lower and upper bounds of patella Pb levels. The strongest associations were observed between mortality and baseline patella Pb concentration. Baseline tibia Pb levels were more weakly associated with CVD mortality. Tibia bone Pb level is thought to reflect a longer cumulative exposure period than is patella bone Pb level because the residence time of Pb in trabecular bone is shorter than that in cortical bone.

Concentration response information was provided in a small number of studies of Pb-related nephrotoxicity in the occupational setting (Weaver et al., 2003b; Ehrlich et al., 1998). 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., 2003b).

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 effects. 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). 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. The 2006 Pb AQCD also 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 lognormally 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 factors contributing to the supralinear relationship between blood Pb levels and neurocognitive function in children 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 (Lanphear et al., 2005; Lanphear et al., 2000). 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 overwhelmingly larger effects of these other risk factors (Schwartz, 1994). Several studies 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 5-7). Few studies have compared Pb effect estimates among groups in different sociodemographic strata, and the limited data are mixed. Greater Pb-associated neurocognitive deficits were reported in low-SES groups 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 level 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 concentration-response relationship.

In support of epidemiologic findings, toxicological studies provided some evidence that compared with control or higher Pb exposures (e.g., 150 ppm), lower Pb exposures (e.g., 50 ppm) during gestation and lactation (possibly with offspring stress co-exposure) impaired learning and memory as indicated by increased FI responses on schedule controlled behavior tests (Rossi-George et al., 2011; Cory-Slechta, 1994). The 2006 Pb AQCD did not identify a biological mechanism for a steeper slope at lower than at higher blood Pb levels but such a mechanism was not ruled out. In fact, several lines of toxicological evidence support the possibility of lower and higher Pb exposures acting through differentially activating mechanisms underlying cognition.

As detailed in Section 5.3.10, the shape of the C-R relationship for dopamine varied across studies. However, relative to higher Pb exposures, lower Pb exposures were found to reduce LTP (Gilbert et al., 1999) and glutamate release in the hippocampus (Lasley and Gilbert, 2002). LTP is one indication of synaptic plasticity (Section 5.3.8.4) and is considered to mediate learning and memory. Glutaminergic neurotransmission via its NMDA receptor has been implicated in learning and memory (Section 5.3.8.8). Thus, these differential responses to lower versus higher Pb exposures may provide mechanistic understanding and additional biological plausibility for the nonlinear associations of Pb

exposure or blood Pb levels with neurodevelopmental outcomes observed in animals and children.

While epidemiologic studies have not examined widely the shape of the concentration-response relationship for other nervous system effects, as detailed in Section 5.3.10, toxicological studies have found nonlinear C-R relationships for diverse outcomes such as rod and retinal effects in the visual system (Giddabasappa et al., 2011; Fox et al., 2010), motor function (Leasure et al., 2008), and hippocampal neurogenesis (Fox et al., 2008; Gilbert et al., 2005).

The supralinear concentration-response relationship widely documented for Pb is consistent with the lack of a threshold for Pb-associated nervous system effects in children because a smaller effect estimate would be expected at lower blood Pb levels if a threshold existed. However, an important limitation of previous studies in terms of identifying whether a threshold exists, is 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 in 1999-2002 NHANES to 0.025 µg/dL in 2003-2004 NHANES allowing categorization of children in multiple 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 nervous system effects. 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). However. the large proportions of adolescents in NHANES analyses who were born the 1970s may have had higher past Pb exposures that contributed to associations observed with concurrent blood Pb levels. Nonetheless, several recent studies reported associations between blood Pb levels and deficits in cognitive and behavioral endpoints in children ages 8-11 years with mean or quantile blood Pb levels <2 µg/dL (Cho et al., 2010; Kim et al., 2009b; Miranda et al., 2009). In comparisons of various quantiles of blood Pb, 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. Collectively, these new findings in children, as summarized in this document, do not provide evidence for a threshold for the nervous system effects of Pb in the ranges of blood Pb levels examined to date.

2.9.5 Pb Exposure and Nervous System Effects in Children

As discussed in various sections across this document, there are uncertainties related to the frequency, timing, duration and level of Pb exposure contributing to the health effects observed in epidemiologic studies of adults and older children who are likely to have higher past than recent Pb exposure. Both blood Pb and bone Pb, which are the most common metrics of exposure/dose used in epidemiologic studies, are integrative measures and do not allow these aspects of exposure to be distinguished. Bone Pb is a measure of cumulative exposure in adults and is not typically used in studies of children. As discussed in Section 4.3.5, blood Pb may reflect both recent exposures and Pb released from the bone; the relative proportion of blood Pb from recent versus past exposure is uncertain in the absence of information on exposure timing and duration. Young children, however, do not have lengthy exposure histories and consequently the interpretation of associations with blood Pb levels for this age group may be less complicated compared to older age groups. Several lines of evidence, which are discussed below, inform the interpretation of study findings as they relate to aspects of exposure that can be attributed to the neurocognitive and behavioral effects of Pb observed in young children.

A common limitation of epidemiologic studies is the potentially high correlation between Pb exposure metrics at different ages in childhood and with maternal Pb exposure metrics. In longitudinal studies of the effect of Pb in children, blood Pb levels remained relatively stable over time. Thus, it is difficult to distinguish effects of past and concurrent exposures. Further, concurrent blood Pb levels in children, although highly affected by recent exposure, are also influenced by their past exposure (including prenatal exposure) due to the rapid growth-related bone turnover in children. Thus, concurrent blood Pb level in children also may reflect cumulative dose (Section 4.3.5.1). There is some evidence that the influence of maternal Pb levels on postnatal blood Pb level is substantially reduced soon after birth. Simon et al. (2007) followed a cohort of 13 children living near an Australian smelter from birth through 36 months. In general, immediately after birth, blood Pb levels fell for 1-2 months to approximately 47% of birth blood Pb level. After this initial fall, all infants' blood Pb levels rose with age until approximately 12 months old. There was a good correlation between child blood Pb level and child hand Pb loading ($R^2 = 0.70$) in this study, indicating the influence of concurrent Pb exposures on blood Pb during the early childhood years.

In epidemiologic studies, associations of cognitive function and behavior have been observed with prenatal, early-childhood, lifetime average, and concurrent blood Pb levels as well as with childhood tooth Pb levels and it is difficult to ascertain which lifestage within childhood is associated with the greatest risk of Pb-associated effects on cognition

(Section 5.3.9, Table 5-13, Table 5-14). Epidemiologic studies of Bayley MDI following children beginning in utero and through three years of age indicate that short-duration exposures in utero or during early childhood are associated with cognitive decrements in young children. Among studies with prenatal and concurrent blood Pb measurements, some found stronger associations for prenatal blood Pb levels (Hu et al., 2006; Bellinger et al., 1984), and other found stronger associations for concurrent blood Pb levels (Wasserman et al., 1998; Wasserman et al., 1992). Studies that found associations with concurrent blood Pb levels also tended to find associations with prenatal cord or maternal blood Pb levels. Thus, both postnatal child and maternal Pb exposures may contribute to lower cognitive function in young children. In addition, exposures that are reflected by concurrent blood Pb measured when children are older, by cumulative blood Pb levels or by tooth Pb levels have also been demonstrated to be associated with neurodevelopmental deficits throughout school-age and into adolescence. These findings are consistent with the understanding that the nervous system continues to develop throughout childhood. However, the weight of epidemiologic evidence supports associations of concurrent blood Pb level with neurodevelopmental effects (i.e., cognition and behavior) in children (Sections 5.3.2.1 and 5.3.3.1).

The persistence of neurodevelopmental effects from 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. The toxicological evidence continues to support a range of effects with prenatal and early postnatal Pb exposures which persist to adulthood (Sections 5.3.2.1 and 5.3.2.4). A number of mechanisms, including changes in neurogenesis, synaptogenesis and synaptic pruning, long term potentiation, and neurotransmitter function have been identified that provide biological plausibility for epidemiologic and toxicological findings of persistent cognitive and behavioral effects that result from short-term Pb exposures during prenatal and early childhood periods. The persistence of effects appears to depend on the duration and window of exposure as well as other factors that may affect an individual's ability to recover from an insult.

Toxicological studies in the 2006 Pb AQCD highlighted the importance of Pb exposure during early life in promoting Alzheimer's like pathologies in the adult rodent brain, with Pb-induced neurodegeneration and formation of neurofibrillary tangles in aged animals in which blood Pb levels had returned to control levels after an earlier life Pb exposure (U.S. EPA, 2006b). 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. The same Pb exposure (i.e., dose and duration equivalent to the early life exposure, to adult rodents) did not induce neurofibrillary tangles in the aged animals. However, blood Pb is not generally associated with Alzheimer's disease in

epidemiologic studies of adults. In other studies, recent evidence indicates associations between early life ALAD activity, a biomarker of Pb exposure, and schizophrenia later in adulthood when schizophrenia typically manifests. Consistent with these findings, toxicological studies have shown NMDA pathway disruption with Pb exposure; manipulation of this pathway is known to be associated with schizophrenia. There is also toxicological evidence for Pb-induced emotional changes in males and the occurrence of depression changes in females. Sensitive windows of early life Pb exposure have been associated with persistent changes in adulthood as demonstrated with animal models of neurodegeneration, i.e., neurofibrillary tangle formation, and with epidemiologic findings for the psychotic disorder schizophrenia. These effects are not reflective of concurrent blood-Pb levels at the age of manifestation of the pathology but instead are associated with an earlier life Pb exposure.

2.9.6 Populations Potentially At-Risk for Health Effects

The NAAQS are intended to provide an adequate margin of safety for both the population as a whole and those groups with unique factors that make them potentially at increased risk for health effects in response to ambient air pollutants. 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. Epidemiologic studies reporting results from stratified analyses designed to identify effect measure modifiers comprise the overall weight of evidence for conclusions related to the increased risk of specific populations to the Pb-related health effects presented in Section 6.3. Supporting evidence from toxicological studies provide biological plausibility for conclusions and summarizes factors that potentially influence Pb levels within the body (Section 6.1) and factors related to differential Pb exposures (Section 6.2). The factors that were evaluated are listed in Table 2-8.

Table 2-8 Factors evaluated that may determine populations potentially at increased risk from lead

Physiological Factors that Influence the Internal Distribution of Lead	Population Characteristics Potentially Related to Differential Lead Exposure	Factors Potentially Related to Increased Risk of Lead Induced Health Effects
Age (Section 6.1 and Section 4.4)	Age (Section 6.2.1)	Age (Section 6.3.1, and Section 5.3.8)
	Sex (Section 6.2.2)	Sex (Section 6.3.2)
Genetics (Section 6.1, Section 4.1, Section 4.2)		Genetics (Section 6.3.3)
		Pre-existing Disease (Section 6.3.4)
	Race and Ethnicity (Section 6.2.3)	Race and Ethnicity (Section 6.3.6)
	Socioeconomic Status (Section 6.2.4)	Socioeconomic Status (Section 6.3.7)
	Proximity to Pb Sources (Section 6.2.5, Section 3.2, Section 3.3, Section 3.5, and Section 4.1)	
	Residential Factors (Section 6.2.6)	
		Lifestyle Factors
		Smoking (Section 6.3.5)
		Body Mass Index (Section 6.3.8)
		Alcohol Consumption (Section 6.3.9)
Nutrition (Section 6.1 and Section 4.2)		Nutrition (Section 6.3.10)
		Stress (Section 6.3.11)
		Cognitive Reserve (Section 6.3.12)
Co-exposure (Section 6.1 and Section 4.2)		Other metals (Section 6.3.13)

Studies are included in Section 6.3 only if analyses were conducted to identify the presence or absence of effect measure modification. 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 at-risk populations and lifestages appearing throughout this document. Also, physiological factors that influence the internal distribution of Pb, population characteristics potentially related to differential Pb exposure and factors potentially related to increased risk of Pb induced health effects are considered together in this integrative summary.

2.9.6.1 Age and/or Lifestage

Infancy and Early Childhood

It is recognized that Pb can cross the placenta to affect the developing nervous system of the fetus (Section 4.2.2.4). Further, elevated blood Pb levels among mothers present a potential exposure to their children through breast milk. This transfer of Pb from mother to fetus is observed to occur partly from the remobilization of the mother's bone stores (Section 4.2.2.4). Other biokinetic factors that vary by age, including bone turnover and absorption, also affect blood Pb levels. Typically, children have increased exposure to Pb compared with adults because children's behaviors and activities include increased hand-to-mouth contact, crawling, outdoor play, and poor hand-washing that typically result in increased ingestion compared with adults. Blood Pb among different age groups are shown in Table 6-1. Blood Pb levels are highest among the young children and decrease with increasing age of the child.

There is evidence of increased susceptibility to the neurocognitive effects of Pb exposure during several lifestages throughout childhood (Section 5.3.2.1 to Section 5.3.8, and Section 6.3.1.1). Exposure to environmental toxicants 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 (Section 5.3.9). Synaptic pruning, which is active throughout early childhood (ages 1-4 years), may underlie the elevated risk of young children to environmental exposures. Overall evidence indicates early childhood as a lifestage of increased risk for Pb-related health effects with epidemiologic studies report associations with cognition (Section 5.3.9) among the youngest age groups (6 months to 3 years). Toxicological studies provide support for the younger age groups being especially sensitive to Pb exposure. Toxicological studies have reported that younger animals, whose nervous systems are developing (i.e., laying down and pruning neuronal circuits) and whose junctional barrier systems in the brain (i.e., the blood brain barrier) and GI system (i.e., gut closure) are immature, are more at risk from the effects Pb exposure (Fullmer et al., 1985). Further, blood Pb level is associated with effects to the immune system (Section 5.6) of children. Findings of a recent study indicate that very young children may be at increased risk for Pb-associated activation of humoral immune responses and perturbations in cell-to-cell interactions that underlie allergic, asthma, and inflammatory responses (Section 5.6.2.1 and Section 5.6.3).

Adolescents

The 2007-2008 NHANES data suggest that sex-based differences in blood Pb levels are not substantial until adolescence at which time blood Pb levels of boys are higher than girls. Anatomical understanding of brain development in the typically or normally developing child also helps to inform findings with associated pathologies by providing grounds for comparison. Findings from MRI studies have been used to detail anatomical changes within separate brain segments in the normally developing child and adolescent, from age 3-30 (Section 5.3.9). Throughout adolescence, normal brain development remains dynamic. Volumes of specific regions vary largely by sex and age, but also include inter-individual variation. Studies have linked concurrent blood Pb level as well as other blood Pb metrics) in adolescents to decrements in cognitive function, delinquent or criminal behavior (Section 5.3.3.1). Delays in puberty onset (Section 5.8.1.3) and renal effects (Section 5.5.1.1) are also observed in association with concurrent blood Pb level in cross-sectional studies of adolescents. However, the populations of older children studied generally had higher past exposures, which may have influenced the findings of these studies.

Adulthood

There is uncertainty regarding the frequency, duration, timing and magnitude of exposure contributing to the blood Pb levels among the adult population studied. Associations of both blood Pb and/or bone Pb with blood pressure, hypertension 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. Higher average and median bone and blood Pb levels among older adults could potentially be due to a shared experience of higher historical Pb exposures stored in bone in conjunction with remobilization of stored Pb during bone loss (Section 4.2). In recent studies, age was specifically examined as an effect modifier of the association of Pb with mortality (Section 5.4.5), cognition (Section 5.3.2.5) and blood pressure (Section 5.4.2) in adults. Results for age-related modification of the association between Pb and mortality were mixed and no difference by age was observed for the associations between Pb and cognitive function or blood pressure. Toxicological studies have shown increases in Pb-related health effects by age that may be relevant to nervous system effects in humans (Section 5.3.1).

2.9.6.2 Sex

Multiple associations between Pb and various health endpoints have been examined for effect measure modification by sex (Section 6.3.2). Some studies reported differences between the associations for males and females; overall, the findings were mixed. For example, studies on cognition from the Cincinnati Lead Study cohort and a study in Poland reported males to be an at-risk population, whereas studies from Australia pointed to females as an at-risk population. Toxicological studies continue to demonstrate increased susceptibility of males to Pb for specific endpoints such as sensory function, balance, liver hyperplasia, obesity, memory and gross motor skills. Males and females show differential susceptibility in other toxicological studies across a wide array of endpoints including of behavior, stress hormone homeostasis, and depression with some endpoints having greater effects in one sex versus the other based on the exposure paradigm.

2.9.6.3 Genes

The 2006 Pb AQCD observed that genetic polymorphisms s have been implicated as influencing the absorption, retention, and toxicokinetics of Pb in humans, with the majority of discussion focused on the aminolevulinate dehydratase (ALAD) and vitamin D receptor (VDR) polymorphisms (U.S. EPA, 2006b). Studies published since then continue to indicate that variants of the VDR and the ALAD polymorphism have been associated with varied internal Pb concentrations. Overall, studies of ALAD observed increased Pb-related health effects associated with certain gene variants. Other genes, such as VDR, MTHFR, APOE, HFE, DRD4, GSTM1, TNF-α, and eNOS, may also affect the risk of Pb-related health effects but conclusions are limited due to the small number of studies (Section 6.3.3).

2.9.6.4 Pre-existing Conditions

Studies have also been performed to examine whether certain morbidities increase an individual's risk of Pb-related effects on health. Recent studies have explored relationships for autism, atopy, diabetes, and hypertension; however, there were generally few studies available that evaluated conditions such as autism and atopy. The 2006 Pb AQCD concluded that diabetes and hypertension may result in increased risk of Pb-associated declines in renal function (U.S. EPA, 2006b). However, a recent study indicates that diabetes was not related to increased risk of Pb-related cardiovascular outcomes, and there were mixed results regarding the potential for hypertension to

increase the risk of Pb-related effects on renal function and cardiovascular effects (Section 6.3.4).

2.9.6.5 Race and Ethnicity

Higher blood Pb and bone Pb levels among African Americans has been well documented (U.S. EPA, 2006b). Recent studies are consistent with those previous findings (Section 6.2.3). Greater tibia Pb, but not patella Pb or blood Pb levels, was reported in the Baltimore population studied by Theppeang et al. (2008b). This finding may indicate greater historical exposure among African Americans compared to Caucasians. In addition, a higher proportion of blacks compared with other race-ethnicity groups has been reported in Census blocks where soil Pb concentration is higher, whereas the proportion of whites was higher in low soil Pb Census blocks for a study of New Orleans soil exposure (Campanella and Mielke, 2008). The results of recent epidemiologic studies suggest that there may be race/ethnicity-related increased risk for cardiovascular effects and delayed puberty, although the overall understanding of potential effect measure modification by race/ethnicity is limited by the small number of studies. Additionally, these results may be cofounded by other factors, such as socioeconomic status (Section 6.3.6).

2.9.6.6 Socioeconomic Status

Socioeconomic factors have sometimes been associated with Pb biomarkers, although these relationships have not always been consistent (U.S. EPA, 2006b). On a national level, the gap between income levels with respect to blood Pb has been decreasing. However, blood Pb level of children remains higher depending on income, enrollment in Medicaid and poverty income ratio (PIR), which is the ratio of family income to the poverty threshold appropriate for a given family size (Section 6.2.4). Although there are a limited number of studies, there is some evidence that Pb-associated neurocognitive effects may be larger in magnitude among lower SES populations (Section 6.3.7). 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 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

2.9.6.7 Proximity to Pb Sources and Residential Factors

Proximity to an industrial Pb source or Superfund site likely contributes to higher Pb exposures (U.S. EPA, 2006b). Recent studies show that soil Pb concentrations may be higher in some urban areas as a result of contemporaneous and/or historical Pb sources (Section 6.2.5). Consistent with findings of the 2006 Pb AQCD (U.S. EPA, 2006b), child blood Pb level was significantly associated with renovation activities in older homes as well as housing factors (e.g., year of construction, floor and windowsill condition). It has also been observed that windowsill dust Pb was significantly associated with the presence of deteriorated indoor paint. Other studies of renovation activities involving removal of Pb paint have included measurements of highly elevated blood Pb among children and adult workers. Living in a home built prior to 1950, where old home age is a surrogate for presence of Pb paint, has also been shown to be a significant predictor of blood Pb. (Sections 4.1.3.2 and 6.2.6).

2.9.6.8 Lifestyle Factors

Body mass index (BMI), obesity and alcohol consumption have been examined in epidemiologic and toxicological studies. Modification of associations between Pb with mortality and HRV was not observed by BMI or obesity (Section 6.3.8). There is limited evidence on the potential for alcohol consumption as a modifier of Pb-related effects (Section 6.3.9) and mixed findings on whether smoking modifies the relationship between Pb and health effects (Section 6.3.5).

2.9.6.9 Nutritional Factors

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 (Section 6.1). 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, 2006b), and recent epidemiologic and toxicological studies continue to observe greater Pb-related health effects among individuals with low levels of iron (Section 6.3.10). Calcium intake from diet and vitamin D supplement can modify blood Pb levels in women of various ages. Calcium supplementation during pregnancy and lactation may decrease the amount of Pb to which the developing fetus of infant is exposed. The evidence for this seems especially strong for protection during pregnancy and more mixed for protective effects of calcium during lactation. There is little recent evidence available on the potential for zinc or other as modifiers of Pb-related

health effects associations. A recent study in China reported that children who regularly consumed breakfast had lower blood Pb levels than those children that did not eat breakfast (Liu et al., 2011b). Recent toxicological studies r indicate that diets designed to limit or reduce caloric intake and induce weight loss have been associated with increased blood Pb levels and an epidemiologic analysis reported that regular consumption of breakfast reduced blood Pb levels in children, compared with children who did not eat breakfast (Section 6.1).

2.9.6.10 Stress and Cognitive Reserve

Animal toxicology findings described in the 2006 Pb AQCD demonstrated interactions between Pb exposure and stress. 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. Although examined in a limited number of studies, recent epidemiologic studies observed modification of the association between Pb and various nervous system and cardiovascular effects by stress-level and also indicated that maternal self-esteem may attenuate the negative effects observed of Pb on MDI and PDI scores. Toxicological studies have demonstrated that early life exposure to Pb and maternal stress can result in altered responses in multiple systems, including altered corticosterone and neurotransmitter levels. Additionally, toxicological studies have demonstrated that immune stress also affects associations with Pb (Section 6.3.11).

2.9.6.11 Co-exposure of Lead with Metals and Chemicals

Other exposures have also been studied to assess how they affected the uptake and absorption of Pb. Recent toxicological studies that have examined the addition of arsenic (As) to Pb and Cd mixtures report increases in bioavailability of Pb (Section 6.1). The 2006 Pb AQCD reported that the majority of studies examined other metals as potential confounders rather than effect measure modifiers (U.S. EPA, 2006b). Recent epidemiologic studies have, however, begun to explore the possible interaction between Pb and other metals. These studies report some stronger associations between Pb and various health endpoints with co-exposure to Cd, As and Mn (Section 6.3.13); some studies indicate that coexposures may increase bioavailability of Pb (Section 6.1). Since Pb is acid soluble, fluoridation may increase Pb concentration in water through leaching from pipes and Pb solder (Section 4.1.3.3)

2.9.6.12 Summary of At-Risk Populations

As shown in Table 2-9 (Table 6-4) the most well-substantiated at risk population for the effects of Pb exposure is children. Evidence for all other factors was limited. Among children, the youngest age groups were observed to be most at risk of elevated blood Pb levels, with levels decreasing with increasing age of the children. Recent epidemiologic studies of infants/children detected increased risk of Pb-related health effects, and this was supported by toxicological studies. Synaptic pruning, which is active throughout early childhood (ages 1-4 years), may underlie the elevated risk of neurodevelopmental effects in young children to environmental exposures. Other factors that have been studied with regard to the influence on Pb-related risk are listed in Table 2-9 below.

Table 2-9 Summary of evidence for factors that potentially increase the risk of lead-related health effects

Factor Evaluated	Potentially Related to Increased Risk
Age (Section 6.3.1)	Children
Sex (Section 6.3.2)	Males,[a] Females[a]
Genetics (Section 6.3.3)	ALAD[a], VDR[a]*, DRD4[a]*, GSTM1[a], TNF-α[a], eNOS[a], APOE[a], HFE[a]
Pre-existing Disease (Section 6.3.4)	Autism[a], Atopy[ab], Hypertension[b]
Smoking (Section 6.3.5)	Smokers[a]
Race/Ethnicity (Section 6.3.6)	Non-Hispanic Blacks[a], Hispanics[a]
Socioeconomic Status (SES) (Section 6.3.7)	Low SES[a]
Nutrition (Section 6.3.10)	Iron deficiency[a]
Stress (Section 6.3.11)	High stress[a]
Cognitive Reserve (Section 6.3.12)	Low cognitive reserve[ab]
Other Metals (Section 6.3.13)	High/co-exposure to Cd[a], As[a], Mn[a]

[a] Evidence for this factor was limited.
[b]Possible mediator

2.10 Summary

Table 2-10 summarizes the main conclusions from the 2006 Pb AQCD and from this assessment, including causality determinations, regarding the health and ecological effects of Pb.

Table 2-10 Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb

Outcome/Effect	Conclusions from the 2006 Pb AQCD	Conclusions from the (2012-2nd Draft) Pb ISA
Health Outcomes:		
Nervous System Effects	The 2006 Pb AQCD did not integrate the evidence across specific endpoints within the nervous system to make conclusions regarding role of Pb in causing effects for the organ system as a whole.	Based most heavily on cognitive function decrements and behavioral problems in children, the collective body of evidence is sufficient to conclude that there is a causal relationship between Pb exposures and nervous system effects.
Neurocognitive Function and Learning	**Children:**	
	The overall weight of the available evidence provides clear substantiation of neurocognitive decrements being associated in young children with blood-Pb concentrations in the range of 5-10 µg/dL, and possibly lower. Prenatal, early childhood, lifetime average, and concurrent blood Pb levels were associated with decrements in IQ and specific indices of learning and memory; however, concurrent blood Pb level was the strongest predictor.	Recent epidemiologic studies in children continue to demonstrate associations of concurrent blood Pb level with IQ decrements; most new evidence emphasizes associations of concurrent blood Pb levels with specific indices of neurocognitive function (e.g., verbal skills, memory, learning visuospatial processing). The weight of evidence supports associations in populations with mean blood Pb levels 2-7 µg/dL.
	Adults**:**	
	Among environmentally-exposed adults, bone Pb levels but not blood Pb levels were associated with poorer cognitive performance. These findings point to an effect of long-term cumulative Pb exposure.	Consistent with previous studies, the weight of evidence in new studies indicates associations of cumulative Pb exposure metrics with cognitive performance in environmentally-exposed adults. However, as these outcomes are observed in adults with likely higher past Pb exposures, uncertainty exists as to the Pb exposure level, frequency, duration, and timing contributing to the observed associations
Neurobehavioral Effects	Children:	
	Several epidemiologic studies reported associations between Pb exposure and effects that ranged from inattention and aggression in children to delinquent behaviors and criminal activities in adolescents and young adults. Most studies examined blood Pb levels measured earlier in childhood (means ~ 10 µg/dL), tooth Pb, or bone Pb. There was little examination of concurrent blood Pb levels. Uncertainty 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 withdrawn behavior and anxiety symptoms in children.	Recent studies in children continue to support associations of Pb exposure with a range of effects, largely inattention and hyperactivity, and also misconduct delinquent behavior. In new studies, the weight of evidence supports associations with concurrent blood Pb in populations with lower mean blood Pb levels (2–5 µg/dL) than those in previous studies. New evidence indicates associations of concurrent blood Pb levels with ADHD diagnosis and contributing diagnostic indices in populations with mean blood Pb levels 2-4 µg/dL.

Outcome/Effect	Conclusions from the 2006 Pb AQCD	Conclusions from the (2012-2nd Draft) Pb ISA
Neurobehavioral, Mood and Psychiatric Effects	Adults:	
	Associations of Pb exposure with behavioral outcomes were not widely examined in environmentally-exposed adults.	In the few available new studies of environmentally-exposed adults, blood and bone Pb levels are associated with symptoms of depression and anxiety.
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 extensive animal and the limited available human literature reporting associations between concurrent blood Pb levels (population means 7-12 µg/dL) and decreased auditory function in children.	The limited available new epidemiologic studies on sensory organ function in children examined children with high blood Pb levels ((means > 30 µg/dL. A recent toxicological study found retinal effects in male rodents who had been exposed at gestation through 10 days of age at exposures resulting in lower blood Pb levels (~12 µg/dL) than those at which effects had been previously reported.
Neurodegenerative Diseases	In the limited body of epidemiologic studies, blood and bone Pb levels were not associated with Alzheimer's disease or Amyotrophic Lateral Sclerosis among environmentally-exposed adults. Each study had sufficient limitations.	A few case-control studies report associations between bone Pb levels and PD in environmentally-exposed adults. Recent toxicological evidence suggests that early life Pb exposure may be associated with neurodegeneration in adult animals.
Cardiovascular Effects	The 2006 Pb AQCD did not integrate the evidence across specific endpoints within the cardiovascular system to make conclusions regarding role of Pb in causing effects for the organ system as a whole.	The weight of the evidence continues to support and expand upon the strong body of evidence that Pb exposure is associated with increased blood pressure and hypertension in adults. The evidence is sufficient to conclude that there is a causal relationship between Pb exposures and cardiovascular effects; however, as these outcomes are observed in adults with likely higher past Pb exposures, uncertainty exists as to the Pb exposure level, frequency, duration, and timing contributing to the observed associations.
Blood Pressure	A meta-analysis of numerous epidemiologic studies estimated that a doubling of blood Pb level (e.g., from 5 to 10 µg/dL) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP."	Recent epidemiologic and toxicological studies continue to support associations between long-term Pb exposure and increased BP. Associations of increased BP with blood and bone Pb concentrations are observed in populations with lower mean blood Pb levels.
Hypertension	Epidemiologic studies consistently demonstrated associations between Pb and incidence of hypertension with suggestive evidence that bone Pb may be associated with hypertension. Animal studies demonstrated that long-term exposure to Pb resulted in hypertension that persisted after cessation of exposure.	Recent studies, including those using bone Pb as a metric of cumulative exposure, continue to demonstrate associations of hypertension with Pb levels in adults at lower population Pb concentrations. Recent studies have emphasized the interaction of cumulative exposure to Pb with other factors including stress.
Cardiovascular Mortality	The evidence for an association of Pb with cardiovascular mortality is limited but supportive.	Recent studies address limitations of previous studies and provide additional evidence for an association of Pb with cardiovascular mortality in adults. Specific causes of mortality that were associated with Pb could be related to increased BP and hypertension.
Renal Effects	The 2006 Pb AQCD did not integrate the evidence across specific endpoints within the renal system to make conclusions regarding role of Pb in causing effects for the organ system as a whole. Circulating and cumulative Pb was associated with longitudinal decline in renal function in adults. Toxicological studies demonstrated that initial accumulation of absorbed Pb occurred primarily in the kidneys and noted a hyperfiltration phenomenon during the first 3 months of exposure, followed by decrements in kidney function.	Recent epidemiologic and toxicological studies evaluated in the current review support or expand upon the strong body of evidence indicating that Pb exposure is associated with kidney dysfunction (e.g., lower creatinine clearance, higher serum creatinine, and lower GFR) in nonoccupationally-exposed adults. The evidence is sufficient to conclude that there is a causal relationship between Pb exposures and renal health effects; however, as these outcomes are most often observed in adults with likely higher past Pb exposures, uncertainty exists as to the Pb exposure level, frequency, duration, and timing contributing to the observed associations.

Outcome/Effect	Conclusions from the 2006 Pb AQCD	Conclusions from the (2012-2nd Draft) Pb ISA
Immune System Effects	The 2006 Pb AQCD did not integrate the evidence across specific endpoints within the immune system to make conclusions regarding role of Pb in causing effects for the organ system as a whole.	The consistency and coherence of findings across the continuum of related immune parameters that demonstrate a stimulation of Th2 responses in toxicological studies combined with the supporting epidemiologic evidence in children are sufficient to conclude that there is a causal relationship between Pb exposures and immune system effects.
	Children:	
	Several epidemiologic studies suggested that Pb exposure may be associated with effects on cellular and humoral immunity in children. The principal effects demonstrated were decreases in T cell abundance and increases in serum immunoglobulin E levels with concurrent blood Pb levels > 10 µg/dL. Toxicological evidence supported these findings with extensive evidence for prenatal and early postnatal Pb exposures skewing toward Th2 cytokine production and affecting downstream events such as inflammation and decreased responses to antigens (e.g., delayed type hypersensitivity, bacterial resistance).	A small body of new studies supports the previous evidence that Pb exposure is associated with immune effects in children. New studies in children added to the evidence for associations of blood Pb levels with asthma, allergy, and IgE. The consistency and coherence of findings among related immune effects that support a shift from a Th1 to a Th2 phenotype establishes the biological plausibility for epidemiologic observations of associations with asthma, allergy and inflammation-related effects in other organ systems.
	Adults:	
	In the large body of studies in occupationally-exposed adults, the most consistent findings were reduced neutrophil functionality in workers with blood Pb levels > 30 µg/dL. Pb exposure-associated immune effects were not widely examined in environmentally-exposed adults. Several toxicological studies found a Pb-induced shift to Th2 cytokine production and a hyperinflammatory phenotype of macrophages in adult animals with long-term (> weeks) exposure.	A small body of available studies provides new evidence for immune effects in environmentally-exposed adults. Specific endpoints varied among studies but were consistent with increased inflammation. Associations were observed in populations with a wide range of mean concurrent blood Pb levels (3-22 µg/dL). However, as these outcomes are observed in adults with likely higher past Pb exposures, uncertainty exists as to the Pb exposure level, frequency, duration, and timing contributing to the observed associations. A few available toxicological studies find Pb-associated immune effects in adult mice.
Heme Synthesis and RBC Function	Children:	
	Pb exposure was associated with disruption in heme synthesis with increases in blood Pb levels of approximately 20 µg/dL sufficient to halve ALAD activity and inhibit ferrochelatase. Risk of clinical anemia in children becomes apparent at high blood Pb levels: 10% probability of anemia was estimated to be associated with ~ 20 µg/dL Pb at 1 year of age, 50 µg/dL at 3 years of age, and 75 µg/dL at 5 years of age.	Recent epidemiologic studies provide strong evidence that exposure to Pb is associated with numerous deleterious effects on the hematological system in children, including altered hematological parameters (Hb, MCV, MCH, RBC count), perturbed heme synthesis mediated through decreased ALAD and ferrochelatase activities, and oxidative stress. Associations were observed in populations with mean (or median) blood Pb concentrations as low as approximately 5 µg/dL.
	Adults:	
	Pb exposure was associated with disruption in heme synthesis with increases in blood Pb levels of approximately 20 µg/dL sufficient to halve ALAD activity and inhibit ferrochelatase. Exposures to Pb resulting in blood concentrations < 40 µg/dL appear to be tolerated without decreases in blood hemoglobin or hematocrit, however changes in erythropoiesis do occur at these blood levels.	Recent epidemiologic studies provide strong evidence exposure to Pb is associated with numerous deleterious effects on the hematological system, including altered hematological parameters (Hb, MCV, MCH, RBC count), perturbed heme synthesis mediated through decreased ALAD and ferrochelatase activities, decreased erythropoiesis, and oxidative stress.
Reproductive Effects and Birth Outcomes	The 2006 Pb AQCD did not integrate the evidence across specific endpoints within the reproductive system to make conclusions regarding role of Pb in causing effects for the organ system as a whole.	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 and developmental effects.
	Children:	

Outcome/Effect	Conclusions from the 2006 Pb AQCD	Conclusions from the (2012-2nd Draft) Pb ISA
	No epidemiologic studies of delayed onset of puberty were reviewed.	Recent toxicological and epidemiologic studies provide strong evidence for delayed onset of puberty in males and females. The weight of evidence for delayed onset of puberty was among children ages 6-18 years with mean/median blood Pb levels less than 5 µg/dL.
	Adults:	
	Epidemiologic evidence suggested small associations between Pb exposure and male reproductive outcomes including perturbed semen quality and increased time to pregnancy. Associations between Pb exposure and male reproductive endocrine status were not observed in the occupational populations studied. Toxicological studies provided evidence that Pb produced effects on male and female reproductive junction and development and disrupts endocrine function.	Recent toxicological and epidemiologic studies provide strong evidence for 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 occupational populations suggest a relationship between Pb and cancers of the lung and the stomach; however the evidence is limited by the presence of various potential confounders, including metal coexposures (e.g., to arsenic, cadmium), smoking, and dietary habits. The 2003 NTP and 2004 IARC reviews concluded that Pb and Pb compounds were probable carcinogens, based on limited evidence in humans and sufficient evidence in animals. Based on animal data and inadequate human data Pb and Pb compounds would be classified as likely carcinogens according to the EPA Cancer Assessment Guidelines for Carcinogen Risk Assessment.	The toxicological literature continues to provide the strongest evidence for Pb exposure and cancer with supporting evidence provided by the epidemiologic literature. Epidemiologic studies of cancer incidence and mortality reported inconsistent results.The evidence i is sufficient to conclude that there is a likely causal relationship between Pb exposure and cancer.
Ecological/Welfare Effects:		
	Terrestrial Organisms:	
Physiological Stress	Pb exposure may cause lipid peroxidation and changes in glutathione concentrations. There are species differences in resistance to oxidative stress.	Recent studies continue to support previous associations of Pb exposure with physiological stress. New evidence includes upregulation of antioxidant enzymes, production of reactive oxygen species and increased lipid peroxidation associated with Pb exposure in additional species of terrestrial plants, invertebrates and vertebrates. Experimental exposures increasing from background concentrations to concentrations higher than near point sources result in increasing effects. The evidence is sufficient to conclude a causal relationship between Pb exposure and physiological stress in terrestrial plants, invertebrates and vertebrates.
	Aquatic Organisms:	
	Pb exposure associated with alterations in enzymes involved in physiological stress responses.	Recent studies continue to support previous associations of Pb exposure with physiological stress. New evidence includes upregulation of antioxidant enzymes, production of reactive oxygen species and increased lipid peroxidation associated with Pb exposure in additional species of aquatic plants, invertebrates and vertebrates and decreased glycogen levels in freshwater snails. Observed effects generally occurred at concentrations that typically exceed Pb levels in U.S. waters with limited evidence for effects associated with Pb at polluted sites. The evidence is sufficient to conclude a causal relationship between Pb exposure and physiological stress in aquatic plants.

Outcome/Effect	Conclusions from the 2006 Pb AQCD	Conclusions from the (2012-2nd Draft) Pb ISA
Hematological Effects	Terrestrial Organismps:	
	Pb effects on heme synthesis were documented in the 1986 Pb AQCD and continue to be studied in terrestrial biota. Changes in ALAD are not always related to adverse effects but may simply indicate exposure. The linkage between effects of Pb on blood parameters is well documented, however, the linkage between hematological indicators and ecologically relevant effects is less well understood.	Consistent with previous studies, the weight of the evidence in new studies continues to support findings of Pb effects on heme synthesis and ALAD enzyme activity. New studies in birds near historical mining areas and altered serum profiles and blood cell counts in vertebrates provide evidence for additional species and hematological endpoints potentially effected by Pb. The evidence is sufficient to conclude a causal relationship between Pb exposure and hematological effects in invertebrates and vertebrates in terrestrial ecosystems.
	Aquatic Organisms:	
	In metal impacted habitats, ALAD is a recognized biomarker of Pb exposure. Changes in ALAD are not always related to adverse effects but may simply indicate exposure. In fish, Pb effects on blood chemistry have been documented with Pb concentrations ranging from 100 to 10,000 µg Pb/L.	Consistent with previous studies, the weight of the evidence in new studies continues to support findings of Pb effects on ALAD and expands this evidence to additional species of bacteria, invertebrates, and vertebrates as well as new studies on altered blood cell counts in vertebrates. The evidence is sufficient to conclude a causal relationship between Pb exposure and hematological effects in invertebrates and vertebrates in aquatic ecosystems.
Neurobehavioral Effects	Terrestrial Organisms:	
	Exposure to Pb in laboratory studies and simulated ecosystems may alter species competitive behaviors, predator-prey interactions and contaminant avoidance behaviors.	Recent studies continue to support previous evidence that Pb exposure is associated with behavioral alterations. New studies identify possible molecular targets for Pb neurotoxicity in invertebrates and there is new evidence in a few invertebrate and vertebrate species for behavioral effects associated with Pb exposure (i.e., feeding and escape behaviors). The evidence is sufficient to conclude that there is a likely causal relationship between Pb exposure and neurobehavioral effects in terrestrial invertebrates and vertebrates.
	Aquatic Organisms:	
	Exposure to Pb has been shown to affect brain receptors in fish and may alter avoidance behaviors and predator-prey interactions.	Recent studies continue to support previous evidence that Pb exposure is associated with behavioral alterations. New studies identify possible molecular targets for Pb neurotoxicity in fish and provide additional evidence for Pb effects on behaviors in aquatic organisms that may impact predator avoidance (swimming). The evidence is sufficient to conclude that there is a likely causal relationship between neurobehavioral effects in aquatic invertebrates and vertebrates.

Outcome/Effect	Conclusions from the 2006 Pb AQCD	Conclusions from the (2012-2nd Draft) Pb ISA
Developmental and Reproductive Effects	Terrestrial Organisms:	
	No information on reproduction in plants.	There are an insufficient number of studies that consider Pb effects on plant reproduction. Thus the evidence is inadequate to infer a causal relationship for terrestrial plants
	Limited evidence in invertebrates and vertebrates.	Recent studies in a few taxa expand the evidence for Pb effects on developmental and reproductive endpoints for invertebrates and vertebrates at concentrations that generally exceed Pb levels in U.S. soils. In some organisms, exposure-dependent responses in development and reproductive outcomes are observed in experiments where exposure increases from background level to levels greater than near point sources. Data on terrestrial species is supported by toxicological data from mammals in the context of human health research. The evidence is sufficient to conclude a causal relationship in terrestrial invertebrates and vertebrates.
	Aquatic Organisms:	
	No reviewed studies on reproductive effects in aquatic plants. Reproductive and developmental effects reported in a few species of invertebrates at <50 µg Pb/L and in fish at <150 µg Pb/L	Recent evidence supports previous findings of reproductive and developmental effects of Pb in aquatic biota and differential lifestage response at near ambient concentrations of Pb in some organisms. The evidence is inadequate to conclude a causal relationship for plants, and sufficient to conclude a causal relationship in invertebrates and vertebrates.
Growth	Terrestrial Organisms:	
	Pb inhibits photosynthesis and respiration in plants. Limited evidence for growth effects in soil invertebrates, avian and mammalian consumers.	Recent studies support previous findings of Pb effects on plant growth with some evidence for exposure-dependent decreases in some plant species' biomass in soil amended with 30 mg Pb/kg soil. The evidence is adequate to conclude a causal relationship in plants. Limited studies considered effects on growth in invertebrates and vertebrates. The evidence is inadequate to conclude a causal relationship for terrestrial invertebrates and vertebrates.
	Aquatic Organisms:	
	Evidence for growth effects in algae, aquatic plants and aquatic invertebrates Most primary producers experience EC_{50}s for growth in the range of 1,000 to 100,000 µg Pb/L	The weight of the evidence continues to support growth effects of Pb in aquatic plants and invertebrates.Recent studies on growth in invertebrates find effects of Pb at lower concentrations than previously reported. The evidence is sufficient to conclude a causal relationship between Pb exposure and growth in aquatic plants and invertebrates. Growth inhibition in one species of freshwater snail was observed at <4 µg Pb/L in juveniles. Lowest genus mean chronic value for Pb reported at 10 µg Pb/L in a freshwater mussel. The evidence is inadequate to conclude a causal relationship between Pb exposure growth effects in aquatic vertebrates.

Outcome/Effect	Conclusions from the 2006 Pb AQCD	Conclusions from the (2012-2nd Draft) Pb ISA
Survival	Terrestrial Organisms:	
	No information on mortality in plants. Effects of Pb on invertebrates and vertebrates include decreased survival. In terrestrial and avian species toxicity was observed in laboratory studies over a wide range of doses (<1 to >1,000 mg Pb/kg body weight•day) (U.S. EPA, 2005b).	Recent studies in invertebrates and vertebrates support previous associations between Pb exposure and mortality. The evidence is inadequate to conclude a causal relationship between Pb exposure and survival for terrestrial plants. The evidence is sufficient to conclude a causal relationship in terrestrial vertebrates and invertebrates.
	Aquatic Organisms:	
	No studies reviewed on mortality in plants at current concentrations of Pb in the environment. Pb impacted survival of some aquatic invertebrates at <20 µg Pb/L dependent upon water quality variables (i.e., DOC, hardness, pH). Range of 96-hour LC_{50} values in fathead minnow: 810->5,400 µg Pb/L	The weight of evidence continues to support Pb effects on survival of aquatic invertebrates and vertebrates and indicates that there are effects in a few species at lower concentrations than previously reported. New evidence for effects in a few invertebrates: at <20 µg Pb/L New evidence in fish for impacts to survival at <100 µg Pb/L dependent upon water quality parameters and lifestage 96- hour LC_{50} values as low as 41 µg Pb/L in fathead minnows tested in natural waters from across the U.S. The evidence is inadequate to conclude a causal relationship between Pb exposure and survival for aquatic plants. The evidence is sufficient to conclude a causal relationship between Pb exposure and survival for aquatic invertebrates and vertebrates.
Community and Ecosystem Level Effects	Terrestrial Ecosystems:	
	Effects of Pb difficult to interpret because of the presence of other stressors including metals. The 1986 Pb AQCD reported shifts toward Pb-tolerant communities at 500 to 1,000 mg Pb/kg soil. In the 2006 Pb AQCD, decreased species diversity and changes in community composition were observed in ecosystems surrounding former smelters.	New evidence for effects of Pb in soil microbial communities add to the body of evidence for effects at higher levels of biological organization. However, most evidence for Pb toxicity to terrestrial biota is from single-species assays. Uncertainties exist in generalizing effects observed under small-scale, predicted conditions up to effects at the ecosystem level however, uptake of Pb into terrestrial organisms and subsequent effects on reproduction, growth and survival at the species level is likely to lead to effects at the population, community and ecosystem level. The evidence is sufficient to conclude that there is a likely causal relationship between Pb exposure and the alteration of species richness, species composition and biodiversity in terrestrial ecosystems.
	Aquatic Ecosystems:	
	Most evidence of community and ecosystem level effects is from near point sources, usually mining effluents. Effects of Pb difficult to interpret because of the presence of other stressors including metals. Generally, there is insufficient information available for single materials in controlled studies to permit evaluation of specific impacts on higher levels of organization (beyond the individual organism).	New evidence for Pb effects on sediment-associated and aquatic plant communities add to the body of evidence of effects at higher levels of biological organization. However, most evidence for Pb toxicity to aquatic biota is from single-species assays. Uncertainties exist in generalizing effects observed under small-scale, predicted conditions up to effects at the ecosystem-level however, uptake of Pb into aquatic organisms and subsequent effects on reproduction, growth and survival at the species level is likely to lead to effects at the population, community and ecosystem level. The evidence is sufficient to conclude that there is likely to be a causal relationship between Pb exposure and the alteration of species richness, species composition and biodiversity in aquatic ecosystems.

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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 and analysis methodologies, and recent data describing Pb concentrations as a function of size range. The chapter focuses on Pb concentrations in the U.S. but includes non-U.S. studies to the extent that they are informative regarding current conditions in the U.S. Description of the chemical forms of Pb is not provided here, however, because this information is well established. The reader is referred to the 2006 Pb AQCD for a description of the chemical forms of Pb (U.S. EPA, 2006b).

Section 3.2 provides an overview of the sources of ambient 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), associations with copollutants and background Pb concentrations 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

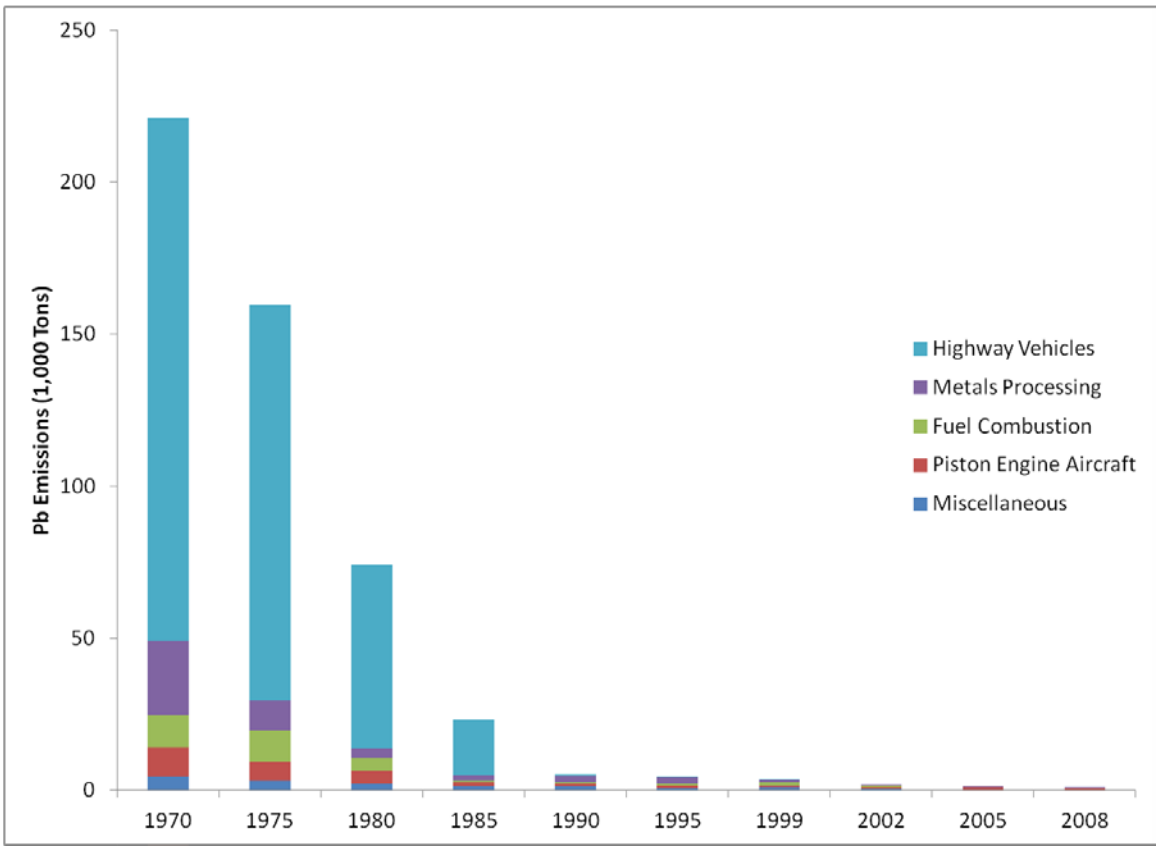
The following section reviews updated National Emissions Inventory (NEI) data from 2008 (U.S. EPA, 2011a), which is the most recently available quality-assured Pb emissions data and compares these emissions data with those from previous years. This section also reviews updated information from the peer-reviewed literature regarding sources of ambient Pb. Detailed information about processes for anthropogenic emissions and naturally-occurring emissions can be found in the 2006 Pb AQCD (U.S. EPA, 2006b). The papers cited herein generally utilized PM sampling data, because ambient airborne Pb readily condenses to PM. The mobile source category included combustion products from organic Pb antiknock additives used in piston-engine aircraft (hereafter referred to piston-engine aircraft emissions).

3.2.1 National Emissions Inventory

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

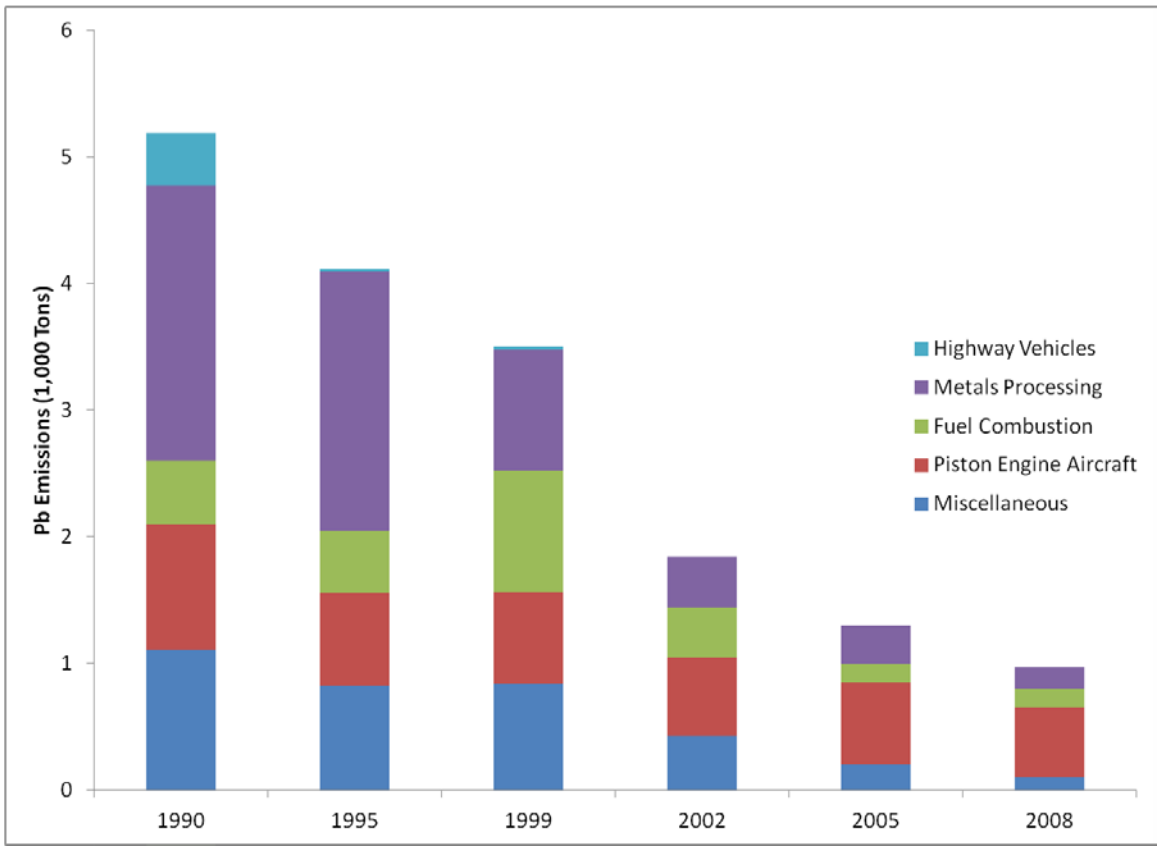
Emissions of Pb have dropped substantially over the past forty years, as shown in Figure 3-1 and Figure 3-2. The reduction before 1990 is largely due to the phase-out of Pb as an anti-knock agent in gasoline for on-road automobiles, as discussed in the 2006 Pb AQCD (U.S. EPA, 2006b). This action resulted in a 98% reduction in Pb emissions from 1970-1995. Total Pb emissions over the period 1995-2008 decreased an additional 76%, from 4,100 tons in 1995 to 970 tons in 2008. Additional emissions reductions are related to enhanced control of the metals processing industry. In 1995, metals processing accounted for 42% (2,200 tons) of total Pb emissions. By 2008, metals processing accounted for 18% (170 tons) of total emissions. This represented more than an order of magnitude decrease in Pb emissions from metals processing. Emissions from piston-engine aircraft decreased 34% over this time period. In 1990, nonroad Pb emissions were 990 tons, 830 tons of which were generated from piston-engine aircraft, and represented 19% of total Pb emissions. In 2008, nonroad Pb emissions from piston-engine aircraft were slightly lower at 550 tons,[1] which represented 56% of all Pb emissions. 2008 piston-engine aircraft emissions were comprised of 254 tons of Pb from emissions at or near airports and 296 tons of Pb emitted in flight (i.e., outside the landing and take-off cycles). "Miscellaneous" emissions from other industrial processes, solvent utilization, agriculture, and construction constituted 10% of emissions (100 tons) in 2008 (U.S. EPA, 2011a, 2008a).

[1] This is the most recent version of the 2008 National Emissions Inventory and is posted separately from version 1 and version 1.5. The piston-engine aircraft emissions inventory can be obtained from the following site: http://www.epa.gov/ttnchie1/net/2008inventory.html under the link for Aircraft, Locomotive, and Commercial marine Vessel sources.



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

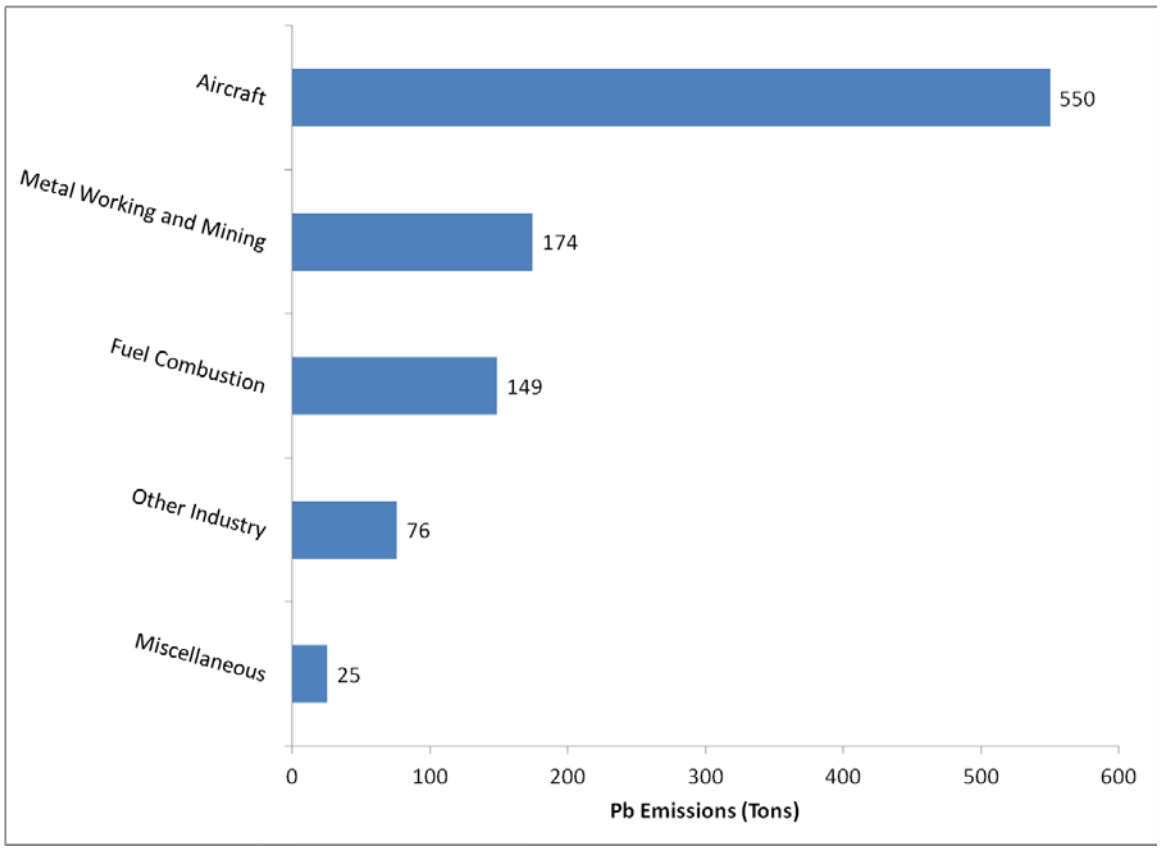
Figure 3-1 Trends in Pb emissions (thousand tons) from stationary and mobile sources in the U.S., 1970-2008.



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

Figure 3-2 Trends in Pb emissions (thousand tons) from stationary and mobile sources in the U.S., 1990-2008.

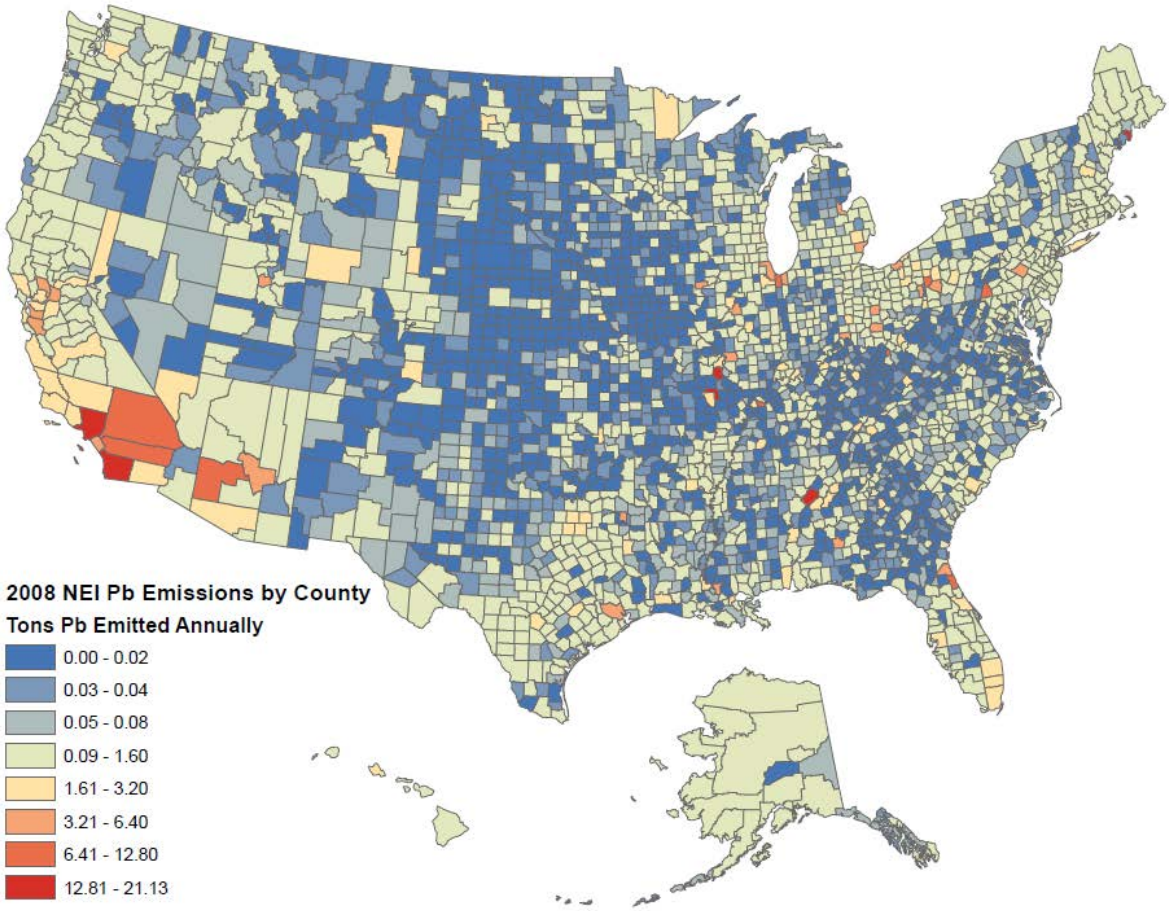
Direct emissions of Pb into the atmosphere primarily come from piston-engine aircraft, fuel combustion, and industrial activities. Direct Pb emissions estimated by the 2008 NEI are shown in Figure 3-3. Piston-engine aircraft produced more than half of all emissions (550 tons). Metal working and mining contributed 170 tons (18%) of Pb emissions in 2008, followed by industrial fuel combustion (15%), other industry (8%), and miscellaneous contributions from agriculture, solvent utilization, and operation of commercial marine vessels and locomotives (3%) (U.S. EPA, 2011a). Pb emissions from the "metal working and mining" category include the single primary Pb smelter in the U.S., the Doe Run facility in Herculaneum, MO; secondary Pb smelters, mostly designed to reclaim Pb for use in Pb-acid batteries; and smelters for other metals.



Source: U. S. EPA (2011a)

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

There is substantial variability in Pb emissions across U.S. counties, as shown in Figure 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. This category included all counties emitting more than 20 tons of Pb in 2008. Jefferson County, MO was the highest emitting county, with over 34 tons of airborne Pb emissions in 2008. Jefferson County is home to the Doe Run primary Pb smelting facility, which is the only remaining operational primary Pb smelter in the U.S. and is planning to cease the existing smelter operations at this site by April, 2014 (DRRC, 2010). Pb emissions from piston-engine aircraft operating on leaded fuel occur at approximately 20,000 airports across the U.S. Airports tend to be more numerous around highly populated metropolitan regions, which suggests that emissions from piston-engine aircraft may be higher in these locations compared with rural areas. In twenty-five counties, piston-engine aircraft are estimated to emit cumulatively greater than one ton of Pb in 2008 U.S. EPA (2011a).



Source: U.S. EPA (2011a)

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

3.2.2 Anthropogenic Sources

Anthropogenic Pb source categories are organized below in order of magnitude with regard to the sum of emissions nationally reported on the 2008 NEI (U.S. EPA, 2011a). Pb sources were reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) by species. Forms of Pb commonly observed in the environment are carried forward from the 2006 Pb AQCD (U.S. EPA, 2006b) and are presented in Table 3-1 to serve as a reference for the categories of Pb sources described in Sections 3.2.1 and 3.2.2.

Table 3-1 Pb compounds observed in the environment

Emission Source	Observed Pb Compounds
Minerals	PbS (Galena)
	PbO (Litharge, Massicot)
	Pb_3O_4 ("Red Pb")
	$PbSO_4$ (Anglesite)
Smelting aerosols	Pb^0, PbS
	$PbSO_4$, PbO
	$PbCO_3$
	Pb silicates
Coal combustion aerosols	PbS
	PbSe
Coal combustion flue gases	Pb^0, PbO, PbO_2 (Above 1,150 K)
	$PbCl_2$ (Low rank coals, above 1,150 K)
	$PbSO_4$ (Below 1,150 K)
Wood combustion	$PbCO_3$
Waste incineration aerosols	$PbCl_2$, PbO
Soils near mining operations	$PbCO_3$
	$PbSO_4$
	$[PbFe_6(SO_4)_4(OH)_{12}]$
	$[Pb_5(PO_4)_3Cl]$
	$[Pb_4SO_4(CO_3)_2(OH)_3]$
	$PbS\text{-}Bi_2S_3$
	Pb oxides, silicates
Piston-engine aircraft emissions, racing vehicle exhaust (combustion of leaded fuel)	$PbBr_2$
	Alkyl Pb
	$PbBrCl\text{-}NH_4Cl$, $PbBrCl\text{-}_2NH_4Cl$
Roadside dust	$PbSO_4$, Pb^0, $PbSO_4(NH_4)SO_4$, Pb_3O_4, $PbO\text{-}PbSO_4$ and $_2PbCO_3\text{-}Pb(OH)_2$
Brake wear, wheel weights	Pb^0
Aircraft engine wear	Pb^0

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

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, in terms of total emissions nationally, is emissions from piston-engine aircraft operating on leaded aviation gasoline (U.S. EPA, 2011a). Levin et al. (2008) point out that emissions from piston-engine aircraft are exempt from reporting to the EPA Toxic Release Inventory. As outlined in Table 3-1, there are several forms of Pb emitted from engines operating on leaded fuel. Dynamometer testing has indicated that Pb emissions from piston engines operating on leaded fuel can occur in the particulate and gaseous forms. For example, Gidney et al. (2010) performed dynamometer testing on automobiles operating on standard gasoline and on gasoline with low levels of organometallic additives. Tetraethyl Pb was included since it is still used in

piston-engine aircraft fuel. Gidney et al. (2010) point out that, where tetraethyl Pb is used as an additive in piston-engine aircraft fuel, the fuel also contains ethylene dibromide, which reacts with Pb to form Pb bromide and Pb oxybromides. Pb bromides and Pb oxybromides are more volatile than elemental Pb at combustion temperatures and are therefore exhausted from the engine. After being exhausted, the brominated Pb compounds cool to ambient temperatures and condense to form solid particles. In contrast, emissions of organic Pb would remain largely in the vapor phase at ambient temperatures. Studies of Pb emissions within enclosed microenvironments where automobiles were the dominant Pb source cited within the 1986 Pb AQCD (U.S. EPA, 1986a), reported that organic Pb vapors contributed less than 20% of total vehicular Pb emissions. A more recent study supports this (Shotyk et al., 2002). The 20% estimate of organic Pb emissions from the previous studies of on-road Pb emissions may potentially provide an upper bound for organic Pb emissions from current piston-engine aircraft.

Pb emission rates from piston aircraft vary with fuel consumption rates, which depend on the engine/airframe combination and the mode of operation of the aircraft. The ASTM specification for the maximum Pb content in "100 Low Lead", the most commonly used leaded piston-engine aircraft fuel, is 2.12 g of elemental Pb/gallon (ASTM, 2007). Fuel consumption rates can be obtained for some engine/aircraft combinations by running FAA's Emissions and Dispersion Modeling System (FAA, 2011). Fuel consumption for piston-engine aircraft operating at one airport in the U.S. were estimated to range from 1.6 g/sec of fuel during taxi-out to 15.3 g/sec of fuel during run-up preflight check for single-engine aircraft and 5.1 g/sec during taxi and 50 g/sec during preflight run-up check for twin-engine aircraft (Carr et al., 2011). Fuel consumption rates for aircraft listed in FAA's Emissions and Dispersion Modeling System were used to develop the Pb emissions inventory for piston aircraft that are discussed in Section 3.2.1. EPA estimates that on average, 7.34 g of Pb is emitted during a landing and take-off cycle conducted by piston-engine aircraft (ERG, 2011).

3.2.2.2 Emissions from Metals Processing and Mining

High Pb emissions were observed in the 2008 NEI (U.S. EPA, 2011a) in Herculaneum, MO, where the Doe Run Pb smelter is operated. Although it is set to cease smelting operations in 2014 (2010), it is of interest to consider studies of primary smelter emissions in the context of the data analyzed in this ISA. Batonneau et al. (2004) and Sobanska et al. (1999) found that the Pb content in PM emitted from a primary Pb smelter was 56.6% by weight, and the Pb content in PM from a Pb/Zn smelter was 19.0% by weight. Choel et al. (2006) confirmed that Pb was strongly associated with sulfur in Pb-Zn smelter emission PM, and that Pb sulfates and Pb oxy-sulfates were the most

abundant species, with important contributions from Pb oxides. Pb concentrations downwind of the smelter were roughly thirty-five times higher than upwind in this study (0.625-0.880 µg/m^3).

Fugitive emissions from secondary Pb processing can be substantial over the course of a year, but they are difficult to estimate. Thurston et al. (2011) performed source apportionment of $PM_{2.5}$ found that Pb-$PM_{2.5}$ concentrations from the Chemical Speciation Network (CSN) were associated with the metals industry along with Zn-$PM_{2.5}$. Goyal et al. (2005) estimated fugitive emissions using concentration data obtained from samplers sited in close vicinity of secondary Pb 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 8.8×10^{-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. Recently, speciation of emissions from a battery recycling facility indicated that PbS was most abundant, followed by Pb sulfates ($PbSO_4$ and $PbSO_4$-PbO), PbO and Pb^0 (Uzu et al., 2009).

In addition to secondary Pb smelting, Pb emissions occur from processing of other metals. For example, a recent study examined Pb emissions from a sintering plant, a major component of the steel making process in southern France (Sammut et al., 2010). Cerussite, a Pb carbonate ($PbCO_3$-$_2H_{2O}$), was observed to be the most abundant species and contributed 20 g Pb/kg measured PM. In another example, Reinard et al. (2007) used a real-time single particle mass spectrometer to characterize the composition of PM_1 collected in Wilmington, Delaware in 2005 and 2006. Strong Pb-Zn-K-Na associations were observed within 13% of PM samples. Comparison with stack emissions revealed that a nearby steel manufacturing facility was an important source of Pb. Ambient PM classes containing only a subset of such elements, e.g., Zn only, Pb-K only were non-specific and so could not be mapped to individual sources. Ogulei et al. (2006) observed that 6% of Pb in $PM_{2.5}$, along with some O_3, Cu, and Fe, was attributed to steel processing in Baltimore, MD. Murphy et al. (2007) conducted a detailed study of the distribution of Pb in single atmospheric particles during the fifth Cloud and Aerosol Characterization Experiment in the Free Troposphere campaign at the Jungfraujoch

research station in Switzerland and found that the predominant type of urban Pb-bearing aerosols contained Pb together with K and Zn. The mode of the size distribution for this type was around 200 nm.

Waste from current or defunct mines has been shown to present an additional fugitive source of Pb. For example, 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

Murphy et al. (2007) found that the volatility of Pb and its compounds such as PbO may result in its presence at high concentration in the submicron fraction of PM emitted from coal emissions. $PbSO_4$, also derived from coal combustion, has low water solubility (Barrett et al., 2010). 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. At the time of the Murphy et al. (2007) study, there were no emission factors for crude oil or residual oil but these represent potentially large sources (up to 100-500 tons/year and up to 25-700 tons/year, respectively). As part of recent rulemaking, EPA has developed a draft Pb emission factor of 1.3×10^{-5} lb/MMBtu for boilers larger than 25 MW that use #2 or #6 fuel oil (U.S. EPA, 2011b).The amounts of Pb emitted from these U.S. sources, however, are several orders magnitude smaller than those estimated to arise from coal combustion in China.

Coal combustion is considered to be a major source of Pb in the atmosphere now that leaded gasoline has been phased out for use in on-road vehicles (Diaz-Somoano et al., 2009). Global Pb estimates are considered here to inform understanding of U.S. Pb emissions from coal combustion. Rauch and Pacyna (2009) constructed global metal cycles using anthropogenic data from 2000. They confirmed that the largest anthropogenic airborne Pb emissions arise from fossil fuel combustion, and they quantified Pb emissions at 85,000 tons/year worldwide. Globally, Pb emissions from stationary sources have been increasing and the north-south gradient in aerosol Pb concentrations over the Atlantic Ocean has disappeared as a result of industrialization of the southern hemisphere (Witt et al., 2006; Pacyna and Pacyna, 2001). The Pb isotope

ratio values (mainly $^{206}Pb/^{207}Pb$) for coal from around the world have been compared with those for atmospheric aerosols. In most parts of the world, there has been a difference between the signature for aerosols and that for coal, where the atmospheric $^{206}Pb/^{207}Pb$ ratio values are lower, indicative of additional contributions from other sources. Zhang et al. (2009a) used single particle aerosol mass spectrometry (ATOFMS) to find that PM containing Pb along with OC and/or EC was attributed to coal combustion processes in Shanghai, China; this accounted for roughly 45% of Pb-bearing PM.

Seasonal effects of the contributions of Pb emissions from coal combustion have been observed. For example, in Tianjin, northern China, the winter heating period starts in November, and the contribution from coal combustion to the Pb aerosol becomes high during the winter. This leads to both a high Pb content and a high $^{206}Pb/^{207}Pb$ ratio. Coal consumption and Pb-bearing PM concentrations declined during the summer months, and Pb from other sources, mainly vehicle exhaust emissions, became relatively more pronounced (Wang et al., 2006c). This seasonal relationship contrasts with observations for the U.S. described in the 2006 Pb AQCD (U.S. EPA, 2006b) 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 Waste Incineration

Waste incineration studies suggest that the Pb content vary by industrial or municipal waste stream. For example, Ogulei et al. (2006) performed positive matrix factorization of $PM_{2.5}$ and gaseous copollutants for Baltimore, MD and observed that 63% of Pb in $PM_{2.5}$ was attributed to waste incineration. Other prevalent compounds associated with incineration included NO_3^-, EC, Cd, Cu, Fe, Mn, Se, Zn, O_3, and NO_2 (note that Cl was not observed in this study). A study by Moffet et al. (2008a) found that Pb-Zn-Cl-containing particles in $PM_{2.5}$ samples collected from an industrial area in Mexico City represented as much as 73% of fine PM. These were mainly in the submicron size range and were typically mixed with elemental carbon (EC), suggesting a combustion source. Zhang et al. (2009a) also observed high correlation between Pb and Cl associated with waste incineration in Shanghai, China. Several Pb isotope studies have also been used to distinguish contributions to incineration from industrial sources. Isotope analysis is discussed in more detail in Section 3.4.1.5. Novak et al. (2008) evaluated changes in the amounts and sources of Pb emissions in the U.K. and Czech Republic during the 19th and 20th centuries and found uncertainty in the amount and the isotope composition of Pb emanating from incineration plants. The isotopic signature of Pb recycled into the atmosphere by incineration of various industrial wastes could have shifted from relatively

high $^{206}Pb/^{207}Pb$ ratios consistent with local Variscan ores to lower values reflecting imported Precambrian ores. However, other environmental studies concerning incineration have given highly consistent values for the Pb isotope ratio for European incineration sources. For example, Cloquet et al. (2006) showed that the Pb isotopic composition of urban waste incineration flue gases in northeastern France was ~1.16. de la Cruz et al. (2009) reported that waste incineration was an important source of Pb and showed that the $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios for waste incineration Pb emitted in European countries were 1.14-1.16 and 2.43 respectively (de la Cruz et al., 2009).

3.2.2.5 Wood Burning

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

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

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

3.2.2.6 Roadway-Related Sources

Contemporary Emissions from Vehicle Parts

Contemporary Pb emissions from motor vehicles may occur because several vehicle parts still contain Pb. Wheel weights, used to balance tires, are clipped to the rims of tire wheels in order to balance the tires, and may become loose and fall off. Pb wheel weights have been banned in several states including Washington, Maine, and Vermont with legislation considered in Iowa, California, and Maryland. However, Pb wheel weights are a source in most states for the period of time covered in this assessment. Ambient air Pb concentrations near heavily trafficked areas may be related to use of Pb-based wheel weights that are prone to dislodgement. On pavement they may be ground into dust 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 Pb-$PM_{2.5}$ concentration did not exceed 17% of the Pb-PM_{10} concentration in any of the runs. Schauer et al. (2006) suggested that enrichment in the coarse fraction may have been related to wheel weights. Additionally, Schauer et al. (2006) measured PM_{10} and $PM_{2.5}$ composition from brake dust and found concentrations that were low but statistically significantly greater than zero for Pb in PM_{10} (0.02 ± 0.01 mg/g) and Pb in $PM_{2.5}$ (0.01 ± 0.00 mg/g) for semi-metallic brake pads and for Pb in PM_{10} (0.01 ± 0.00 mg/g) for low-metallic brake pads. Song et al. (2011) speciated coarse and fine PM samples obtained next to the New Jersey Turnpike in winter and summer of 2007-2008. Using principal component analysis, they found that Pb was prevalent in the factor including automobile exhaust and brake wear. Pb was observed to have a similar size distribution as Zn in the winter and Zn and Cd in the summer, with higher concentrations in the fine fraction at a mode of 0.18-0.32 µm. 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 is usually found in coarser size ranges (Chon et al., 2010), while those in the submicron range are more typically associated with combustion and incineration sources.

Unleaded Fuel

Unleaded fuel contains Pb as an impurity within crude oil (Pacyna et al., 2007). Schauer et al. (2006) measured Pb in $PM_{2.5}$ from tailpipe emissions and observed quantities in on-road gasoline emissions that were statistically significantly different from zero (83.5 ± 12.80 mg/kg), whereas emissions of Pb from diesel engines were not statistically significantly different from zero. Hu et al. (2009a) investigated the heavy metal content of diesel fuel and lubricating oil. They found <1-3 mg/kg Pb in samples of lubricating oil. Hu et al. (2009a) also measured the size distribution of Pb emissions during dynamometer testing of heavy duty diesel vehicles with different driving patterns and control technologies. An urban 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.

3.2.2.7 Deposited Lead

Soil Pb can serve as a reservoir for deposited Pb. The following subsections describe studies of previously deposited Pb that originated from industrial activities, historical use of leaded on-road gasoline, and urban sources such as paint and building materials. The 2006 Pb AQCD (U.S. EPA, 2006b) cited an estimate by Harris and Davidson (2005) that more than 90% of airborne Pb emissions in the South Coast Basin of California were from soil resuspension. This value was obtained by constructing mass balances rather than from direct measurements of Pb along roads, and hence it is an estimate. Currently, measured data are not available with sufficient spatial resolution to discern the specific contribution of soil Pb resuspension to air Pb concentration, but resuspended soil Pb cannot be eliminated as a potential notable source of airborne Pb. The concentration of historically deposited Pb in soil is expected to be the greatest in soil next to roads. Section 3.5.1.2 includes recent information on ambient air concentrations of Pb-TSP, sampled at 6 m AGL at a distance of 500 m from the heavily trafficked I-405 and 10 m from a busy arterial road in Los Angeles. From this monitor, average concentrations were not substantially higher than the local urban background concentration (Sabin et al., 2006b). Insufficient data are available to ascertain if the near road Pb-TSP concentrations would be higher at lower monitor heights. The 2006 Pb AQCD (U.S. EPA, 2006b) also noted a smaller estimate of 40% for the Southern California Air Basin (Lankey et al., 1998).

In a recent paper, Laidlaw and Filipelli (2008) analyzed Interagency Monitoring of Protected Visual Environments (IMPROVE) data to explore conditions under which $PM_{2.5}$ particles estimated to be of crustal origins that may contain Pb may become airborne. They observed a seasonal pattern in the concentration of $PM_{2.5}$ of crustal origins in the atmosphere, and they also found that at one IMPROVE site in central Illinois, 83% of the variability in concentrations of crustal $PM_{2.5}$ was predicted by variability in meteorology and soil moisture content. The authors concluded that seasonality and climate parameters could not be eliminated in relation to ambient Pb concentrations. Such mechanisms are described in more detail in Section 3.3. As described in Sections 3.2.2.6 and 3.6.1, there are many contemporary contributions of Pb to soil in urban areas, and studies summarized here have not quantitatively differentiated the contributions of these various sources to Pb concentrations in urban areas.

Lead from Industrial Activities

Several studies have indicated elevated levels of Pb are found in soil exposed to industrial emissions, including brownfield sites (Dermont et al., 2010; Verstraete and Van Meirvenne, 2008; Jennings and Ma, 2007; Van Herwijnen et al., 2007; Deng and

Jennings, 2006). Pb in industrial soils is described in Section 3.6.1. Recent Pb speciation results also indicate a contribution from resuspended soils in areas with previous major emission sources, but without current major sources. Data from airborne PM in the vicinity of an inactive smelter in El Paso, TX were 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).

Lead from Paint and Building Materials

Exterior structures painted with Pb-based paint have long been known to be a source of Pb in outdoor dust or grit (U.S. EPA, 2006b). Recent studies support 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 in the grit under the steel structures (median: 1,480 mg/kg) collectively being 4.4 times higher than median Pb concentrations in the roadway grit not near a structure (median: 340 mg/kg).

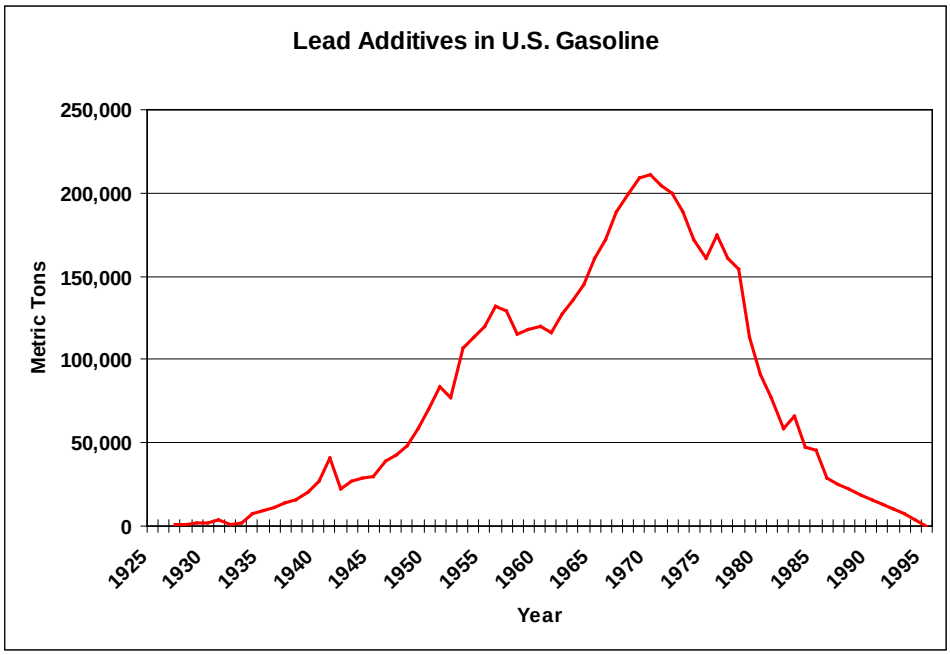
The studies described above considered paint as a source of Pb in outdoor dust through gradual abrasion of the painted surfaces. However, atmospheric conditions may also affect the availability of Pb in paints. Edwards et al. (2009) performed experiments to simulate one week of exposure of Pb-based paints to highly elevated levels of O_3 (11.3 ± 0.8 mg/kg or 150 times the level of the 8-hour NAAQS) and NO_2 (11.6 ± 0.9 mg/kg, or 220 times the level of the annual NAAQS). Following NO_2 exposure, the Pb 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% ($p = 0.004$).

Building demolition was listed as a source of Pb in urban dust in the 2006 Pb AQCD (U.S. EPA, 2006b). In a follow-up study to previous work cited therein, Farfel et al. (2005) observed that surface loadings of dust containing Pb increased by 200% in streets, by 138% in alleys, and by 26% in sidewalks immediately following demolition of an old building. 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 in some areas may serve as a potential source of airborne Pb. The historical use of leaded on-road gasoline has been estimated from documents submitted by Ethyl Corporation to the U.S. Senate (1984) and a report by the U.S. Geological Survey (USGS, 2005); see Mielke et al. (2010a). These estimates are presented in Figure 3-5. The peak U.S. use of Pb additives occurred between 1968 and 1972 with an annual amount of over 200,000 metric tons. According to Ethyl Corporation, the 1970 use of Pb additives was 211,000 metric tons. By 1980, the annual use of Pb additives to on-road gasoline decreased to about 91,000 metric tons or a 57% reduction from its 1970 peak. From 1970 to 1990 there was a 92% decline in Pb additive use. In 1990, the annual U.S. use of Pb additives decreased to 16,000 metric tons, a further 82% decline in Pb additive use from 1980. The final U.S. ban on the use of Pb additives for highway use in on-road gasoline occurred in 1996. After that time, Pb additives were only allowed in nonroad applications, including piston-engine aircraft fuel, racing fuels, farm tractors, snowmobiles, and boats.



Source: Reprinted with permission of Pergamon Press, Mielke et al. (2010a).

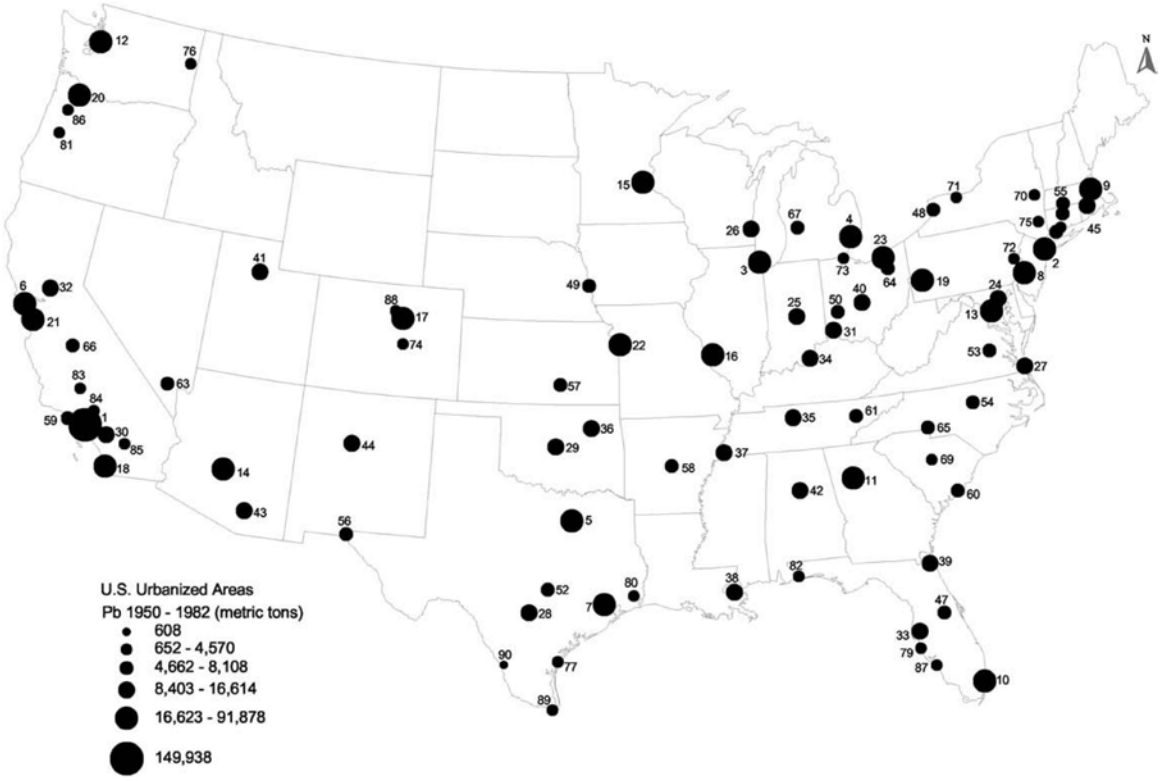
Note: Estimates were derived from the proceedings of the U.S. Senate hearings on the Airborne Pb Reduction Act of 1984, S. 2609 (1984) and the U.S. Geological Survey Pb end use statistics (USGS, 2005).

Figure 3-5 Total U.S. Pb additives in on-road gasoline used in on-road vehicles, 1927-1995.

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

The use of Pb additives resulted in a national scale of influence. For example, variously sized urbanized areas of the U.S. have different amounts of vehicle traffic associated with Pb (Mielke et al., 2010b). Figure 3-6 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 in these areas 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 3-6, depending on the amount of highway construction in those cities. As noted in Section 3.2.2.6 , there have historically been, and are currently, many additional sources of Pb contributing to near-roadway soil Pb concentrations. Data are lacking that quantify the range of airborne Pb concentrations originating from historic Pb in resuspended soil particles, but data on airborne concentrations near roadways indicate measured air Pb concentrations (from all contributing sources) to be generally less than 0.02 µg/m^3 (Section 3.5.3.2).



Source: Reprinted with permission of Pergamon Press, Mielke et al. (2010a)

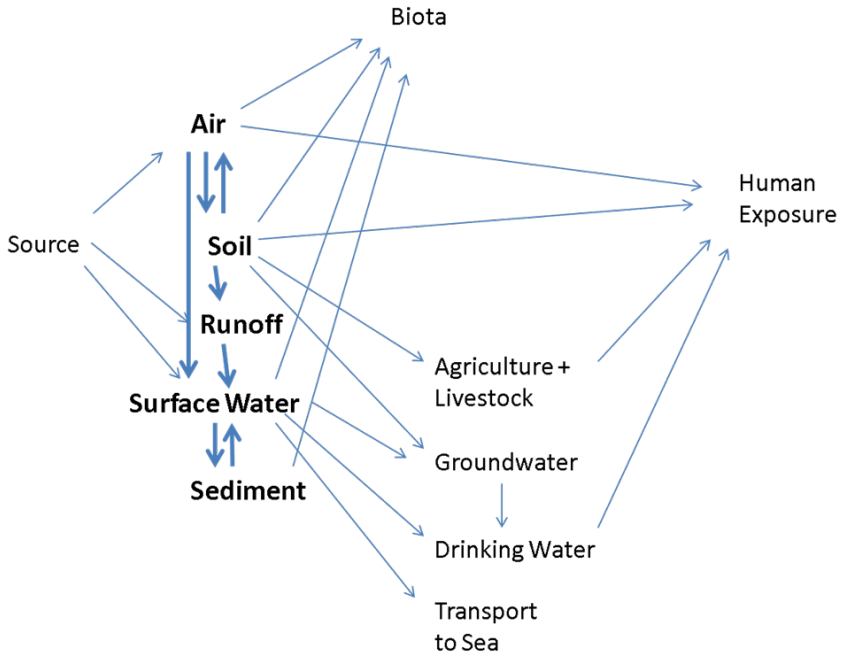
Note: The numbers on the map are rankings of each UA. The size of each dot refers to the magnitude of motor vehicle gasoline-related emissions for each group of UAs.

Figure 3-6 Estimated Pb aerosol inputs from on-road gasoline into 90 U.S. urbanized areas (UAs), from 1950 through 1982.

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 as the contribution of atmospheric Pb to total Pb exposure. Figure 3-7 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.



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

Figure 3-7 Fate of atmospheric lead.

3.3.1 Air

The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that Pb was primarily present in submicron aerosols, but that bimodal size distributions were frequently observed. Pb-PM in the fine fraction is transported long distances, found in remote areas, and can be modeled using Gaussian plume models and Lagrangian or Eulerian continental transport models as reported by several studies. Good agreement between measurements and these models have been reported. Historical records of atmospheric deposition to soil, sediments, peat, plants, snowpacks, and ice cores have provided valuable information on

trends and characteristics of atmospheric Pb transport. Numerous studies using a variety of environmental media indicated a consistent pattern of Pb deposition peaking in the 1970s, followed by a more recent decline. These findings indicated that the elimination of leaded gasoline for motor vehicles 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, 2006b) documented that soluble Pb was mostly removed by wet deposition, and most of the insoluble Pb was mostly removed by dry deposition. As a result, dry deposition was the major removal mechanism for Pb in coarse PM (which is mainly insoluble) and 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 U.S. 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, 2006b) concluded that resuspension by wind and traffic contribute to airborne Pb near sources.

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, 2006b), much of the recent research on Pb transport has focused on interactions between anthropogenic and coarser geogenic PM that leads to incorporation of Pb into coarse PM as well as subsequent transformation on exposure to mineral components of coarse PM. Using scanning electron microscopy (SEM), Schleicher et al. (2010) observed interactions of anthropogenic soot and fly ash particles on the surfaces

of coarse geogenic mineral particles 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. $PbSO_4$ is one of the main constituents of Pb-containing aerosols resulting from coal combustion (Gieré et al., 2006) and it has been shown to react with calcite, $CaCO_3$, a PM mineral component, to form $Pb_3(CO_3)_2(OH)_2$, $Pb(CO_3)$ and $Ca(SO_{4)2}{\cdot}H_2O$ on the surface of the calcite (Falgayrac et al., 2006). In laboratory experiments, (Ishizaka et al., 2009) also showed that $PbSO_4$ could be converted to $PbCO_3$ in the presence of water. Approximately 60-80% was converted after only 24 hours for test samples immersed in a water droplet. This compared with only 4% conversion for particles that had not been immersed. As a result of recent research, there is considerable evidence that appreciable amounts of Pb can accumulate on coarse PM during transport, and that the physical and chemical characteristics of Pb can be altered by this process due to accompanying transformations.

3.3.1.2 Deposition

Wet Deposition

The 2006 Pb AQCD (U.S. EPA, 2006b) documented that dry deposition was the major removal mechanism for Pb in coarse PM and wet deposition as the most important removal mechanism for fine PM. Which process is most important for atmospheric removal of metals by deposition is largely controlled by solubility in rain water. Metal solubility in natural waters is determined by a complex multicomponent equilibrium between metals and their soluble complexes and insoluble ionic solids formed with hydroxide, oxide, and carbonate ions. This equilibrium is strongly dependent on pH and ionic composition of the rain water. As pH increases, Pb solubility is reduced. As a consequence, it is possible that efforts to reduce acidity of precipitation could also reduce wet deposition of Pb. Recent research confirms the general trend described in the 2006 Pb AQCD (U.S. EPA, 2006b) that Pb associated with fine PM is usually more soluble in

rain water than Pb associated with coarse PM, leading to a relatively greater importance of wet deposition for fine Pb and of dry deposition for coarse Pb. 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

Recent research on dry deposition has focused differences between urban or industrial sites and rural or less industrial areas. For locations outside of industrial areas, new measurements of Pb dry deposition fluxes are similar to those reported in the 2006 Pb AQCD (U.S. EPA, 2006b), but in industrialized urban areas, they are considerably greater. The following studies presented measurements of dry deposition flux obtained by capturing deposited particles onto a sampling substrate. Hence, these measurements did not provide information on net deposition following resuspension of deposited material. Resuspension processes and measurements thereof are described in Section 3.3.1.3. For example, Yi et al. (2006) calculated dry deposition fluxes for trace elements including Pb in New York-New Jersey harbor and observed much greater dry deposition fluxes for this urban industrial site in Jersey City (mean: 50 $\mu g/m^2 \cdot d$) than for suburban New Brunswick (mean: 8 $\mu g/m^2 \cdot d$). Sabin and Schiff (2008) (2008) measured dry Pb deposition flux along a transect from Santa Barbara to San Diego, CA in 2006 and observed and observed a range of 0.52-14 $\mu g/m^2 \cdot d$ for the median values across the eight sites. The highest median Pb flux was observed at Los Angeles Harbor, which is downwind of a harbor with a mix of industrial (harbor-related) and urban activities (14 $\mu g/m^2 \cdot d$). The second highest median Pb flux was observed at San Diego Bay, a military port (3.3 $\mu g/m^2 \cdot d$). 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 and 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 Pb AQCD (U.S. EPA, 2006b). They also reported that dry deposition fluxes were greater in industrially impacted urban areas, ranging from 12-17 mg/m^2·yr, more than 10 times the upper bound of the range reported in the 2006 Pb AQCD (U.S. EPA, 2006b).

Recent results also confirmed the trend of decreasing overall deposition fluxes after removal of Pb from on-road gasoline, as described in the 2006 Pb AQCD (U.S. EPA, 2006b). Watmough and Dillon (2007) found that the bulk annual deposition of Pb in a central Ontario forested watershed during 2002-2003 was 0.49 mg/m^2·yr; this was lower than the value of 1.30-1.90 mg/m^2·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.

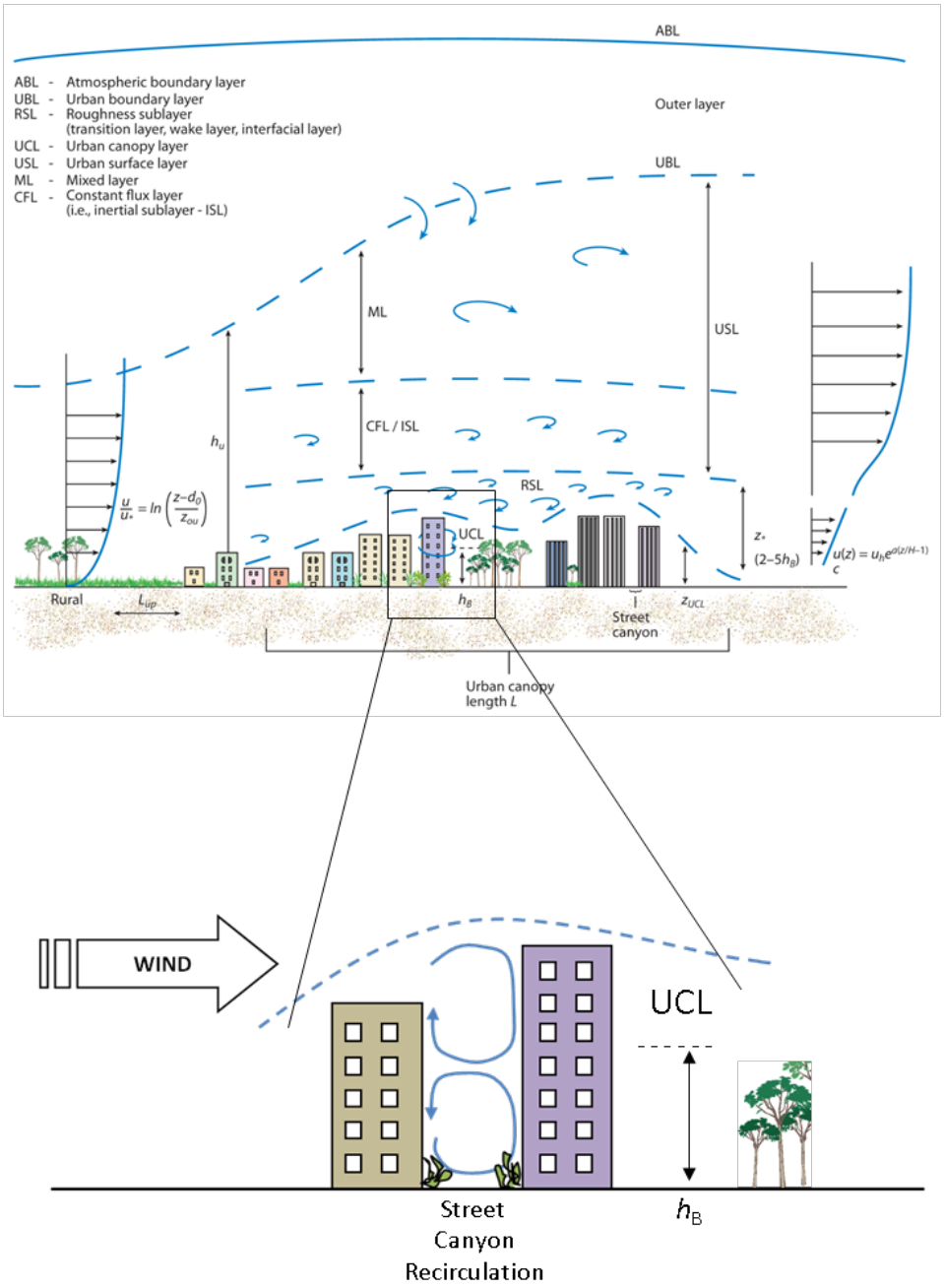
3.3.1.3 Resuspension of Lead from Surface Soil to Air after Deposition

The following information focuses on issues regarding the transport processes affecting resuspended soil Pb and dust Pb in urban environments. As described in Section 3.2.1, the greatest point source Pb emissions in the U.S. occur in locations near specific major facilities, such as secondary smelters, and other industrial operations involving large

scale metal processing or fuel combustion. However, in the absence of such sources and in the vicinity of previous major sources, the 2006 Pb AQCD (U.S. EPA, 2006b) concluded that resuspension by mechanical stressors such as traffic, construction, and wind can be a source of airborne Pb above background levels near sources, with Pb accounting for between 0.002 to 0.3% of the mass of resuspended PM_{10}.

Results from several studies have suggested a minor contribution from resuspension to airborne Pb concentration is indicated by a smoothed soil Pb concentration profile that decreases with distance from various sources, including city centers (Laidlaw and Filippelli, 2008), major freeways (Sabin et al., 2006b), and steel structures with abrading paint (Weiss et al., 2006). The smoothed profile is suggested to be consistent with continual Pb resuspension and deposition due to atmospheric turbulence. As noted in the 2006 Pb AQCD (U.S. EPA, 2006b), the contribution of resuspended soil and dust to the airborne burden may be significant from highly contaminated sites (e.g., active or abandoned industrial facilities and Superfund sites). In contrast, as summarized in Section 3.5.3, Pb concentrations near roads in urban areas are one to two orders of magnitude below the current Pb NAAQS.

The urban environment can be considered quite different from natural landscapes because it has been highly modified by human activity, including above- and below-ground infrastructure, buildings, and pavement, and a high density of motorized transportation. These factors may influence the distribution and redistribution of Pb-bearing PM. As shown in Figure 3-8, urban turbulence occurs on several scales. Transport and dispersion of urban grit is subject to air movement within the urban canopy layer, where air movement is driven by air velocity within the urban boundary layer and urban topographical conditions such as building shape, building façade, and street canyon aspect ratio (Fernando, 2010). Within a street canyon, air circulates and tends to form counter-rotating eddies along the height of the canyon (Figure 3-8), which result in lower mean components of air movement, higher turbulence components, and higher shear stress within the canyon compared with open field conditions (Kastner-Klein and Rotach, 2004; Britter and Hanna, 2003). Recirculation around intersection corners and two-way traffic conditions can also enhance turbulence levels, while one-way traffic conditions increase air velocity along the street (Soulhac et al., 2009; Kastner-Klein et al., 2003; Kastner-Klein et al., 2001). Sedefian et al. (1981) measured the length scales of turbulent eddies resulting from passing 50 mph (22.2 m/s) traffic on a test road and observed scales of 0.6-2.7 m when winds were perpendicular to the test road and scales of 1.8-2.7 m when winds were parallel to the road.



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

Figure 3-8 Scales of turbulence within an urban environment.

Recent research on urban PM transport is highly relevant to Pb transport and dispersion because Pb is most prevalently particle-bound. Relevant results for Pb exposure in these areas include observations that PM concentration peaks dissipate more rapidly on wider streets than in narrow street canyons (Buonanno et al., 2011); concentrations are typically

low next to a building because either less source material is available or less material penetrates the boundary layer of the building (Buonanno et al., 2011); and there are stronger inverse 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 (i.e., rock salt) was applied to a road and then the grit's dispersion by traffic was measured over time to simulate resuspension and transport of road dust. During the experiments, 0.039% of the tracer grit was measured to move down the road with each passing vehicle, 0.0050% was estimated to be swept across the road with each passing vehicle, and 0.031% was estimated to become airborne when a vehicle passed.

Harris and Davidson (2008) developed a model of resuspension of single particles initially at rest on a solid surface based on the balance of lift, drag, gravity, torque, and adhesion forces on the particle in addition to turbulent wind fluctuations within a simulated urban boundary layer. In their model simulations showed that 2.5 µm and 10 µm particles reached a maximum height of 0.04-0.06 m above ground level (AGL), while 50 µm particles reached a maximum of 0.2 m AGL and 75 µm particles reached at least 0.4 m AGL. Empirical analysis has shown that lift force is proportional to particle diameter to the power of approximately 1.5, so that large particles actually have larger initial displacement than smaller particles. At the same time, lateral travel distance following resuspension tended to decrease linearly with increasing particle size (0.5 µm particle: median lateral distance estimated at 3.75 m; 100 µm particle: median lateral distance estimated at 0.5 m), reflecting the counteracting force of gravity. Harris and Davidson (2008) estimated that the combination of forces caused a maximum height of 0.05 m to occur for particles with diameters around 75-100 µm, depending on turbulent wind conditions. For all cases simulated, the resuspension and deposition were estimated to occur over time frames on the order of seconds.

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

In urban environments the transport distance that must be traversed to penetrate indoors can be very short, and at the same time resuspension and dispersion of larger particles may be caused by motor vehicles. Resuspension of road dust by traffic becomes more difficult with decreasing particle size because adhesive forces are stronger than shear force that is imparted by traffic-induced turbulent air movement (Harris and Davidson,

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

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

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.

3.3.2 Water

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), 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 removal by flushing, evaporation, and sedimentation occurs. Transport in surface water is largely controlled by exchange with sediments, and the cycling of Pb between water and sediments is governed by chemical, biological, and mechanical processes that are affected by many factors, including salinity, organic complexation, oxidation-reduction potential, and pH. As described in the 2006 Pb AQCD (U.S. EPA, 2006b), metals in waterways are transported primarily as soluble chelates and ions, or adsorbed on colloidal surfaces, including secondary clay minerals, iron and manganese oxides or hydroxides, and organic matter, and adsorption on organic or inorganic colloids is particularly important for Pb. The extent of sorption strongly depends on particle size as smaller particles have larger collective surface areas. Aqueous Pb concentrations also increase with increasing salinity. Pb is found predominantly as PbO or $PbCO_3$ in aqueous ecosystems. Pb is relatively stable in sediments, with long residence times and limited mobility. However, Pb-containing sediment particles can be remobilized into the water column. As a result trends in sediment concentration tend to follow those in overlying waters. Fe and Mn oxides are especially susceptible to recycling with the overlying water column. Although resuspension of sediments into overlying waters is generally small compared to sedimentation, resuspension of contaminated sediments is often a more important source than atmospheric deposition. Organic matter (OM) in sediments has a high capacity for accumulating trace elements. In an anoxic 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, 2006b), runoff from storms was also identified as an important source. A substantial portion of Pb susceptible to runoff originates from 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 and von der Kammer, 2008), which is consistent with previous research using cross flow filtration (Pokrovsky and Schott, 2002; Ross and Sherrell, 1999) and SEM examination of single particles (Taillefert et al., 2000).

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

Observations in boreal rivers and soil pore waters in permafrost dominated areas of Central Siberia indicated that Pb was transported with colloids in Fe-rich waters. Trace elements that normally exhibited limited mobility (including Pb) had 40-80% of their annual flux in the nominal dissolved phase, operationally defined as material that passes through a 0.45 µm pore-size filter, and that these metals had a higher affinity for organo-mineral Fe-Al colloids (Pokrovsky et al., 2006). Pokrovsky et al. (2006) postulated that during the summer, rainwater interacts with degrading plant litter in the top soil leading to the formation of Fe-Al-organic colloids with incorporated trace elements. Migration of trace element-Fe-Al-OM colloids may result in export of Pb and other elements to riverine systems. Most of the transport occurred after thawing had commenced. This contrasts with permafrost free areas where trace elements such as Pb are incorporated into 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). 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, 2006b) described rivers and lakes as temporary reservoirs with Pb lifetimes of months or less, estuaries can present a substantial barrier to transport into the open ocean.

3.3.2.2 Deposition of Lead within Bodies of Water and in Sediment

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), in general Pb is relatively stable in sediments, with long residence times and limited mobility. As described in previous sections, Pb enters and is distributed in bodies of water largely in PM form. In rivers, particle-bound metals can often account for ≥ 75% of the total load, e.g. (Horowitz and Stephens, 2008). 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 (Horowitz et al., 2008; Chalmers et al., 2007). Indeed, Chalmers et al. (2007) revealed that in river and lake sediments in New England, there was an order of magnitude difference between Pb sediment concentrations in rural versus urbanized areas.

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

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

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

The accurate modeling of Pb behavior in marine waters (including estuaries) requires consideration of many parameters such as hydrodynamics, salinity, pH, suspended PM, fluxes between PM and dissolved phases (Hartnett and Berry, 2010). Several new advances in the study of Pb cycling in these complex environments have been described in recent publications. Li et al. (2010a) used particle organic carbon (POC) as a surrogate for the primary sorption phase in the water column to describe and model the partitioning of Pb between PM and dissolved forms. Huang and Conte (2009) observed that considerable change in the composition of PM occurs as they sink in the marine environment of the Sargasso Sea, with mineralization of OM resulting in increased Pb-PM concentration with increased depth. As a result of this depletion of OM in sinking particles, geochemical behavior at depth was dominated by inorganic processes, e.g. adsorption onto surfaces, which were largely independent of Pb source. Sinking rates in marine environments can vary, but a rate approximating 1 m/day has been used in some models of Pb transport and distribution in aquatic-sediment systems (Li et al., 2010a). Surface sediment Pb concentrations for various continental shelves were collated and compared by Fang et al. (2009); (Table 3-2).

Table 3-2 Surface sediment Pb concentrations for various continental shelves

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

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

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

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. (Roulier et al., 2010) determined that Pb flux from sediments originated mostly from organic fractions, but also partially from Mn and Fe components undergoing reductive dissolution. The rate of release was controlled by OM content, particle size, clay type and content, and silt fraction (Roulier et al., 2010). The importance of sediment particle size, OM content and acid volatile sulfide concentration in relation to metal release was similarly identified (Cantwell et al., 2008). The effect of pH change on Pb release from lake sediments has also been examined, revealing that 1.8 protons (H^+) were exchanged per divalent metal cation released (Lee et al., 2008a). Processes governing Pb release from lake sediments, including microbial reductive dissolution of Fe, biogenic sulfide production and metal sorption-desorption, have been investigated and results indicated that release of Pb from suboxic and anoxic zones of the sediment act as a Pb source to the overlying water of the lake (Sengor et al., 2007). Bacardit et al. (2010a, b) performed a mass balance of Pb, Zn, and As for three lakes in the Central Pyrenees in France to identify dominant metals distribution processes. They estimated that flux from the catchment accounted for 91-99% of the lakes' Pb inputs, while sediment flux accounted for 98-99% of Pb outputs. In this paper, sediment was only modeled as an output.

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 (Li et al., 2010a). In a related way, a half-life for sediment Pb (considering benthic flux alone as the loss mechanism) of 5.3 years was estimated for marine sediments off the Belgian coast (Gao et al., 2009). Atkinson et al. (2007) conducted experiments in an area contaminated by metal smelters, Lake Macquarie, Australia, to assess the factors that influence flux of metals from marine sediment. Low pH (pH = 6 ± 1), bioturbation, and other mixing processes were found to have stronger influence over flux than binding to sulfides, which were thought to be sequestered in deeper sediments.

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

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

Overall, recent research on Pb flux from sediments in natural waters provided greater detail on resuspension processes than was available in the 2006 Pb AQCD (U.S. EPA, 2006b), and 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 watersheds, which must take into account direct atmospheric deposition, runoff of atmospherically deposited Pb, and runoff of Pb from sources such as mine tailings or paint chips that are shed from outdoor structures. The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that runoff was consistently mostly PM, with a relatively small dissolved fraction, and that dissolution of carbonate and related compounds were important contributors to Pb pollution in runoff waters. It also described 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 the flux of Pb from roadway and urban runoff and snow melt to watersheds.

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

New research has improved the understanding of suspended PM size ranges, speciation, and impacts of Pb runoff from urban soil and road dust. Soil and road dust have been identified as major sources of Pb pollution to near-coastal waters, leading to high Pb concentrations in stormwater runoff that became associated with dissolved and suspended PM phases as well as bedload, material moved by rolling, sliding, and saltating along the bottom of a stream (Birch and McCready, 2009).

Several 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 and Lottermoser, 2007). Pb in roadside dusts in Thessaloniki, Greece was characterized by Ewen et al. (2009) and no difference in Pb concentration was found between <75 µm and 75-125 µm PM size ranges, although a difference in the chemical form of Pb between slightly versus highly contaminated areas was observed.

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

Murakami et al. (2007) also emphasized the importance of $PbCrO_4$ as an important species of Pb from road surfaces. That study identified individual particles containing high levels of Pb and Cr ($\geq$ 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, 2006b), most recent studies have concluded that, during storm events, Pb is transported together with large PM. Some studies, however, found that Pb was concentrated in the fine PM fraction and, occasionally, Pb was found predominantly in the dissolved fraction. Tuccillo (2006) found that Pb was almost entirely in the >5 µm size range and, indeed, may be associated with PM larger than 20 µm. (Sansalone et al., 2010) compared Pb-containing PM size distributions from New Orleans, LA; Little Rock, AR; North Little Rock, AR; and Cincinnati, OH and found no common distribution pattern. Pb was associated with Cincinnati PM mainly in the <75 µm fractions, at Baton Rouge and Little Rock Pb mainly in the 75-425 µm PM fractions, and at North Little Rock Pb predominantly in the >425 µm PM fractions. New Orleans Pb was almost uniformly distributed among the smaller size PM fractions. McKenzie et al. (2008) found that Pb was enriched in the finest PM (0.1-0.3 µm) in stormwater samples collected in California, particularly for storms that occurred during and after an extended dry period.

Guo et al. (2006a) investigated the effect of engineered partial exfiltration reactor (PER) systems on the partitioning and speciation of Pb in rainfall-runoff at the upstream end of an urban source area catchment that is part of the much larger urbanized and industrial Mill Creek watershed in Hamilton County, Ohio. The catchment is paved to a large extent with asphalt and is used for transportation. Guo et al. (2006a) investigated a catchment that drained toward a wide grassy area and found that Pb was mainly associated with dissolved organic matter (DOM). 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. (2006a) 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, 2006b). 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 (Sansalone and Buchberger, 1996). The melt process usually occurs in a sequence: pavement melt, followed by roadside (impervious) and finally pervious area melt. As part of this sequence, rain-on-snow can transport high loads of PM-associated pollutants (Oberts, 2000). Westerlund and Viklander (2006) investigated differences in PM and Pb concentrations between rainfall events occurring during snowmelt and rain periods. Runoff events occurring during the snowmelt period (i.e. rain-on-snow) had about five times higher numbers of particles (in the size range 4 to 120 µm)/liter of runoff. The first rain-on-snow event was characterized by an increase in the number of particles in the 4 to 25 µm size range. The rain-on-snow gave a "flush" through the snow but this was still not sufficient to transport the larger sized particles. Only the highest energy rain-on-snow events increased transport of PM across the entire size spectrum. There was no difference in particle size distributions between snowmelt and rain on snow events, although more was transported during snowmelt. Pb concentrations were most strongly associated with the smaller PM size fractions.

Overall, there was a significant difference between the melt period and the rain period in terms of concentrations, loads, transportation and association of heavy metals with particles in different size fractions (Westerlund and Viklander, 2006). Over a 4-year period, Magill and Sansalone (2010) analyzed the distribution of metal in snow plowed to the edge of roads in the Lake Tahoe catchment in Nevada, and concluded that metals including Pb were mainly associated with 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 and Sansalone, 2008). For adsorbed species that form outer sphere complexes, a decrease in adsorption and an increase in aqueous complexes for pollutant metals is a likely consequence of higher deicing salt concentrations. If metals form inner-sphere complexes directly coordinated to adsorbent surfaces, background deicing salt ions would have less impact. It is thought that physical and outer-sphere complexes predominate for coarse PM, as was the case in Nevada, and so leaching would be likely to cause an increase in dissolved phase Pb concentrations.

Rural runoff has also been extensively studied since publication of the 2006 Pb AQCD (U.S. EPA, 2006b), including several recent publications on a forested watershed (Lake Plastic) in central Ontario (Landre et al., 2010, 2009; Watmough and Dillon, 2007) and nearby Kawagama Lake, Canada (Shotyk and Krachler, 2010). Results indicated that

bulk deposition substantially decreased to 0.49 mg/m^2 in 2002 from 1.30-1.90 mg/m^2 in 1989-91. The upland soils retained >95% of the Pb in bulk deposition, i.e. leaching losses to stream water were small. The wetland area was, however, a net source of Pb with annual Pb concentrations in stream water ranging from 0.38 to 0.77 µg/L. Lake sediments were efficient sinks for atmospherically deposited Pb with 80-91% of the Pb input being retained. Up to 68% of the Pb entering the lake was derived from the terrestrial catchment. Overall, the watershed effectively retained atmospherically deposited Pb, but some Pb was then redistributed from the catchment to the lake sediments; and the Pb in the near-surface lake sediments reflected terrestrially transported soil material, rather Pb being deposited from the atmosphere. The highest concentrations of dissolved organic carbon (DOC), Fe and Pb in the wetland draining stream occurred in summer when 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. (2007a) observed stormflow Pb concentrations almost three times higher than those reported by Graham et al. (2006) for northeastern Scotland. The generally high dissolved Pb were due to high soil Pb pools and high stream water

DOC concentrations (Rothwell et al., 2007a). In a separate study, Rothwell et al. (2007b) showed that OM was the main vector for Pb transport in the fluvial system. Some seasonal variability was observed: declining Pb concentrations in autumn stormflow may indicate the exhaustion of DOC from the acrotelm (the hydrologically active upper layer of peat which is subject to a fluctuating water table and is generally aerobic) or a dilution effect from an increasing importance of overland flow.

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

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

Dunlap et al. (2008) studied a large (>160,000 km^2) riparian system (the Sacramento River, CA) and showed that the present day flux of Pb 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. (2007a) commented that although there have been substantial reductions in sulfur deposition to U.K. uplands over the last few decades (Fowler et al., 2005), anthropogenic acidification of upland waters is still possible if there is nitrogen leaching from the surrounding catchment and this may increase with nitrogen saturation (Curtis et al., 2005). Rothwell et al. (2007a) predicted that if an increase in surface water acidification is coupled with further increases in DOC export from organic-rich catchments, metal export from peatland systems will increase. The deterioration of peat soils by erosion is considered to be exacerbated by climatic change. Rothwell et al. (2010) used digital terrain analysis to model suspended Pb concentrations in contaminated peatland catchments. The peat soils of the Peak District are characterized by extensive eroding gullies and so they were combined in an empirical relationship between sediment-associated Pb concentrations and mean upslope gully depth with fine-resolution mapping of the gully areas. This model will enable prediction of metal contamination in receiving waters.

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

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

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

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

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

Overall, research results from the last several years have greatly expanded the extent of the knowledge concerning Pb from runoff. Substantial Pb input to estuarine and marine ecosystems has been well documented. More detail concerning the origin of Pb from roof runoff has led to the conclusion that roof flashing could be especially important. Research on road runoff has provided valuable insight into 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. 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, 2006b).

3.3.3 Soil

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

3.3.3.1 Deposition of Lead onto Soil from Air

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), a considerable amount of Pb has been deposited from air onto soils in urban areas and near stationary sources and mines, and soil Pb concentrations are typically on the order of 100 mg/kg (Mielke et al., 2010a). Removal and translocation of Pb in soil is an ongoing process.

High Pb soil concentrations were observed near stationary sources such as smelters and battery disposal operations, and soil Pb concentrations decreased rapidly with distance from the source. Several recent studies continue to document high concentrations of Pb in soil. A study of soil Pb concentrations in Queensland, Australia described atmospheric transport and deposition of Pb in 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 (Shotbolt et al., 2008; Watmough and Dillon, 2007; Fowler et al., 2006; Graham et al., 2006).

There has been considerable interest in the response of soils to the decreasing aerosol Pb concentrations and Pb deposition rates that have been recorded in recent years. Kaste et al. (2006) resampled soils at 26 locations in the Northeast U.S. (during a 2001-2002 survey of soil sites originally sampled in 1980), and found no significant change in the amount of Pb in the O-horizon at high altitude sites. 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. High Pb concentrations at greater altitudes were also found in Japan, especially above 600 m (Takamatsu et al., 2010).

Further support for the use of mosses as bioindicators or monitors for atmospheric Pb inputs to peat bogs have recently been published by Kempter et al. (2010) who found that high moss productivity did not cause a dilution of Pb concentrations in peat bogs. They also found that productive plants were able to accumulate particles from the air and that rates of net Pb accumulation by the mosses were in excellent agreement with the atmospheric fluxes obtained by direct atmospheric measurements at nearby monitoring

stations. In addition, Bindler et al. (2008) used Pb isotopes to compare the distribution of Pb in the forest soils with that of lake sediments where no "plant pumping" processes could be invoked, and used Pb isotope ratios to demonstrate that observations were consistent with anthropogenic Pb deposition to the soils rather than intermixing of natural Pb from underlying mineral soil horizons.

Overall, recent studies provided deposition data that was consistent with deposition fluxes reported in the 2006 Pb AQCD (U.S. EPA, 2006b), and demonstrated consistently that Pb deposition to soils has decreased since the phase-out of leaded on-road gasoline. Follow-up studies in several locations 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.

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, 2006b). It also described a complex variety of factors that influence Pb retention, including hydraulic conductivity, solid composition, OM content, clay mineral content, microbial activity, plant root channels, geochemical reactions, colloid amounts, colloidal surface charge, and pH.

Recent research in this area has provided more insight into the details of the Pb sequestration process. Importance of leaf litter was further investigated, and it was observed that the absolute Pb content can be substantial because rain events cause splashing of the leaf litter with soil thus placing the litter in direct contact with soil metals. The resulting increase in leaf litter metal concentrations suggests that the litter can act as a temporary sink for metals from the soil around and below leaves on the ground. The low solubility of Pb in the leaf litter indicates that the Pb is tightly bound to the decomposing litter, making the decomposing leaves act as an efficient metal storage pool (Scheid et al., 2009). Differences between throughfall (i.e., water depositing onto the soil following collection on leaves) and litterfall (i.e., deposition of leaves, bark, and other vegetative debris onto soil) in forested areas have been investigated in forested areas, and the combined input of Pb to the forest floor from throughfall and litterfall was approximately twice that measured in bulk deposition (Landre et al., 2010). The difference was attributed to a substantial contribution from internal forest cycling and indicates that bulk deposition collectors may underestimate the amount of Pb reaching the forest floor by about 50% (Landre et al., 2010).

New research has also provided details about the complexity of Pb sequestration during soil OM decomposition. Schroth et al. (2008) investigated Pb sequestration in the surface layer of forest soils and the transformation of Pb speciation during soil OM decomposition. The pH range for forest floor soils in the Northeast U.S. is typically 3.5-5 and, under these conditions, dissolved Pb would adsorb strongly to soluble OM and to Fe/Al/Mn oxides and oxyhydroxides. It had been thought that the high affinity of Pb for organic ligands meant that sequestered atmospheric Pb would be preferentially bound to soluble OM. As a consequence, decomposition of OM would lead to Pb migration to the underlying mineral layers where it would be precipitated with the dissolved OC or adsorbed to pedogenic mineral phases. However, recent research has revealed a more complicated picture of gasoline-derived Pb associations in the forest floor. More recent research indicates that, as decomposition progresses, Pb and Fe become more concentrated in "hotspots" and Pb likely becomes increasingly distributed on surfaces associated with Fe and Mn (and to some extent Ca). It was postulated that Pb was initially bound to labile organic 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 soil O-horizons of the Northeast U.S. differed considerably from those that were originally deposited from fossil fuel combustion (including on-road gasoline). $PbSO_4$ was considered to be the main form of Pb that had been delivered from the atmosphere to the surface of the Earth and it was postulated that the presence of sulfate may have facilitated the adsorption of Pb to colloidal Fe phases within the organic-rich horizons.

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

Recent research also addressed 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 3-9 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 and Giammar, 2007). Overall, in agreement with the 2006 Pb AQCD (U.S. EPA, 2006b), the addition of phosphate can enhance immobilization of Pb under certain conditions in the field but may cause desorption and mobilization of anionic species of As, Cr and Se.

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, 2006b). The fate of any metal transport in soil is in response to a complex set of parameters including soil texture, mineralogy, pH and redox potential, hydraulic conductivity, abundance of OM and oxyhydroxides of Al, Fe, and Mn, in addition to climate, situation and nature of the parent material. As a consequence, it is impossible to make general conclusions about the final fate of anthropogenic Pb in soils. Indeed, Shotyk and LeRoux (2005) contend that the fate of Pb in soils may have to be evaluated on the basis of soil type. Some generalizations are, however, possible: Pb migration is likely to be greater under acidic soil conditions (Shotyk and Le Roux, 2005). In this respect, it would be expected that there should be considerable mobility of Pb in the surface layers of certain types of forest soils. This section reviews recent research on movement of Pb through soil types by first focusing on forest soils, followed by a broader treatment of a more diverse range of soils.

Forest Soils and Wetlands

Several studies confirmed the slow downward movement of Pb within the soil column. Kaste et al. (2006) found that the amount of Pb in O-horizon soils had remained constant at 15 of 26 sites in remote forested areas of the Northeast U.S. that had been re-sampled after a 21-year time period had elapsed, but that measured soil Pb concentrations were lower than predicted concentrations from total deposition, strongly suggesting that the O-horizon had not retained all of the atmospheric Pb, and that a proportion of the atmospheric deposition must have leached into the underlying mineral layers. At some sites, mainly those at the southern latitudes and lower altitude sites, the proportion of Pb that had been leached downward from the O-horizon was quite considerable. Relative

retention of Pb was influenced by the rate of OM decomposition, depth of soil O-horizon, and pH. For soils where Pb was strongly retained by the O-horizon, a relationship between Pb and Fe-rich phase was observed, but Pb was also significantly correlated with other metals. XANES data suggested a possible interaction with an amorphous Fe oxide, but spectra were not entirely explained by Fe and oxygen and an additional spectral feature suggested the presence of a 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.

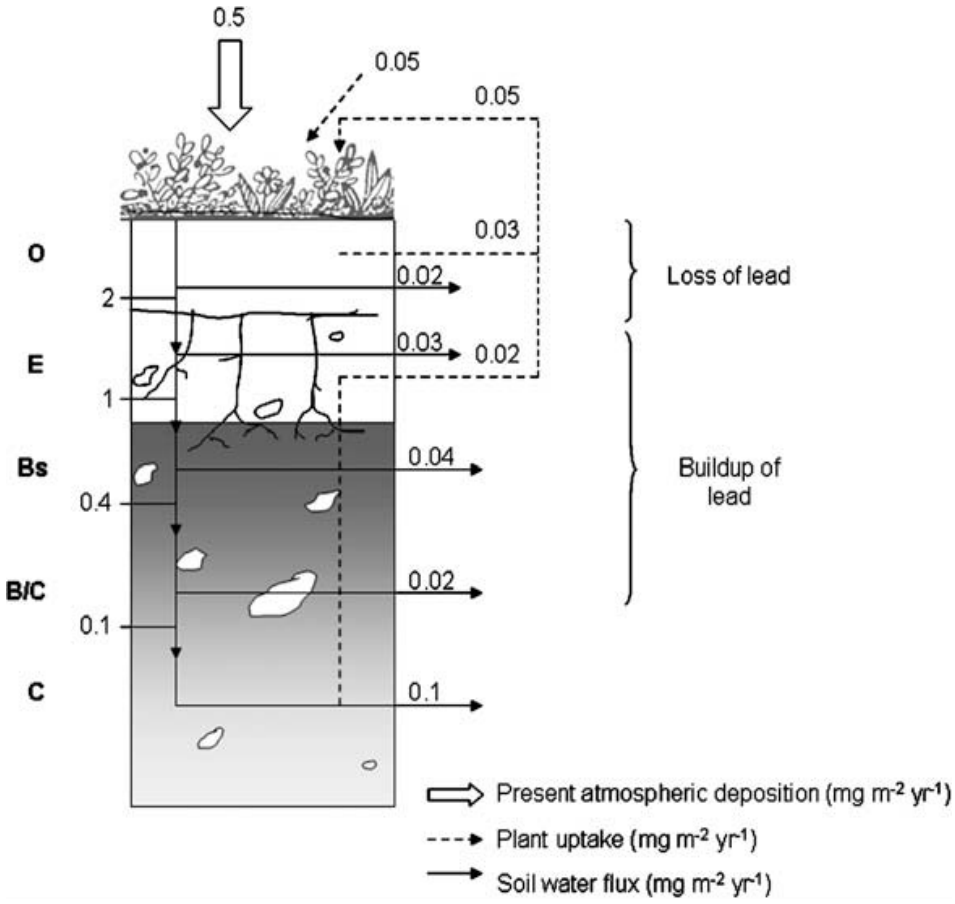
Klaminder et al. (2006b) also considered the transfer of Pb from the O-horizon to the underlying mineral horizons (including the C-horizon). They concluded that atmospheric pollution-derived Pb migrated at a rate about 10-1,000 times slower than water. They assumed that Pb was mainly transported by dissolved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

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

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

Klaminder et al. (2008a) considered the biogeochemical behavior of atmospherically derived Pb in boreal forest soils in Sweden (Figure 3-9). The estimated annual losses via

percolating soil water were ~2 mg/m^2·yr (Klaminder et al., 2008a) 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 et al., 2006a). These values were greater than the values of 2-150 years determined for U.S. forest soils, e.g. (Watmough et al., 2004; Kaste et al., 2003) but the difference was attributed to the lower decomposition rates of OM within the northern boreal forests of Sweden. They concluded that more research was needed to determine the processes occurring within the mor layer that control the release of Pb from this horizon.



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

Figure 3-9 Schematic model summarizing the estimated flux of Pb within a typical podzol profile from northern Sweden using data from Klaminder et al. (2006a).

Klaminder et al. (2008b) 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). In another study of Pb mobility within Mediterranean soils, Pb infiltration velocity was measured to be 0.005 m/yr (Erel, 1998). The authors attributed Pb movement within the soil column to advection and concluded that the soil profile of Pb is similar to the anthropogenic air Pb emissions trend. Pedrot et al. (2008) studied colloid-mediated trace element release at the soil/water interface and showed that Pb was mobilized by Fe nanoparticles that were bound to humic acids.

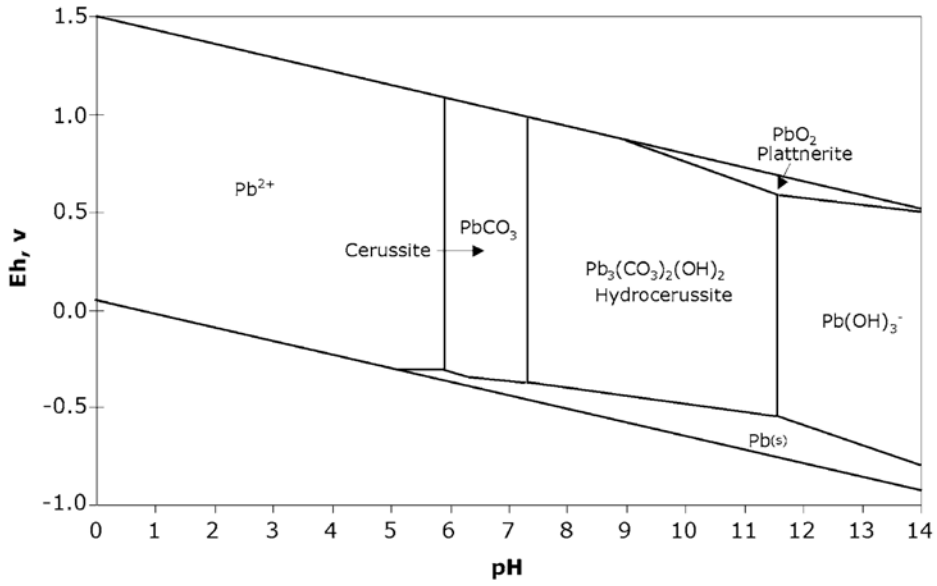
Soil pH value is probably the single most important factor affecting solubility, mobility and phytoavailability but reducing conditions also results in increased Pb mobility, with the release of Pb into an anoxic soil solution due to the combined effect of Fe(III) reductive dissolution and dissolved OM release. Dissolved OM is more important than Fe oxyhydroxides in determining Pb mobility. Under oxic conditions, Fe-Mn-hydroxides often play an important role in the sorption of Pb to the solid phase soil (Schulz-Zunkel and Krueger, 2009). In an agricultural soil, fate of Pb in soils is related to agricultural management. Although Pb was found to be strongly sorbed to the soil, downward migration was observed and the movement of Pb to deeper soils was due to the soil mixing activities of earthworms (Fernandez et al., 2007). Thus in relatively unpolluted non-forested soils, as in forested soils, colloidal Fe and OM, pH, and biophysical transport all enhance Pb mobility in soil. Pb transport in more highly contaminated soils has also been the subject of recent research. In a vegetated roadside soil, Pb was leached from the upper 50 cm of the soil even though the pH was 7.2. Pb was transported on mobile particles and colloids in the soil solution. Some of the colloids may have formed

from OM produced by roots and decaying shoots. The transport process was enhanced by preferential flow triggered by intense rainfall events. This study suggested that the value of the effective sorption coefficient estimated under dynamic conditions was unrelated to values measured in conventional batch studies. This indicates that the use of batch studies to derive input values for sorption coefficients in transport models requires caution. It was concluded that the primary control of Pb transport in the long term was the degree of preferential flow in the system (Roulier et al., 2008b).

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

Williams et al. (2006) characterized Pb speciation in a mine waste-derived fertilizer, ironite. It was thought that PbS would be the main form of Pb, but instead was the predominant form was $PbSO_4$, which may move more easily through soil and enter proximal waters. In contrast, Courtin-Nomade et al. (2008) showed that Pb was incorporated into barite rather than goethite in waste rock pile materials. The high-stability phase formed was an anglesite-barite solid solution.

In weathering flotation residues of a Zn-Pb sulfide mine 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 3-10, which shows an Eh-pH diagram indicating the solubility, equilibrium, and stability of these corroded Pb molecules in terms of the activity of hydrogen ions (pH) versus the activity of electrons (Eh [in volts]). The solubilized Pb was largely re-adsorbed by the Fe and Mn oxides and carbonate soil fractions. The minimum solubility of hydrocerussite lies in the pH range 8-9 but solubility increases by several orders of magnitude at pH below 6 (Scheetz and Rimstidt, 2009).



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

Figure 3-10 Eh-pH diagram for Pb in shooting range soils, Jefferson National Forest, VA.

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

While in some studies the flux of Pb, from the soil through aquatic ecosystems to lakes has peaked and declined. In other studies, no recovery of lake sediments in response to emission reductions was observed (Norton, 2007). For example, Klaminder et al. (2010) has shown that the Pb concentrations in sub-Arctic lake sediments remain unchanged in recent years, with the lack of recovery linked to the effects of soil warming, which affect

Pb-OM transport from soil to the receiving lake systems. Shotyk and Krachler (2010) also reported a disconnect between atmospheric deposition and recent changes in Pb concentration and isotope ratios in the lake sediments. Simulations of future metal behavior suggest that the more strongly sorbing metals such as Pb will respond to changes in metal inputs or acidification status only over centuries to millennia (Tipping et al., 2006).

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

3.4 Monitoring of Ambient Lead

3.4.1 Ambient Measurement Techniques

3.4.1.1 Sample Collection

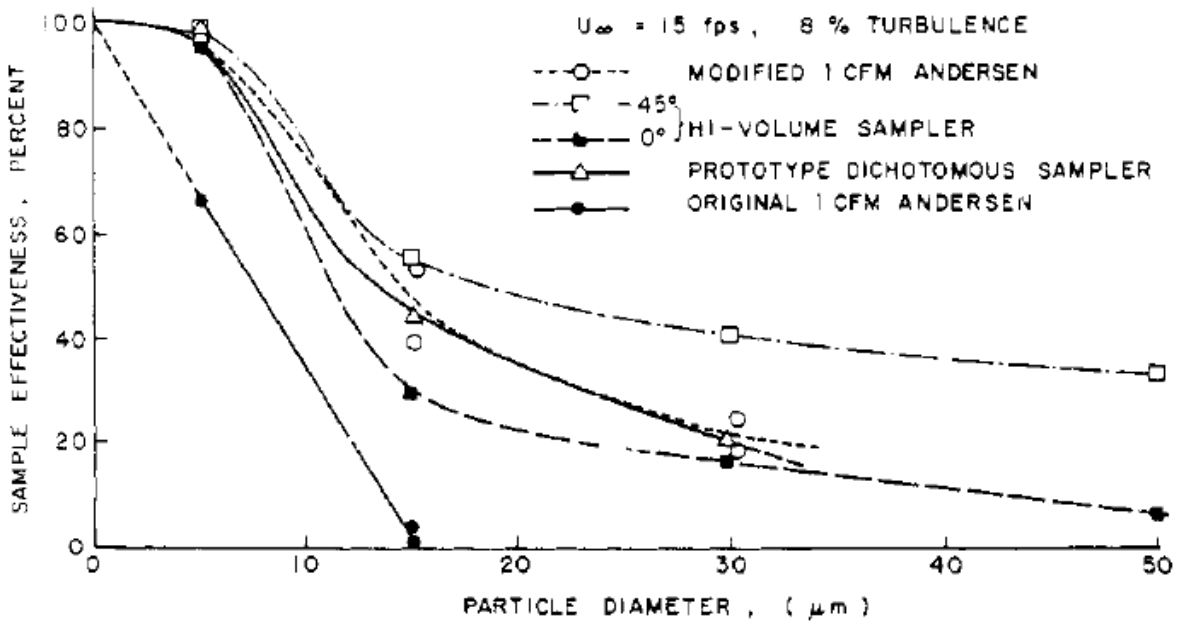
Federal Reference Methods

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

There are two FRMs for sample collection in the Pb NAAQS monitoring network (described in Section 3.4.2 below): (1) Reference Method for the Determination of Lead in Suspended Particulate Matter Collected From Ambient Air (40 CFR part 50 Appendix G), and (2) Reference Method for the Determination of Lead in Particulate Matter as PM_{10} Collected From Ambient Air (40 CFR part 50, Appendix G). The Pb-TSP FRM sample collection method is required for all source-oriented NAAQS monitors, and the

FRM for Pb-PM_{10} is accepted for Pb NAAQS monitoring at non-source-oriented monitors in specified situations.

The Pb-TSP FRM sample collection method specifies use of a high-volume TSP sampler that meets specified design criteria (40 CFR part 50 Appendix B). Ambient airborne PM is collected on a glass fiber filter for 24 hours using a high volume air sampler. It has long been recognized that there is notable variability in high-volume TSP sample measurements associated with the effects of wind speed and wind direction on collection efficiency. This variability is predominantly associated with the capture efficiency for particles larger than 10 µm, but the sampler's size selective performance is known to be affected by wind speed and direction. For example, a directional difference of 45 degrees can result in a nearly two-fold difference in 15 µm particle collection efficiency and a nearly five-fold difference in 50 µm particle collection efficiency (Wedding et al., 1977). Effective D_{50} (size at 50% efficiency) was observed to decrease from 50 µm at a 2 km/h wind speed to 22 µm at 24 km/h (Rodes and Evans, 1985). Figure 3-11 illustrates the effect of sampler orientation on collection efficiency as a function of particle size.



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

Figure 3-11 Comparison of particle collection efficiency among different TSP sampler types (Modified Andersen Sampler, Hi-volume Sampler (for different incident wind direction (45°, 0°), Prototype Dichotomous Sampler, and Original Andersen Sampler).

Some existing commercially available sampler inlets are designed to collect particles larger than 10 µm with greater than 50% efficiency (Kenny et al., 2005), and these inlets can be tested as potential replacements for TSP sampling. Efficient collection of particles much larger than 10 µm is considerably more challenging because their greater inertia and higher settling velocities hinder their efficient intake by samplers. The sampling difficulties and the long history of research to develop adequate sampling technology for large particles have been thoroughly reviewed (Garland and Nicholson, 1991). High intake velocities and large inlet openings are necessary to minimize sampling bias for sampling ultra-coarse particles. At this time, no alternative to the FRM TSP sampler has been identified that has been adequately characterized. As such, there is a continued need to assess the feasibility of a revised TSP sampler design with improved control on collection efficiency over a wider range of particle sizes, including ultra-coarse particles (which are not captured with PM_{10} samplers).

The spatial scale for which ambient air Pb samples are representative varies depending on particle sizes present, as discussed further in Section 3.5.3. Concentrations of particles larger than 10 µm are likely to be very spatially and temporally heterogeneous, with higher concentrations in the vicinity of their emissions sources. Under typical conditions, $PM_{10-2.5}$ particles travel much shorter distances before settling out than finer particles (U.S. EPA, 2009). As a result, spatial and temporal heterogeneity is greater for $PM_{10-2.5}$ than for $PM_{2.5}$, because coarser particles have greater settling velocities (Hinds, 1999), and settling velocities are even greater for particles larger than 10 µm. Thus, spatial gradients are steepest near sources, such that measured concentrations of larger particle sizes tend to be most representative of the ambient air in areas in close proximity to the monitor, with higher concentrations likely to occur closer to the source and decreasing concentrations with increasing distance from the source. This issue has been thoroughly discussed in the previous AQCD (U.S. EPA, 2006b). It has also been acknowledged in previous AQCDs, with a lengthy discussion appearing in the 1977 AQCD (U.S. EPA, 1986b, 1977).

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

Other Sample Collection Methods

In addition to the FRMs for ambient Pb sample collection, a range of other PM sampling methods are available for collecting samples for Pb analysis. These include FRM sampling methods for PM that have also been used for collection of samples for Pb analysis, sampling methods in use in other sampling networks such as the CSN, IMPROVE and National Air Toxics Trends Stations (NATTS) networks described in Section 3.4.2, and other sampling methods that have been used to measure airborne Pb concentrations in research studies unrelated to network applications. Some of the more commonly used methods are listed in Table 3-3. Most of these methods have been described in considerable detail in the 2004 PM AQCD (U.S. EPA, 2004). Table 3-3 also lists key conditions of capture for each method, including particle size, inlet type, collection medium, and flow rate.

Table 3-3 Airborne Pb sampling methods

Sampler	Network	Sampler Type	Mass Median Aerodynamic Diameter	Inlet Type	Collection Medium	Typical Flow Rate	Reference
High Volume TSP	Pb-FRM	Single Channel	TSP	None	Glass	1.13 m^3/min	U.S. EPA (2011e)
Low Volume PM_{10}	PM-FRM, NATTS	Single Channel	< 10 μm	Louvered Inlet + PM_{10} Impactor	Teflon	16.67 L/min	U.S. EPA (2011e)
$PM_{2.5}$	PM-FRM	Single Channel	< 2.5 μm	WINS Impactor	Teflon	16.67 L/min	U.S. EPA (2011e)
Met One SASS	CSN	Multiple Channel	< 2.5 μm	Cyclone	Teflon	6.7 L/min	MetOne (2009)
IMPROVE	IMPROVE	Multiple Channel	< 2.5 μm	Cyclone	Teflon	22.8 L/min	IMPROVE (2001)
MOUDI	None	Multistage Impactor	8 stages 0.056-18 μm	Impactor	Teflon	30 L/min	(Marple et al., 1991)
Noll Impactor	None	Multistage Impactor	4 stages < 108 μm	Impactor	Coated Mylar	Rotating arm	(Noll, 1970)
SEAS	None	Slurry	< 1.2 μm	Impactor	Slurry	90 L/min	(Pancras et al., 2006)

Size discrimination is usually accomplished with impactors or cyclones. With impactors, PM is forced through a jet at high speed, and particle inertia carries particles above a given size into a collection surface downstream of the jet, while smaller particles follow the air stream around the collector. In multistage impactors, a series of successive stages of jets are used to collect a range of particle sizes. The micoro-orifice uniform deposit impactor (MOUDI) is a widely used multistage impactor. The impaction process and performance of various impactors, including the WINS and MOUDI, has been described in detail in the 2004 PM AQCD (U.S. EPA, 2004). The biggest concern in collection by

impaction is particle bounce, which occurs when particles collide with the collection surface but bounce off the collection stage into the air stream and are not actually collected. Considerable effort has been devoted to minimizing errors due to bounce in FRM samplers, and this has been thoroughly discussed in the 2004 PM AQCD (U.S. EPA, 2004). An alternative to impaction that also eliminates particle bounce is the use of an air sampling cyclone. In the CSN and IMPROVE networks, cyclones are used to remove particles larger than 2.5 μm. An air sampling cyclone brings air into a tangential jet and directs flow against a circular wall, where particles larger than a given size are removed by centrifugal and gravitational forces.

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

3.4.1.2 Sample Analysis: Federal Reference and Federal Equivalence Methods

As described in Section 3.4.1.1., measurements for determinations of NAAQS compliance must be made with FRMs or FEMs. As of October 12, 2011, 1 manual reference method and 25 manual equivalent methods for sample analysis had been approved for Pb (http://www.epa.gov/ttn/amtic/files/ambient/criteria/reference-equivalent-methods-list.pdf). The FRM for Pb (Pb-TSP) was promulgated in 1979 and is based on flame atomic absorption spectroscopy (AAS) (40 CFR Part 50, Appendix G). Ambient air suspended in PM is collected on a glass fiber filter for 24 hours using a high volume air sampler. Pb in PM is then solubilized by extraction with nitric acid (HNO_3), facilitated by heat, or by a mixture of HNO_3 and hydrochloric acid (HCl) facilitated by ultrasonication. The Pb content of the sample is analyzed by atomic absorption

spectrometry using an air-acetylene flame, using the 283.3 or 217.0 nm Pb absorption line, and the optimum instrumental conditions recommended by the manufacturer. Several FEMs have been approved based on a variety of principles of operation have been approved, including inductively coupled plasma optical emission spectrometry, and inductively-coupled plasma mass spectrometry (ICPMS).

Atomic Absorption Spectrometry

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

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

Inductively-Coupled Plasma Mass Spectrometry

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

a suite of elements simultaneously. An additional advantage is low detection limits of 50-100 parts/trillion for Pb.

Inductively-Coupled Atomic Emission Spectroscopy

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

Energy Dispersive X-ray Fluorescence

In energy dispersive X-ray fluorescence spectrometry a beam of X-ray photons from an external excitation source is applied to a sample, causing ejection of inner shell electrons from elements in the sample. Because inner shell electrons have higher electron binding energies than outer shell electrons, the ejection of the inner shell electron induces an energetically favorable electronic transition of an outer shell electron to replace the ejected electron. The energy released as a result of this transition is in the form of electromagnetic radiation, corresponding to the difference in electronic binding energies before and after the transition. The energy released is typically in the X-ray portion of the electromagnetic spectrum. The release of electromagnetic radiation as a result of an electronic transition is defined as fluorescence. Fluorescence energies associated with electronic transitions depend on atomic structure, and vary between elements. As a result, X-ray fluorescence energy is uniquely characteristic of an element, and X-ray intensity at a given energy provides a quantitative measurement of elemental concentration in the sample. The X-rays are detected by passing them through a semiconductor material, resulting in an electrical current that depends on the energy of the X-ray.

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

XPS

X-ray photoelectron spectroscopy (XPS), also called electron spectroscopy for chemical analysis (ESCA) has been used to determine Pb concentrations on materials surfaces, including atmospheric PM (Finlayson-Pitts and Pitts, 2000). A fixed frequency X-ray beam causes inner shell electrons to be emitted and kinetic energy of ejected electrons is measured. Binding energy characteristic of an element can be calculated from the measured kinetic energy, allowing identification of the element. XPS can also provide information about an element's chemical environment or oxidation states because of chemical shifts in binding energy caused by differences in chemical environment. There have been some recent applications of XPS to airborne PM, concluding that Pb was mostly in the form of Pb sulfate (Qi et al., 2006). XPS analysis is a surface technique that is suitable only to a depth of 20-50Å.

Other Total Lead Methods

Anodic stripping voltammetry, atomic emission spectroscopy, and colorimetry have also been used for measurement of atmospheric Pb (Finlayson-Pitts and Pitts, 2000). In anodic stripping voltammetry, metal ions are reduced to metallic form and concentrated as an amalgam on a suitable electrode (e.g. a mercury 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.

3.4.1.4 Sequential Extraction

Sequential extraction has been widely used to further classify Pb for various purposes, including bioavailability, mobility, and chemical speciation. In general the more easily extractable Pb is considered more mobile in soil and is more bioavailable to organisms. This approach has also been used widely in characterization of airborne PM. In its original application (Tessier et al., 1979) metals extraction solvents were selected to correspond to common species present in soil, and metals were classified as exchangeable, bound to carbonates, bound to iron 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 and Lum, 1983).

Tessier's scheme was modified and optimized for airborne PM over time (Fernandez Espinosa et al., 2002) and additional extraction schemes were also developed (Chester et al., 1989), including the simplest case of two fractions corresponding to soluble and insoluble fractions (Falta et al., 2008; Canepari et al., 2006; Voutsa and Samara, 2002). The variety of methods in current use was recently thoroughly reviewed by Smichowski et al. (2005). With the recognition that biological processes involving deposited PM metals were related to their solubility (U.S. EPA, 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 and Iwatsuki, 2002; Wang et al., 2002b). Compared to other elements, a large fraction of total Pb is soluble (Graney et al., 2004). Recent advances in this area have included application to size fractionated PM (Dos Santos et al., 2009; Birmili et al., 2006), time resolved measurements (Perrino et al., 2010), and an extensive comparison of different fractionation schemes (Canepari et al., 2010). Sequential extraction with two or more fractions is becoming more widely used for characterization of Pb-PM in a variety of sources (Canepari et al., 2008; Smichowski et al., 2008; Poykio et al., 2007; Sillanpaa et al., 2005) and locations (Perrino et al., 2010; Dos Santos et al., 2009; Cizmecioglu and Muezzinoglu, 2008; Dahl et al., 2008; Sato et al., 2008; Annibaldi et al., 2007; Richter et al., 2007; Al-Masri et al., 2006; Canepari et al., 2006; Fujiwara et al., 2006; Wang et al., 2006c; Yadav and Rajamani, 2006;

Gutierrez-Castillo et al., 2005; Heal et al., 2005), leading to a better understanding of mobility characteristics of Pb in airborne PM.

3.4.1.5 Speciation Techniques

XAFS

There have been few attempts to speciate Pb in atmospheric PM. However, recently X-ray absorption fine structure (XAFS) has been applied to PM and road dust to obtain Pb speciation data from direct analysis of particle surfaces. In XAFS the absolute position of the absorption edge can be used to determine the oxidation state of the absorbing atom, and scattering events that dominate in the near edge region provide data on vacant orbital energies, electronic configurations, and site symmetry of the absorbing atom that can be used to determine the geometry of the atoms surrounding the absorbing atom. XAFS can be divided into two spectral regions. X-ray absorption near edge structure (XANES) is the region of the x-ray absorption spectrum up to 50 eV above the absorption edge observed when an inner shell electron is electronically excited into unoccupied states, and Extended X-ray Absorption Fine Structure (EXAFS) up to 1 keV above the absorption edge. Both have been applied recently to Pb in PM. XANES spectra of Pb coordination complexes with a wide range of environmentally relevant ligands have been reported (Swarbrick et al., 2009). XANES has been used to show that several different Pb species are probably present in urban airborne PM (Funasaka et al., 2008) and urban road dust (Barrett et al., 2010). XANES has been used to differentiate between Pb chromate, Pb-sorbed minerals, Pb chloride, Pb oxide, Pb carbonate, Pb sulfide and Pb sulfate 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 (Guo et al., 2006b). EXAFS has been applied to emission sources to show Pb from a sinter plant was mainly carbonate (Sammut et al., 2010). XAFS has only been applied to airborne PM very recently and shows promise for chemical speciation of airborne metals, including Pb.

GC- 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 system capable of analyzing a wide range of elements along with Pb, and these approaches have also been recently reviewed (Hirner, 2006). Chromatographic systems in common use are gas chromatography 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 and Heumann, 1997), which could be relevant for resuspended soil and road dust. GC-ICPMS has been more widely used for separation and analysis of methyl and ethyl Pb species in atmospheric PM (Poperechna and Heumann, 2005; Jitaru et al., 2004; Leal-Granadillo et al., 2000).

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 and Hu, 2010). Isotope ratio analysis was first applied to airborne PM in 1965 to identify the impact of motor vehicle exhaust on marine and terrestrial Pb deposition in the Los Angeles area (Chow and Johnstone, 1965). More recently, high resolution ICPMS has also proved to be a sensitive tool for isotope ratio analysis. High resolution ICPMS was first applied to geological samples (Walder and Freedman, 1992), and has since been widely used for determination of Pb isotope ratios in airborne PM samples. Pb isotope ratios have been measured in a number of recent studies in a variety of locations to investigate the origin of airborne Pb (Knowlton and Moran, 2010; Noble et al., 2008; Hsu et al., 2006; Widory, 2006). Shotyk and Krachler

(2010) also used Pb isotopes to demonstrate that the fate of Pb from runoff can be different from Pb with different origins. They observed that humus PM impacted by leaded on-road gasoline that are derived from soil surfaces are likely to be more easily transferred to sediments than Pb of other origins, with substantial amounts retained by lakes.

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

3.4.1.6 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 (Raabe et al., 1988; Cahill et al., 1987) collects PM samples with a cascade impactor on Mylar film substrate on a slowly rotating drum, with samples analyzed by XRF. It has been used to measure size and time resolved Pb and other elements with a time resolution of less than 6 hours using x-ray fluorescence (Cahill, 2003; Bench et al., 2002). The University of Maryland Semi-continuous Elements in Aerosol Sampler (Kidwell and Ondov, 2004, 2001) uses direct steam injection to promote condensational growth of sampled 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). Preferential loss as a function of particle size is a concern with this method, but considerable effort has been devoted to optimizing transfer from atmospheric pressure down to time of flight operating pressures with minimal particle loss (Prather et al., 1994). This technique can also be considered as an effective method for real time Pb measurement in PM, including size-resolved measurements from 0.1 to 4.0 µm (Silva and Prather, 1997). Progress has continued in the development of single particle mass spectrometry to quantify elements and organic ion fragments and a number of recent applications that included (Snyder et al., 2009; Johnson et al., 2008; Bein et al., 2007; Reinard et al., 2007; Pekney et al., 2006) or specifically targeted (Salcedo et al., 2010; Moffet et al., 2008b; Murphy et al., 2007) Pb measurements.

3.4.2 Ambient Network Design

Four national monitoring networks collect data on Pb concentrations in ambient air and report it to the Air Quality System (AQS).1 State and local agencies carry out the monitoring at state and local monitoring stations (SLAMS) using FRMs and FEMs and report data to these national networks, which have been established for various purposes. Although these data may be used for other scientific purposes, the SLAMS network is designed primarily with the goal of evaluating compliance with the Pb NAAQS. In addition to FRM monitoring, Pb is also measured within the Chemical Speciation Network (CSN), IMPROVE, and the NATTS networks as described in Section 3.4.2.2. Measurements among these networks are not directly comparable in all cases because of method differences, including the PM size range sampled (TSP, PM_{10}, or $PM_{2.5}$). Data from these various networks are presented in Section 3.5.1 to provide information on ambient Pb concentrations in different size ranges.

3.4.2.1 NAAQS Monitoring Network

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

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

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

per year.1 The emission monitoring threshold was established to ensure monitoring near Pb air sources with the greatest potential to cause ambient air concentrations to exceed the Pb NAAQS. The Pb NAAQS measurements required at these sites may be as Pb-TSP or Pb-PM_{10} (75 FR 81126).

Monitoring agencies are also required to conduct non-source-oriented Pb monitoring at each National Core multipollutant monitoring network (NCore)2 site in a Core Based Statistical Area (CBSA) with a population of 500,000 or more. While non-source-oriented monitoring data can be used for purposes of NAAQS attainment designations, the main objective for non-source-oriented monitoring is to gather information on neighborhood-scale lead concentrations that are typical in urban areas so to better understand ambient air-related Pb exposures for populations in these areas.

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

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

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

[2] 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.

Source oriented monitors near sources estimated to emit 1.0 tons/year Pb were required to be operational by January 1, 2010, and the remainder of the newly required monitors, including the non-source-oriented NCore sites, are required to be operational by December 27, 2011 (75 FR 81126). When the December 2010 Pb network requirements are fully implemented, the Pb NAAQS monitoring network is expected to consist of approximately 270 required monitors including approximately 210 source-oriented monitors and 60 non-source-oriented monitors. Figure 3-12 shows the estimated geographic distribution of Pb NAAQS monitors in the current Pb NAAQS monitoring network. This includes monitors that previously existed and are still in operation, along with those that are newly required.

With the December, 2010 regulations, EPA also required one year of Pb-TSP (FRM) monitoring near 15 airports in order to gather additional information on the likelihood of NAAQS exceedances near airports due to the combustion of leaded aviation gasoline (75 FR 81126). These airports were selected based on three criteria: annual Pb inventory between 0.5 tons/year and 1.0 tons/year, ambient air within 150 meters of the location of maximum emissions (e.g., the end of the runway or run-up location), and airport configuration and meteorological scenario that leads to a greater frequency of operations from one runway. These characteristics were selected because they are expected, collectively, to identify airports with the highest potential to have ambient Pb concentrations approaching or exceeding the Pb NAAQS. Data from this monitoring study will be used to assess the need for additional Pb monitoring at airports. These 15 sites (Figure 3-13 and Table 3-14) are required to be operational no later than December 27, 2011.

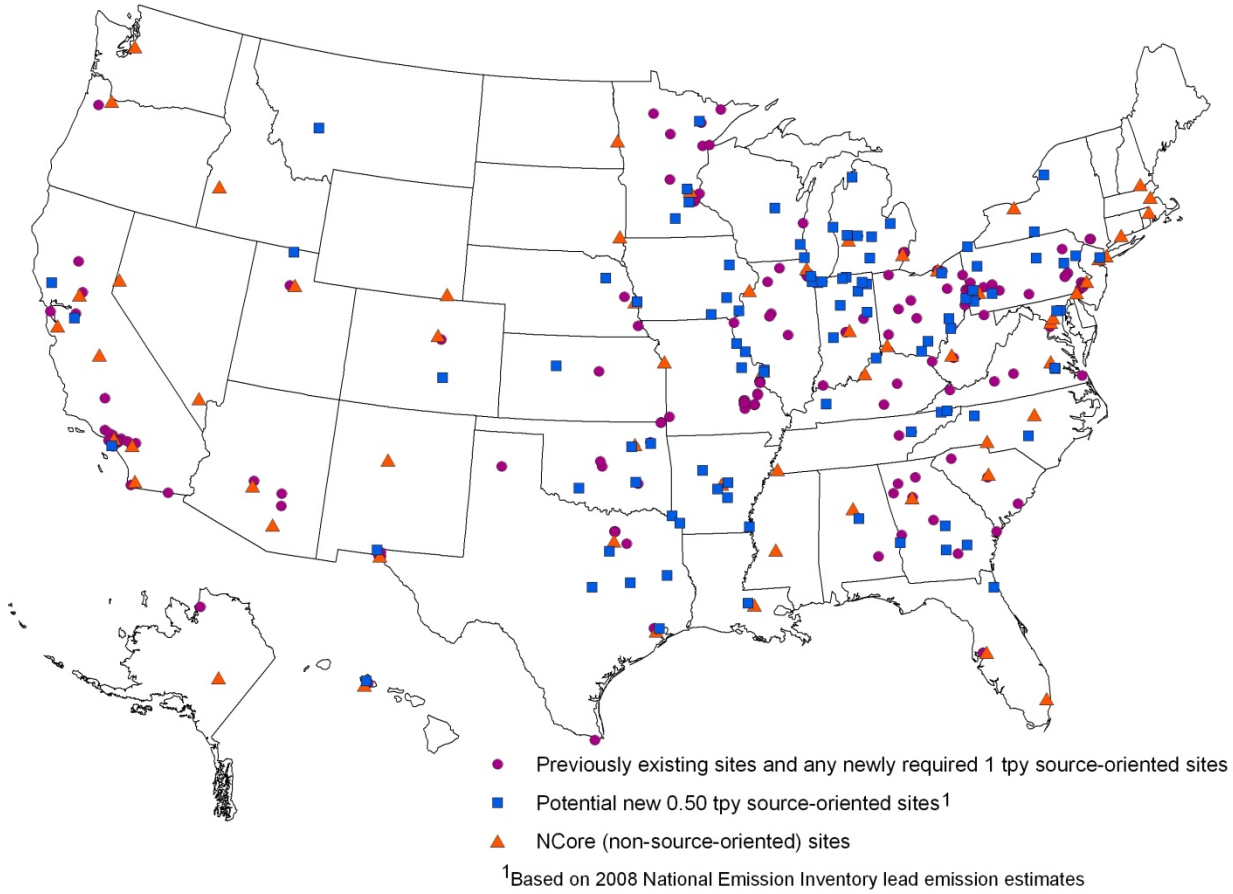
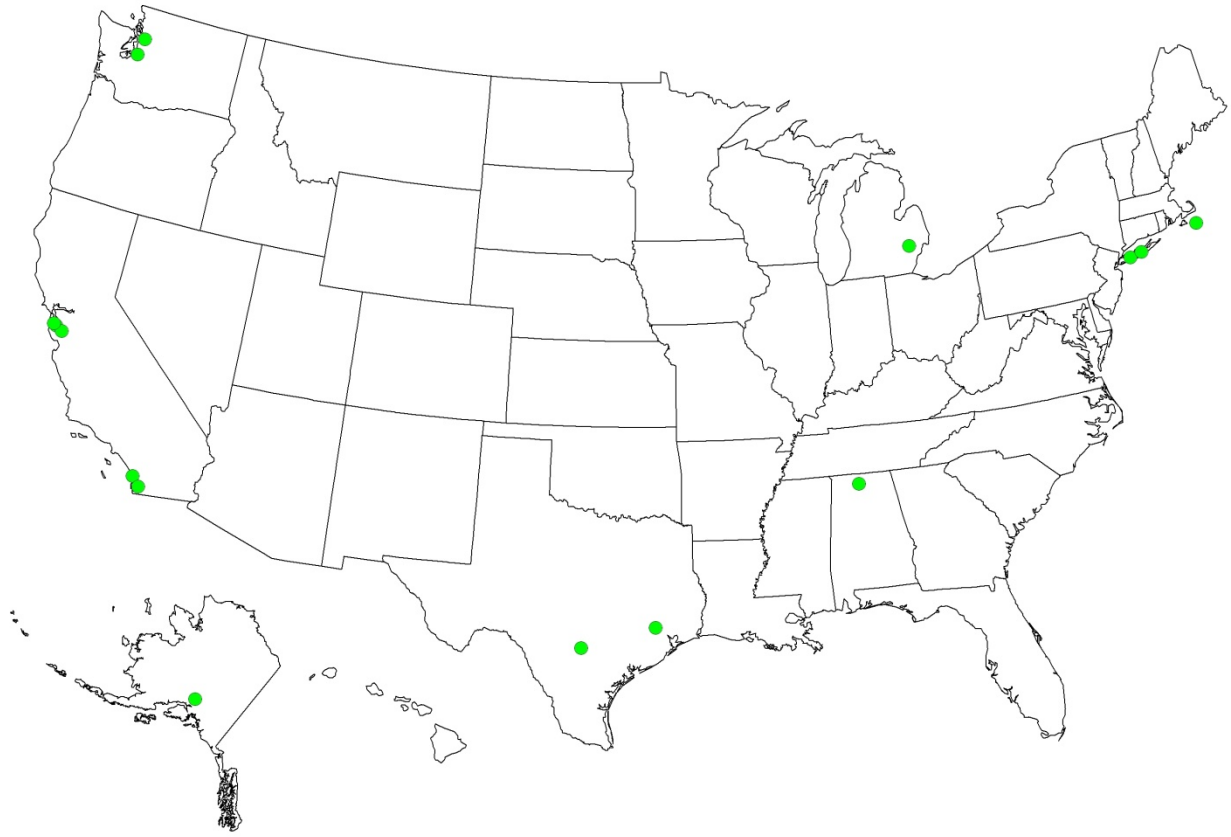


Figure 3-12 Map of Monitoring Sites in Current Pb NAAQS Monitoring Network.

[1] Estimates for source-oriented monitors are based on Pb emissions estimates in the 2008 National Emissions Inventory.



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

Figure 3-13 Fifteen U.S. locations where a study is currently being performed on airport Pb emissions.

Table 3-4 List of 15 airports included in the airport study

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

3.4.2.2 Other Lead Monitoring Networks

In addition to FRM monitoring, Pb is also measured within the Chemical Speciation Network (CSN), Interagency Monitoring of Protected Visual Environments (IMPROVE), and the National Air Toxics Trends Station (NATTS) networks. Pb in $PM_{2.5}$ is monitored as part of the CSN and IMPROVE networks, and Pb in PM_{10} as a part of the National Air Toxics Trends (NATTS) networks (Figure 3-14 and Figure 3-15). These networks are designed to meet different objectives than those of the Pb NAAQS monitoring network.

The purpose of the CSN is to monitor $PM_{2.5}$ species to assist in understanding $PM_{2.5}$ chemistry and for spatial and temporal analyses including annual, seasonal, and sub-seasonal trends (http://www.epa.gov/ttn/amtic/specgen.html). The CSN consists of about 50 long-term trends sites (commonly referred to as the Speciation Trends Network or STN sites) and about 150 supplemental sites, all operated by state and local monitoring agencies. Higher spatial and temporal resolution of the CSN facilitates increased utility in the scientific community, and the data from the CSN also assists states in formulating their emission control strategies, even if the network is not compliance-oriented. Pb is one of 33 elements in $PM_{2.5}$ collected on Teflon filters every third day and analyzed by energy dispersive XRF spectrometry.

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

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

Pb monitoring is also conducted at NCore monitoring sites. Monitoring for Pb-$PM_{2.5}$ is currently being conducted at NCore sites as part of the larger CSN (described above). As described in Section 3.4.2, monitoring for Pb-PM_{10} is required to be operational at NCore sites by December 27, 2011. Methods for Pb in $PM_{10-2.5}$ are being developed as part of the $PM_{10-2.5}$ speciation pilot project and may be implemented at some NCore sites in the future.

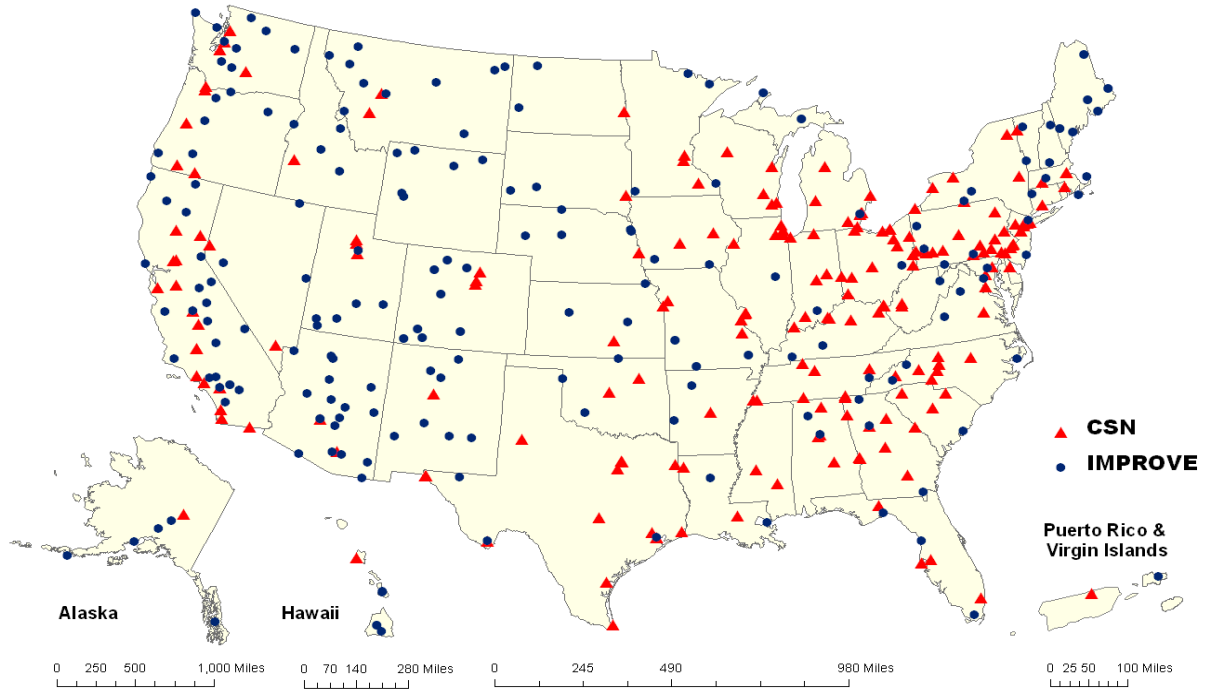


Figure 3-14 Pb-$PM_{2.5}$ monitoring sites for CSN and IMPROVE networks.

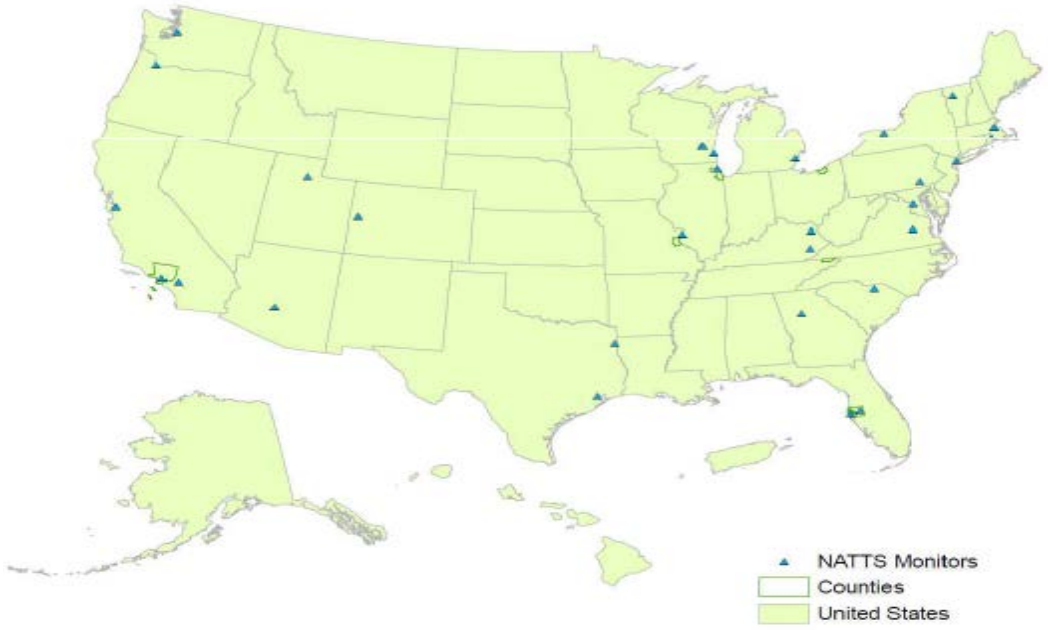


Figure 3-15 Pb-PM_{10} monitoring sites for NATTS network.

3.5 Ambient Air Lead Concentrations

The following section summarizes data on ambient air Pb concentrations during the years 2008-2010. Data used in this chapter are from the period 2008-2010. Data from SLAMS (i.e., source-oriented and non-source-oriented Pb-TSP) are presented. Data from other networks, such as the CSN, IMPROVE, and the NATTS networks, are also presented for scientific purposes to characterize ambient PM component concentrations in different size fractions and for different settings. Measurements among these networks are not directly comparable in all cases because there are method differences, including the PM size range sampled.

The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 Appendix R and, as such, cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS. For the purpose of analyses within this ISA, monitors were initially designated to be source-oriented if either (1) they were designated in AQS as source-oriented, (2) they were located within one mile of a 0.5 ton/year or greater source as noted in the 2005 NEI (U.S. EPA, 2008a), or (3) they were located within one mile of a 0.5 tons/year or greater source as noted in the 2008 NEI (U.S. EPA, 2011a). The remainder of FRM monitors reporting to the AQS were classified as non-source-oriented. Following this initial classification, staff from the EPA Regional offices tasked with acting as liaisons to the states reviewed all monitors listed to fall within their Regions and reported any discrepancies between the initial classification and ground observations of the sites made by EPA Regional or state staff. The source and non-source monitor listing was edited accordingly. The definition of source-oriented monitoring is applied flexibly with input from regions in this ISA because 2008 data were obtained before the latest monitor designation requirements were implemented. For this analysis, 120 FRM monitors were considered source-oriented, while 184 were considered to be non-source-oriented.[1,2] However, the number of source-oriented and non-source-oriented monitors differed for each analysis year because there were changes in monitor siting.

The section begins with a description of concentrations observed in Pb-TSP measurements obtained for NAAQS compliance at source-oriented and non-source-oriented monitors across the U.S. Additionally, concentrations of Pb-PM_{10} and Pb-$PM_{2.5}$

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

[2] The requirement for monitoring near sources emitting 0.5 or 1.0 tons/year may be waived if it can be shown that the source will not contribute to a maximum 3-month average Pb concentration in ambient air in excess of 50 percent of the NAAQS level based on historical monitoring data, modeling, or other means (40 CFR, part 58, Appendix D, Section 4.5(a)(ii)).

are presented. 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 the peer-reviewed literature is provided to evaluate the size distribution of Pb-bearing airborne PM under varied ambient conditions. The relationship between Pb concentration and concentrations of copollutants are presented. Finally, estimates of transcontinental transport of Pb to the U.S. and current background Pb levels are provided. Summary information is presented within this section, and detailed data are included in an Appendix (Section 3.8) 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 Pb-TSP FRM monitors from 2008-2010 and PM_{10} and $PM_{2.5}$ monitors for 2007-2009. The source and non-source-oriented Pb-TSP FRM monitors present data pertaining to compliance with the current level of the NAAQS. The Pb-PM_{10} data are obtained from the NATTS network, and the Pb-$PM_{2.5}$ data are from the CSN. Although the Pb-PM_{10} and Pb-$PM_{2.5}$ data are not from compliance networks, data are presented from these networks because the additional data presents a picture of the nationwide distribution of Pb concentration in different classes of particle size. This information is useful to develop a sense of variability in Pb concentrations at a national scale.

Concentrations of Pb Measured using Pb-TSP Monitors (Source-Oriented and Non-Source-Oriented Monitors)

Maximum 3-month average Pb concentrations[1] were calculated for source-oriented Pb-TSP monitors for 50 counties across the U.S. (1.6% of U.S. counties) during the period 2008-2010. Figure 3-16 illustrates that the level of the NAAQS was exceeded in twenty counties where source-oriented monitoring was performed. Summary statistics for the monitor-specific one-month and three-month averages for these monitors are presented below in Table 3-5, and detailed statistics for the one-month and three-month averages are provided in Table 3-13, Table 3-15, Table 3-17, Table 3-19, Table 3-21, and Table 3-23 in the Appendix (Secton 3.8). The mean was skewed toward the 75th percentile of

[1] Maximum 3-month average Pb concentrations are calculated as the maximum 3-month average of 3 consecutive monthly averages within the 2008-2010 time period.

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 one-month or three-month annual site maxima1 were in the upper 90th percentile for 2008-2010 are presented in Table 3-6. The highest monthly and three-month average concentrations occurred in Iron Co., MO, Herculaneum, MO (Jefferson Co.), and Los Angeles, CA. The highest one-month annual site max value occurred in Cook County, IL in 2008 followed by Iron County, MO in 2008. The highest three-month annual site max concentrations occurred in Herculaneum in 2008, Los Angeles in 2008, and Iron County, MO in 2008. The majority of monitors reported data that did not exceed the NAAQS during this three-year period, but the generally higher values at a subset of the source-oriented monitoring locations tended to skew the nationwide distribution of Pb concentrations upwards.

[1] The one-month annual site max is defined as the highest one-month average for a monitoring site over a given year, and the three-month annual site max is defined as the highest three-month average for a monitoring site over a given year.

Table 3-5 Summary data for source-oriented Pb monitors across the U.S., 2008-2010

	Mean, µg/m3	Median, µg/m3	95th%, µg/m3	99th%, µg/m3	Max, µg/m3
Monthly	0.20	0.063	0.86	1.6	4.4
3-mo rolling avg	0.21	0.079	0.88	1.6	2.9

Table 3-6 Summary data for sites at which source-oriented statistics for one-month and three-month annual site max are in the upper 90th percentile, 2008-2010

County	AQS	Highest 1-mo Mean, µg/m^3	Highest 3-mo Mean[a], µg/m^3	Highest 1-mo Annual Site Max, µg/m^3 (Year)	Highest 3-mo Annual Site Max, µg/m^3 (Year)
Pike, AL	011090003	1.3	1.2		1.2 (2008)
Los Angeles, CA	060371405	2.9	2.5	2.9 (2008)	2.5 (2008)
Hillsborough, FL	120571066				1.8 (2008)
Cook, IL	180350009			4.4 (2008)	2.2 (2008)
Iron, MO	290930016	4.2	2.5	4.2 (2008)	2.5 (2008), 2.1 (2009)
Iron, MO	290930021	2.6	1.9	2.6 (2008), 2.4 (2009)	1.9 (2009)
Jefferson, MO	290990004	2.4	2.0	2.4 (2008), 1.6 (2009), 1.6 (2010)	2.0 (2008)
Jefferson, MO	290990011			1.5 (2008)	
Jefferson, MO	290990015	3.1	2.9	3.1 (2008)	2.9 (2008)
Jefferson, MO	290990020	2.2	0.99	2.2 (2008)	
Jefferson, MO	290990021	1.6	1.1	1.6 (2009), 1.6 (2010)	
Jefferson, MO	290990022	0.86			
Jefferson, MO	290999001	1.6	1.2	1.6 (2009)	1.2 (2009)

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.

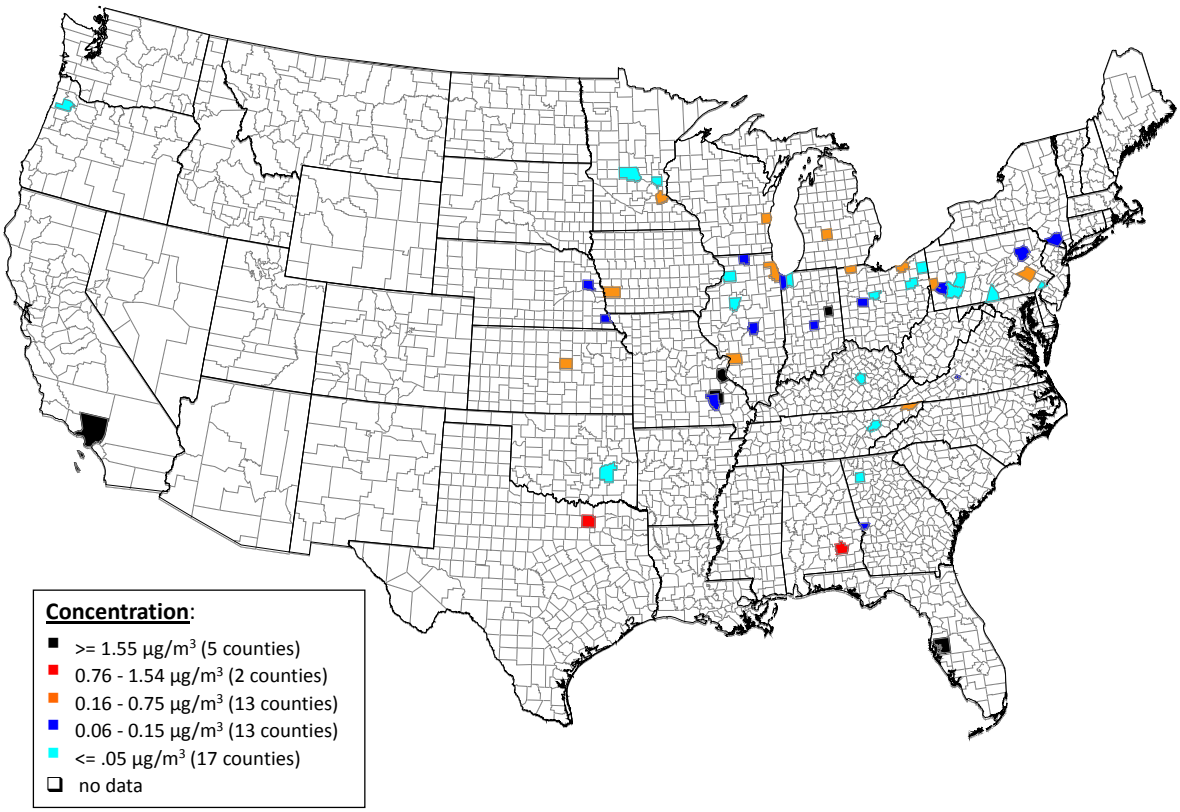


Figure 3-16 Highest county-level source-oriented Pb-TSP concentrations (µg/m^3), maximum 3-month average, 2008-2010.

Maximum 3-month average Pb concentrations were calculated for non-source-oriented Pb-TSP monitors for 47 counties across the U.S. (1.5% of U.S. counties) during the period 2008-2010. Figure 3-17 illustrates that the level of the NAAQS was never exceeded at non-source-oriented monitors. Summary statistics are presented below in Table 3-7, and detailed statistics for the one-month and three-month average and maxima non-source-oriented Pb-TSP concentrations are provided in Table 3-14, Table 3-16, Table 3-18, Table 3-20, Table 3-22, and

Table 3-24 in the Appendix (Section 3.8). The mean was slightly higher than the median 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 maxima of the distributions. Data for sites at which national maxima were reached for 2008-2010 are presented in Table 3-8. This table shows that all non-source-oriented monitor results were below the NAAQS. The highest monthly and three-month average concentrations

occurred at the same site in Cambria County, PA. The highest annual site max 1-month value occurred in Columbiana County, OH in 2010, followed by Cambria County, PA in 2009. The Columbiana and Cambria sites were also above the 90th percentile annual 3-month site max Pb concentrations.

Table 3-7 Summary data for non-source-oriented Pb monitors across the U.S., 2008-2010

	Mean, µg/m3	Median, µg/m3	95th%, µg/m3	99th%, µg/m3	Max, µg/m3
Monthly	0.012	0.010	0.040	0.052	0.14
3-mo rolling avg	0.012	0.010	0.037	0.048	0.073

Table 3-8 Summary data for sites at which non-source-oriented statistics for one-month and three-month annual site max are in the upper 90th percentile, 2008-2010

County	AQS	Highest Monthly Mean, µg/m^3	Highest 3-mo Mean[a], µg/m^3	Highest Monthly Annual Site Max, µg/m^3 (Year)	Highest 3-mo Annual Site Max, µg/m^3 (Year)
Cook, IL	170310022	0.070	0.051	0.070 (2009), 0.062 (2010)	0.048 (2008), 0.047 (2009), 0.051 (2010)
Cook, IL	170310026	0.052	0.046		0.046 (2008)
Cook, IL	170316003	0.040	0.036		
Madison, IL	171193007			0.066 (2008)	
Saint Francois, MO	291870006	0.089	0.054	0.089 (2010)	0.054 (2010)
Saint Francois, MO	291870007	0.054	0.041		
Saint Louis, MO	291892003		0.055	0.066 (2008)	0.055 (2008)
Columbiana, OH	390290019			0.14 (2010)	0.057 (2010)
Columbiana, OH	390290022			0.065 (2010)	0.044 (2010)
Cambria, PA	420210808	0.13	0.073	0.058 (2008), 0.13 (2009)	0.049 (2008), 0.073 (2009)
Delaware, PA	420450002	0.048	0.047		0.047 (2010)
Westmoreland, PA	421290007	0.053	0.048		0.048 (2008)
El Paso, TX	481410002	0.087		0.087 (2010)	
El Paso, TX	481410033			0.057 (2009)	

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.

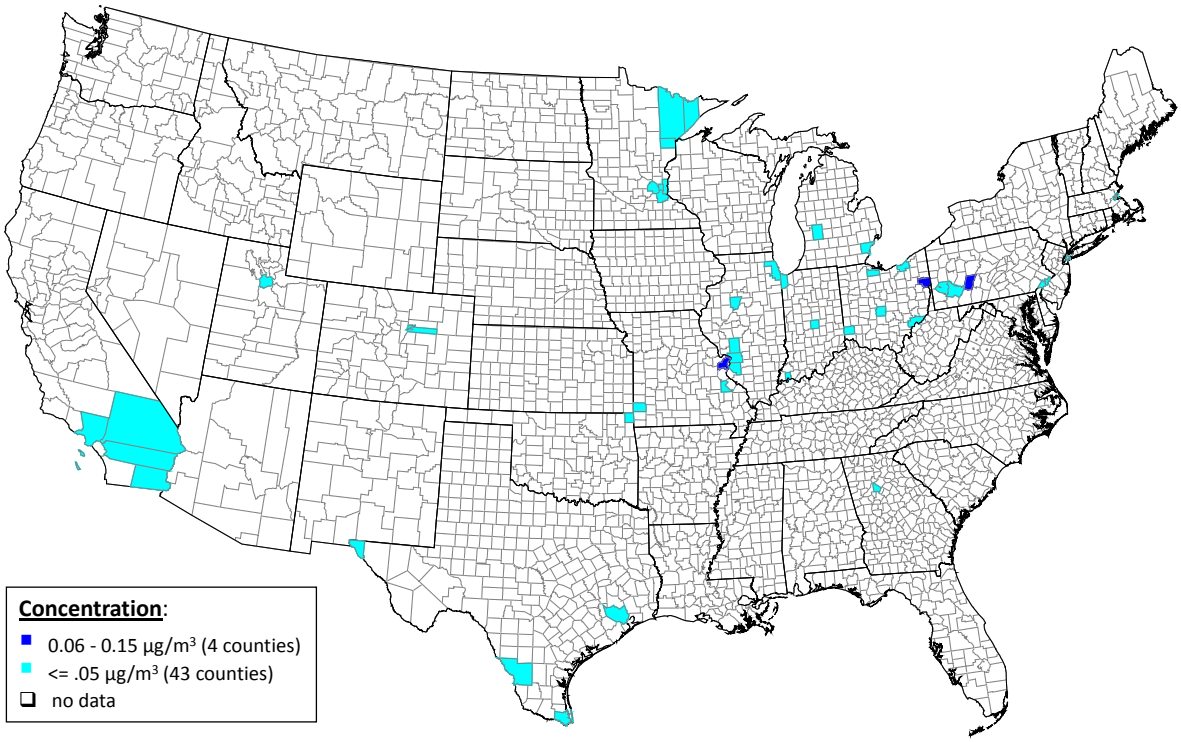


Figure 3-17 Highest county-level non-source-oriented Pb-TSP concentrations (µg/m^3), maximum 3-month average, 2008-2010.

Concentrations of Pb Measured using PM_{10} Monitors (for HAP Concentrations and Trends)

Figure 3-18 displays maximum 3-month averages for Pb-PM_{10} concentrations for 36 counties in which measurements were obtained. Among the 36 counties in which PM_{10} monitoring was conducted, only one county, Gila County, AZ, reported concentrations above 0.076 µg/m^3. Three other counties reported concentrations greater than 0.016 µg/m^3: Wayne County, MI, Boyd County, KY, and the county of St. Louis City, MO.

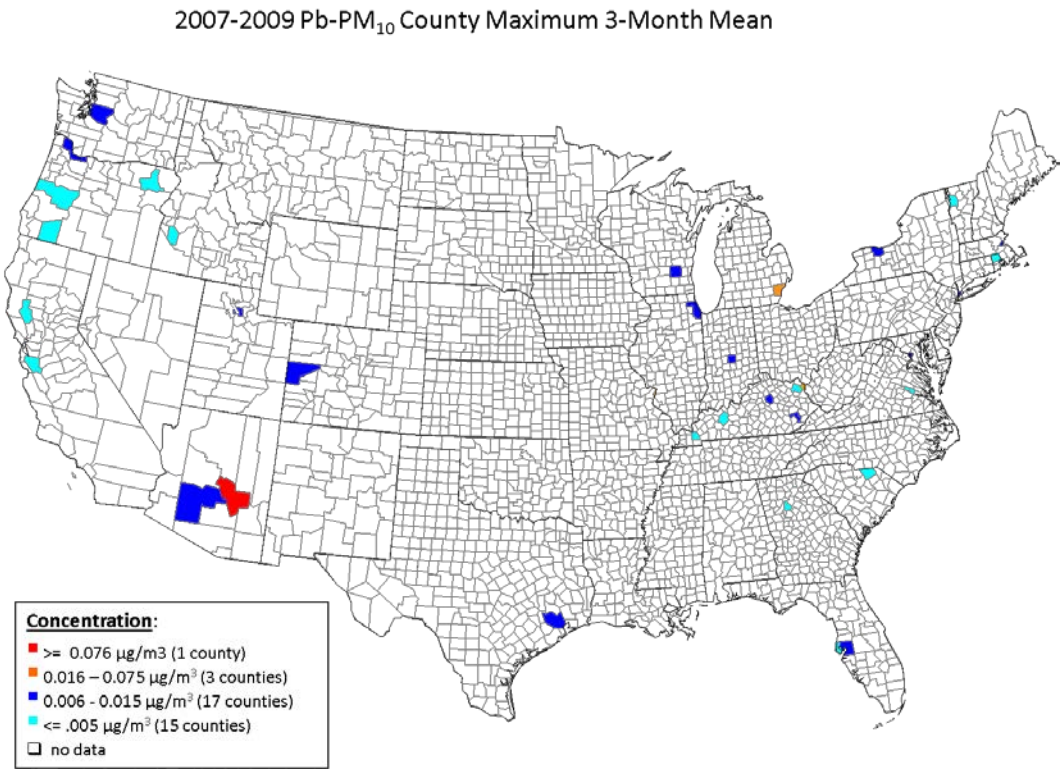


Figure 3-18 Highest county-level Pb-PM_{10} concentrations (µg/m^3), maximum 3-month average, 2007-2009.

Concentrations of Pb Measured using $PM_{2.5}$ Monitors (for Speciation Concentrations and Trends)

Figure 3-19 displays maximum 3-month average county-level data for Pb in $PM_{2.5}$ concentrations for 323 counties in which $PM_{2.5}$ measurements were obtained for speciation in the CSN and IMPROVE networks. The data presented here are not compared to the NAAQS because $PM_{2.5}$ monitors are not deployed for the purpose of evaluating compliance for the NAAQS. Among the 323 counties in which $PM_{2.5}$ monitoring was conducted, only eleven counties reported concentrations greater than 0.016 µg/m^3: Jefferson, AL, San Bernardino, CA, Imperial, CA, Wayne, MI, Jefferson, MO, Erie, NY, Lorain, OH, Allegheny, PA, Berks, PA, Davidson, TN, and El Paso, TX.

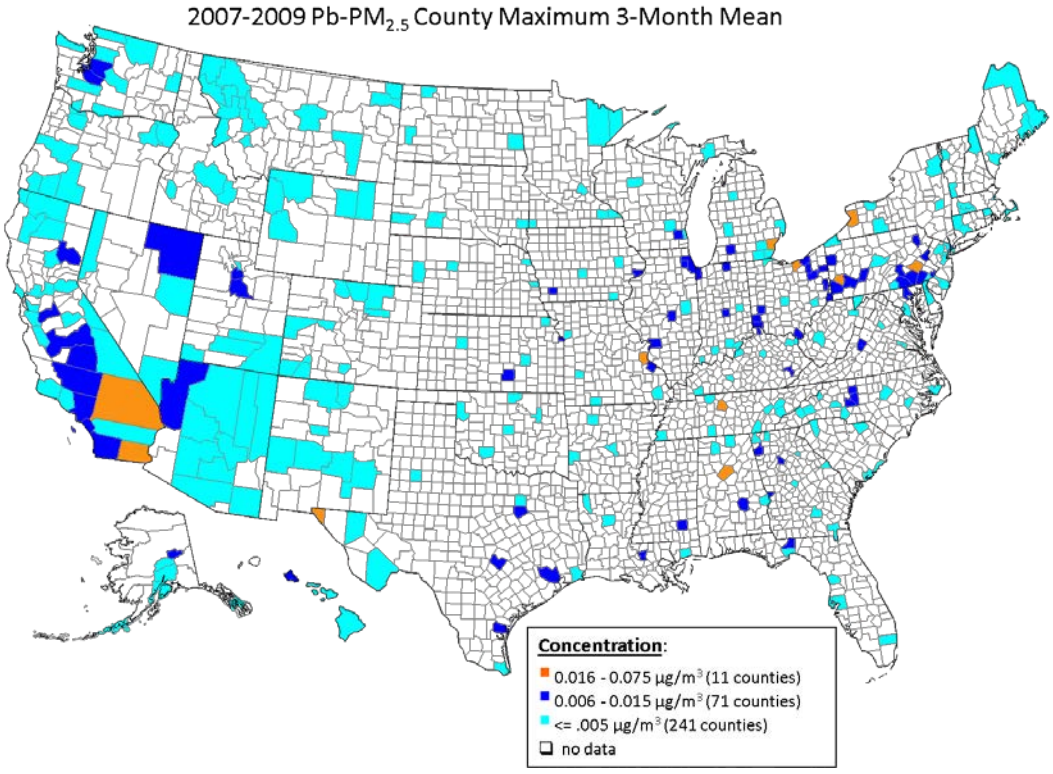


Figure 3-19 Highest county-level Pb-$PM_{2.5}$ concentrations (µg/m^3), maximum 3-month average, 2007-2009.

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 in urban areas, spatial variability of Pb concentrations in urban areas may also be high. Moreover, Pb-PM tends to settle quickly over short distances after becoming airborne because Pb has relatively high density; short settling distances also contribute to high spatio-temporal variability in ambient air Pb concentrations. Such variability may not be detected if one or a small number of central site monitors is in use, so cities with multiple monitors are used to characterize intra-urban variability. Intra-urban variability in Pb concentrations reported to AQS was described in detail in the Appendix in Section 3.8.1 Los Angeles County, CA (Los Angeles), Hillsborough and Pinellas Counties, FL (Tampa), Cook County, IL (Chicago), Jefferson County, MO (Herculaneum), Cuyahoga County, OH (Cleveland), and Sullivan County, TN (Bristol) were selected for this assessment to illustrate the variability in Pb concentrations measured across different metropolitan regions with varying Pb source characteristics. Four of the counties encompass large cities (Los Angeles, Tampa, Chicago, and

Cleveland). All six counties contain source-oriented monitors. Maps and wind roses 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, including at sites near roads. 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.

Airborne Pb near Roads

Five monitors were selected from the TSP network to examine Pb concentrations in the near road environment. The monitors were located in Los Angeles County, CA (06-037-4002), Riverside County, CA (06-065-1003), Cook County, IL (17-031-0052, 17-031-6003), and Suffolk County, MA (25-025-0002). These monitors were selected because they are located in the vicinity of major roadways in urban areas with different characteristics and because they each have long-term data are available. Further, based on reviews of emissions inventory information as well as satellite image searches, these sites are not known to be near metals-related industrial sites. The monitoring sites are described in Table 3-9, and time series of Pb-TSP monthly concentration for all five monitors are shown in Figure 3-20. The annual average over the two sites that were reporting data in 1980, the first year presented in Figure 3-20, was 0.90 $\mu g/m^3$. This Pb-TSP concentration from 1980 likely reflected the influence of Pb emissions from leaded automobile gasoline (Figure 3-6 for annual national consumption of leaded motor vehicle gasoline). By 1986, when all five monitors were reporting data, the annual average of Pb-TSP concentration over all five monitors dropped to 0.18 $\mu g/m^3$. Over 2001-2010, the annual average Pb-TSP concentration over all sites was 0.02 $\mu g/m^3$ with a standard deviation of 0.01 $\mu g/m^3$. The highest 2008-2010 design value was 0.04 $\mu g/m^3$, which occurred at the Chicago site (17-031-6003) located less than 10 m to Interstate-290 at a monitor height of 2 AGL. The multi-site average was not substantially larger than the

maximum three-month rolling average of 0.012 μg/m^3 for non-source-oriented monitors for the period 2008-2010, and the Pb-TSP concentration varied little over the period 2001-2010. Note that the monitor heights were 2-6 m AGL, which may be higher than the vertical distance likely traveled by some particles (depending on particle size) following initial resuspension (see Section 3.3.1.3).

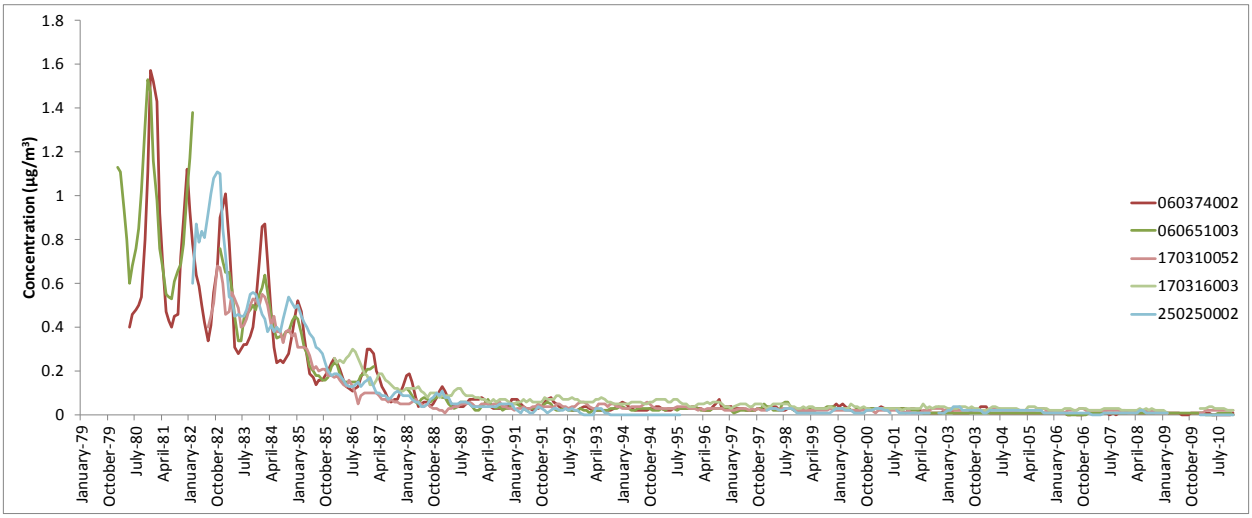
Table 3-9 Sample of U.S. near road Pb TSP monitors

County, State	Site ID	Latitude	Longitude	2008-2010 Design Value (μg/m^3)[a,b]	Monitor Height (m AGL)	Distance from Roads	Surrounding Area
Los Angeles, CA	06-037-4002	33.82376	-118.18921	0.01	6	500 m to I-405 (San Diego Freeway), 10 m to Long Beach Blvd	High intensity residential, urban
Riverside, CA	06-065-1003	33.94603	-117.40063	0.01	4	Within 20 m of intersection of Magnolia and Arlington Ave.	High intensity residential, mixed use urban
Cook, IL	17-031-0052	41.96548	-87.749928	0.02[c]	5	Near to intersection of I-90 and I-94, 80 m to I-90, 200 m to I-94, 70 m to railroad	Located at public utilities water pumping station, high density residential urban
Cook, IL	17-031-6003	41.872202	-87.826165	0.04[c]	2	Less than 10 m to I-290 (Dwight D. Eisenhower Expressway)	Parking lot of Circuit Court of Cook County, ¾ surrounded by Concordia Cemetary
Suffolk, MA	25-025-0002	42.348873	-71.097163	0.02[c]	5	95 m to I-90, inside median of Commonwealth Ave.	High intensity urban, mixed use residential and commercial

[a]The level of the 2008 NAAQS for lead is 0.15 micrograms per cubic meter (μg/m^3) not to be exceeded in any 3-month period. The design value for the 2008 Pb NAAQS is the maximum rolling 3-month Pb-TSP average within the 3-year design period.

[b]The design values shown here are computed for the latest design value period using Federal Reference Method or equivalent data reported by States, Tribes, and location agencies to EPA's Air Quality System (AQS) as of 7/12/2011. Concentrations flagged by States, Tribes, and local agencies as exceptional events (e.g., high winds, wildfires, volcanic eruptions, construction) and concurred by the associated EPA Regional Office are not included in the calculation of these design values. Although the indicator for the 2008 Pb NAAQS is Pb-TSP at "local conditions" (i.e., actual temperature and pressure; parameter 14129), 2008 Pb-TSP data reported in "standard temperature and pressure" (i.e., 25 ° C, 760 mmHg; parameter 12128) are also considered valid for NAAQS comparisons and related attainment/nonattainment determinations if the sampling and analysis methods that were utilized to collect that data were consistent with previous or newly designated FRMs or FEMs and quality assurance requirements were met.

[c]Fewer than 36 rolling 3-month lead-TSP average data are available at this site for this 3-year period; the value shown here is the highest valid 3-month mean.



Note: Monitor IDs: Los Angeles, CA: 06-037-4002; Riverside, CA: 06-065-1003; Cook, IL: 17-031-0052, 17-031-6003; Suffolk, MA: 25-025-0002.

Figure 3-20 Time series of monthly average Pb-TSP concentration at five near-road monitors.

Airborne Pb near Airports

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. Data presented in the Canadian report yielded median air Pb-PM_{10} level of 0.01 for ten sample days (with 37 24-hour and 3 11-hour samples) compared with an average reported background level of 0.007 µg/m^3 (Conor Pacific Environmental Technologies Inc, 2000). Median and average values were derived from all samples collected at the airport, both upwind and downwind from aircraft activity. The maximum 24-hour concentration measured in this 10-day study was 0.13 µg/m^3. The Illinois report noted that air Pb concentrations were elevated downwind of O'Hare airport compared with upwind levels (Illinois Environmental Protection Agency, 2002).

More recently, Carr et al. (2011) performed TSP monitoring and dispersion modeling of Pb emissions at the Santa Monica Airport and surrounding neighborhood in Santa Monica, CA. Ambient sampling was conducted in March and July, 2009. During winter, measurements of 24-hour Pb-TSP concentrations upwind of the airport ranged from 0.003 to 0.011 µg/m^3 and at the two downwind locations, concentrations ranged from 0.025 to 0.083 µg/m^3 approximately 70m from the location where piston engine aircraft emit the majority of Pb during ground-based operations and 0.028 to 0.050 µg/m^3 in an adjacent neighborhood roughly 85m further downwind. During summer, 24-hour Pb-TSP concentrations upwind from aircraft operations ranged from 0.001 to 0.006 µg/m^3. Three

locations downwind were monitored, the first of which was the same location as the winter site 70m from the location where piston engine aircraft emit the majority of Pb during ground-based operations. This location had concentrations ranging from 0.034 to 0.062 µg/m^3. At a site 100 m further downwind from this location, concentrations ranging from 0.027 to 0.044 µg/m^3 and at a distance of 175 m downwind, a 24-hour concentration of 0.023 µg/m^3 was measured. Modeling results suggest that three-month average Pb concentrations above local background extended beyond the airport property and that the preflight runup check, taxi, and takeoff emissions were the most important contributors to Pb concentrations. This airport had a Pb emissions inventory of 0.3 tons/year and is therefore not subject to Pb monitoring to evaluate compliance with the Pb NAAQS, which is described further in Section 3.4.2.

Airborne Pb at Urban and Rural Sites

A number of studies have characterized how Pb-bearing PM is distributed over the neighborhood scale in the air. Yu et al. (2011) measured Pb-PM_{10} concentration at four rooftop sites (10-13 m AGL) within Paterson, NJ: background, near-road, industrial, and commercial. Interstate-80 and Route 19 were both within 0.8 km from the near-road site. The industrial site was located 0.1-1 km from a metal recovery plant, a plating facility that emits Ni and Zn compounds, and another facility emitting Cu compounds. The commercial site was proximal to several restaurants and dry cleaners. Average concentrations at each site were: 2.95 ng/m^3 (background), 5.61 ng/m^3 (near road), 6.48 ng/m^3 (industrial), and 6.58 ng/m^3 (commercial) to yield a coefficient of variation coefficient of variation (CV, defined as the standard deviation of site measurements divided by the average) of 31.3%. Weekday and weekend ambient Pb-PM_{10} concentrations were not significantly different ($p = 0.45$). Martuzevicius et al. (2004) examined the spatial variability of Pb-$PM_{2.5}$ samples obtained in the greater Cincinnati, OH area at 6 urban, 4 suburban, and 1 rural site and found that Pb-$PM_{2.5}$ had a CV of 33.8%, compared with a CV for $PM_{2.5}$ of 11.3% over all sites. Average Pb-$PM_{2.5}$ concentration among the sites varied from 1.79-28.4 ng/m^3. Martuzevicius et al. (2004) suggested that differences between mass and Pb spatial variability implied that Pb originated primarily from local sources. Sabin et al. (2006a) measured Pb-PM with 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. (2006a) 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. Li et al. (2009a) observed that Pb concentration in $PM_{2.5}$ samples was 2.2-3.0 times higher near a bus depot than next to a rural-suburban road; in this study, the authors provided ratios but not actual concentrations. Ondov et al. (2006) measured Pb-

$PM_{2.5}$ concentration at three Baltimore sites. Average Pb-$PM_{2.5}$ concentrations at the different sites were 8.3 ng/$m^{3,}$ 7.2 ng/$m^{3,}$ and 1.9 ng/m^3, with the two higher concentration sites located within two miles of industrial facilities. The industrial sites include a major steel plant; several chemical manufacturing plants; and incinerators for municipal waste, medical waste, and sludge. However, the authors do not specify which industrial facilities were proximal to each monitor. Although these concentrations are low, they agree with the body of literature to suggest that intra-urban variability is most strongly related to source type, strength, and location.

3.5.2 Temporal Variability

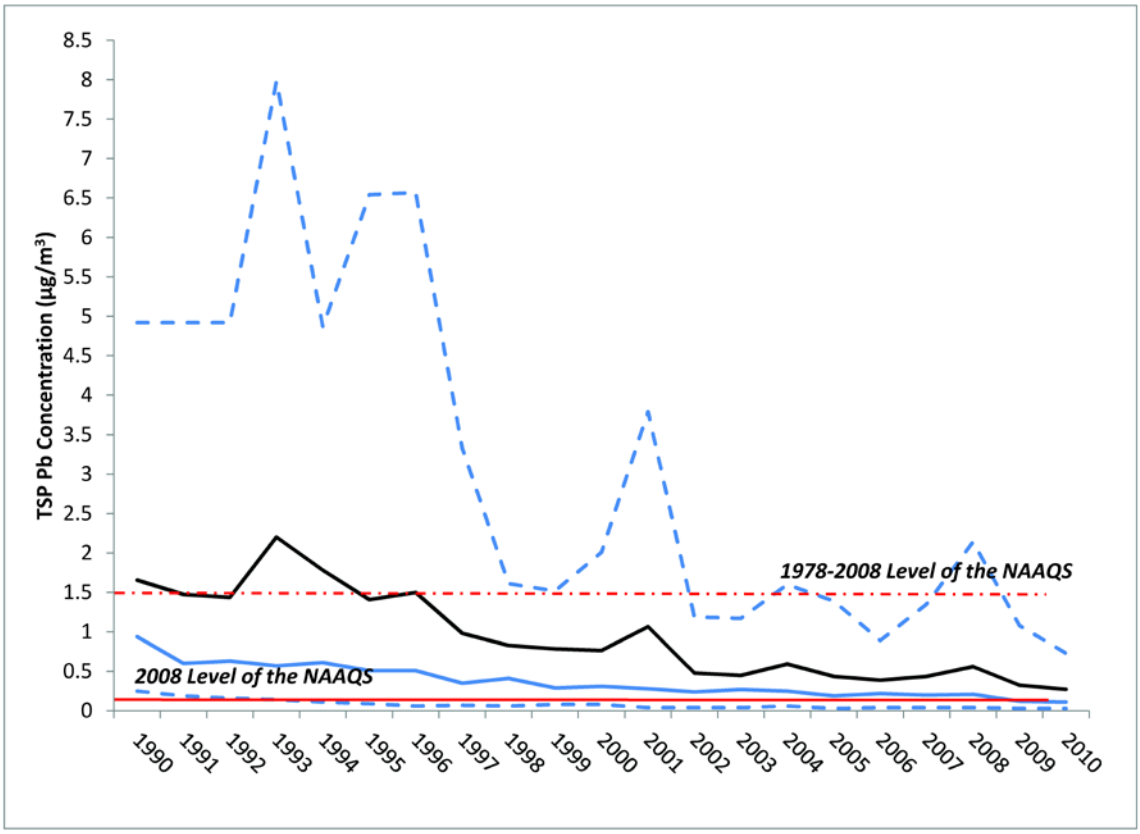
The following section presents data for multi-year trends and seasonal variability of Pb concentrations on a nationwide basis. The data presented here provide information on the success of Pb reduction efforts over past decades as well as on areas for continued attention with respect to Pb monitoring. 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

Pb-TSP concentrations have declined substantially during the years 1980-2010. For source and non-source monitors combined, the annual average across Pb-TSP monitors reporting three-month average site max air Pb concentrations have dropped by 89% from 1.3 μg/m^3 in 1980 to 0.14 μg/m^3 in 2010. The median concentrations have declined by 97% from 0.87 μg/m^3 in 1980 to 0.03 μg/m^3 in 2010. 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 2010 following reductions in industrial use and processing of Pb, as described in Section 3.2.1. In 1990, the average Pb concentration was 0.84 μg/m^3 and the median Pb concentration was 0.25 μg/m^3 to yield 84% and 71% reductions, respectively, from 1990 to 2010.

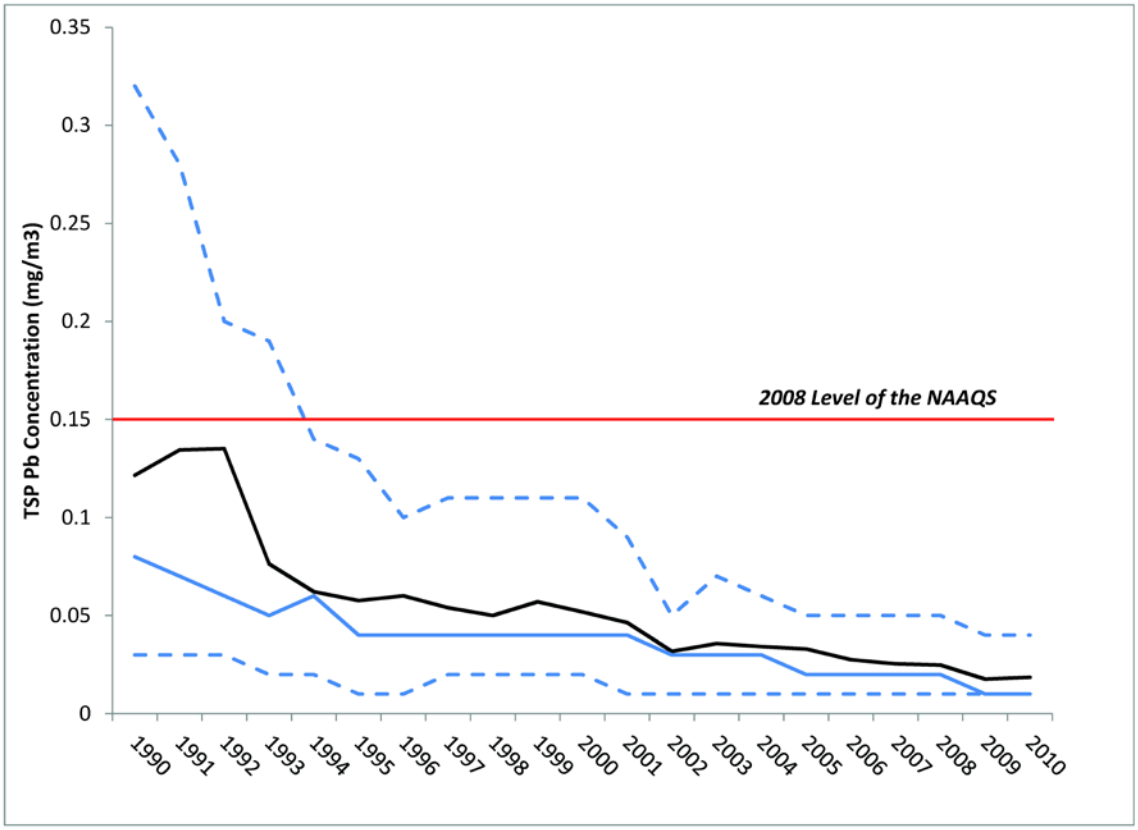
Average concentrations in these calculations are heavily influenced by the source-oriented monitors in the network. New Pb concentration data from expansion of the source-oriented portion of the network in 2010 will allow for greater assessment of changes of Pb concentrations on nationwide statistics and trends. Figure 3-21 and Figure 3-22 show ambient Pb concentrations from 1990 to 2010 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.



Note: Annual average of Pb-TSP monitors reporting three-month average site max concentration is shown by the solid black line, median concentration is shown by the solid blue line, and the 10th and 90th percentiles are shown by the dashed lines.

Figure 3-21 National trends in Pb concentration (µg/m^3), source-oriented FRM monitors, 1990-2010.



Note: Annual average of Pb-TSP monitors reporting three-month average site max. Concentration is shown by the solid black line, median concentration is shown by the solid blue line, and the 10th and 90th percentiles are shown by the dashed lines.

Figure 3-22 National trends in Pb concentration (µg/m^3), non-source-oriented FRM monitors, 1990-2010.

For source-oriented monitors, the annual average across Pb-TSP monitors reporting three-month average site max decreased from 1.7 µg/m^3 to 0.27 µg/m^3 (84% decline) and upper 90th percentile concentrations decreased from 4.9 µg/m^3 to 0.73 µg/m^3 (85% 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, 2010c). An abrupt decrease in average concentrations between these years is evident in Figure 3-21.

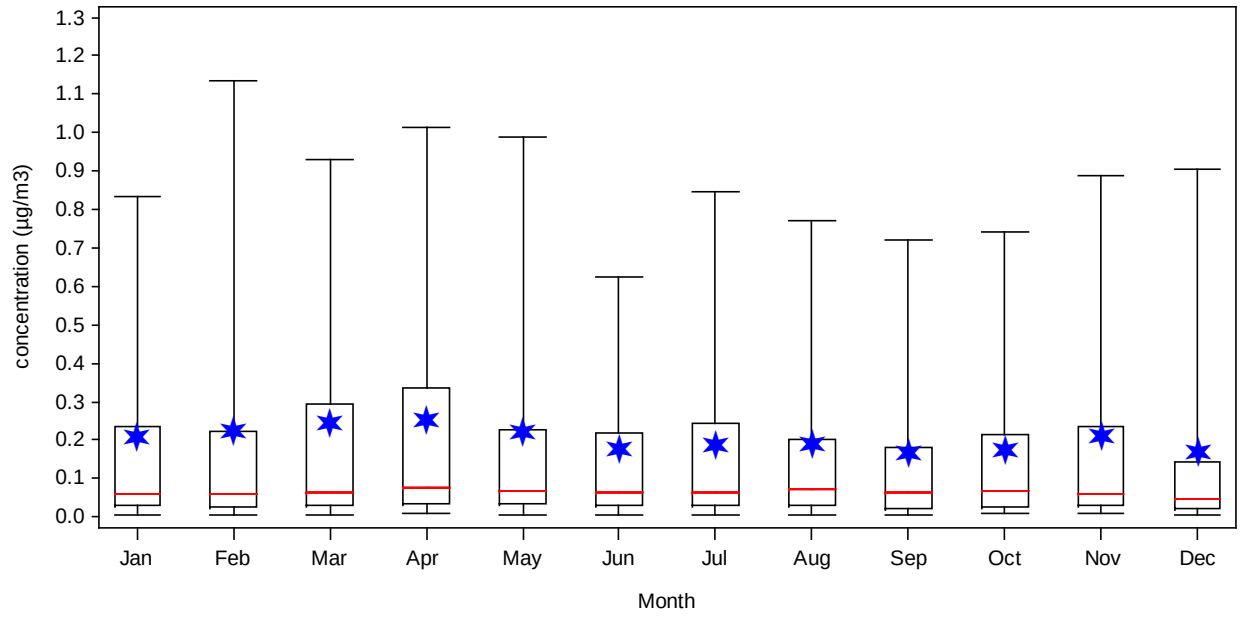
For non-source-oriented monitors, the annual average across Pb-TSP monitors reporting three-month average site max decreased from 0.12 µg/m^3 to 0.018 µg/m^3 (85% decline) and upper 90th percentile concentrations decreased from 0.32 µg/m^3 to approximately 0.04 µg/m^3 (88% decline) over the 20-year period. Average concentrations near stationary sources were 5 to 8 times typical concentrations from non-source-oriented monitoring locations between 1990 and 1999; during the subsequent decade, average source-oriented Pb concentrations were 13 to 24 times higher than non-source

concentrations (U.S. EPA, 2010c). This differential likely reflects the absence of Pb emissions from automobiles during 2000-2009.

3.5.2.2 Seasonal Variations

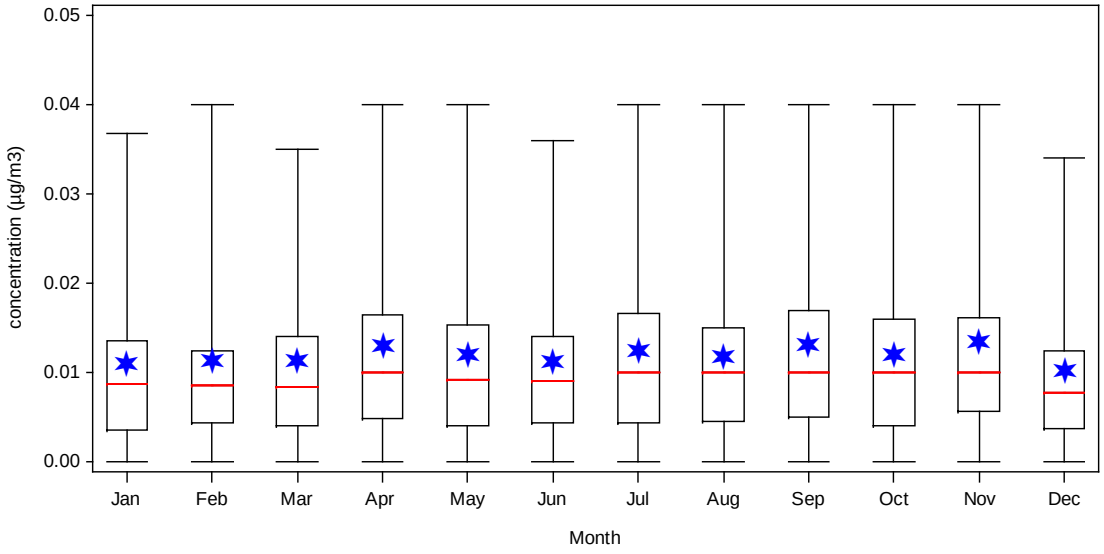
This section outlines seasonal variability among Pb monitors on a nationwide basis. Seasonal variation may provide insight related to differential influences of sources and climate throughout a year. Additionally, the magnitude of concentrations within the monthly data distributions and of variations 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.

Monthly average Pb concentrations averaged over multiple sites and over 3 years from 2008-2010 are shown for Pb-TSP from source-oriented monitors (Figure 3-23), Pb-TSP from non-source-oriented monitors (Figure 3-24), Pb-PM_{10} (Figure 3-25), and Pb-$PM_{2.5}$ (Figure 3-26). For source-oriented Pb-TSP (Figure 3-23), monthly average concentrations were determined from between 146 and 154 samples in each month. For non-source-oriented TSP (Figure 3-24), 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. In both cases, there is little seasonal variation. Minor variations in monthly averages are probably driven by exceptional events. Monthly median concentrations are very similar for all months.



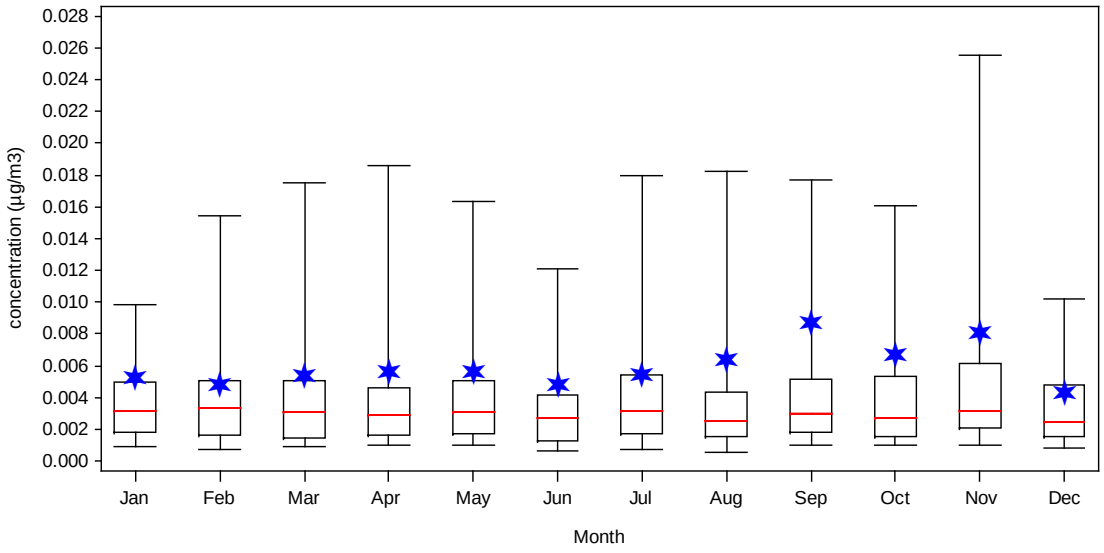
Note: Box and whisker plots are used for each month, with the box comprising the interquartile range of the data and the whiskers comprising the range within the 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

Figure 3-23 Monthly source-oriented Pb-TSP average (µg/m^3) over 12 months of the year, 2008-2010.



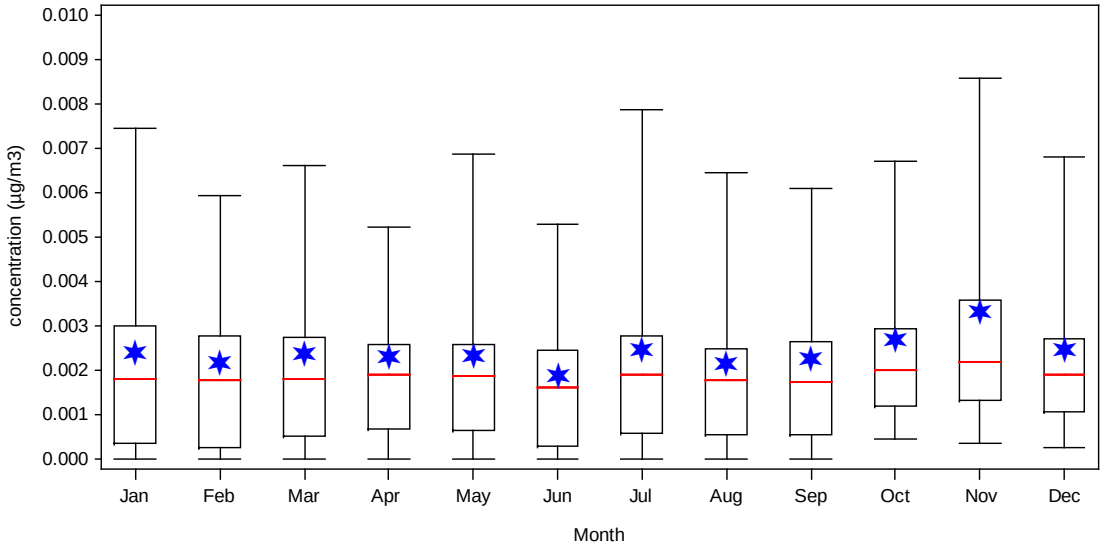
Note: Box and whisker plots are used for each month, with the box comprising the interquartile range of the data and the whiskers comprising the range within the 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

Figure 3-24 Monthly non-source-oriented lead-TSP average (µg/m^3) over 12 months of the year, 2008-2010.



Note: Box and whisker plots are used for each month, with the box comprising the interquartile range of data and the whiskers comprising the range from 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

Figure 3-25 Monthly lead-PM_{10} average (µg/m^3) over 12 months of the year, 2007-2009.



Note: Box and whisker plots are used for each month, with the box comprising the interquartile range of the data and whiskers comprising the range from 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

Figure 3-26 Monthly lead-$PM_{2.5}$ average (µg/m^3) over 12 months of the year, 2007-2009.

For both Pb-PM_{10} (Figure 3-25) and Pb-$PM_{2.5}$, (Figure 3-26) there is also little seasonal variation, with minor fluctuations in monthly averages probably driven by exceptional events, and similar monthly median concentrations for all months. Pb-PM_{10} monthly average concentrations were determined from between 100 and 109 samples and Pb-$PM_{2.5}$ from between 866 and 1,034 samples each month.

3.5.3 Size Distribution of Lead-Bearing PM

The diverse nature of the main source types of ambient air Pb contributes to variations in Pb-PM size distribution. Such variation in the size distribution, along with size-dependent biases in Pb-TSP collection efficiency (Section 3.4.1), can lead to uncertainties in the interpretation of results from Pb-PM measurements. Accordingly, depending on the locations and magnitudes of nearby sources, ambient air Pb may be 1) mainly Pb in PM_{10}, for which good sampler performance is well established, 2) Pb-PM with a size distribution that ranges up to slightly larger than 10 µm, in which case the existing Pb-TSP FRM could potentially be subject to wind related bias, or 3) a Pb-PM size range that extends well above 10 µm, or too large to be efficiently collected even by an improved Pb-TSP method. In the latter case, air sampling is likely to be less representative of actual concentrations of Pb.

Because atmospheric lifetime is dependent on particle size, as described in Section 3.3.1.3 and in U.S. EPA (2009), sampling is likely to be representative only on a very small spatial scale. Ultra-coarse particles have a sharp concentration gradient with distance from source, because coarser particles have greater settling velocities. Hence, concentrations of particles larger than 10 µm are likely to be very spatially and temporally heterogeneous compared with finer particles (U.S. EPA, 2009; Hinds, 1999). As a consequence, in locations near sources of ultra-course particles, measurements may reflect true concentrations only in small areas in close proximity to the monitor. This issue has been thoroughly discussed in the 2006 Pb AQCD (U.S. EPA, 2006b), as well as in the 1977 Pb AQCD (U.S. EPA, 1977).

Size-selective monitoring data from the literature are examined in this section. Size distribution data enhances understanding of the relationship between sources and characteristics of airborne Pb-bearing PM and hence informs monitoring strategies. Several studies in the literature since the last review have been designed to characterize the size distribution of Pb concentrations in the vicinity of sources. In the subsections below, the currently available information is presented for locations in the vicinity of industrial sources (active and closed), near roadways, and in other urban and rural environments.

3.5.3.1 Airborne Pb Near Metals Industries

Size distributions of Pb-bearing PM have been measured near several active and closed industrial sites. Yi et al. (2006) collected Pb-PM size distribution in an industrial area of Jersey City, NJ and contrasted it with the Pb-PM size distribution in suburban New Brunswick, NJ, which is influenced only by traffic. Yi et al. (2006) sampled size distribution for Pb-bearing particles with a Multi-Orifice Uniform Deposit Impactor (MOUDI) (cut point range: 0.18-18 µm) along with a coarse particle rotary impactor (CPRI) collecting particles ranging in size from 14.4-100 µm. In the industrial area, 27% of Pb-PM were larger than PM_{10} (avg. Pb-TSP: 9.7 ng/m^3), while in the suburban area 7% of Pb-PM were larger than PM_{10} (avg. Pb-TSP: 6.6 ng/m^3). Bein et al. (2006) measured the size distribution of Pb in PM from the Pittsburgh Supersite using rapid single particle mass spectrometry and a MOUDI. The Pittsburgh, PA, Supersite had seventeen major PM sources within a 24-km radius; source apportionment illustrated that Pb was contained in a sub-population of particles of almost every major particle-containing class in this study, emanating from point sources including fuel combustion, steel processing, incinerators, foundries, battery manufacturing, and glass manufacturing (Pekney et al., 2006). Bein et al.'s (2006) measurements yielded different results on different days, with a bimodal distribution with modes around 140 nm and 750 nm during

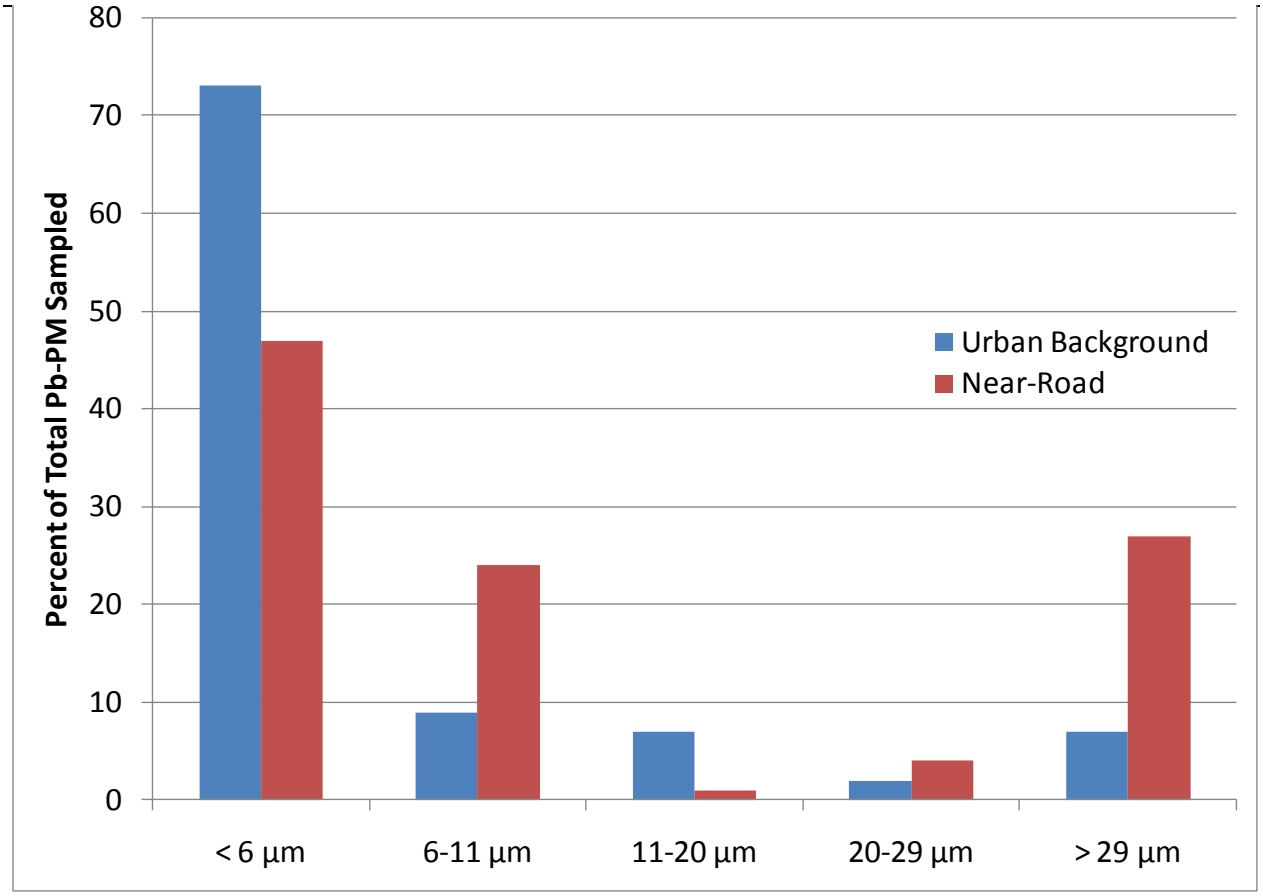
an October, 2001 measurement and a single dominant mode around 800 nm during a March, 2002 measurement. Differences in the size distributions could have been related to differences among wind speed, wind direction, and source contributions on the respective dates. Concentrations of Pb-$PM_{3.2}$ measured by the MOUDI were 5.6 ng/m^3 in October, 2001 and 5.0 ng/m^3 in March, 2002; measurements by the hi-vol were 4.4 ng/m^3 in October, 2001 and 6.5 ng/m^3 in March, 2002. Singh et al. (2002) measured the mass distribution of Pb-PM_{10} in the coarse and fine PM size ranges (cut points range: 0.10-10 µm) for the Downey 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_{10} size distribution was unimodal with a concentration peak in the 100-350 nm size range with 34% of the particles in this size bin. The sum of the geometric mean concentrations in each size bin was 13 ng/m^3 for the Downey data. At the downwind site, a bimodal distribution was observed with peaks in the 2.5-10 µm bin and the 350 nm-1 µm bin, 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 sum of the geometric mean concentrations in each size bin was 7 ng/m^3 for the Riverside data. The authors suggested that higher wind speeds in Riverside compared with the Downey site are effective in resuspending larger particles from the ground to create a peak in the coarse mode of the distribution.

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 Pb concentrations in the coarse to fine PM size range (cut points range: 0.196-18 µm). The size distribution was multimodal with a primary mode around 1 µm at a concentration of 40 ng/m^3, a secondary mode around 300 nm at a concentration of 25 ng/m^3, and a very small additional mode around 5 µm at a concentration of 7 ng/m^3. 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 (avg. Pb-$PM_{2.5}$: 53 ng/m^3) , 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.

3.5.3.2 Airborne Pb Near Roadways

Traffic-induced turbulence can be a cause of resuspension of Pb-bearing particles from deposited contemporaneous wheel weights, industrial emissions, or historic sources. Pb in resuspended road dust exhibits a bimodal size distribution, with fine mode likely from vapor phase condensation from combustion engines and the coarse mode from vehicle wear (U.S. EPA, 2006b). However, mass is predominantly associated with coarse PM. The Pb fraction in resuspended dust generally ranges from 0.002 to 0.3%, with the highest fractions observed for paved road dust and lowest for agricultural soil. Sabin et al. (2006b) compared the size distribution of coarse Pb-PM measured using a Noll rotary impactor at an urban background site and at a location 10 m from the I-405 Freeway in the southern California air basin; data from Sabin et al. (2006b) are displayed in Figure 3-27; note that the Noll rotary impactor has been shown to sample with high efficiency for particles up to 100 µm in diameter (Noll, 1970). 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. Overall 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. (2006b) point out that the freeway tends to be a source of very large particles that are dispersed via the turbulent motion of the vehicular traffic. Song et al. (2011) used an eight-stage MOUDI (cut point range: 0.18-18 µm) to measure roadside PM 5 m from the New Jersey Turnpike in Carlstadt, NJ and speciated the samples. They observed a bimodal distribution of the Pb concentration in summer and a trimodal distribution in winter. 85% of the Pb-PM mass smaller than 2.5 µm during the summer and 68% was within the 2.5 µm fraction in the winter. Pb-PM mass measured in this study ranged from 1.2-2.8 ng/m^3. Similarly, Zereini et al. (2005) observed that roughly 80% of particle-bound Pb measured with a MOUDI was smaller than 5.8 µm for an urban main street (avg. conc.: 33 ng/m^3), and more than 90% were smaller than 5.8 µm for a rural area included in that study (avg. conc.: 12 ng/m^3). However, in a study of automotive emissions in a traffic tunnel, Lough et al. (2005) measured that 85% of Pb measured with a MOUDI was in the PM_{10}, with just 39% in the $PM_{2.5}$ fraction and 20% in the PM_1 fraction. In a near-road study conducted in Raleigh, NC, Hays et al. (2011) note that the concentration of Pb in ultrafine, fine, and coarse size ranges was roughly constant at 50 mg/kg; similar to Lough et al. (2005), mass concentrations were 0.4 ± 0.4 ng/m^3, 1.4 ± 0.6 ng/m^3, and 0.1 ± 0.02 ng/m^3 for $PM_{10-2.5}$, $PM_{2.5-0.1}$, and $PM_{0.1}$, respectively. The Pb-PM_{10} samples from Hays et al. (2011) were highly correlated with As samples ($\rho = 0.7$, $p < 0.0001$); both Pb and As are found in wheel weights (see Section 3.2.2.6). Likewise, the Pb samples were not well correlated with crustal elements in the coarse size distribution, so it is more likely that resuspended Pb originated from contemporary roadway sources rather than historic Pb

on-road gasoline emissions. Chen et al. (2010b) measured Pb in $PM_{10-2.5}$, $PM_{2.5-0.1}$, and $PM_{0.1}$ using a MOUDI at a roadside location and in a tunnel in Taipei, Taiwan in 2008. While roadside and tunnel concentrations of PM_{10} and $PM_{2.5}$ were roughly equivalent around 20-30 ng/m^3, Pb in $PM_{0.1}$ was approximately 15 times higher in the tunnel (during the hours 9:00 AM – 9:00 PM) than by the roadside (tunnel: 20 µg/m^3; roadside: 1 ng/m^3). The authors suggest that particle-bound Pb was emitted from on-road gasoline and diesel engines. This could possibly be attributed to trace levels of Pb in diesel fuel and lubricating oil. Birmili et al. (2006) compared concentrations of Pb in PM 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-$PM_{1.5-3.0}$. 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-$PM_{1.5-3.0}$. At night, roadside concentrations reduced to 0.17 ng/m^3 for Pb-$PM_{0.5}$ and 0.6 ng/m^3 for Pb-$PM_{1.5-3.0}$. In contrast, urban background was more enriched in the finer size fraction, with concentrations of 5.4 ng/m^3 for Pb-$PM_{0.5}$ and 0.84 ng/m^3 for Pb-$PM_{1.5-3.0}$. Remote background concentrations were on 0.16 ng/m^3 for Pb-$PM_{0.5}$ and 0.03 ng/m^3 for Pb-$PM_{1.5-3.0}$. 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. 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.



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

Figure 3-27 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 sources other than from the road. Harrison et al. (2003) measured the distribution of Pb in PM_{10} at a roadside sampler in Birmingham, U.K.. The size distribution was unimodal with approximately 2% of the Pb mass (0.5 ng/m^3) above the 10 µm cut point, 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 NO_X ($\beta = 0.067$, $R^2 = 0.38$) as well as PM_{10} ($\beta = 0.26$, $R^2 = 0.35$). Reactivity of NO_X may contribute to the somewhat low values for R^2 in these models. Brüggemann et al. (2009) observed a unimodal Pb size distribution with 51% of the mass in the 0.42-1.2 µm size bin. Observed Pb-PM_{10} concentration was 17 ng/m^3. During winter, Pb concentrations were more than twice as high as during the summer (0.42-1.2 µm mode, winter: 50 ng/m^3;

summer: 20 ng/m^3), and they were also higher when winds blew from the east (0.42-1.2 µm mode, east: 60 ng/m^3; west: 25 ng/m^3). Brüggemann et al. (2009) suggested that this finding reflected coal burning sources rather than road dust resuspension. Wang et al. (2006d) used a nine-stage cascade impactor (cut point range: 0.43-11 µm) to measure the Pb-PM size distribution in a heavily trafficked area of Kanazawa, Japan with incineration and generation facilities nearby. They observed a bimodal distribution with modes at the 0.65-1.1 µm and the 3.3-4.7 µm size bins. Average concentration in the coarse mode was 2.1 ng/m^3, while fine mode average concentration was 3.7 ng/m^3. Wang et al.'s (2006d) source apportionment work in this study suggested that the fine mode derives from incineration and combustion of oil and coal.

3.5.3.3 Airborne Pb at Other Urban and Rural Sites

Spatial and temporal concentration variability is also reflected in varying Pb-PM size distributions within and between cities. Martuzevicius et al. (2004) measured the size distribution of Pb in Cincinnati, OH at the city center site using a MOUDI and showed it to be bimodal with a primary peak at 0.56 µm and a slightly smaller secondary peak at 5.6 µm. 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.

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 material from pulverized wheel weights or contaminated soil. Additionally, seasonality can influence correlations, potentially from differences among sources or the contaminants' responses to climate differences.

Pb concentrations exhibit varying degrees of association with other criteria pollutant concentrations. Spearman correlations of monitored Pb-TSP concentrations with concentrations of other criteria pollutants are summarized in Figure 3-28 for 2007-2008 data from 129 monitoring sites, and in Figure 3-29 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 3-28, and fewer for each copollutant in Figure 3-29. Each of these figures illustrates co-pollutant correlations across the U.S. Additionally, seasonal correlations between Pb and co-pollutants are provided in Figure 3-60 through Figure 3-65 in the Section 3.8, with seasonal co-pollutant measurement data from the literature (Table 3-31). As evident in each figure, there were considerably fewer source-oriented sites available for co-located comparisons.

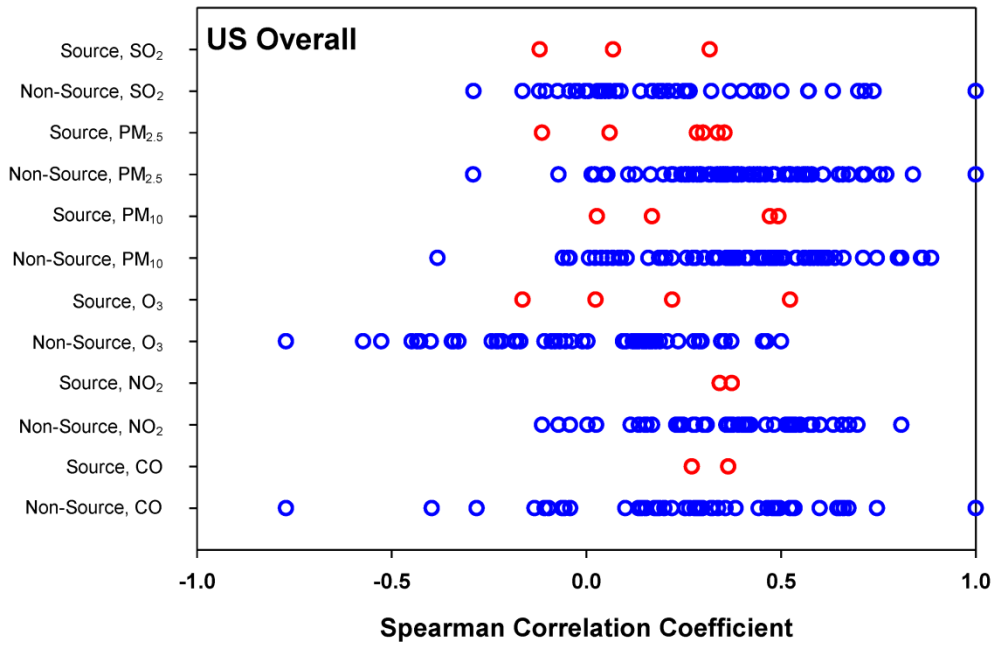


Figure 3-28 Correlations of monitored Pb-TSP concentration with copollutant concentrations, 2007-2008.

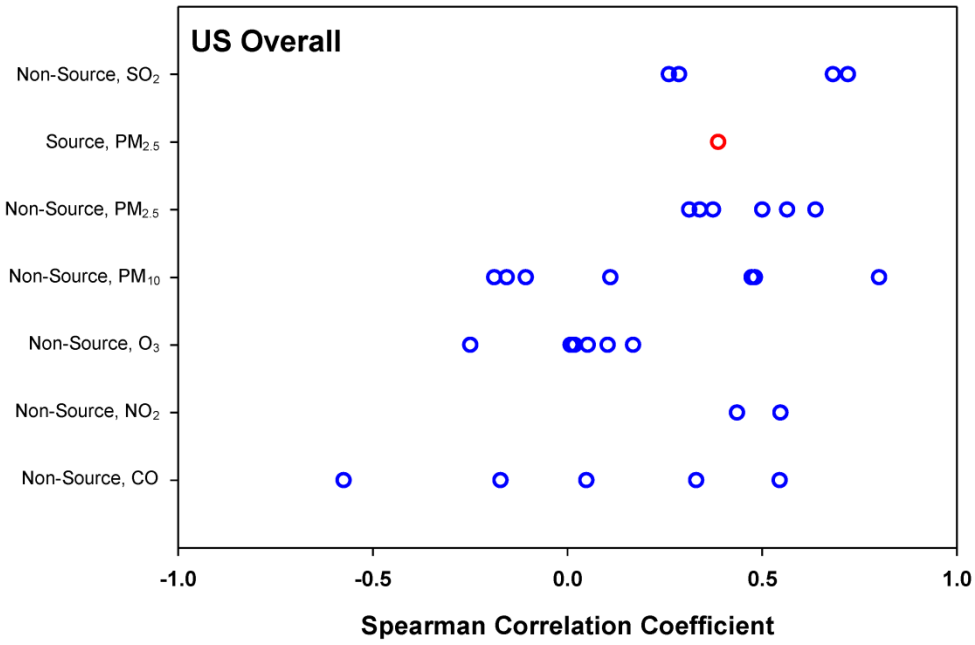


Figure 3-29 Correlations of monitored Pb-TSP concentration with co-pollutant concentrations, 2009.

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 ρ = 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 ρ = 0.29 for CO, 0.17 for SO_2). The poorest associations were observed between Pb and O_3 (median ρ = 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 3-30, which describes data from 3 years of CSN results using Pearson R. These data provide a national perspective on relationships between the various bulk and elemental species monitored in the CSN network. The strongest association was with Zn (median R = 0.51). K, Cu, and Br 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. For example, as described in Section 3.2.2.1, piston-engine aircraft emit Pb as $PbBr_2$ so this source may explain the covariation in Pb and Br concentrations at the CSN sites. Other species more useful as diagnostic indicators of crustal, general combustion, industrial emission, and coal combustion processes exhibited weaker, but still remarkable associations with Pb, including Fe (median R = 0.34), EC (median R = 0.32), crustal elements (median R = 0.32), Mn (median R = 0.32), and OC (median R = 0.30). Summer associations between Pb and other species tended to be weaker than in other seasons, with a Correlation Coefficient greater than R = 0.35 observed only for Zn (median R = 0.37). The weakest associations were with non-volatile NO_3^-, Cl, As, Na^{+2}, Hg, Na, and Ni (median R = -0.02 to 0.10).

A few recent studies have used speciation techniques to characterize Pb and other components of PM_{10}, $PM_{2.5}$, and PM_1. Pingitore et al. (2009) used XAFS to speciate air samples obtained near a defunct smelter in El Paso, TX, in 1999 and 2005 and found that air Pb-TSP concentrations of 0.10 to 0.50 $\mu g/m^3$ could largely be attributed to Pb-humate. 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 correlation varied substantially with other elements across size distributions. Correlations were very high between Pb and several PM_{10} metals, and only Pb was only highly correlated with Mg in $PM_{2.5}$. These results suggested that Pb and copollutants emanated from a variety of sources including road dust and fuel combustion (Table 3-10). Variations in the relative

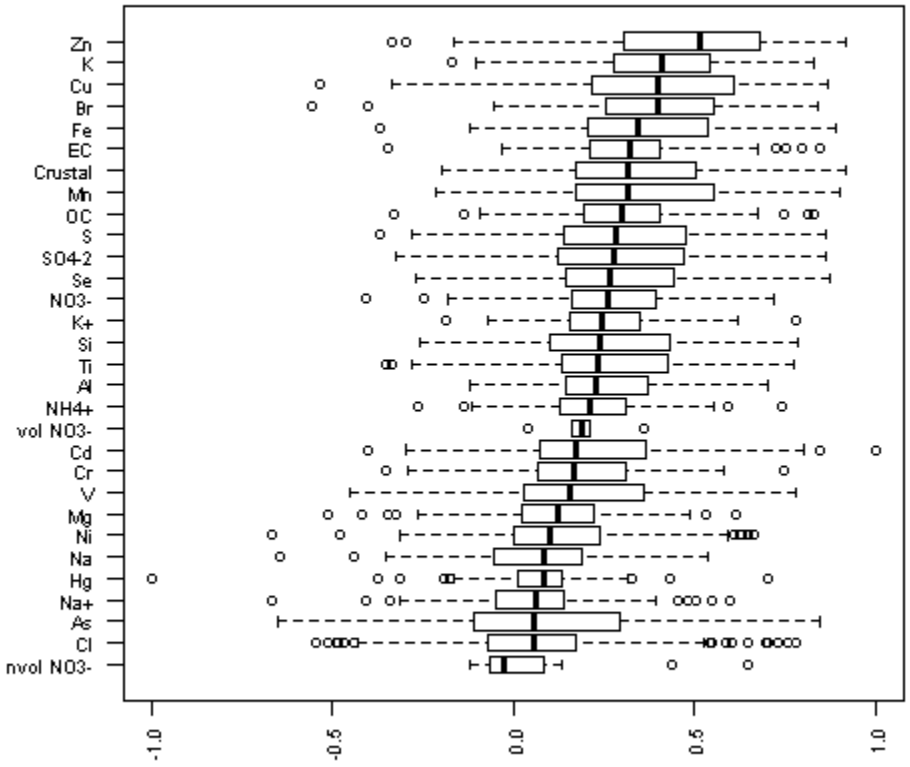
proportions of Pb-containing compounds may account for the difference in Pb solubility in aerosols (von Schneidemesser et al., 2010; Tan et al., 2006; Fernández Espinosa and Ternero-Rodríguez, 2004).

Murphy et al. (2008) studied weekly patterns of metals and other aerosol components using data collected from 2000 to 2006 at Interagency Monitoring of Protected Visual Environments (IMPROVE) sites that are described further in Section 3.4.2.2. The authors concluded that Pb concentrations were impacted by piston aircraft emissions, particularly on weekends when there is typically a peak in general aviation flights. The authors also note that Zn and Pb were highly correlated in atmospheric samples and they suggest that this is due to similar sources (i.e., electric utility and industrial sources). Murphy et al. (2007) also carried out a detailed study of the distribution of Pb in single atmospheric particles. During the fifth Cloud and Aerosol Characterization Experiment in the Free Troposphere (CLACE 5) campaign conducted at the Jungfraujoch 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.

Table 3-10 Correlations between Pb and copollutants, measured in TSP, PM_{10}, and $PM_{2.5}$

Element[a]	TSP	PM_{10}	$PM_{2.5}$
Al	0.54	-0.21	0.20
As	0.14	-0.24	-0.03
Ca	0.4	***0.96***	-0.52
Cd	***0.95***	***0.93***	0.20
Co	0.59	-0.19	0.47
Cr	0.04	***0.99***	0.20
Cu	***0.85***	***0.84***	-0.74
Fe	0.43	***0.99***	-0.51
K	***0.81***	***0.90***	NA
Mg	0.43	***0.97***	***0.80***
Mn	***0.71***	***0.97***	NA
Mo	0.69	***0.99***	0.04
Ni	0.08	***0.99***	-0.67
Sb	***0.81***	***0.85***	-0.84
Si	0.36	***0.99***	0.26
V	0.28	-0.17	-0.43
Zn	***0.92***	***0.99***	0.27

[a]High correlations ($r \geq 0.7$) are shown in bold italics
Source: Wojas and Almquist (2007)



Note: "nvol" = non-volatile, "vol" = volatile, and organic carbon (OC) samples were blank-adjusted.

Figure 3-30 Correlations of monitored lead-$PM_{2.5}$ concentration with copollutant concentrations, 2007-2009.

3.5.5 Background Lead Concentrations

To inform NAAQS decisions, EPA has historically estimated risk due to pollutant concentrations above background concentrations. In previous NAAQS reviews, a specific definition of background concentrations was used and referred to as policy relevant background (PRB). In those previous reviews, PRB concentrations were defined by EPA as those concentrations that would occur in the U.S. in the absence of anthropogenic emissions in continental North America (CNA), defined here as the U.S., Canada, and Mexico. For this document, we have focused on the sum of those background concentrations from natural sources everywhere in the world and from anthropogenic sources outside CNA. Background concentrations so defined facilitate separation of

pollution that can be controlled by U.S. regulations or through international agreements with other countries from those that are judged to be generally uncontrollable by the U.S. Over time, consideration of potentially broader ranging international agreements may lead to alternative determinations of which Pb source contributions should be considered by EPA as part of background.

The 2006 and 1986 Pb AQCDs evaluated evidence on Pb emissions from natural sources, such as volcanoes, sea-salt spray, biogenic sources, wild forest fires and wind-borne soil particles in rural areas without elevated Pb soil concentration. The 1986 Pb AQCD concluded that the natural particulate Pb concentration was probably lower than the concentration of 0.076 ng/m^3 reported at the South Pole [see pp 1-14, (U.S. EPA, 1986a)]. A 1980 National Academy of Sciences report estimated that average natural background levels of airborne Pb might range from 0.02 to 0.5 ng/m^3 (NAS Committee on Lead in the Human Environment, 1980).

Global transport can carry airborne Pb to remote areas with no industrial activity, thus it is difficult to estimate a natural background concentration of Pb. Hong et al. (1994) found that Pb concentrations in Greenland ice cores remained nearly constant (at about 0.55 pg Pb/g ice) from about 7,760 years ago to about 3,000 years ago. Ratios of Pb to major crustal elements were not enriched in this section of the ice core suggesting that Pb was natural in origin, produced by rock and soil dust. At about 2,500 years ago, Pb concentrations started to increase (to about 100 pg Pb/g snow averaged from 1930 to 1990) (Boutron et al., 1991) corresponding to an enrichment of ~ 200 times natural background levels.

Chemistry-transport models are not available for estimating background concentrations of airborne Pb is problematic because chemistry-transport model calculations are not available as they are for O_3 or for $PM_{2.5}$ and they would have to include the entire size range for particle-bound Pb. The only data that might be relevant are from the IMPROVE network, but only in the $PM_{2.5}$ size fraction. Pb in this size fraction is most amenable to long range transport because particles in the $PM_{2.5}$ size fraction have a much longer atmospheric lifetime compared to larger size fractions. It is impossible to obtain reliable estimates of background concentrations solely on the basis of measurements of $PM_{2.5}$, $PM_{10-2.5}$, or PM_{10}. It is preferable to quantify contributions from both background and non-background sources by using compositional data in techniques such as source apportionment modeling.

Measurements of Pb from IMPROVE sites and source apportionment modeling have been used to assess the potential input from intercontinental transport. Liu et al. (2003) used positive matrix factorization to attribute sources of Asian dust to the measurements at two western IMPROVE sites at high elevations, Crater Lake and Lassen Volcanic Park

from 1988 to 2000. Geometric mean concentrations of 0.34 and 0.48 ng/m^3 were found in the samples with only a few percent of these values attributable to transport from Asia. No enrichment in Pb and other metals (As, Cr, Cu, Ni, Pb, V and Zn) above reference Asian dust material was found. Their results suggest either that arriving air masses did not entrain contributions from Asian pollution sources or that these contributions were preferentially scrubbed out during transport. Large enrichments in S were found, however, which might have been due to pollution sources but also due to model artifacts. However, other studies have found some evidence of trans-Pacific transport. Murphy et al. (2007) measured single Pb particles off the coast of California in a NOAA aircraft elevated more than 2 km AGL. Given the elevation of the measurement and the timing of trans-Pacific plume events, the authors concluded that these Pb-bearing $PM_{2.5}$ originated in Asia. They also noted Pb/Zn ratios in $PM_{2.5}$ at the Mount Zirkel, CO IMPROVE site of 0.6 corresponding to measurements at Mauna Loa, HI in spring, when measurements at other times of year produced Pb/Zn ratios of 0.3-0.4. Ewing et al. (2010) used time series analysis of Pb isotope measurements to estimate Asian and local contributions to Pb-$PM_{2.5}$ concentrations measured at two observatories near San Francisco, CA. They estimated a springtime contribution of Asian dust to Pb-$PM_{2.5}$ measurements. In both the Murphy et al. (2007) and Ewing et al. (2010) studies, the authors conclude that the Asian contribution is still generally less than 1 ng/m^3.

The use of data for $PM_{2.5}$, $PM_{10-2.5}$, and PM_{10} from monitoring sites in the East will generally result in gross overestimates of background concentrations because anthropogenic sources will cause extensive contamination. Intercontinental transport of African dust contributes to PM and is observed mainly in the Southeast but is apparent on an episodic basis elsewhere in the eastern U.S. [see e.g., 2004 PM CD (U.S. EPA, 2004) and 2009 PM ISA (U.S. EPA, 2009)]. Data obtained at four eastern IMPROVE sites (Moosehorn NWR, ME; Acadia NP, ME; Swanquarter, NC; Cape Romain NWR, SC) from 2007 to 2009 indicate a median Pb-$PM_{2.5}$ concentration of 1.0 ng/m^3 with a 95th percentile value of 2.5 ng/m^3. As noted above, these sites are likely to be affected by upwind anthropogenic sources within the U.S.

Rough estimates for the natural source of Pb in different size fractions of Pb-PM can be made by multiplying the abundance of Pb in soils by the crustal component of PM in the different size fractions. It is assumed that there is no fractionation between size ranges in this approach. The mean abundance of Pb in surface rocks is ~ 20 mg/kg (Potts and Webb, 1992); the 2006 Pb AQCD (U.S. EPA, 2006b) reported Pb concentrations in different types of rocks to range from 3.5 to 32 ppm (Reuer and Weiss, 2002). There is substantial variation with location depending on composition, in particular on the abundances of U and Th, since Pb is produced mainly by radioactive decay of these elements. The mean Pb concentration of 863 soil samples taken across the U.S. at 2 m

depth is ~16 mg/kg; this value was derived by sampling residual Pb of the weathered rocks on which they formed (Wedepohl (1978) and references therein).

Concentrations of the Pb content of soils can be used with estimates of the crustal component of $PM_{2.5}$, $PM_{10-2.5}$ (which is mainly crustal), PM_{10}, and TSP produced by wind erosion of natural surfaces to estimate contributions to Pb concentrations in these size fractions. U.S. annual average PM_{10} concentrations in some arid counties most affected by windblown dust in the western U.S. are ~ 20 $\mu g/m^3$. If it is assumed that these levels of PM_{10} are entirely due to natural wind erosion without any anthropogenic contribution, an estimate of ~ 0.3 ng/m^3 for the contribution of wind erosion on natural surfaces to Pb in PM_{10} is obtained; however, it must be observed that the natural contribution is probably lower than this estimate. An assumed ratio 3.5 for TSP to PM_{10} in dust storms, derived by Bacon et al. (2011), indicates a contribution of ~ 1 ng/m^3 for Pb from natural sources in TSP. These estimates exceed estimates of natural background presented in the 1986 AQCD (U.S. EPA, 1986a) and the National Academy of Sciences Report (NAS Committee on Lead in the Human Environment, 1980) by a factor of 2 to 20. The more recent estimate still indicates that background airborne Pb concentrations are well below current ambient concentrations.

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 across a variety of environmental media and biota. In general these concentrations reflect the decreases observed in atmospheric Pb concentrations due to reduced on-road Pb emissions.

The 2006 Pb AQCD (U.S. EPA, 2006b) describes several studies showing higher Pb concentrations in plants grown in Pb contaminated soil related to mine spoils, smelting operations, sludge amendment, contaminated irrigation water, and Pb containing agro-chemicals. In general, metal accumulation occurs more readily for Pb salts applied to soils than for the same quantity of metal in sewage sludge or fly ash. Root uptake is the dominant means of accumulation, and it is strongly influenced by pH. Root vegetables are the most strongly affected, and fruits and grains are the least susceptible. More Pb is also generally found in roots than in other parts of the plant.

The 2006 Pb AQCD (U.S. EPA, 2006b) identified ingestion and water intake as major routes of Pb exposure for aquatic organisms, and it identified food, drinking water, and inhalation as major routes of exposure for livestock and terrestrial wildlife. The 2006 Pb AQCD (U.S. EPA, 2006b) reports data from the U.S. Geologic Service National Water-Quality Assessment (NAWQA), which are updated every ten years. In the NAWQA

survey, maxima concentrations in surface waters, sediments, and fish tissues were 30 µg/L, 12,000 mg/kg, and 23 mg/kg, respectively, compared with median values of 0.50 µg/L, 28 mg/kg, and 0.59 mg/kg. Some of the highest levels of Pb contamination occur near major sources, like smelters, and fatal doses have been measured in tissue from sheep and horses near sources. High levels in cattle have also been observed. Wildlife in urban areas tend to contain higher Pb concentrations than in rural areas, and higher Pb accumulations have been observed for aquatic organisms living in polluted coastal zones than in the open sea. Ingestion of deposited Pb-PM on plant surfaces was consistently observed to be more important than Pb accumulated from soil. Some important variations between animals have been observed, and ruminants appear to be less susceptible to Pb uptake than other animals. Uptake of Pb by lowest trophic levels, including invertebrates, phytoplankton, krill, were described as the most important means of introduction into food chains. Elevated Pb levels have been observed in aquatic organisms that feed from sediments when the sediments contain appreciable Pb. In shrimp, a substantial fraction of Pb can be absorbed from prey, and considerably more accumulated Pb from food has been observed to be irreversibly retained than is the case for dissolved Pb from water. These examples all illustrated that substantial Pb uptake by livestock and wildlife readily occurs in Pb contaminated environments.

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. (2010a) 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 189 mg/kg. Soil Pb was thought to originate from present-day sources, such as industry, debrided paint, and piston-engine aircraft emissions, as well as historic sources, such as on-road gasoline emissions, as described in Section 3.2. At the same time, soils in remote or rural areas tend to have lower Pb concentrations. The most extensive survey of background soil Pb concentration in the conterminous U.S. was conducted between 1961 and 1976 and comprised 1,319 non-urban, undisturbed sample locations, where 250 cm^3 of soil was collected at a depth of 20 cm (Shacklette and Boerngen, 1984). The lower detection limit was 10 mg/kg, and 14% of the 1,319 samples were below it. The mean Pb concentration was 19.3 mg/kg, the median 15 mg/kg, and the 95th percentile was 50 mg/kg. Sixteen locations had Pb concentrations between 100 and 700 mg/kg. These results were in agreement with 3 previous surveys. When creating the Eco-SSL guidance document, the U.S. EPA (U.S. EPA, 2007d, 2003b) augmented these data with observations from an additional 13 studies conducted between 1982 and 1997, most of them limited to one

state. The resulting data were summarized using state means for each of the fifty states. Those means ranged between 5 and 38.6 mg/kg, with an overall national mean of 18.9 mg/kg. This is reasonably close to the values reported by Wedepohl (1978) and references therein with a mean soil Pb concentration of roughly 16 mg/kg when samples were taken at 2 m depths. Aelion et al. (2009) compared Pb soil levels in urban and two rural locations in the southeastern U.S. (specific sites were not disclosed). The urban and one of the rural areas had significantly high prevalence of mental retardation or developmental disabilities (MMDD), while the other urban area did not. Aelion et al. (2009) found that soil Pb concentration in the rural area without prevalence of MMDD ranged from 2.1-53 mg/kg dry basis, while in the rural area with high prevalence of MMDD, soil Pb ranged from 1.6-140 mg/kg; in the urban area with high MMDD, soil Pb ranged from 2.4-288 mg/kg. Biasioli et al. (2006) contrasted urban and rural soils of the same alluvial composition near Torino, Italy to assess the influence of anthropogenic inputs. The urban soils had a median Pb concentration of 117 mg/kg, while the median Pb concentration for rural soil was 19 mg/kg.

In North American forest soils, Pb concentrations have decreased substantially since the phase out of leaded motor vehicle gasoline. Evans et al. (2005) observed Pb concentrations ranging from 60 to 200 mg/kg in Vermont and Quebec, with lower concentrations in Quebec than in southern Vermont in 1979, but in 1996 concentrations had decreased to between 32 and 66 mg/kg with no spatial trend. Johnson and Richter (2010) also observed a substantial decrease in Pb concentrations in soil between 1978 and 2004 in West Virginia, Maryland, Pennsylvania, New Jersey, New York, and Connecticut, with a median change of -65%. However, elevation also appears to be an important factor in determining whether appreciable decreases in Pb concentration have occurred since the phase out of leaded gasoline (Kaste et al., 2006). At sites above 800 m in the northeastern U.S. concentrations ranged from 11 to 29 kg Pb/ ha, and little change in Pb concentration was observed between 1980 and 2000. In contrast, concentrations ranged from 10 to 20 kg Pb/ha at low elevation sites and decreased to 2 to 10 kg Pb/ha by 2000. This difference was likely due to greater organic turnover increasing Pb mobility at the lower elevations (Kaste et al., 2006).

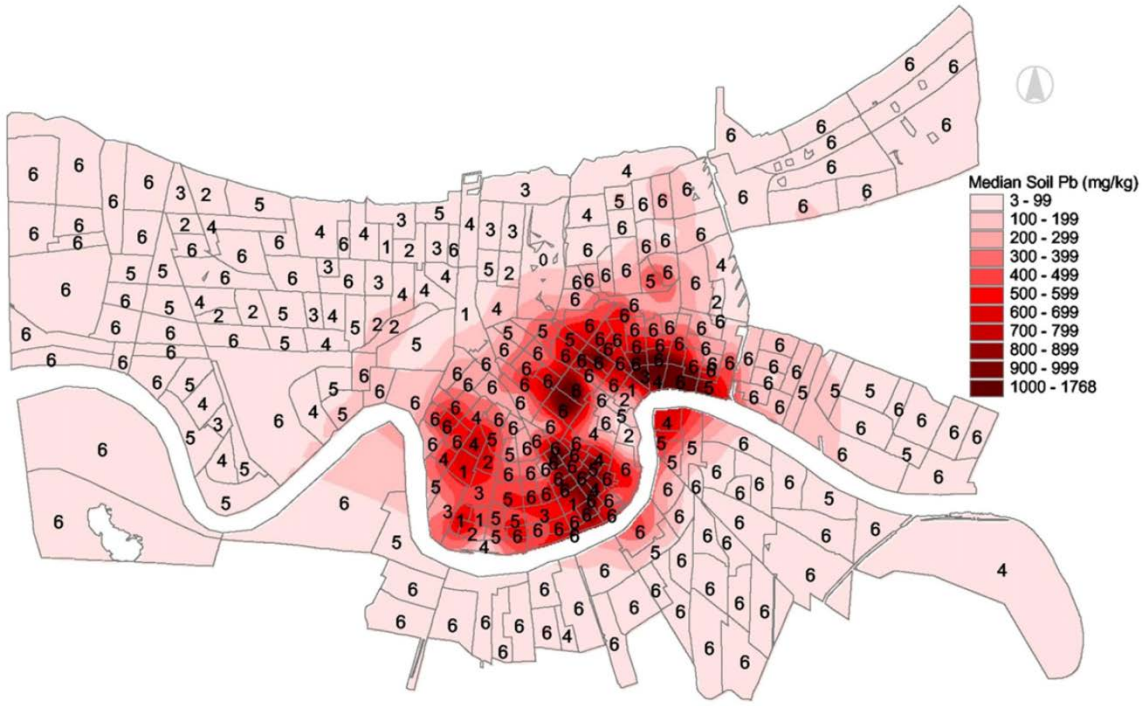
Emissions trends have shown that industrial activities are now one of the largest sources of soil Pb following phase out of Pb in on-road gasoline. Pruvot et al. (2006) compared urban and agricultural soils near a closed Pb smelter with soils in similar environments not exposed to smelter emissions in northern France. For samples near the smelter, Pruvot et al. (2006) observed that median soil Pb levels in lawns were roughly 2 times higher, while kitchen garden soil Pb concentrations were 10 times higher and agricultural soil Pb was almost 15 times higher than soil not exposed to smelter emissions. Bonnard and McKone (2009) reported surface soil Pb concentrations of 66-493 mg/kg outside

homes of children living within 1 km of a Pb smelter in France; air Pb levels reported by Bonnard and McKone (2009) for this town ranged from 0.025-0.20 μg/m^3. The air samples Pingitore et al. (2009) obtained near a defunct El Paso, TX smelter (described in Section 3.5.4) found that the air Pb-TSP concentrations could largely be attributed to Pb-humate, which is created by sorption of Pb onto humic substances in soil and can be resuspended. Spalinger et al. (2007) compared soil Pb samples 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.

Recent studies of brownfield soils have shown variable Pb concentrations. Van Herwijnen et al. (2007) measured soils near a defunct Zn smelter in Avonmouth, UK in areas termed low and high contamination by the authors. Total soil Pb concentration in the low contamination area was 315 mg/kg, while soil Pb concentration in the high contamination area was 1,688 mg/kg. Deng and Jennings (2006) tested various Pb extraction methods on soils obtained from over 50 brownfield sites in the greater Cleveland, OH area. Comparison of twelve extraction methods for three samples produced a range of 1,780-2,636 mg/kg for one sample, 283-491 mg/kg for a second sample, and 273-499 mg/kg for a third sample. Verstraete and Van Meirvenne (2008) measured Pb in soils at a remediated brownfield site in Belgium and reported average Pb concentrations to be 188 mg/kg and 224 mg/kg in two sampling campaigns. Dermont et al. (2010) fractionated soil by particle size class and measured the Pb concentration in each. Pb concentrations by size bin were as follows: 125-250 μm: 1,132 mg/kg; 63-125 μm: 1,786 mg/kg; 38-63 μm: 1,712 mg/kg; 20-38 μm: 2,465 mg/kg; 0-20 μm: 3,596 mg/kg. Hence, the highest concentration was in the smallest soil particle fraction. Bulk Pb concentration over 0-250 μm particle sizes was 2,168 mg/kg.

Several studies explore the relationship between soil Pb concentration and land use. Laidlaw and Filippelli (2008) displayed data for Indianapolis, IN showing the Pb concentration at the soil surface 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 and 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 (Section 3.2.2.6) and Pb paint (Section 3.2.2.7).

In this paper, soil Pb concentrations were also shown to decrease with distance from roadways, but the levels were roughly four times higher in urban areas compared with suburban areas. This is also illustrated for urban scale Pb accumulation in New Orleans, LA in Figure 3-31. 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: Reprinted with permission of Elsevier Publishing, Mielke et al. (2007a)

Note: At the urban scale, Pb quantities are largest within the inner-city residential communities that surround the Central Business District where pavement and concrete cover the soil. Note the several orders of magnitude difference between the interior and the exterior areas of the city. Note that the number on each census tract indicates the number of blood Pb samples taken from that tract during the six years from which the study data were obtained.

Figure 3-31 Map of median Pb content in soil in New Orleans.

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. (2007b) 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 an area of Boston, MA with no large industrial sources of Pb 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% was attributed to historic Pb on-road gasoline emissions in a suburban area. The remainder of the Pb was attributed to paint degradation. However, the Clark et al. (2006) model assumed that the only two sources of soil Pb were paint and historic Pb on-road gasoline emissions. So, the percentages provided are upper limits of the contribution of those two sources. Additional discussion of historic sources of Pb is provided in Section 3.2.2.7. Isotope ratios for paint and gasoline references used in the Clark et al. (2006) study were obtained from Rabinowitz (1986).

Several studies have examined the effects of roadway attributes on Pb content in roadside dust. Yesilonis et al. (2008) measured metal content in surface soil samples (0-10 cm) at selected land parcels throughout Baltimore based on a stratified random sampling design that accounted for land use factors. They compared soil metals within 100 m buffers of roadways and outside those buffers and found that median soil Pb concentration inside the buffer was significantly higher than median soil Pb concentration outside the buffers (outside: 38.7 kg/ha; inside: 134 kg/ha; $p < 0.0001$). In an analysis of the relationship between land use parameters and Pb concentration in soil in Los Angeles, Wu et al. (2010) observed that soil Pb concentration was higher near freeways and major traffic arteries compared with other locations. The (square-root transformed) age of the building on a sampled land parcel, length of highway within a 1,000 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. However, Wu et al. (2010) acknowledged uncertainty in historical roadway and traffic count data, which introduces uncertainty into that conclusion. Study areas were classified as residential, commercial, park, and industrial (not specific to Pb emissions), although the authors were not able to distinguish the relative effects of each area on Pb content in roadside dust. Wu et al. (2010) reported that the highest median measured concentrations of Pb content in roadside dust were in residential freeway samples (112 mg/kg), followed by residential arterial samples (98 mg/kg), and industrial freeway samples (90 mg/kg). Additional sources of Pb to soil near roadways, such as traces of Pb in unleaded gasoline and Pb-containing wheel weights (described in Section 3.2.2.6) were not considered in this study. 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 mg/kg) and in the city center (225 mg/kg),

followed by demolition and construction sites (177 mg/kg) and near a harbor (100 mg/kg). Joshi et al. (2009) also observed Pb dust concentrations to be highest at industrial sites (260 mg/kg) followed by commercial sites (120 mg/kg) and residential sites (60 mg/kg) in Singapore.

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., 2006b; Weiss et al., 2006). Caravanos et al. (2006b) 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. (2005, 2003) 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. One month following demolition, Pb dust levels dropped by a factor of 45% for the street (48,000 µ/m^2), compared with post-demolition concentrations, 67% for alleys (21,000 µg/m^2), and 41% for sidewalks (14,000 µg/m^2). At another demolition site, smaller increases were observed: 29% for streets (29,000 µg/m^2), 18% for alleys (19,000 µg/m^2) and 18% for sidewalks (22,000 µg/m^2). No values were reported for the 1-month follow-up for the second site (Farfel et al., 2005).

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. 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.

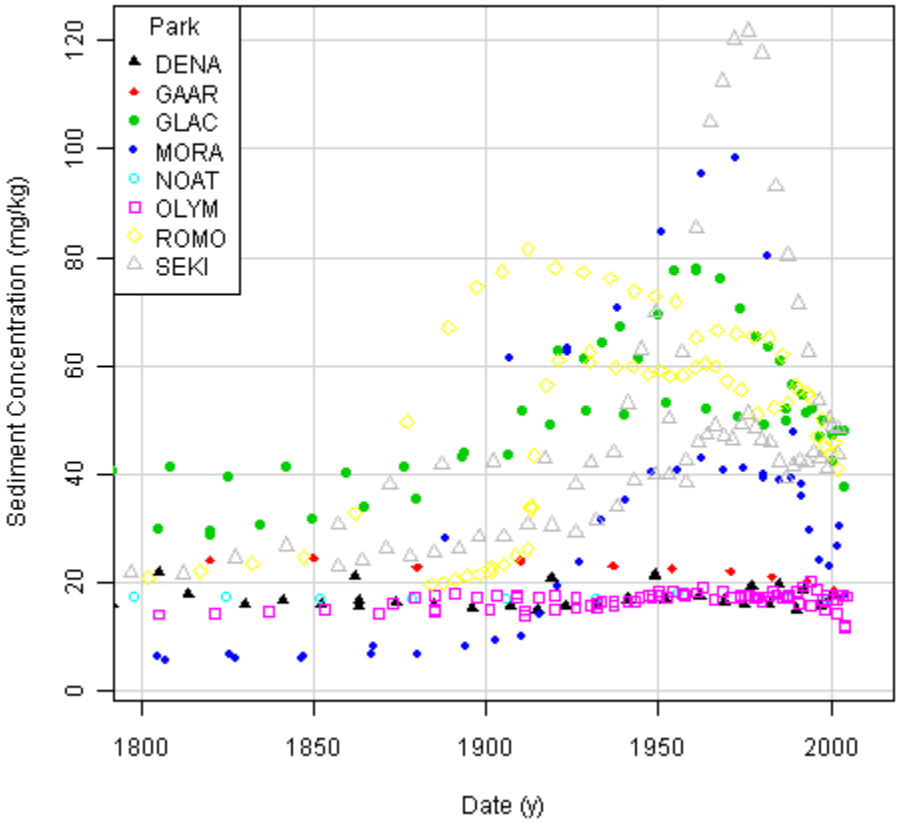
Pb can be elevated in soils located where ammunition is used for military or hunting purposes. In a study of Pb content in sand used to cover a firing range, Lewis et al. (2010) found that 93% of bullet mass was recovered in the top 0.3 m of the sand, and 6.4% was recovered at a depth of 0.3-0.45 m. 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.

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. Pb was measured in sediments, as well as snow, water, lichen, fish, and moose during the multiyear project, and although Pb was not measured in air as a part of this study, routine monitoring find particle Pb was monitored at IMPROVE sites in the majority of national parks included in the study.

Pb concentrations in sediments from all lakes in which Pb was measured in the conterminous 48 states exhibited higher Pb concentrations near the surface relative to 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, as shown in Figure 3-32. A clear decline in Pb concentrations in sediments after the discontinued use of leaded on-road gasoline was observed at almost all WACAP locations, of nearly all

WACAP sites in the western U.S. Sediment Pb concentrations averaged over the year in which they were obtained correlated moderately well with annual average Pb-TSP concentrations from the AQS with R = 0.63 for 1980-2004, in which WACAP data were available (Landers et al., 2008). Pb concentrations in sediments were much lower in Alaska, and no such decline was observed. Pb in sediments was mainly attributed to on-road gasoline use, but for some lakes a strong influence from other local sources of Pb to lake sediments was shown to be important, including Pb mining, smelting, logging, and other industrial activities. The reduction in sediment Pb concentrations shown in Figure 3-32 for recent years coincides with declines in air Pb concentrations following the phase-out of Pb anti-knock agents in gasoline and reductions of air Pb emissions from industrial activities. Elevated Pb deposition at the Glacier, Rocky Mountain, and Sequoia and Kings Canyon National Park and Preserve sites was thought by Landers et al. (2008) to reflect regional scale bioaccumulation of airborne contaminants in remote ecosystems in the Western U.S. Accumulation of contaminants was shown to vary geographically; Landers et al. (2008) lists potentially influential factors causing variation in Pb deposition including proximity to individual sources or source areas, primarily agriculture, mining, and smelting operations. This finding was counter to the original working hypothesis that most of the contaminants found in western parks would originate from eastern Europe and Asia.



Source: (Landers et al., 2008)

Note: (DENA = Denali, GAAR = Gates of the Arctic, GLAC = Glacier, MORA = Mount Ranier, NOAT = Noatak, OLYM = Olympic, ROMO = Rocky Mountain, SEKI = Sequoia and Kings Canyon)

Figure 3-32 WACAP data for Pb concentration in sediment at eight National Parks and/or Preserves.

In a survey of 35 reservoirs and lakes in 16 continental U.S. states, Van Metre et al. (2006) collected data from sediment cores extending back as far as the early 1800s, and up to 2001. For most locations, they were able to match at least three bodies of water in rural (designated as 'reference'), light urban, and dense urban settings. In reference bodies of water, the median sediment Pb concentration corresponding to the 1990s was 48 mg/kg. It was 56 mg/kg in sediments from light urban bodies, and 214 mg/kg in dense urban ones (Mahler et al., 2006). Using the most distant past sediment records, Mahler et al. (2006) provided approximations of concentrations attributable to anthropogenic inputs. The median of these values for the 1990s were 28, 22, and 194 mg/kg in the reference, light urban, and dense urban bodies of water, respectively.

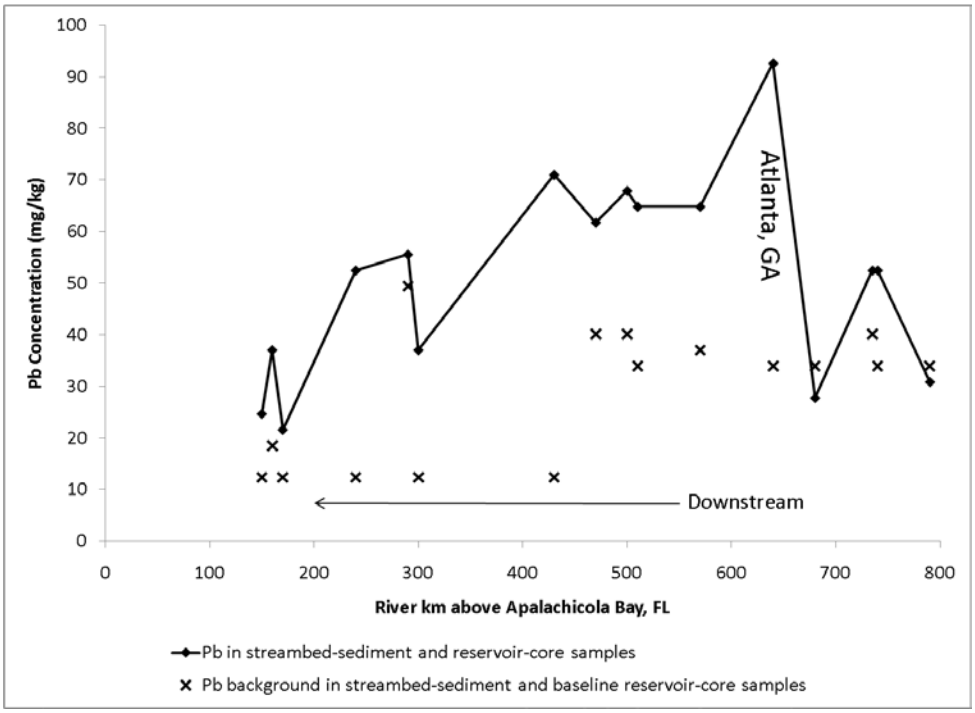
Data from select regions of the U.S. illustrate that Pb concentrations in surface waters and sediment are likely to be higher in urbanized areas compared with rural locations. Table 3-11 presents data from seven metropolitan areas (Cobb et al., 2006). Differences among the intraurban concentration ranges illustrate a high level of spatial variability within individual cities as well as high inter-urban variability. The rural New Orleans site reported relatively low Pb sediment concentrations, and the highest average Pb sediment concentrations were reported for the city of New Orleans. Figure 3-33 and Figure 3-34 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. Consistent with the WACAP trends shown in Figure 3-32, the data also illustrated that Pb concentrations in sediment have declined in the U.S. since 1975 (Figure 3-34), prior to the phase-out of on-road leaded gasoline.

Table 3-11 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	25-1,612
Mt. Pleasant, MI	320	100-840
New Orleans, LA	784	31.7-5,195
New Orleans, LA (rural outskirts)	11	4.8-17.3
St. Louis, MO	427	35-1,860
Syracuse, NY	80	20-800

[a]Dry weight basis.

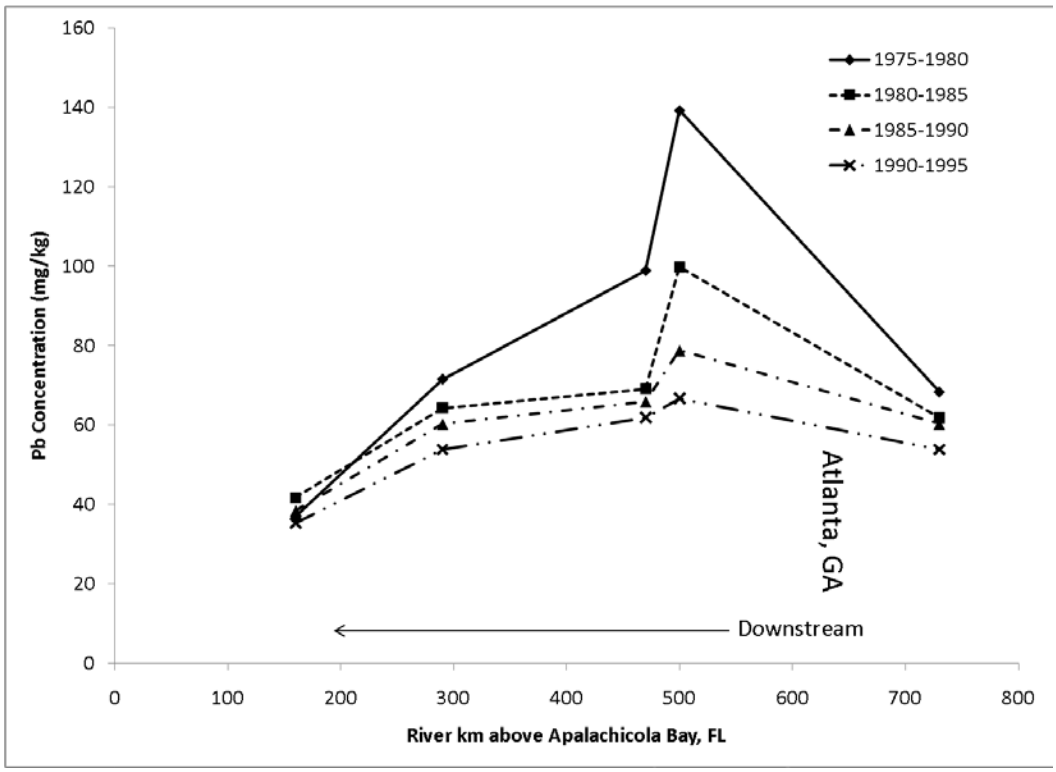
Source: Reprinted with permission of the American Chemical Society, (Cobb et al., 2006).



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

Note: The background refers to concentrations from undeveloped geographic regions and baseline samples are obtained from the bottom of the sediment core to minimize anthropogenic effects on the sample.

Figure 3-33 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.



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

Note: The background refers to concentrations from undeveloped geographic regions and baseline samples are obtained from the bottom of the sediment core to minimize anthropogenic effects on the sample.

Figure 3-34 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.

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

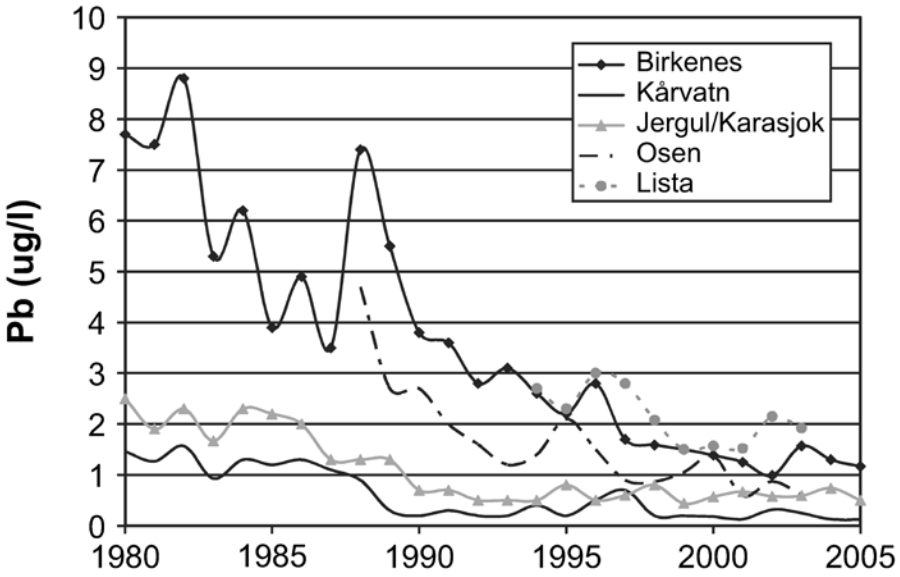
sediments (Abel et al., 2010; Abel et al., 2007; Schwab et al., 2007; Cobb et al., 2006; Presley et al., 2006). Shi et al. (2010) analyzed Lake Pontchartrain sediment samples using a factored approach and found that most Pb was sequestered in carbonate-rich, iron oxide-rich, and magnesium oxide-rich 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

The only network that provides a long-term record of precipitation chemistry across the U.S. is the National Trend Network operated by the National Atmospheric Deposition Program, a cooperative effort between EPA and other federal as well as state, tribal, and local government agencies, educational institutions, private companies, and non-governmental agencies. Precipitation is monitored for pH, conductance, and major cations and anions. A separate network, the Mercury Deposition Network, is operated by the same organization and monitors Hg concentrations in rain and a separate network. Neither of these networks includes Pb monitoring.

Recent results from locations outside the U.S. were consistent with decreasing rain water concentrations described in the 2006 Pb AQCD, reflecting the elimination of Pb from on-road gasoline in most countries. From the 2006 Pb AQCD (U.S. EPA, 2006b), volume weighted Pb concentrations in precipitation collected in 1993-94 from Lake Superior, Lake Michigan and Lake Erie ranged from ~0.7 to ~1.1 µg/L (Sweet et al., 1998). These values fit well with the temporal trend reported in Watmough and Dillon (2007), who calculated annual volume-weighted Pb concentrations to be 2.12, 1.17 and 0.58 µg/L for 1989-90, 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 and Krachler, 2010).

There have also been a few recently published, long-term European studies of Pb concentration in precipitation including Berg et al. (2008) and Farmer et al. (2010). Berg et al. (2008) compared the trends in Pb concentration in precipitation at Norwegian background sites in relation to the decreasing European emissions of Pb over the period 1980-2005. The Birkenes site at the southern tip of Norway is most affected by long-range transport of Pb from mainland Europe but there had been a 97% reduction in the concentration of Pb in precipitation over the 26-year time period. This was similar to the reductions of 95% and 92% found for the more northerly sites, Karvatn and Jergul/Karasjok, respectively (Figure 3-35). 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; Figure 3-35.



Source: Reprinted with permission of Pergamon Press, Berg et al. (2008)

Figure 3-35 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

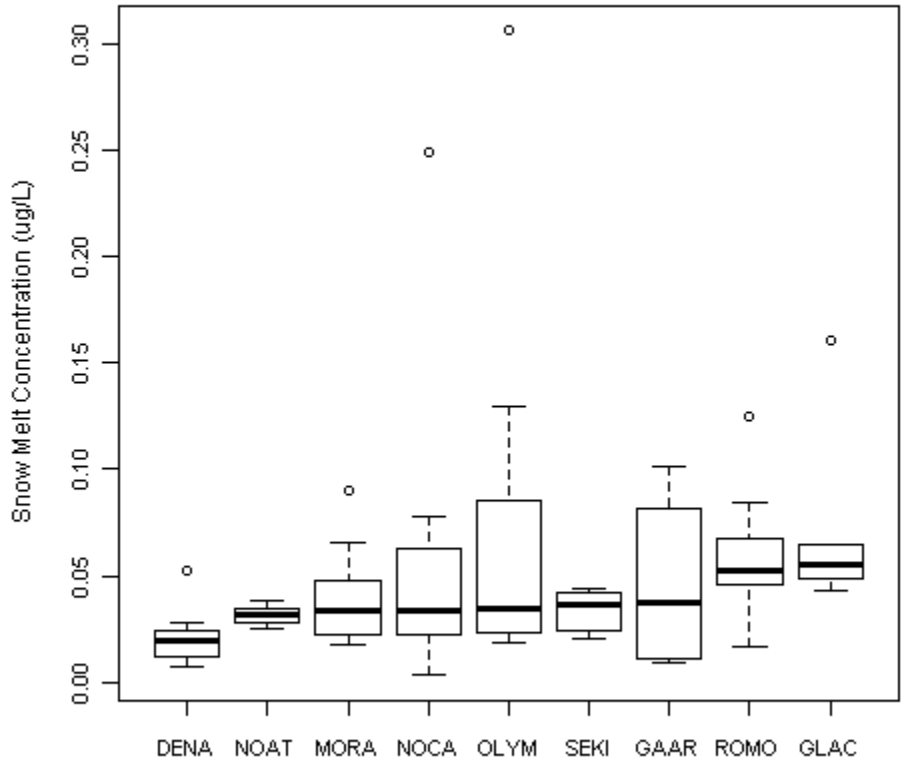
decline in mean concentration from 4.92 µg/L (1989-1991) to 1.88 µg/L (1999) and then to 0.63 µg/L (2006-2007) is qualitatively but not quantitatively in line with the sixfold decline in annual total U.K. emissions of Pb to the atmosphere over each of these time periods. Since the outright ban on the 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, 2006b), and similar to concentrations reported for those studies conducted after the removal of Pb from on-road gasoline. Overall, recent studies of wet deposition tended to confirm the conclusions of the 2006 Pb AQCD (U.S. EPA, 2006b) that wet deposition fluxes have greatly decreased since the removal of Pb from on-road gasoline.

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. Measurements of Pb in snowmelt during the WACAP study, showed that median Pb concentration ranged form 20-60 ng/L, with 95th percentile values ranging from 30-130 ng/L; Figure 3-36 (Landers et al., 2008). Measurements in WACAP of Hg and particulate carbon deposition onto snow were thought to reflect coal combustion, and Pb was not significantly correlated with Hg in snow samples of concentration or of calculated enrichment factors normalized to Al concentrations. Shotyk and Krachler (2009) reported considerably higher concentrations at two North American sites, Johnson and Parnell, in Ontario, Canada. Mean Pb concentration for contemporary snow was 672 (Johnson, n = 6; Parnell, n = 3) ng/L. Shotyk et al. (2010) presented additional values for Pb in contemporary snow samples 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.

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.



Source: (Landers et al., 2008)

(DENA = Denali, GAAR = Gates of the Arctic, GLAC = Glacier, MORA = Mount Ranier, NOCA = North Cascades, NOAT = Noatak, OLYM = Olympic, ROMO = Rocky Mountain, SEKI = Sequoia and Kings Canyon)

Figure 3-36 Box plots illustrating Pb concentration in snow melt at nine National Parks and Preserves.

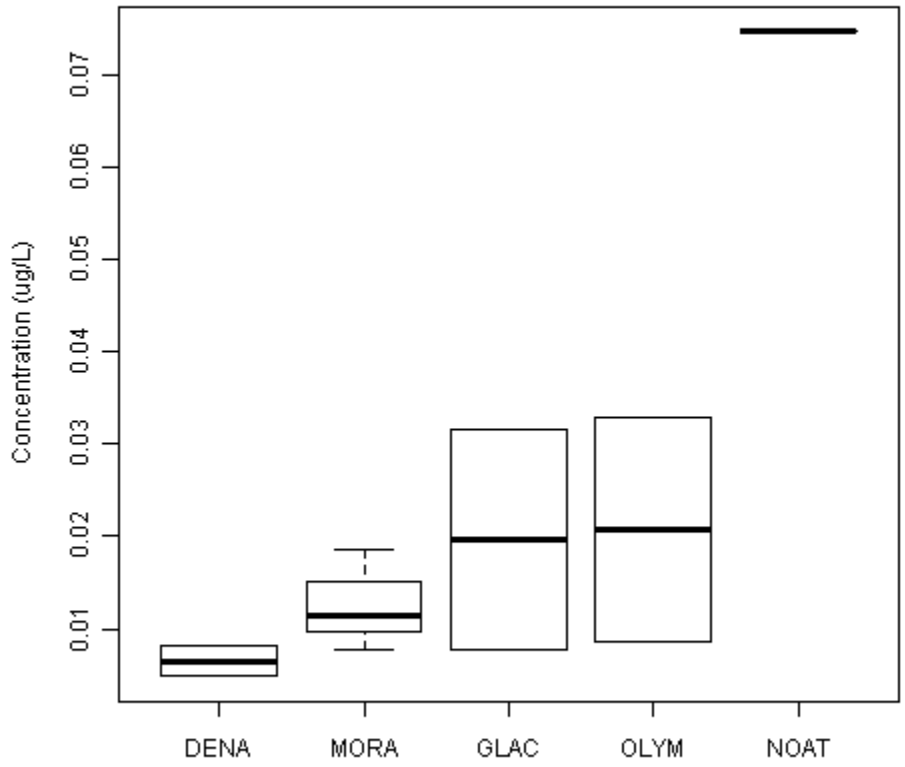
Lee et al. (2008b) collected 42 snow samples during the period autumn 2004-summer 2005 from a 2.1 m snow pit at a high-altitude site on the northeast slope of Mount Everest, Himalayas. Pb concentrations ranged from 5-530 pg/g with a mean value of 77 pg/g. The mean value is clearly higher than the Hur 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; Lee et al. (2008b)]. The mean Pb concentration for Mount Everest snow was lower during the monsoon (28 pg/g) compared with the non-monsoon periods (137 pg/g). From calculated enrichment factors (Pb/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. (2008b) noted that their conclusions

differ from those in Kang et al. (2007), who stated that anthropogenic contributions of Pb to Mount Everest snow were negligible because the Everest concentrations were similar to those in Antarctica. Kang et al. (2007) did not take account of the difference in accumulation rates at the two sites and had also used Pb concentrations for Antarctic snow from a study by Ikegawa et al. (1999). Lee et al. (2008b) suggested that these Pb concentrations were much higher than expected and that their snow samples may have suffered from contamination during sampling and analysis.

3.6.5 Natural Waters

Monitoring data for streams, rivers and lakes are summarized in periodic national assessments of surface waters are carried out periodically by EPA, and they include measurement of major biological and chemical stressors. Pb concentrations in natural waters also may reflect deposition of Pb even in remote locations. WACAP data at five National Parks and Preserves show median Pb concentrations in surface waters to range from 6 to 75 ng/L (Landers et al., 2008); Figure 3-37. Four sites (Denali, Mt. Ranier, Glacier, and Olympic National Parks) were in the lower range of 6 to 20 ng/L. One site (Noatak) reported a single value of 75 ng/L. With the exception of the Noatak site, the WACAP values were in line with measurements by Shotyk and Krachler (2007) of Pb concentrations in six artesian flows in Simcoe County, near Elmvale, Ontario, Canada. The values ranged from 0.9 to 18 ng/L with a median (n = 18) of 5.1 ng/L. These are comparable with reports of a range of 0.3-8 ng/L for Lake Superior water samples (Field and Sherrell, 2003). Shotyk and Krachler (2007) also commented that such low concentrations for ground and surface waters are not significantly different from those (5.1 ± 1.4 ng/L) reported for Arctic ice from Devon Island, Canada, dating from 4,000-6,000 years ago. In a separate study, Shotyk and Krachler (2009) reported concentrations of Pb in groundwater (from two locations, Johnson and Parnell), surface water (Kawagama Lake) and contemporary snow (Johnson and Parnell, as described in Section 3.6.4). The lowest mean dissolved Pb concentrations were found for groundwater: 5.9 (Johnson, n = 11) and 3.4 (Parnell, n = 12) ng/L. For lake water the mean Pb concentration was 57 (Kawagama Lake, n = 12) ng/L. The extremely low concentrations of Pb in the groundwaters were attributed to natural removal processes. Specifically, at the sampling location in Canada, there is an abundance of clay minerals with high surface area and high cation exchange capacity and these, combined with the elevated pH values (pH=8.0) resulting from flow through a terrain rich in limestone and dolostone, provide optimal circumstances for the removal of trace elements such as Pb from groundwater. Although such removal mechanisms have not been demonstrated, the vast difference between Pb concentration in snow and that in the groundwaters indicate

that the removal process is very effective. Shotyk and Krachler (2010) speculate that even at these very low Pb concentrations, much if not most of the Pb is likely to be colloidal, as suggested by the 2006 Pb AQCD (U.S. EPA, 2006b). Finally, Shotyk et al. (2010) suggest that the pristine groundwaters from Simcoe County, Canada, provide a useful reference level against which other water samples can be compared.



Source: (Landers et al., 2008)
Note: (DENA = Denali, GLAC = Glacier, MORA = Mount Ranier, NOAT = Noatak, OLYM = Olympic)

Figure 3-37 Boxplots of Pb concentration in surface waters measured at five National Parks and Preserves.

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 inconsistent with those expected for natural Pb (the clay fraction from the lake

sediments dating from the pre-industrial period had values of 1.19-1.21) and it was concluded that most of the dissolved Pb in the lake water was of industrial origin. Shotyk and Krachler (2010) found that the full range of isotope ratios for Kawagama Lake water samples (Ontario, Canada) was 1.09 to 1.15; this was not only much lower than the stream water values entering the lake but also lower than the values attributed to leaded on-road gasoline in Canada (~1.15). The streamwater ratio values were ~1.16 to 1.17, while those for rainwater were as low as 1.09; in good agreement with the lower lake water values. This means that there must be an additional atmospheric source of Pb, which has a lower $^{206}Pb/^{207}Pb$ ratio than leaded on-road gasoline. Supporting evidence came from contemporary samples such as near surface peat, rainwater and snow, all of which confirmed a trend away from natural Pb (1.191 to 1.201) to lower $^{206}Pb/^{207}Pb$ ratios. The local smelting activities (Sudbury) were unlikely to be the source of anthropogenic Pb as Sudbury-derived emissions exhibit a typical $^{206}Pb/^{207}Pb$ ratio of ~1.15, similar to leaded on-road gasoline. Instead, it was suggested that long-range transport of Pb from the smelter at Rouyn-Noranda (known as the "Capital of Metal," NW Quebec) may still be impacting on Kawagama Lake but no Pb isotope data was quoted to support this supposition. Several studies, summarized in Mager (2012), reported Pb concentrations in matched reference and mining-disturbed streams in Missouri and the Western U.S. They are summarized in Table 3-12.

The range of Pb levels in saltwater are available from several studies although the values are not specific to the U.S. A range of 0.005-0.4 µg Pb/L for salt water was reported by Leland and Kuwabara (1985) and 0.01 to 27 µg Pb/L by Sadiq (1992). In general, Pb in seawater is higher in coastal areas and estuaries since these locations are closer to sources of Pb contamination and loading from terrestrial systems (U.S. EPA, 2008b).

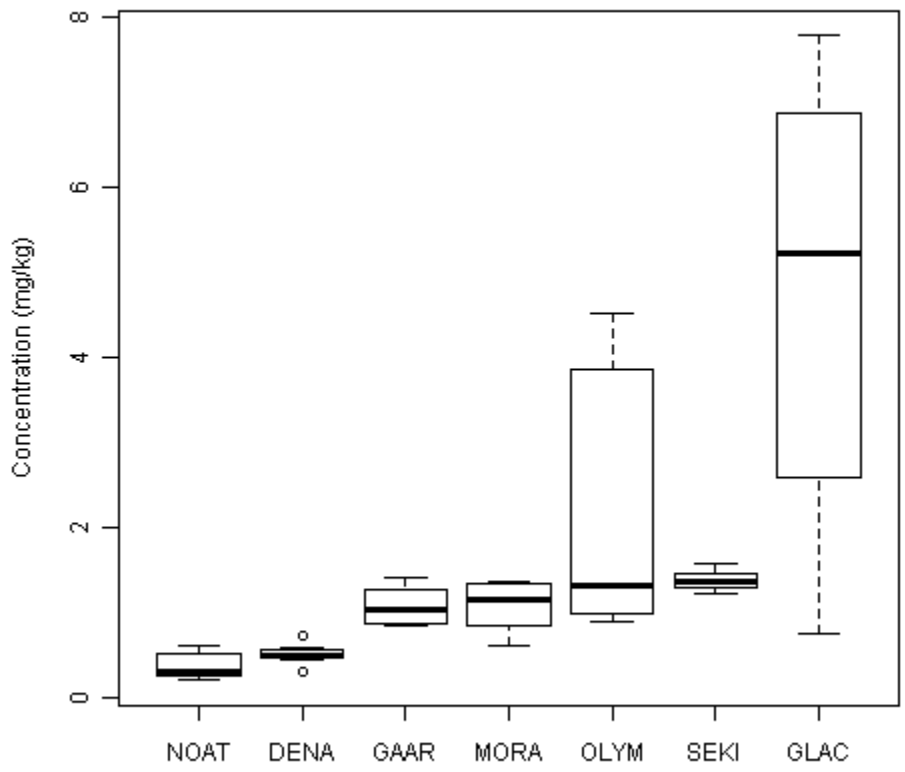
Table 3-12 Pb concentrations from stream food webs in mining-disturbed areas of Missouri and the western United States

Area	Total Pb in water (µg/L)	Dissolved Pb (µg/L)
Animas River, CO (Besser et al., 2001):		
Reference Streams	<1.8	<0.2
Mining-disturbed areas	0.9–8.6	<0.1–6.9
Boulder River, MT (Farag et al., 2007):		
Reference Streams	0.4 (colloidal)	0.3–0.4
Mining-disturbed areas	0.1–44	0.1–2
Coeur d'Alene River, ID (Clark, 2003; Farag et al., 1998):		
Reference Streams	2–20	0.01–2
Mining-disturbed areas	6–2000	2–50
New Lead Belt, MO (Besser et al., 2007; Brumbaugh et al., 2007):		
Reference Streams	.	<0.01-1.6
Mining-disturbed areas	.	0.02–1.7

Adapted from: Mager (2012)

3.6.6 Vegetation

The 2006 Pb AQCD (U.S. EPA, 2006b) presented data on Pb in vegetation. The main conclusions were that Pb uptake was strongly affected by pH, and acidic soils are most likely to have Pb in solution for absorption by plants. Additionally, the 2006 Pb AQCD (U.S. EPA, 2006b) states that most Pb stored within vegetation is stored in roots rather than fruits or shoots. Recent measurements from the WACAP study (Landers et al., 2008) have shown some Pb storage in lichens. Median Pb concentrations ranged from 0.3 mg/kg in Noatak National Park to 5 mg/kg in Glacier National Park, with substantial variation in the Glacier and Olympic National Park samples; Figure 3-38. Landers et al. (2008) state that lichen Pb concentrations have decreased substantially from the 1980s and that metal concentrations were within background levels for these remote Western sites.



Source: (Landers et al., 2008)

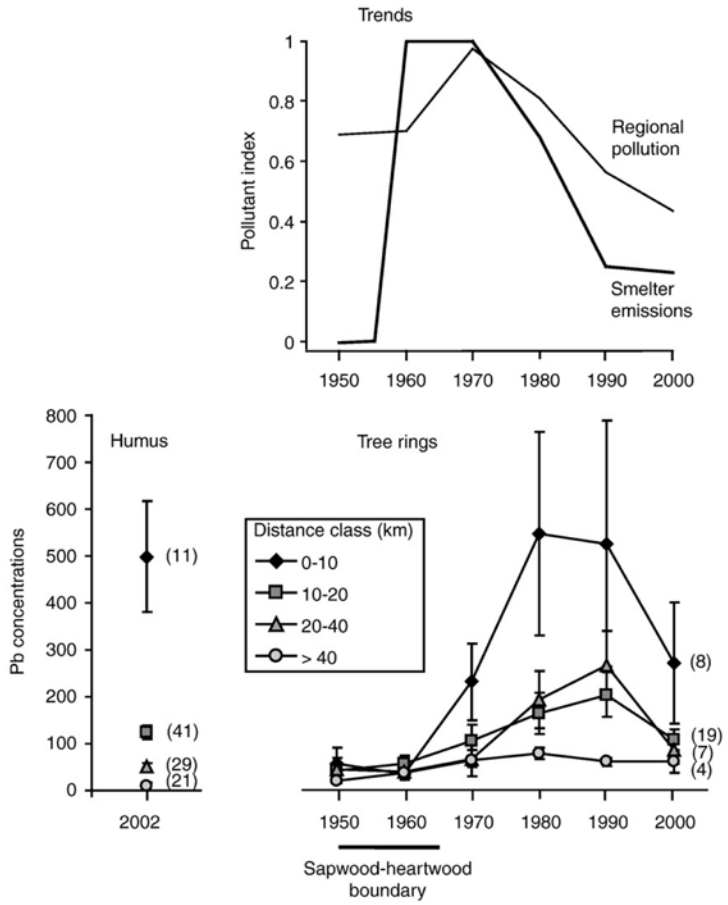
Note: (DENA = Denali, GAAR = Gates of the Arctic, GLAC = Glacier, MORA = Mount Ranier, NOAT = Noatak, OLYM = Olympic, SEKI = Sequoia and Kings Canyon)

Figure 3-38 Boxplots of Pb concentration in lichen measured at seven National Parks and Preserves.

Mosses can be used effectively for monitoring trends in Pb deposition as demonstrated in many studies (Harmens et al., 2010; Harmens et al., 2008). For example, Harmens et al. (2008) showed that a 52% decrease in deposited Pb concentrations corresponded to a 57% decrease in Pb concentrations in moss. Farmer et al. (2010) showed that there was good agreement between the $^{206}Pb/^{207}Pb$ ratio for precipitation and mosses collected in northeast Scotland. A study in the Vosges mountains 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].

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. (2008b) observed that the decline in Pb concentrations in the outer level of tree rings corresponded with the decline in Cu smelter emissions in Gaspé Peninsula in Canada; Figure 3-39. Both Pb concentrations and Pb isotope ratios declined with distance from the smelter (Aznar et al., 2008a; Aznar et al., 2008b).



Source: Reprinted with permission of Elsevier Publishing, Aznar et al. (2008b)

Figure 3-39 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.

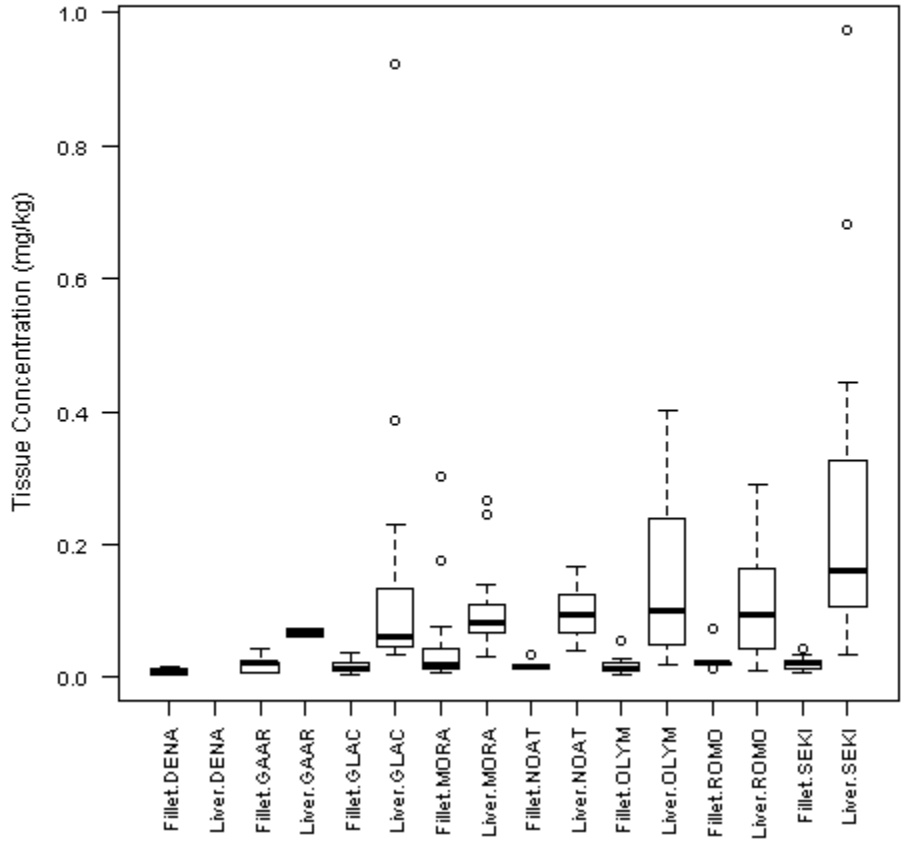
3.6.7 Aquatic Bivalves

Data from invertebrate waterborne populations can serve as in indicator of Pb contamination because animals such as mussels and oysters take in contaminants during filter feeding. Kimbrough et al. (2008) surveyed Pb concentrations in mussels, zebra mussels, and oysters along the coastlines of the continental U.S. In general, they observed the highest concentrations of Pb in the vicinity of urban and industrial areas. Company et al. (2008) measured Pb concentrations and Pb isotope ratios in bivalves along the Guadiana River separating Spain and Portugal. Analysis of Pb isotope ratio data suggested that high Pb concentrations were related to historical mining activities in the region. Elevated Pb concentrations were also observed by Company et al. (2008) in the vicinity of more populated areas. Couture et al. (2010) report data from a survey of the

isotopic ratios of Pb in *Mytilus edulis* blue mussel, collected off the coast of France from 1985-2005. The results indicated that the likely source of Pb in mussel tissue is from resuspension of contaminated sediments enriched with Pb runoff from wastewater treatment plants, municipal waste incinerators, smelters and refineries rather than from atmospheric deposition (Couture et al., 2010).

3.6.8 Vertebrate Populations

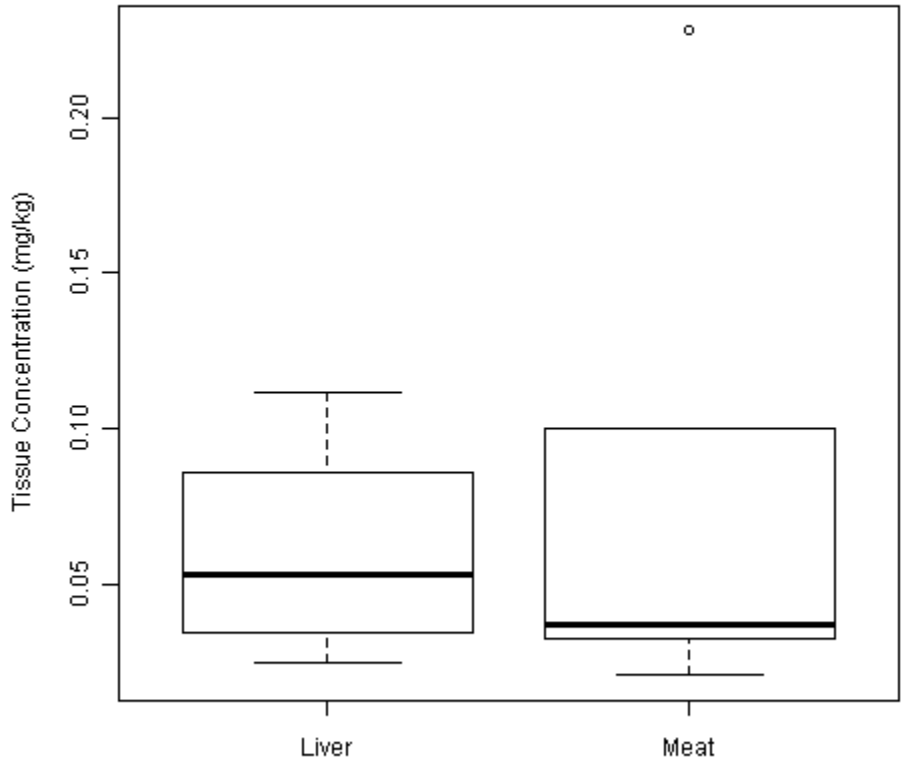
Pb concentrations in fish fillet and liver were measured through the WACAP study in eight National Parks and Preserves (Landers et al., 2008). For fish fillet, Pb concentrations ranged from 0.0033-0.30 mg/kg, with a median of 0.016 mg/kg. Liver stores were several times higher, with Pb concentrations ranging from 0.011-0.97 mg/kg and a median of 0.096 mg/kg. Pb concentrations in moose meat and liver were also measured at the Denali National Park and Preserve as part of WACAP (Landers et al., 2008). Moose meat Pb concentrations ranged from 0.021-0.23 mg/kg with a median of 0.037 mg/kg. Pb concentrations in moose liver ranged from 0.025-0.11 mg/kg with a median of 0.053 mg/kg. Boxplots of measured Pb concentrations in fish fillet and liver are shown in Figure 3-40, and boxplots of measured Pb concentrations for moose meat and liver are shown in Figure 3-41. For fish and meat tissues, median and maximum Pb concentrations were substantially lower than values reported in the 2006 Pb AQCD (U.S. EPA, 2006b). Still, the WACAP findings suggest some Pb accumulation in fish and moose in these remote locations.



(DENA = Denali, GAAR = Gates of the Arctic, GLAC = Glacier, MORA = Mount Ranier, NOAT = Noatak, OLYM = Olympic, ROMO = Rocky Mountain, SEKI = Sequoia and Kings Canyon)

Source: (Landers et al., 2008)

Figure 3-40 Boxplots of Pb concentration in fish fillet and liver measured at eight National Parks and Preserves.



Source: (Landers et al., 2008)

Figure 3-41 Boxplots of Pb concentration in moose meat and liver measured at Denali National Park and Preserve.

3.7 Summary and Conculusions

3.7.1 Sources of Atmospheric Lead

The 2006 Pb AQCD (U.S. EPA, 2006b) documented the decline in ambient air Pb emissions following the ban on alkyl-Pb additives for on-road gasoline. Pb emissions declined by 98% from 1970 to 1995 and then by an additional 76% from 1995 to 2008, at which time national Pb emissions were 970 tons/year. As was the case for the 2008 NAAQS review, piston-engine aircraft emissions currently comprise the largest share (56%) of total atmospheric Pb emissions nationally; the 2008 NEI (U.S. EPA, 2011a) estimated that 550 tons of Pb were emitted from piston-engine aircraft. Other sources of

ambient air Pb, in approximate order of importance with regard to national totals, include metals processing, fossil fuel combustion, other industrial sources, roadway related sources, and historic Pb.

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. Atmospheric Pb deposition peaked in the 1970s, followed by a decline. 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. Deposition is not an ultimate sink for Pb because particles are potentially resuspended and redeposited many times before reaching a site where further transport is unlikely, especially for dry deposition. Resuspension modeling has suggested that larger particles (10-100 μm) become more readily resuspended compared with smaller particles, because smaller particles must overcome stronger adhesion forces to be resuspended by air movement and lift forces are proportional to particle size to the approximate power of 1.5.

In water, Pb is transported as free ions, soluble chelates, or on surfaces of iron and organic rich colloids. 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 binding to sulfides is a particularly important process that affects Pb bioavailability. Pb is relatively stable in sediments, with long residence times and limited mobility. However, Pb-containing sediment particles can be remobilized into the water column. Resuspended Pb is largely

associated with OM or 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 to the water column than atmospheric deposition. Resuspension of sediments largely occurs during discrete events related to storms.

A complex variety of factors influence Pb retention in soil, including hydraulic conductivity, solid composition, OM content, clay mineral content, microbial activity, plant root channels, animal holes, geochemical reactions, colloid amounts, colloidal surface charge, and pH. Leaf litter can be an important temporary sink for metals from the soil around and below leaves, and decomposition of leaf litter can reintroduce substantial amounts of Pb into soil "hot spots," where re-adsorption of Pb is favored. A small fraction of Pb in soil is present as the free Pb^{2+} ion. The fraction of Pb in this form is strongly dependent on soil pH.

In summary, environmental distribution of Pb occurs mainly through the atmosphere, from where it is deposited into surface waters and soil. Pb associated with coarse PM deposits to a great extent near sources, while fine Pb-PM can be transported long distances. Surface waters act as an important reservoir, with half-lives of Pb in the water column largely controlled by rates of deposition to and resuspension from bottom sediments. Pb retention in soil depends on Pb speciation and a variety of factors intrinsic to the soil.

3.7.3 Ambient Lead Monitoring

Since the publication of the 2006 Pb AQCD for Pb (U.S. EPA, 2006b) there has been little progress in the state of the science regarding monitoring technology and monitor siting criteria for representation of population exposures to airborne Pb and Pb of atmospheric origin. Our understanding of sampling errors in existing measurements, our understanding of possible alternatives to existing Pb-TSP sampling technology, and our understanding of particle size ranges of Pb particles occurring in different types of locations have changed little in that time.

The current Pb monitoring network design requirements include two types of monitoring sites: source-oriented and non-source-oriented. Source-oriented monitoring sites are required near sources of air Pb emissions which are expected to or have been shown to contribute to ambient air Pb concentrations in excess of the NAAQS. With the December 2010 completion of action on regulatorily required Pb monitoring, one-year of Pb-TSP monitoring is also required near 15 specific airports to gather additional information on the likelihood of NAAQS exceedances near airports due to combustion of leaded aviation

gasoline. Non-source-oriented monitoring is also required at NCore sites in CBSAs with a population of at least 500,000.

In addition to Pb-TSP monitoring for the purposes of judging attainment with the NAAQS, Pb is also routinely measured in smaller PM fractions in the CSN, IMPROVE, and the NATTS networks. While monitoring in multiple networks provides extensive geographic coverage, measurements between networks are not directly comparable in all cases because different PM size ranges are sampled in different networks. Depending on monitoring network, Pb is monitored in TSP, PM_{10}, or $PM_{2.5}$ using high-volume or low-volume samplers.

3.7.4 Ambient Air Lead Concentrations

Ambient air Pb concentrations have declined drastically over the period 1980-2010. The median annual concentrations for all monitors have dropped by 97% from 0.87 $\mu g/m^3$ in 1980 to 0.03 $\mu g/m^3$ in 2010. While the sharpest drop in Pb concentration occurred during 1980-1990, a declining trend was observed between 1990 and 2010. Slightly smaller reductions were observable among source-oriented Pb concentration (83%) and non-source-oriented Pb data (91%) for 2000-2010. For source-oriented monitoring over the period 2008-2010, the 3-month rolling average was measured to be above the level of the NAAQS in twenty 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 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.

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. Since the phase-out of Pb from on-road gasoline, concentrations in these media have decreased to varying degrees. In rain, snowpack, and surface waters, Pb concentrations have decreased considerably. Declining Pb concentrations in tree foliage, trunk sections, and grasses have also been observed. In contrast, Pb is retained in soils and sediments, where it provides a historical record of deposition and associated ambient concentrations. In remote lakes, sediment profiles indicate higher Pb concentrations in near surface sediment as compared to pre-industrial era sediment from greater depth and indicate peak concentrations between 1960 and 1980, when leaded on-road gasoline was at peak use. Concentrations of Pb in moss, lichens, peat, and aquatic bivalves have been used to understand spatial and temporal distribution patterns of air Pb concentrations. Ingestion and water intake are the major routes of Pb exposure for aquatic organisms, and food, drinking water, and inhalation are major routes of exposure for livestock and terrestrial wildlife. Overall, Pb concentrations have decreased substantially in media through which Pb is rapidly transported, such as air and water. Substantial Pb remains in soil and sediment sinks. In areas less affected by major local sources, the highest concentrations are below the surface layers and reflect the previous use of Pb in on-road gasoline and emissions reductions from other sources.

3.8 Chapter 3 Appendix (Supplemental Material)

3.8.1 Variability across the U.S.

Table 3-13 Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010

Year	Sea-son	State/ County	Stat	County name	Site ID	N mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics																			
2008-2010						2,318		0.202	0.000	0.003	0.006	0.010	0.029	0.063	0.217	0.578	0.856	1.576	4.440
2008						548		0.318	0.004	0.004	0.013	0.024	0.050	0.110	0.348	0.841	1.240	2.557	4.440
2009						629		0.212	0.002	0.004	0.008	0.013	0.038	0.084	0.256	0.611	0.856	1.357	2.438
2010						1141		0.141	0.000	0.002	0.005	0.008	0.018	0.045	0.136	0.408	0.625	1.233	1.828
	Winter					554		0.202	0.000	0.002	0.006	0.008	0.026	0.055	0.184	0.502	0.883	2.438	3.103
	Spring					579		0.239	0.000	0.003	0.007	0.012	0.034	0.070	0.272	0.738	0.977	1.905	3.123
	Summer					601		0.186	0.001	0.003	0.006	0.010	0.030	0.066	0.212	0.559	0.755	1.233	4.440
	Fall					584		0.184	0.000	0.004	0.007	0.011	0.026	0.064	0.206	0.505	0.758	1.178	4.225
Nationwide statistics, pooled by site																			
2008-2010							111	0.161	0.002	0.003	0.008	0.013	0.031	0.056	0.177	0.441	0.687	0.997	1.275
2008							47	0.323	0.007	0.007	0.022	0.028	0.055	0.148	0.419	0.890	1.205	1.540	1.540
2009							54	0.214	0.007	0.007	0.013	0.018	0.043	0.090	0.343	0.669	0.849	0.921	0.921
2010							101	0.140	0.002	0.003	0.005	0.013	0.030	0.052	0.165	0.392	0.586	0.888	1.185
	Winter						108	0.156	0.000	0.003	0.006	0.009	0.021	0.048	0.160	0.475	0.879	1.130	1.488
	Spring						110	0.185	0.002	0.002	0.010	0.015	0.027	0.057	0.210	0.568	0.921	1.189	1.548
	Summer						111	0.148	0.002	0.003	0.006	0.012	0.025	0.050	0.153	0.430	0.696	0.882	1.031
	Fall						110	0.152	0.002	0.004	0.009	0.013	0.034	0.062	0.168	0.421	0.616	1.081	1.189
Statistics for individual counties (2008-2010)																			
		04013	AZ	Maricopa		6	1	0.0218	0.009	0.009	0.009	0.009	0.014	0.021	0.028	0.038	0.038	0.038	0.038
		06025	CA	Imperial		33	1	0.016	0.004	0.004	0.006	0.009	0.011	0.015	0.019	0.025	0.032	0.035	0.035
		06037	CA	Los Angeles		224	8	0.0098	0.000	0.000	0.000	0.002	0.006	0.010	0.012	0.017	0.020	0.038	0.044
		06065	CA	Riverside		72	2	0.0077	0.000	0.000	0.003	0.004	0.006	0.008	0.010	0.010	0.012	0.014	0.014
		06071	CA	San Bern-ardino		71	2	0.0091	0.001	0.001	0.003	0.004	0.007	0.010	0.012	0.014	0.014	0.022	0.022
		08005	CO	Arapahoe		9	1	0.0120	0.004	0.004	0.004	0.004	0.007	0.012	0.016	0.018	0.018	0.018	0.018
		08031	CO	Denver		12	1	0.006	0.003	0.003	0.003	0.004	0.005	0.005	0.006	0.008	0.008	0.008	0.008
		13089	GA	DeKalb		10	1	0.003	0.002	0.002	0.002	0.002	0.003	0.003	0.004	0.005	0.006	0.006	0.006
		17031	IL	Cook		288	8	0.020	0.010	0.010	0.010	0.010	0.012	0.016	0.025	0.034	0.040	0.060	0.070
		17117	IL	Macoupin		24	1	0.0101	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.012
		17119	IL	Madison		36	1	0.019	0.010	0.010	0.010	0.010	0.012	0.016	0.020	0.032	0.053	0.066	0.066
		17143	IL	Peoria		36	1	0.011	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.013	0.013	0.014	0.014
		17163	IL	Saint Clair		36	1	0.0206	0.010	0.010	0.010	0.012	0.014	0.018	0.026	0.032	0.038	0.054	0.054
		18089	IN	Lake		36	1	0.015	0.005	0.005	0.005	0.005	0.008	0.014	0.019	0.030	0.033	0.049	0.049
		18097	IN	Marion		35	1	0.006	0.002	0.002	0.002	0.003	0.004	0.005	0.008	0.010	0.012	0.013	0.013
		18163	IN	Vander-burgh		33	2	0.0045	0.001	0.001	0.001	0.002	0.003	0.004	0.005	0.006	0.010	0.010	0.010

Year	Season	State/ County	Stat	County name	Site ID	N mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
		25025	MA	Suffolk		31	2	0.009	0.004	0.004	0.004	0.005	0.007	0.008	0.010	0.013	0.016	0.020	0.020
		26081	MI	Kent		12	1	0.005	0.003	0.003	0.003	0.003	0.005	0.005	0.006	0.008	0.008	0.008	0.008
		26163	MI	Wayne		36	2	0.011	0.003	0.003	0.003	0.004	0.005	0.009	0.015	0.021	0.023	0.032	0.032
		27017	MN	Carlton		12	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		27037	MN	Dakota		118	5	0.004	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.008	0.010	0.017	0.036
		27053	MN	Hennepin		126	4	0.0032	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.006	0.008	0.010	0.044
		27075	MN	Lake		10	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		27123	MN	Ramsey		71	3	0.006	0.000	0.000	0.000	0.000	0.002	0.004	0.008	0.013	0.020	0.028	0.028
		27137	MN	Saint Louis		72	2	0.0015	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.006	0.010	0.010
		27163	MN	Wash-ington		72	3	0.0016	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.004	0.005	0.006	0.006
		29097	MO	Jasper		12	1	0.013	0.007	0.007	0.007	0.007	0.009	0.012	0.017	0.018	0.019	0.019	0.019
		29187	MO	Saint Francois		24	2	0.0327	0.008	0.008	0.009	0.009	0.018	0.032	0.039	0.054	0.080	0.089	0.089
		29189	MO	Saint Louis		33	1	0.0230	0.005	0.005	0.005	0.005	0.006	0.008	0.050	0.050	0.050	0.066	0.066
		36047	NY	Kings		24	1	0.013	0.010	0.010	0.010	0.010	0.011	0.012	0.014	0.018	0.020	0.020	0.020
		39017	OH	Butler		34	1	0.006	0.002	0.002	0.003	0.004	0.004	0.005	0.007	0.008	0.009	0.009	0.009
		39029	OH	Columbia na		107	3	0.0155	0.004	0.004	0.006	0.007	0.008	0.011	0.018	0.027	0.034	0.065	0.136
		39035	OH	Cuyahoga		107	3	0.0143	0.004	0.004	0.006	0.007	0.009	0.012	0.017	0.024	0.030	0.041	0.041
		39049	OH	Franklin		36	1	0.009	0.004	0.004	0.005	0.005	0.007	0.009	0.011	0.013	0.014	0.016	0.016
		39143	OH	Sandusky		12	1	0.0048	0.003	0.003	0.003	0.003	0.004	0.005	0.006	0.006	0.007	0.007	0.007
		39167	OH	Washingt on		54	2	0.0048	0.002	0.002	0.002	0.003	0.003	0.005	0.006	0.007	0.008	0.010	0.010
		40115	OK	Ottawa		16	2	0.012	0.003	0.003	0.003	0.005	0.006	0.013	0.017	0.021	0.025	0.025	0.025
		42003	PA	Allegheny		36	1	0.0105	0.000	0.000	0.000	0.000	0.004	0.009	0.015	0.019	0.024	0.053	0.053
		42021	PA	Cambria		23	1	0.046	0.040	0.040	0.040	0.040	0.040	0.040	0.044	0.054	0.058	0.128	0.128
		42045	PA	Delaware		20	1	0.0432	0.040	0.040	0.040	0.040	0.040	0.043	0.046	0.047	0.048	0.048	0.048
		42101	PA	Philadelp hia		24	1	0.0210	0.011	0.011	0.011	0.012	0.014	0.020	0.027	0.033	0.033	0.039	0.039
		42129	PA	Westmore land		24	1	0.0419	0.037	0.037	0.040	0.040	0.040	0.040	0.042	0.050	0.050	0.053	0.053
		48061	TX	Cameron		35	1	0.0041	0.002	0.002	0.003	0.003	0.003	0.004	0.005	0.006	0.007	0.009	0.009
		48141	TX	El Paso		68	3	0.021	0.014	0.014	0.014	0.014	0.015	0.017	0.019	0.029	0.056	0.087	0.087
		48201	TX	Harris		32	1	0.005	0.003	0.003	0.003	0.004	0.004	0.005	0.006	0.007	0.008	0.010	0.010
		48479	TX	Webb		29	1	0.013	0.004	0.004	0.005	0.006	0.008	0.011	0.018	0.026	0.028	0.035	0.035
		49035	UT	Salt Lake		12	1	0.0173	0.003	0.003	0.003	0.006	0.009	0.011	0.024	0.040	0.043	0.043	0.043
		51087	VA	Henrico		7	1	0.007	0.003	0.003	0.003	0.003	0.003	0.004	0.005	0.024	0.024	0.024	0.024
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2008-2010)																			
					11090003	32		0.525	0.054	0.054	0.083	0.164	0.252	0.402	0.798	1.053	1.117	1.277	1.277
					060371405	36		0.671	0.100	0.100	0.188	0.235	0.285	0.359	0.771	2.086	2.501	2.880	2.880
					290930016	36		0.670	0.166	0.166	0.186	0.219	0.330	0.466	0.726	0.974	2.435	4.225	4.225
					290930021	36		0.681	0.082	0.082	0.084	0.095	0.194	0.650	0.879	1.437	2.438	2.557	2.557
					290990004	36		0.997	0.256	0.256	0.307	0.408	0.598	0.918	1.236	1.690	1.905	2.416	2.416
					290990015	21		1.275	0.340	0.340	0.421	0.646	0.756	1.118	1.349	2.440	3.103	3.123	3.123
					290990020[a]	31		0.687	0.191	0.191	0.195	0.297	0.368	0.620	0.808	1.111	1.280	2.220	2.220
					290990021[a]	21		0.719	0.084	0.084	0.141	0.359	0.572	0.666	0.876	1.164	1.168	1.553	1.553
					290990022[a]	31		0.441	0.140	0.140	0.171	0.208	0.303	0.409	0.599	0.683	0.754	0.861	0.861
					290999001[a]	24		0.850	0.186	0.186	0.208	0.319	0.449	0.845	1.071	1.382	1.558	1.623	1.623
					290999005[a]	24		0.986	0.155	0.155	0.250	0.330	0.558	0.864	1.487	1.802	1.828	1.985	1.985

Year	Sea-son	State/ County	Stat	County name	Site ID	N mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
					480850009[a]	36		0.601	0.137	0.137	0.138	0.185	0.420	0.579	0.757	1.101	1.178	1.564	1.564

[a]Sites listed in the bottom six rows of the table fall in the upper 90th percentile of the data pooled by site.

Table 3-14 Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010

Year	Season	State/ County	State	County name	Site ID	N mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics																			
2008-2010						2290		0.0120	0.000	0.000	0.000	0.002	0.004	0.010	0.015	0.026	0.040	0.052	0.136
2008						685		0.0126	0.000	0.000	0.000	0.002	0.005	0.010	0.015	0.029	0.040	0.052	0.066
2009						768		0.0114	0.000	0.000	0.000	0.002	0.004	0.010	0.014	0.023	0.040	0.048	0.128
2010						837		0.0120	0.000	0.000	0.000	0.000	0.004	0.009	0.016	0.026	0.036	0.054	0.136
	Winter					556		0.0109	0.000	0.000	0.000	0.001	0.004	0.008	0.013	0.022	0.038	0.056	0.087
	Spring					574		0.0122	0.000	0.000	0.000	0.002	0.004	0.009	0.015	0.028	0.040	0.052	0.128
	Summer					584		0.0119	0.000	0.000	0.000	0.002	0.005	0.010	0.016	0.026	0.040	0.050	0.057
	Fall					576		0.0129	0.000	0.000	0.000	0.002	0.005	0.010	0.016	0.026	0.040	0.053	0.136
Nationwide statistics, pooled by site																			
2008-2010							88	0.0120	0.000	0.000	0.001	0.002	0.005	0.011	0.016	0.024	0.033	0.046	0.046
2008							59	0.0125	0.001	0.001	0.002	0.003	0.006	0.010	0.016	0.024	0.043	0.051	0.051
2009							66	0.0116	0.000	0.000	0.001	0.002	0.004	0.010	0.014	0.024	0.032	0.050	0.050
2010							73	0.0119	0.000	0.000	0.001	0.001	0.005	0.010	0.018	0.023	0.028	0.046	0.046
	Winter						88	0.0115	0.000	0.000	0.001	0.001	0.004	0.009	0.016	0.025	0.038	0.048	0.048
	Spring						86	0.0119	0.000	0.000	0.001	0.002	0.004	0.009	0.016	0.027	0.032	0.059	0.059
	Summer						88	0.0117	0.000	0.000	0.000	0.001	0.005	0.010	0.016	0.026	0.034	0.043	0.043
	Fall						88	0.0130	0.000	0.000	0.001	0.003	0.005	0.011	0.017	0.028	0.031	0.054	0.054
Statistics for individual counties (2008-2010)																			
		04013	AZ	Maricopa		6	1	0.0218	0.009	0.009	0.009	0.009	0.014	0.021	0.028	0.038	0.038	0.038	0.038
		06025	CA	Imperial		33	1	0.0162	0.004	0.004	0.006	0.009	0.011	0.015	0.019	0.025	0.032	0.035	0.035
		06037	CA	Los Angeles		224	8	0.0098	0.000	0.000	0.000	0.002	0.006	0.010	0.012	0.017	0.020	0.038	0.044
		06065	CA	Riverside		72	2	0.0077	0.000	0.000	0.003	0.004	0.006	0.008	0.010	0.010	0.012	0.014	0.014
		06071	CA	San Bern-ardino		71	2	0.0091	0.001	0.001	0.003	0.004	0.007	0.010	0.012	0.014	0.014	0.022	0.022
		08005	CO	Arapahoe		9	1	0.0120	0.004	0.004	0.004	0.004	0.007	0.012	0.016	0.018	0.018	0.018	0.018
		08031	CO	Denver		12	1	0.0056	0.003	0.003	0.003	0.004	0.005	0.005	0.006	0.008	0.008	0.008	0.008
		13089	GA	DeKalb		10	1	0.0033	0.002	0.002	0.002	0.002	0.003	0.003	0.004	0.005	0.006	0.006	0.006
		17031	IL	Cook		288	8	0.0195	0.010	0.010	0.010	0.010	0.012	0.016	0.025	0.034	0.040	0.060	0.070
		17117	IL	Macoupin		24	1	0.0101	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.012
		17119	IL	Madison		36	1	0.0188	0.010	0.010	0.010	0.010	0.012	0.016	0.020	0.032	0.053	0.066	0.066
		17143	IL	Peoria		36	1	0.0105	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.013	0.013	0.014	0.014
		17163	IL	Saint Clair		36	1	0.0206	0.010	0.010	0.010	0.012	0.014	0.018	0.026	0.032	0.038	0.054	0.054
		18089	IN	Lake		36	1	0.0150	0.005	0.005	0.005	0.005	0.008	0.014	0.019	0.030	0.033	0.049	0.049
		18097	IN	Marion		35	1	0.0058	0.002	0.002	0.002	0.003	0.004	0.005	0.008	0.010	0.012	0.013	0.013
		18163	IN	Vander-burgh		33	2	0.0045	0.001	0.001	0.001	0.002	0.003	0.004	0.005	0.006	0.010	0.010	0.010
		25025	MA	Suffolk		31	2	0.0087	0.004	0.004	0.004	0.005	0.007	0.008	0.010	0.013	0.016	0.020	0.020
		26081	MI	Kent		12	1	0.0053	0.003	0.003	0.003	0.003	0.005	0.005	0.006	0.008	0.008	0.008	0.008

Year	Season	State/ County	State	County name	Site ID	N mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
		26163	MI	Wayne		36	2	0.0112	0.003	0.003	0.003	0.004	0.005	0.009	0.015	0.021	0.023	0.032	0.032
		27017	MN	Carlton		12	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		27037	MN	Dakota		118	5	0.0035	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.008	0.010	0.017	0.036
		27053	MN	Hennepin		126	4	0.0032	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.006	0.008	0.010	0.044
		27075	MN	Lake		10	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		27123	MN	Ramsey		71	3	0.0062	0.000	0.000	0.000	0.000	0.002	0.004	0.008	0.013	0.020	0.028	0.028
		27137	MN	Saint Louis		72	2	0.0015	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.006	0.010	0.010
		27163	MN	Washington		72	3	0.0016	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.004	0.005	0.006	0.006
		29097	MO	Jasper		12	1	0.0125	0.007	0.007	0.007	0.007	0.009	0.012	0.017	0.018	0.019	0.019	0.019
		29187	MO	Saint Francois		24	2	0.0327	0.008	0.008	0.009	0.009	0.018	0.032	0.039	0.054	0.080	0.089	0.089
		29189	MO	Saint Louis		33	1	0.0230	0.005	0.005	0.005	0.005	0.006	0.008	0.050	0.050	0.050	0.066	0.066
		36047	NY	Kings		24	1	0.0131	0.010	0.010	0.010	0.010	0.011	0.012	0.014	0.018	0.020	0.020	0.020
		39017	OH	Butler		34	1	0.0055	0.002	0.002	0.003	0.004	0.004	0.005	0.007	0.008	0.009	0.009	0.009
		39029	OH	Columbiana		107	3	0.0155	0.004	0.004	0.006	0.007	0.008	0.011	0.018	0.027	0.034	0.065	0.136
		39035	OH	Cuyahoga		107	3	0.0143	0.004	0.004	0.006	0.007	0.009	0.012	0.017	0.024	0.030	0.041	0.041
		39049	OH	Franklin		36	1	0.0092	0.004	0.004	0.005	0.005	0.007	0.009	0.011	0.013	0.014	0.016	0.016
		39143	OH	Sandusky		12	1	0.0048	0.003	0.003	0.003	0.003	0.004	0.005	0.006	0.006	0.007	0.007	0.007
		39167	OH	Washington		54	2	0.0048	0.002	0.002	0.002	0.003	0.003	0.005	0.006	0.007	0.008	0.010	0.010
		40115	OK	Ottawa		16	2	0.0124	0.003	0.003	0.003	0.005	0.006	0.013	0.017	0.021	0.025	0.025	0.025
		42003	PA	Allegheny		36	1	0.0105	0.000	0.000	0.000	0.000	0.004	0.009	0.015	0.019	0.024	0.053	0.053
		42021	PA	Cambria		23	1	0.0463	0.040	0.040	0.040	0.040	0.040	0.040	0.044	0.054	0.058	0.128	0.128
		42045	PA	Delaware		20	1	0.0432	0.040	0.040	0.040	0.040	0.040	0.043	0.046	0.047	0.048	0.048	0.048
		42101	PA	Philadelphia		24	1	0.0210	0.011	0.011	0.011	0.012	0.014	0.020	0.027	0.033	0.033	0.039	0.039
		42129	PA	West-moreland		24	1	0.0419	0.037	0.037	0.040	0.040	0.040	0.040	0.042	0.050	0.050	0.053	0.053
		48061	TX	Cameron		35	1	0.0041	0.002	0.002	0.003	0.003	0.003	0.004	0.005	0.006	0.007	0.009	0.009
		48141	TX	El Paso		68	3	0.0206	0.014	0.014	0.014	0.014	0.015	0.017	0.019	0.029	0.056	0.087	0.087
		48201	TX	Harris		32	1	0.0053	0.003	0.003	0.003	0.004	0.004	0.005	0.006	0.007	0.008	0.010	0.010
		48479	TX	Webb		29	1	0.0134	0.004	0.004	0.005	0.006	0.008	0.011	0.018	0.026	0.028	0.035	0.035
		49035	UT	Salt Lake		12	1	0.0173	0.003	0.003	0.003	0.006	0.009	0.011	0.024	0.040	0.043	0.043	0.043
		51087	VA	Henrico		7	1	0.0066	0.003	0.003	0.003	0.003	0.003	0.004	0.005	0.024	0.024	0.024	0.024
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2008-2010)																			
					170310022	36		0.0330	0.012	0.012	0.014	0.016	0.020	0.033	0.040	0.056	0.062	0.070	0.070
					170310026	36		0.0282	0.014	0.014	0.014	0.018	0.020	0.028	0.034	0.044	0.048	0.052	0.052
					170316003	36		0.0249	0.012	0.012	0.014	0.018	0.020	0.026	0.031	0.033	0.038	0.040	0.040
					291870006[a]	12		0.0383	0.009	0.009	0.009	0.015	0.024	0.035	0.042	0.080	0.089	0.089	0.089
					291870007[a]	12		0.0271	0.008	0.008	0.008	0.009	0.013	0.026	0.035	0.052	0.054	0.054	0.054
					420210808[a]	23		0.0463	0.040	0.040	0.040	0.040	0.040	0.040	0.044	0.054	0.058	0.128	0.128
					420450002[a]	20		0.0432	0.040	0.040	0.040	0.040	0.040	0.043	0.046	0.047	0.048	0.048	0.048
					421290007[a]	24		0.0419	0.037	0.037	0.040	0.040	0.040	0.040	0.042	0.050	0.050	0.053	0.053
					481410002[a]	23		0.0236	0.016	0.016	0.016	0.016	0.017	0.018	0.021	0.033	0.056	0.087	0.087

[a]Sites listed in the bottom six rows of the table fall in the upper 90th percentile of the data pooled by site.

Table 3-15 Distribution of 3-month moving average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010

Year	Season	State/ County	State	County name	Site ID	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics[a]																			
2008-2010						2,112		0.2134	0.000	0.004	0.010	0.014	0.035	0.079	0.250	0.600	0.881	1.555	2.889
2008						537		0.3225	0.005	0.006	0.016	0.028	0.056	0.129	0.385	0.900	1.197	2.452	2.889
2009						600		0.2177	0.004	0.005	0.011	0.016	0.040	0.090	0.292	0.622	0.799	1.217	2.070
2010						975		0.1507	0.000	0.002	0.008	0.012	0.024	0.052	0.173	0.436	0.694	1.055	1.375
	Winter					443		0.2366	0.003	0.004	0.011	0.014	0.040	0.083	0.272	0.647	0.963	2.070	2.621
	Spring					535		0.2376	0.000	0.004	0.011	0.014	0.035	0.078	0.323	0.642	0.999	2.017	2.889
	Summer					572		0.2022	0.002	0.003	0.009	0.015	0.034	0.077	0.240	0.580	0.869	1.261	2.163
	Fall					562		0.1835	0.002	0.004	0.009	0.013	0.033	0.078	0.220	0.521	0.714	1.186	2.456
Nationwide statistics, pooled by site																			
2008-2010							106	0.1671	0.002	0.003	0.012	0.015	0.030	0.059	0.173	0.577	0.717	1.009	1.316
2008							47	0.3309	0.007	0.007	0.024	0.029	0.056	0.154	0.461	0.814	1.284	1.639	1.639
2009							54	0.2203	0.007	0.007	0.013	0.019	0.042	0.086	0.311	0.632	0.840	0.886	0.886
2010							96	0.1415	0.002	0.002	0.008	0.013	0.027	0.053	0.163	0.407	0.619	1.110	1.110
	Winter						104	0.1700	0.003	0.004	0.011	0.013	0.025	0.055	0.171	0.522	0.827	1.097	1.324
	Spring						101	0.1904	0.001	0.002	0.013	0.016	0.028	0.060	0.186	0.502	0.874	1.231	1.740
	Summer						106	0.1597	0.002	0.004	0.010	0.015	0.028	0.058	0.174	0.520	0.788	0.989	1.104
	Fall						105	0.1538	0.002	0.004	0.009	0.012	0.032	0.066	0.170	0.462	0.630	0.960	1.161
Statistics for individual counties (2008-2010)																			
		01109	AL	Pike		25	1	0.5771	0.223	0.223	0.247	0.256	0.302	0.574	0.719	1.088	1.178	1.210	1.210
		06037	CA	Los Angeles	131	4		0.2521	0.023	0.023	0.036	0.041	0.055	0.078	0.237	0.543	0.832	2.452	2.489
		12057	FL	Hillsborough	79	3		0.1940	0.011	0.011	0.015	0.037	0.063	0.110	0.249	0.423	0.582	1.770	1.770
		13015	GA	Bartow		11	1	0.0125	0.009	0.009	0.009	0.009	0.011	0.013	0.014	0.015	0.016	0.016	0.016
		13215	GA	Muscogee	12	1		0.0367	0.014	0.014	0.014	0.020	0.022	0.031	0.052	0.066	0.070	0.070	0.070
		17031	IL	Cook		9	1	0.1364	0.068	0.068	0.068	0.068	0.109	0.135	0.150	0.241	0.241	0.241	0.241
		17115	IL	Macon		10	1	0.0806	0.048	0.048	0.048	0.052	0.067	0.080	0.088	0.117	0.123	0.123	0.123
		17119	IL	Madison		36	1	0.1346	0.027	0.027	0.035	0.036	0.063	0.113	0.207	0.283	0.341	0.416	0.416
		17143	IL	Peoria		20	2	0.0121	0.010	0.010	0.010	0.010	0.011	0.012	0.014	0.015	0.016	0.016	0.016
		17195	IL	Whiteside	10	1		0.0191	0.012	0.012	0.012	0.014	0.016	0.019	0.022	0.025	0.025	0.025	0.025
		17201	IL	Winnebago	9	1		0.0356	0.019	0.019	0.019	0.019	0.021	0.027	0.057	0.063	0.063	0.063	0.063
		18035	IN	Delaware	57	2		0.2866	0.053	0.053	0.059	0.073	0.090	0.159	0.246	0.495	1.867	2.163	2.163
		18089	IN	Lake		46	2	0.0305	0.007	0.007	0.011	0.012	0.016	0.027	0.036	0.040	0.057	0.129	0.129
		18097	IN	Marion		66	2	0.0198	0.005	0.005	0.006	0.007	0.011	0.014	0.025	0.036	0.043	0.079	0.079
		18127	IN	Porter		10	1	0.0131	0.007	0.007	0.007	0.007	0.007	0.013	0.017	0.020	0.022	0.022	0.022
		19155	IA	Pottawattamie	12	1		0.1581	0.034	0.034	0.034	0.067	0.113	0.153	0.220	0.246	0.263	0.263	0.263
		20169	KS	Saline		9	1	0.2286	0.096	0.096	0.096	0.096	0.107	0.231	0.324	0.421	0.421	0.421	0.421
		21151	KY	Madison		10	1	0.0212	0.013	0.013	0.013	0.014	0.015	0.017	0.024	0.037	0.049	0.049	0.049
		26067	MI	Ionia		10	1	0.1980	0.106	0.106	0.106	0.110	0.128	0.212	0.259	0.273	0.284	0.284	0.284
		27003	MN	Anoka		10	1	0.0161	0.006	0.006	0.006	0.008	0.010	0.013	0.022	0.029	0.031	0.031	0.031
		27037	MN	Dakota		36	1	0.2026	0.068	0.068	0.072	0.088	0.104	0.216	0.248	0.357	0.415	0.429	0.429
		27145	MN	Stearns		10	1	0.0032	0.000	0.000	0.000	0.001	0.002	0.004	0.004	0.005	0.005	0.005	0.005
		29093	MO	Iron		158	6	0.3465	0.010	0.011	0.019	0.022	0.033	0.142	0.549	0.901	1.167	2.076	2.456
		29099	MO	Jefferson	423	19		0.4925	0.023	0.033	0.050	0.071	0.187	0.385	0.723	0.989	1.186	2.017	2.889
		29179	MO	Reynolds	40	4		0.0397	0.012	0.012	0.014	0.015	0.017	0.031	0.057	0.087	0.089	0.100	0.100

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
		31053	NE	Dodge		7	1	0.0474	0.019	0.019	0.019	0.019	0.020	0.060	0.067	0.072	0.072	0.072	0.072
		31127	NE	Nemaha		6	1	0.0447	0.019	0.019	0.019	0.019	0.024	0.032	0.075	0.087	0.087	0.087	0.087
		36071	NY	Orange		99	3	0.0271	0.003	0.003	0.004	0.005	0.007	0.027	0.037	0.068	0.075	0.086	0.086
		39035	OH	Cuyahoga	70	3		0.0905	0.006	0.006	0.010	0.011	0.021	0.050	0.122	0.221	0.287	0.531	0.531
		39051	OH	Fulton		30	1	0.1609	0.025	0.025	0.027	0.046	0.054	0.092	0.254	0.354	0.453	0.567	0.567
		39091	OH	Logan		100	4	0.0499	0.004	0.004	0.004	0.006	0.033	0.047	0.072	0.090	0.095	0.100	0.100
		39101	OH	Marion		8	1	0.0379	0.032	0.032	0.032	0.032	0.034	0.037	0.042	0.047	0.047	0.047	0.047
		39151	OH	Stark		9	1	0.0180	0.015	0.015	0.015	0.015	0.016	0.018	0.019	0.023	0.023	0.023	0.023
		39155	OH	Trumbull		6	1	0.0080	0.005	0.005	0.005	0.005	0.006	0.008	0.010	0.011	0.011	0.011	0.011
		40121	OK	Pittsburg		9	1	0.0021	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003
		41071	OR	Yamhill		10	1	0.0166	0.009	0.009	0.009	0.011	0.013	0.016	0.019	0.026	0.027	0.027	0.027
		42003	PA	Allegheny	20	2		0.0414	0.009	0.009	0.011	0.012	0.017	0.030	0.054	0.099	0.120	0.138	0.138
		42007	PA	Beaver		41	3	0.1160	0.043	0.043	0.052	0.056	0.083	0.114	0.159	0.170	0.187	0.206	0.206
		42011	PA	Berks		105	6	0.0995	0.038	0.039	0.041	0.045	0.051	0.078	0.145	0.183	0.197	0.242	0.251
		42045	PA	Delaware	10	1		0.0447	0.043	0.043	0.043	0.043	0.043	0.045	0.046	0.047	0.047	0.047	0.047
		42055	PA	Franklin		7	1	0.0447	0.043	0.043	0.043	0.043	0.043	0.045	0.046	0.046	0.046	0.046	0.046
		42063	PA	Indiana		10	1	0.0447	0.043	0.043	0.043	0.043	0.043	0.044	0.046	0.049	0.049	0.049	0.049
		42079	PA	Luzerne		6	1	0.1078	0.084	0.084	0.084	0.084	0.085	0.103	0.135	0.137	0.137	0.137	0.137
		42129	PA	Westmoreland	10	1		0.0434	0.041	0.041	0.041	0.042	0.042	0.044	0.044	0.046	0.046	0.046	0.046
		47093	TN	Knox		44	2	0.0165	0.007	0.007	0.009	0.009	0.012	0.016	0.020	0.023	0.027	0.035	0.035
		47163	TN	Sullivan		118	4	0.0554	0.030	0.030	0.033	0.035	0.039	0.045	0.060	0.100	0.125	0.134	0.168
		48085	TX	Collin		108	3	0.3101	0.048	0.051	0.070	0.085	0.120	0.217	0.469	0.682	0.753	1.189	1.262
		51770	VA	Roanoke City	10	1		0.0466	0.013	0.013	0.013	0.016	0.019	0.026	0.097	0.108	0.109	0.109	0.109
		55117	WI	Sheboygan	10	1		0.0897	0.012	0.012	0.012	0.034	0.058	0.076	0.126	0.164	0.170	0.170	0.170
		72013	PR	Arecibo		10	1	0.1725	0.059	0.059	0.059	0.068	0.129	0.194	0.213	0.241	0.245	0.245	0.245
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2008-2010)																			
					011090003	25		0.5771	0.223	0.223	0.247	0.256	0.302	0.574	0.719	1.088	1.178	1.210	1.210
					060371405	36		0.7174	0.188	0.188	0.234	0.237	0.309	0.476	0.791	2.178	2.452	2.489	2.489
					290930016	36		0.6682	0.207	0.207	0.258	0.313	0.418	0.543	0.634	1.167	2.076	2.456	2.456
					290930021	36		0.6950	0.173	0.173	0.192	0.218	0.346	0.689	0.954	1.214	1.275	1.937	1.937
					290990004	36		1.0090	0.640	0.640	0.655	0.699	0.775	0.913	1.081	1.555	2.011	2.017	2.017
					290990015[b]	21		1.3162	0.612	0.612	0.632	0.743	0.921	1.074	1.258	2.621	2.634	2.889	2.889
					290990020[b]	29		0.6680	0.452	0.452	0.471	0.482	0.555	0.651	0.754	0.891	0.943	0.989	0.989
					290990021[b]	21		0.7317	0.429	0.429	0.435	0.507	0.547	0.685	0.900	0.999	1.013	1.141	1.141
					290999001[b]	22		0.8413	0.587	0.587	0.592	0.600	0.699	0.845	0.963	1.061	1.100	1.204	1.204
					290999005[b]	22		0.9875	0.612	0.612	0.630	0.644	0.783	0.995	1.220	1.271	1.278	1.375	1.375
					480850009[b]	36		0.6068	0.196	0.196	0.268	0.335	0.469	0.585	0.704	0.965	1.189	1.262	1.262

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.

[b]Sites listed in the bottom six rows of the table fall in the upper 90th percentile of the data pooled by site.

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Table 3-16 Distribution of 3-month moving average Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010

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																			
2008-2010						2,164		0.0120	0.000	0.000	0.001	0.002	0.005	0.010	0.015	0.025	0.037	0.048	0.073
2008						663		0.0130	0.000	0.000	0.001	0.002	0.005	0.011	0.016	0.027	0.040	0.050	0.055
2009						727		0.0114	0.000	0.000	0.001	0.002	0.004	0.009	0.014	0.024	0.038	0.043	0.073
2010						774		0.0118	0.000	0.000	0.001	0.001	0.005	0.010	0.016	0.025	0.035	0.047	0.057
	winter					494		0.0113	0.000	0.001	0.001	0.002	0.005	0.009	0.014	0.023	0.037	0.050	0.055
	spring					548		0.0119	0.000	0.000	0.001	0.002	0.005	0.009	0.015	0.025	0.036	0.050	0.073
	summer					565		0.0121	0.000	0.000	0.001	0.002	0.005	0.010	0.016	0.026	0.037	0.046	0.053
	fall					557		0.0126	0.000	0.000	0.001	0.002	0.005	0.011	0.017	0.027	0.037	0.048	0.057
Nationwide statistics, pooled by site																			
2008-2010							86	0.0120	0.000	0.000	0.001	0.002	0.005	0.010	0.016	0.024	0.034	0.046	0.046
2008							59	0.0127	0.001	0.001	0.002	0.003	0.005	0.011	0.016	0.024	0.043	0.050	0.050
2009							65	0.0117	0.001	0.001	0.001	0.003	0.004	0.010	0.014	0.026	0.031	0.049	0.049
2010							71	0.0118	0.000	0.000	0.001	0.001	0.005	0.010	0.017	0.022	0.028	0.045	0.045
	Winter						84	0.0118	0.000	0.000	0.001	0.002	0.005	0.010	0.015	0.025	0.036	0.048	0.048
	Spring						83	0.0118	0.000	0.000	0.001	0.002	0.004	0.010	0.015	0.025	0.034	0.059	0.059
	Summer						86	0.0118	0.000	0.000	0.001	0.002	0.005	0.009	0.016	0.023	0.037	0.043	0.043
	Fall						86	0.0126	0.000	0.000	0.001	0.002	0.005	0.011	0.016	0.026	0.030	0.046	0.046
Statistics for individual counties (2008-2010)																			
		06025	CA	Imperial		31	1	0.0165	0.007	0.007	0.008	0.011	0.013	0.017	0.021	0.023	0.023	0.023	0.023
		06037	CA	Los Angeles	218	8	0.0100	0.000	0.000	0.002	0.004	0.006	0.009	0.013	0.016	0.020	0.028	0.035	0.035
		06065	CA	Riverside	72	2	0.0078	0.002	0.002	0.004	0.005	0.007	0.008	0.010	0.011	0.011	0.011	0.011	
		06071	CA	San Bernardino	69	2	0.0091	0.003	0.003	0.005	0.006	0.007	0.009	0.011	0.013	0.014	0.017	0.017	
		08005	CO	Arapahoe	7	1	0.0126	0.011	0.011	0.011	0.011	0.011	0.013	0.014	0.014	0.014	0.014	0.014	
		08031	CO	Denver		10	1	0.0054	0.004	0.004	0.004	0.004	0.005	0.006	0.006	0.006	0.006	0.006	0.006
		13089	GA	DeKalb		8	1	0.0035	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.004	0.004	0.004	0.004
		17031	IL	Cook		287	8	0.0196	0.010	0.010	0.010	0.010	0.012	0.017	0.025	0.033	0.038	0.047	0.051
		17117	IL	Macoupin	24	1	0.0101	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011	0.011	0.011	0.070
		17119	IL	Madison		36	1	0.0188	0.010	0.010	0.010	0.011	0.014	0.016	0.022	0.036	0.036	0.039	0.039
		17143	IL	Peoria		36	1	0.0105	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.012	0.012	0.013	0.013
		17163	IL	Saint Clair	36	1	0.0204	0.012	0.012	0.012	0.014	0.016	0.020	0.024	0.029	0.033	0.036	0.036	0.014
		18089	IN	Lake		36	1	0.0149	0.007	0.007	0.007	0.007	0.010	0.014	0.018	0.024	0.032	0.037	0.037
		18097	IN	Marion		33	1	0.0056	0.003	0.003	0.003	0.003	0.004	0.005	0.007	0.009	0.010	0.011	0.011
		18163	IN	Vanderburgh	31	2	0.0047	0.002	0.002	0.003	0.003	0.004	0.005	0.005	0.006	0.007	0.007	0.007	0.013
		25025	MA	Suffolk		24	2	0.0093	0.005	0.005	0.006	0.006	0.008	0.009	0.011	0.013	0.015	0.016	0.016
		26081	MI	Kent		10	1	0.0055	0.004	0.004	0.004	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006
		26163	MI	Wayne		32	2	0.0119	0.004	0.004	0.004	0.005	0.005	0.012	0.017	0.021	0.023	0.024	0.024
		27017	MN	Carlton		10	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		27037	MN	Dakota		112	5	0.0036	0.000	0.000	0.001	0.001	0.001	0.003	0.005	0.007	0.012	0.013	0.015
		27053	MN	Hennepin	124	4	0.0033	0.000	0.001	0.001	0.001	0.002	0.003	0.004	0.006	0.006	0.015	0.016	0.036
		27075	MN	Lake		8	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		27123	MN	Ramsey		65	3	0.0061	0.001	0.001	0.001	0.001	0.002	0.005	0.008	0.014	0.016	0.017	0.017
		27137	MN	Saint Louis	70	2	0.0016	0.000	0.000	0.000	0.000	0.001	0.001	0.002	0.004	0.004	0.005	0.005	0.028
		27163	MN	Washington	70	3	0.0017	0.000	0.000	0.000	0.000	0.001	0.001	0.003	0.004	0.004	0.005	0.005	

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
		29097	MO	Jasper		10	1	0.0135	0.009	0.009	0.009	0.011	0.012	0.014	0.015	0.016	0.017	0.017	0.017
		29187	MO	Saint Francois	21	2	0.0337	0.011	0.011	0.012	0.012	0.027	0.035	0.042	0.048	0.053	0.054	0.054	0.019
		29189	MO	Saint Louis	33	1	0.0243	0.005	0.005	0.005	0.006	0.007	0.008	0.050	0.050	0.050	0.055	0.055	
		36047	NY	Kings		24	1	0.0131	0.011	0.011	0.011	0.011	0.012	0.013	0.014	0.016	0.018	0.019	0.019
		39017	OH	Butler		30	1	0.0055	0.003	0.003	0.004	0.004	0.005	0.006	0.006	0.007	0.007	0.008	0.008
		39029	OH	Columbiana	105	3	0.0148	0.005	0.005	0.007	0.008	0.010	0.013	0.017	0.021	0.028	0.054	0.057	0.009
		39035	OH	Cuyahoga	105	3	0.0144	0.005	0.006	0.006	0.008	0.010	0.013	0.018	0.023	0.027	0.033	0.035	
		39049	OH	Franklin		36	1	0.0092	0.005	0.005	0.005	0.005	0.008	0.010	0.011	0.011	0.012	0.012	0.012
		39143	OH	Sandusky	10	1	0.0052	0.004	0.004	0.004	0.004	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.016
		39167	OH	Washington	48	2	0.0047	0.002	0.002	0.002	0.003	0.004	0.004	0.006	0.007	0.007	0.008	0.008	
		40115	OK	Ottawa		12	2	0.0128	0.005	0.005	0.005	0.006	0.010	0.014	0.016	0.018	0.019	0.019	0.019
		42003	PA	Allegheny	36	1	0.0101	0.000	0.000	0.000	0.000	0.007	0.012	0.014	0.016	0.018	0.025	0.025	0.025
		42021	PA	Cambria		23	1	0.0459	0.040	0.040	0.040	0.040	0.040	0.041	0.046	0.069	0.070	0.073	0.073
		42045	PA	Delaware	14	1	0.0427	0.040	0.040	0.040	0.040	0.040	0.042	0.045	0.046	0.047	0.047	0.047	0.128
		42101	PA	Philadelphia	22	1	0.0214	0.013	0.013	0.014	0.014	0.018	0.022	0.025	0.029	0.029	0.030	0.030	
		42129	PA	Westmoreland	24	1	0.0417	0.037	0.037	0.040	0.040	0.040	0.041	0.043	0.046	0.047	0.048	0.048	
		48061	TX	Cameron	33	1	0.0042	0.002	0.002	0.003	0.003	0.004	0.004	0.005	0.005	0.006	0.006	0.006	
		48141	TX	El Paso		56	3	0.0212	0.014	0.014	0.014	0.015	0.016	0.018	0.023	0.038	0.040	0.040	0.040
		48201	TX	Harris		30	1	0.0051	0.004	0.004	0.004	0.004	0.005	0.005	0.006	0.006	0.007	0.007	0.007
		48479	TX	Webb		23	1	0.0121	0.006	0.006	0.007	0.007	0.008	0.010	0.016	0.021	0.022	0.026	0.026
		49035	UT	Salt Lake	10	1	0.0145	0.007	0.007	0.007	0.007	0.008	0.011	0.016	0.032	0.036	0.036	0.036	
		48479	TX	Webb		29	1	0.0134	0.004	0.004	0.005	0.006	0.008	0.011	0.018	0.026	0.028	0.035	0.035
		49035	UT	Salt Lake	12	1	0.0173	0.003	0.003	0.003	0.006	0.009	0.011	0.024	0.040	0.043	0.043	0.043	
		51087	VA	Henrico		7	1	0.0066	0.003	0.003	0.003	0.003	0.003	0.004	0.005	0.024	0.024	0.024	0.024
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2008-2010)																			
					170310022	36		0.0335	0.016	0.016	0.018	0.026	0.028	0.032	0.038	0.047	0.048	0.051	0.051
					170310026	36		0.0281	0.018	0.018	0.019	0.022	0.023	0.026	0.032	0.038	0.043	0.046	0.046
					170316003	36		0.0245	0.015	0.015	0.015	0.017	0.020	0.025	0.028	0.031	0.035	0.036	0.036
					291870006[b]	10		0.0412	0.017	0.017	0.017	0.026	0.035	0.043	0.048	0.054	0.054	0.054	0.054
					291870007[b]	11		0.0268	0.011	0.011	0.011	0.012	0.012	0.028	0.035	0.036	0.041	0.041	0.041
					291892003[b]	33		0.0243	0.005	0.005	0.005	0.006	0.007	0.008	0.050	0.050	0.050	0.055	0.055
					420210808[b]	23		0.0459	0.040	0.040	0.040	0.040	0.040	0.041	0.046	0.069	0.070	0.073	0.073
					420450002[b]	14		0.0427	0.040	0.040	0.040	0.040	0.040	0.042	0.045	0.046	0.047	0.047	0.047
					421290007[b]	24		0.0417	0.037	0.037	0.040	0.040	0.040	0.041	0.043	0.046	0.047	0.048	0.048

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.
[b]Sites listed in the bottom six rows of the table fall in the upper 90th percentile of the data pooled by site.

Table 3-17 Distribution of annual 1-month site maxima TSP Pb concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics														
2008-2010		111	0.5003	0.003	0.006	0.016	0.032	0.066	0.156	0.575	1.530	2.416	4.225	4.440
2008		47	0.8138	0.012	0.012	0.052	0.057	0.096	0.320	0.850	2.557	3.123	4.440	4.440

2009		54	0.4486	0.016	0.016	0.022	0.050	0.090	0.170	0.618	1.280	1.623	2.438	2.438
2010		101	0.3105	0.003	0.006	0.008	0.024	0.054	0.142	0.347	0.854	1.117	1.576	1.828
Annual site max 1-month means >= national 90th percentile (2008-20010)														
	060371405-2008		2.8800											
	180350009-2008		4.4400											
	290930016-2008		4.2252											
	290930021-2008		2.5566											
	290930021-2009		2.4380											
	290990004-2008		2.4156											
	290990004-2009		1.5599											
	290990004-2010		1.5762											
	290990011-2008		1.5295											
	290990015[a]-2008		3.1228											
	290990020[a]-2008		2.2204											
	290990021[a]-2008		1.5528											
	290999001[a]-2009		1.6228											
	290999001[a]-2010		1.5576											
	290999005[a]-2009		1.9850											
	290999005[a]-2010		1.8278											
	480850009[a]-2008		1.5640											

[a]Sites listed in the bottom eight rows of the table fall in the upper 90th percentile of the data pooled by site.

Table 3-18 Distribution of annual 1-month site maxima TSP Pb concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics														
2008-2010		88	0.0284	0.000	0.000	0.004	0.006	0.010	0.020	0.041	0.057	0.070	0.136	0.136
2008		59	0.0232	0.004	0.004	0.005	0.006	0.010	0.016	0.033	0.053	0.058	0.066	0.066
2009		66	0.0210	0.003	0.003	0.005	0.006	0.008	0.014	0.026	0.040	0.056	0.128	0.128
2010		73	0.0233	0.000	0.000	0.002	0.004	0.008	0.015	0.029	0.049	0.065	0.136	0.136
Annual site max 1-month means >= national 90th percentile (2008-2010)														
	170310022-2009		0.0700											
	170310022-2010		0.0620											
	171193007-2008		0.0660											
	291870006[a]-2010		0.0894											
	291892003[a]-2008		0.0660											
	390290019[a]-2010		0.1360											
	390290022[a]-2010		0.0652											
	420210808[a]-2008		0.0583											
	420210808[a]-2009		0.1280											
	481410002[a]-2010		0.0870											
	481410033[a]-2009		0.0570											

[a]Sites listed in the bottom eight rows of the table fall in the upper 90th percentile of the data pooled by site.

Table 3-19 Distribution of annual 3-month site maxima Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics[a]														
2008-2010		106	0.3605	0.003	0.005	0.016	0.023	0.047	0.109	0.378	1.204	1.937	2.489	2.889
2007		47	0.5831	0.009	0.009	0.038	0.043	0.085	0.242	0.815	2.017	2.456	2.889	2.889
2008		54	0.3611	0.012	0.012	0.017	0.035	0.060	0.121	0.467	1.079	1.258	2.070	2.070
2009		96	0.2112	0.003	0.003	0.011	0.021	0.046	0.091	0.262	0.630	0.865	1.375	1.375
Annual site max 3-month means >= national 90th percentile (2008-2010)														
	011090003-2008		1.2100											
	060371405-2008		2.4890											
	120571066-2008		1.7700											
	180350009-2008		2.1630											
	290930016[b]-2008		2.4560											
	290930016[b]-2009		2.0700											
	290930021[b]-2009		1.9370											
	290990004[b]-2008		2.0170											
	290990015[b]-2008		2.8890											
	290999001[b]-2009		1.2040											
	290999005[b]-2009		1.2580											
	290999005[b]-2010		1.3750											
	480850009[b]-2008		1.2620											

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.
[b]Sites listed in the bottom nine rows of the table fall in the upper 90th percentile of the data pooled by site.

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Table 3-20 Distribution of annual 3-month site maxima Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics[a]														
2008-2010		86	0.0198	0.000	0.000	0.002	0.004	0.007	0.015	0.028	0.044	0.051	0.073	0.073
2008		59	0.0176	0.002	0.002	0.004	0.005	0.007	0.014	0.024	0.039	0.048	0.055	0.055
2009		65	0.0162	0.002	0.002	0.003	0.004	0.006	0.013	0.021	0.038	0.041	0.073	0.073
2010		71	0.0171	0.000	0.000	0.001	0.002	0.006	0.013	0.024	0.037	0.047	0.057	0.057
Annual site max 3-month means >= national 90th percentile (2008-2010)														
	170310022-2008		0.0480											
	170310022-2009		0.0470											

	170310022-2010		0.0510										
	170310026[b]-2008		0.0460										
	291870006[b]-2010		0.0540										
	291892003[b]-2008		0.0550										
	390290019[b]-2010		0.0570										
	390290022[b]-2010		0.0440										
	420210808[b]-2008		0.0490										
	420210808[b]-2009		0.0730										
	420450002[b]-2010		0.0470										
	421290007[b]-2008		0.0480										

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.
[b] Sites listed in the bottom nine rows of the table fall in the upper 90th percentile of the data pooled by site.

Table 3-21 One-month average Pb-TSP for individual county concentrations nationwide (μg/m^3), source-oriented monitors, 2008-2010

Stcou code	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Statistics for individual counties (2008-2010)																
01109	AL	Pike	32	1	0.5252	0.054	0.054	0.083	0.164	0.252	0.402	0.798	1.053	1.117	1.277	1.277
06037	CA	Los Angeles	131	4	0.2380	0.018	0.019	0.026	0.034	0.047	0.085	0.246	0.602	0.905	2.501	2.880
12057	FL	Hillsborough	81	3	0.1755	0.007	0.007	0.017	0.020	0.053	0.104	0.187	0.530	0.567	1.007	1.007
13015	GA	Bartow	12	1	0.0128	0.007	0.007	0.007	0.008	0.008	0.014	0.016	0.017	0.019	0.019	0.019
13215	GA	Muscogee	12	1	0.0361	0.004	0.004	0.004	0.010	0.013	0.027	0.043	0.058	0.140	0.140	0.140
17031	IL	Cook	11	1	0.1515	0.028	0.028	0.028	0.028	0.050	0.074	0.196	0.304	0.580	0.580	0.580
17115	IL	Macon	12	1	0.0800	0.018	0.018	0.018	0.025	0.035	0.074	0.118	0.144	0.168	0.168	0.168
17119	IL	Madison	36	1	0.1367	0.018	0.018	0.022	0.024	0.037	0.068	0.175	0.304	0.363	0.836	0.836
17143	IL	Peoria	24	2	0.0119	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.016	0.023	0.024	0.024
17195	IL	Whiteside	12	1	0.0194	0.010	0.010	0.010	0.012	0.012	0.015	0.024	0.036	0.040	0.040	0.040
17201	IL	Winnebago	11	1	0.0339	0.010	0.010	0.010	0.014	0.020	0.024	0.032	0.050	0.118	0.118	0.118
18035	IN	Delaware	59	2	0.2746	0.034	0.034	0.040	0.049	0.080	0.128	0.241	0.427	1.011	4.440	4.440
18089	IN	Lake	57	3	0.0309	0.004	0.004	0.007	0.008	0.012	0.020	0.035	0.052	0.079	0.298	0.298
18097	IN	Marion	70	2	0.0195	0.003	0.003	0.005	0.005	0.008	0.012	0.025	0.046	0.050	0.125	0.125
18127	IN	Porter	12	1	0.0125	0.004	0.004	0.004	0.005	0.007	0.009	0.021	0.024	0.026	0.026	0.026
19155	IA	Pottawattamie	12	1	0.1536	0.025	0.025	0.025	0.026	0.063	0.164	0.257	0.276	0.282	0.282	0.282
20169	KS	Saline	11	1	0.2020	0.043	0.043	0.043	0.044	0.083	0.133	0.320	0.457	0.488	0.488	0.488
21019	KY	Boyd	7	1	0.0042	0.002	0.002	0.002	0.002	0.004	0.004	0.004	0.007	0.007	0.007	0.007
21151	KY	Madison	12	1	0.0255	0.004	0.004	0.004	0.008	0.013	0.017	0.022	0.032	0.121	0.121	0.121
26067	MI	Ionia	12	1	0.1781	0.016	0.016	0.016	0.023	0.054	0.169	0.279	0.361	0.414	0.414	0.414
27003	MN	Anoka	12	1	0.0157	0.003	0.003	0.003	0.005	0.007	0.011	0.021	0.022	0.054	0.054	0.054
27037	MN	Dakota	36	1	0.1966	0.037	0.037	0.048	0.058	0.084	0.137	0.259	0.424	0.572	0.738	0.738
27145	MN	Stearns	12	1	0.0028	0.000	0.000	0.000	0.000	0.000	0.003	0.005	0.006	0.008	0.008	0.008
29093	MO	Iron	171	7	0.3388	0.007	0.008	0.014	0.018	0.033	0.093	0.518	0.850	1.110	2.557	4.225
29099	MO	Jefferson	453	19	0.4795	0.011	0.015	0.033	0.048	0.141	0.336	0.659	1.118	1.451	2.220	3.123
29179	MO	Reynolds	48	4	0.0428	0.007	0.007	0.008	0.011	0.017	0.027	0.060	0.087	0.099	0.268	0.268
31053	NE	Dodge	9	1	0.0515	0.005	0.005	0.005	0.005	0.021	0.031	0.053	0.149	0.149	0.149	0.149
31127	NE	Nemaha	8	1	0.0476	0.008	0.008	0.008	0.008	0.010	0.024	0.049	0.206	0.206	0.206	0.206
36071	NY	Orange	105	3	0.0281	0.001	0.001	0.003	0.004	0.006	0.018	0.044	0.063	0.081	0.101	0.134
39035	OH	Cuyahoga	72	3	0.0941	0.004	0.004	0.007	0.008	0.014	0.038	0.121	0.210	0.400	0.719	0.719
39051	OH	Fulton	34	1	0.1462	0.009	0.009	0.009	0.026	0.057	0.091	0.170	0.420	0.490	0.510	0.510
39091	OH	Logan	102	4	0.0480	0.003	0.003	0.004	0.005	0.020	0.042	0.070	0.090	0.100	0.120	0.170
39101	OH	Marion	10	1	0.0358	0.025	0.025	0.025	0.026	0.027	0.033	0.041	0.054	0.066	0.066	0.066
39151	OH	Stark	11	1	0.0175	0.008	0.008	0.008	0.009	0.010	0.018	0.024	0.025	0.028	0.028	0.028
39155	OH	Trumbull	8	1	0.0075	0.004	0.004	0.004	0.004	0.005	0.007	0.008	0.017	0.017	0.017	0.017
40121	OK	Pittsburg	11	1	0.0023	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003	0.003
41071	OR	Yamhill	12	1	0.0157	0.006	0.006	0.006	0.007	0.008	0.016	0.020	0.025	0.037	0.037	0.037
42003	PA	Allegheny	24	2	0.0369	0.006	0.006	0.006	0.006	0.010	0.017	0.040	0.121	0.144	0.149	0.149
42007	PA	Beaver	54	3	0.1130	0.042	0.042	0.044	0.047	0.068	0.096	0.128	0.198	0.272	0.286	0.286
42011	PA	Berks	117	6	0.0989	0.034	0.035	0.038	0.042	0.048	0.066	0.119	0.200	0.295	0.347	0.348
42045	PA	Delaware	12	1	0.0452	0.043	0.043	0.043	0.043	0.043	0.045	0.047	0.048	0.048	0.048	0.048
42055	PA	Franklin	11	1	0.0449	0.042	0.042	0.042	0.043	0.043	0.045	0.047	0.047	0.047	0.047	0.047
42063	PA	Indiana	12	1	0.0454	0.042	0.042	0.042	0.043	0.043	0.044	0.046	0.047	0.058	0.058	0.058
42073	PA	Lawrence	8	1	0.0438	0.042	0.042	0.042	0.042	0.043	0.044	0.045	0.046	0.046	0.046	0.046
42079	PA	Luzerne	10	1	0.0953	0.043	0.043	0.043	0.044	0.045	0.071	0.102	0.215	0.268	0.268	0.268
42129	PA	Westmoreland	12	1	0.0439	0.041	0.041	0.041	0.041	0.043	0.044	0.045	0.046	0.047	0.047	0.047
47093	TN	Knox	48	2	0.0165	0.002	0.002	0.005	0.006	0.008	0.012	0.019	0.032	0.038	0.063	0.063

Stcou code	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
47163	TN	Sullivan	120	4	0.0534	0.021	0.023	0.030	0.032	0.037	0.045	0.059	0.083	0.124	0.145	0.156
48085	TX	Collin	108	3	0.3062	0.007	0.028	0.040	0.052	0.104	0.189	0.438	0.717	0.904	1.178	1.564
48375	TX	Potter	6	1	0.0044	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.006	0.006	0.006	0.006
51770	VA	Roanoke City	12	1	0.0412	0.005	0.005	0.005	0.008	0.010	0.015	0.035	0.054	0.272	0.272	0.272
55117	WI	Sheboygan	12	1	0.0802	0.001	0.001	0.001	0.003	0.007	0.054	0.136	0.182	0.279	0.279	0.279
72013	PR	Arecibo	12	1	0.1774	0.038	0.038	0.038	0.064	0.102	0.178	0.264	0.290	0.310	0.310	0.310

Table 3-22 One-month average Pb-TSP for individual county concentrations nationwide (µg/m^3), non-source-oriented monitors, 2008-2010

Stcou code	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Statistics for individual counties (2008-2010)																
04013	AZ	Maricopa	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
06025	CA	Imperial	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
06037	CA	Los Angeles	6	1	0.0218	0.009	0.009	0.009	0.009	0.014	0.021	0.028	0.038	0.038	0.038	0.038
06065	CA	Riverside	33	1	0.0162	0.004	0.004	0.006	0.009	0.011	0.015	0.019	0.025	0.032	0.035	0.035
06071	CA	San Bernardino	224	8	0.0098	0.000	0.000	0.000	0.002	0.006	0.010	0.012	0.017	0.020	0.038	0.044
08005	CO	Arapahoe	72	2	0.0077	0.000	0.000	0.003	0.004	0.006	0.008	0.010	0.010	0.012	0.014	0.014
08031	CO	Denver	71	2	0.0091	0.001	0.001	0.003	0.004	0.007	0.010	0.012	0.014	0.014	0.022	0.022
13089	GA	DeKalb	9	1	0.0120	0.004	0.004	0.004	0.004	0.007	0.012	0.016	0.018	0.018	0.018	0.018
17031	IL	Cook	12	1	0.0056	0.003	0.003	0.003	0.004	0.005	0.005	0.006	0.008	0.008	0.008	0.008
17117	IL	Macoupin	10	1	0.0033	0.002	0.002	0.002	0.002	0.003	0.003	0.004	0.005	0.006	0.006	0.006
17119	IL	Madison	288	8	0.0195	0.010	0.010	0.010	0.010	0.012	0.016	0.025	0.034	0.040	0.060	0.070
17143	IL	Peoria	24	1	0.0101	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.012
17163	IL	Saint Clair	36	1	0.0188	0.010	0.010	0.010	0.010	0.012	0.016	0.020	0.032	0.053	0.066	0.066
18089	IN	Lake	36	1	0.0105	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.013	0.013	0.014	0.014
18097	IN	Marion	36	1	0.0206	0.010	0.010	0.010	0.012	0.014	0.018	0.026	0.032	0.038	0.054	0.054
18163	IN	Vanderburgh	36	1	0.0150	0.005	0.005	0.005	0.005	0.008	0.014	0.019	0.030	0.033	0.049	0.049
25025	MA	Suffolk	35	1	0.0058	0.002	0.002	0.002	0.003	0.004	0.005	0.008	0.010	0.012	0.013	0.013
26081	MI	Kent	33	2	0.0045	0.001	0.001	0.001	0.002	0.003	0.004	0.005	0.006	0.010	0.010	0.010
26163	MI	Wayne	31	2	0.0087	0.004	0.004	0.004	0.005	0.007	0.008	0.010	0.013	0.016	0.020	0.020
27017	MN	Carlton	12	1	0.0053	0.003	0.003	0.003	0.003	0.005	0.005	0.006	0.008	0.008	0.008	0.008
27037	MN	Dakota	36	2	0.0112	0.003	0.003	0.003	0.004	0.005	0.009	0.015	0.021	0.023	0.032	0.032
27053	MN	Hennepin	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
27075	MN	Lake	118	5	0.0035	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.008	0.010	0.017	0.036
27123	MN	Ramsey	126	4	0.0032	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.006	0.008	0.010	0.044
27137	MN	Saint Louis	10	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
27163	MN	Washington	71	3	0.0062	0.000	0.000	0.000	0.000	0.002	0.004	0.008	0.013	0.020	0.028	0.028
29097	MO	Jasper	72	2	0.0015	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.006	0.010	0.010
29187	MO	Saint Francois	72	3	0.0016	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.004	0.005	0.006	0.006
29189	MO	Saint Louis	12	1	0.0125	0.007	0.007	0.007	0.007	0.009	0.012	0.017	0.018	0.019	0.019	0.019
36047	NY	Kings	24	2	0.0327	0.008	0.008	0.009	0.009	0.018	0.032	0.039	0.054	0.080	0.089	0.089
39017	OH	Butler	33	1	0.0230	0.005	0.005	0.005	0.005	0.006	0.008	0.050	0.050	0.050	0.066	0.066

Stcou code	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
39029	OH	Columbiana	24	1	0.0131	0.010	0.010	0.010	0.010	0.011	0.012	0.014	0.018	0.020	0.020	0.020
39035	OH	Cuyahoga	34	1	0.0055	0.002	0.002	0.003	0.004	0.004	0.005	0.007	0.008	0.009	0.009	0.009
39049	OH	Franklin	107	3	0.0155	0.004	0.004	0.006	0.007	0.008	0.011	0.018	0.027	0.034	0.065	0.136
39143	OH	Sandusky	107	3	0.0143	0.004	0.004	0.006	0.007	0.009	0.012	0.017	0.024	0.030	0.041	0.041
39167	OH	Washington	36	1	0.0092	0.004	0.004	0.005	0.005	0.007	0.009	0.011	0.013	0.014	0.016	0.016
40115	OK	Ottawa	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
42003	PA	Allegheny	54	2	0.0048	0.002	0.002	0.002	0.003	0.003	0.005	0.006	0.007	0.008	0.010	0.010
42021	PA	Cambria	16	2	0.0124	0.003	0.003	0.003	0.005	0.006	0.013	0.017	0.021	0.025	0.025	0.025
42045	PA	Delaware	36	1	0.0105	0.000	0.000	0.000	0.000	0.004	0.009	0.015	0.019	0.024	0.053	0.053
42101	PA	Philadelphia	23	1	0.0463	0.040	0.040	0.040	0.040	0.040	0.040	0.044	0.054	0.058	0.128	0.128
42129	PA	Westmoreland	20	1	0.0432	0.040	0.040	0.040	0.040	0.040	0.043	0.046	0.047	0.048	0.048	0.048
48061	TX	Cameron	24	1	0.0210	0.011	0.011	0.011	0.012	0.014	0.020	0.027	0.033	0.033	0.039	0.039
48141	TX	El Paso	24	1	0.0419	0.037	0.037	0.040	0.040	0.040	0.040	0.042	0.050	0.050	0.053	0.053
48201	TX	Harris	35	1	0.0041	0.002	0.002	0.003	0.003	0.003	0.004	0.005	0.006	0.007	0.009	0.009
48479	TX	Webb	68	3	0.0206	0.014	0.014	0.014	0.014	0.015	0.017	0.019	0.029	0.056	0.087	0.087
49035	UT	Salt Lake	32	1	0.0053	0.003	0.003	0.003	0.004	0.004	0.005	0.006	0.007	0.008	0.010	0.010
51087	VA	Henrico	29	1	0.0134	0.004	0.004	0.005	0.006	0.008	0.011	0.018	0.026	0.028	0.035	0.035

Table 3-23 Three-month moving average Pb-TSP for individual county concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010

Stcou code	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Statistics for individual counties (2008-2010)[a]																
01109	AL	Pike	25	1	0.5771	0.223	0.223	0.247	0.256	0.302	0.574	0.719	1.088	1.178	1.210	1.210
06037	CA	Los Angeles	131	4	0.2521	0.023	0.023	0.036	0.041	0.055	0.078	0.237	0.543	0.832	2.452	2.489
12057	FL	Hillsborough	79	3	0.1940	0.011	0.011	0.015	0.037	0.063	0.110	0.249	0.423	0.582	1.770	1.770
13015	GA	Bartow	11	1	0.0125	0.009	0.009	0.009	0.009	0.011	0.013	0.014	0.015	0.016	0.016	0.016
13215	GA	Muscogee	12	1	0.0367	0.014	0.014	0.014	0.020	0.022	0.031	0.052	0.066	0.070	0.070	0.070
17031	IL	Cook	9	1	0.1364	0.068	0.068	0.068	0.068	0.109	0.135	0.150	0.241	0.241	0.241	0.241
17115	IL	Macon	10	1	0.0806	0.048	0.048	0.048	0.052	0.067	0.080	0.088	0.117	0.123	0.123	0.123
17119	IL	Madison	36	1	0.1346	0.027	0.027	0.035	0.036	0.063	0.113	0.207	0.283	0.341	0.416	0.416
17143	IL	Peoria	20	2	0.0121	0.010	0.010	0.010	0.010	0.011	0.012	0.014	0.015	0.016	0.016	0.016
17195	IL	Whiteside	10	1	0.0191	0.012	0.012	0.012	0.014	0.016	0.019	0.022	0.025	0.025	0.025	0.025
17201	IL	Winnebago	9	1	0.0356	0.019	0.019	0.019	0.019	0.021	0.027	0.057	0.063	0.063	0.063	0.063
18035	IN	Delaware	57	2	0.2866	0.053	0.053	0.059	0.073	0.090	0.159	0.246	0.495	1.867	2.163	2.163
18089	IN	Lake	46	2	0.0305	0.007	0.007	0.011	0.012	0.016	0.027	0.036	0.040	0.057	0.129	0.129
18097	IN	Marion	66	2	0.0198	0.005	0.005	0.006	0.007	0.011	0.014	0.025	0.036	0.043	0.079	0.079
18127	IN	Porter	10	1	0.0131	0.007	0.007	0.007	0.007	0.007	0.013	0.017	0.020	0.022	0.022	0.022
19155	IA	Pottawattamie	12	1	0.1581	0.034	0.034	0.034	0.067	0.113	0.153	0.220	0.246	0.263	0.263	0.263
20169	KS	Saline	9	1	0.2286	0.096	0.096	0.096	0.096	0.107	0.231	0.324	0.421	0.421	0.421	0.421
21151	KY	Madison	10	1	0.0212	0.013	0.013	0.013	0.014	0.015	0.017	0.024	0.037	0.049	0.049	0.049

Stcou code	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
26067	MI	Ionia	10	1	0.1980	0.106	0.106	0.106	0.110	0.128	0.212	0.259	0.273	0.284	0.284	0.284
27003	MN	Anoka	10	1	0.0161	0.006	0.006	0.006	0.008	0.010	0.013	0.022	0.029	0.031	0.031	0.031
27037	MN	Dakota	36	1	0.2026	0.068	0.068	0.072	0.088	0.104	0.216	0.248	0.357	0.415	0.429	0.429
27145	MN	Stearns	10	1	0.0032	0.000	0.000	0.000	0.001	0.002	0.004	0.004	0.005	0.005	0.005	0.005
29093	MO	Iron	158	6	0.3465	0.010	0.011	0.019	0.022	0.033	0.142	0.549	0.901	1.167	2.076	2.456
29099	MO	Jefferson	423	19	0.4925	0.023	0.033	0.050	0.071	0.187	0.385	0.723	0.989	1.186	2.017	2.889
29179	MO	Reynolds	40	4	0.0397	0.012	0.012	0.014	0.015	0.017	0.031	0.057	0.087	0.089	0.100	0.100
31053	NE	Dodge	7	1	0.0474	0.019	0.019	0.019	0.019	0.020	0.060	0.067	0.072	0.072	0.072	0.072
31127	NE	Nemaha	6	1	0.0447	0.019	0.019	0.019	0.019	0.024	0.032	0.075	0.087	0.087	0.087	0.087
36071	NY	Orange	99	3	0.0271	0.003	0.003	0.004	0.005	0.007	0.027	0.037	0.068	0.075	0.086	0.086
39035	OH	Cuyahoga	70	3	0.0905	0.006	0.006	0.010	0.011	0.021	0.050	0.122	0.221	0.287	0.531	0.531
39051	OH	Fulton	30	1	0.1609	0.025	0.025	0.027	0.046	0.054	0.092	0.254	0.354	0.453	0.567	0.567
39091	OH	Logan	100	4	0.0499	0.004	0.004	0.004	0.006	0.033	0.047	0.072	0.090	0.095	0.100	0.100
39101	OH	Marion	8	1	0.0379	0.032	0.032	0.032	0.032	0.034	0.037	0.042	0.047	0.047	0.047	0.047
39151	OH	Stark	9	1	0.0180	0.015	0.015	0.015	0.015	0.016	0.018	0.019	0.023	0.023	0.023	0.023
39155	OH	Trumbull	6	1	0.0080	0.005	0.005	0.005	0.005	0.006	0.008	0.010	0.011	0.011	0.011	0.011
40121	OK	Pittsburg	9	1	0.0021	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003
41071	OR	Yamhill	10	1	0.0166	0.009	0.009	0.009	0.011	0.013	0.016	0.019	0.026	0.027	0.027	0.027
42003	PA	Allegheny	20	2	0.0414	0.009	0.009	0.011	0.012	0.017	0.030	0.054	0.099	0.120	0.138	0.138
42007	PA	Beaver	41	3	0.1160	0.043	0.043	0.052	0.056	0.083	0.114	0.159	0.170	0.187	0.206	0.206
42011	PA	Berks	105	6	0.0995	0.038	0.039	0.041	0.045	0.051	0.078	0.145	0.183	0.197	0.242	0.251
42045	PA	Delaware	10	1	0.0447	0.043	0.043	0.043	0.043	0.043	0.045	0.046	0.047	0.047	0.047	0.047
42055	PA	Franklin	7	1	0.0447	0.043	0.043	0.043	0.043	0.043	0.045	0.046	0.046	0.046	0.046	0.046
42063	PA	Indiana	10	1	0.0447	0.043	0.043	0.043	0.043	0.043	0.044	0.046	0.049	0.049	0.049	0.049
42079	PA	Luzerne	6	1	0.1078	0.084	0.084	0.084	0.084	0.085	0.103	0.135	0.137	0.137	0.137	0.137
42129	PA	Westmoreland	10	1	0.0434	0.041	0.041	0.041	0.042	0.042	0.044	0.044	0.046	0.046	0.046	0.046
47093	TN	Knox	44	2	0.0165	0.007	0.007	0.009	0.009	0.012	0.016	0.020	0.023	0.027	0.035	0.035
47163	TN	Sullivan	118	4	0.0554	0.030	0.030	0.033	0.035	0.039	0.045	0.060	0.100	0.125	0.134	0.168
48085	TX	Collin	108	3	0.3101	0.048	0.051	0.070	0.085	0.120	0.217	0.469	0.682	0.753	1.189	1.262
51770	VA	Roanoke City	10	1	0.0466	0.013	0.013	0.013	0.016	0.019	0.026	0.097	0.108	0.109	0.109	0.109
55117	WI	Sheboygan	10	1	0.0897	0.012	0.012	0.012	0.034	0.058	0.076	0.126	0.164	0.170	0.170	0.170
72013	PR	Arecibo	10	1	0.1725	0.059	0.059	0.059	0.068	0.129	0.194	0.213	0.241	0.245	0.245	0.245

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.

Table 3-24 Three-month moving average Pb-TSP for individual county concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010

Stcou code	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Statistics for individual counties (2008-2010)[a]																
06025	CA	Imperial	31	1	0.0165	0.007	0.007	0.008	0.011	0.013	0.017	0.021	0.023	0.023	0.023	0.023
06037	CA	Los Angeles	218	8	0.0100	0.000	0.000	0.002	0.004	0.006	0.009	0.013	0.016	0.020	0.028	0.035
06065	CA	Riverside	72	2	0.0078	0.002	0.002	0.004	0.005	0.007	0.008	0.010	0.011	0.011	0.011	0.011
06071	CA	San Bernardino	69	2	0.0091	0.003	0.003	0.005	0.006	0.007	0.009	0.011	0.013	0.014	0.017	0.017
08005	CO	Arapahoe	7	1	0.0126	0.011	0.011	0.011	0.011	0.011	0.013	0.014	0.014	0.014	0.014	0.014
08031	CO	Denver	10	1	0.0054	0.004	0.004	0.004	0.004	0.005	0.006	0.006	0.006	0.006	0.006	0.006
13089	GA	DeKalb	8	1	0.0035	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.004	0.004	0.004	0.004
17031	IL	Cook	287	8	0.0196	0.010	0.010	0.010	0.010	0.012	0.017	0.025	0.033	0.038	0.047	0.051
17117	IL	Macoupin	24	1	0.0101	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011	0.011	0.011
17119	IL	Madison	36	1	0.0188	0.010	0.010	0.010	0.011	0.014	0.016	0.022	0.036	0.036	0.039	0.039
17143	IL	Peoria	36	1	0.0105	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.012	0.012	0.013	0.013
17163	IL	Saint Clair	36	1	0.0204	0.012	0.012	0.012	0.014	0.016	0.020	0.024	0.029	0.033	0.036	0.036
18089	IN	Lake	36	1	0.0149	0.007	0.007	0.007	0.007	0.010	0.014	0.018	0.024	0.032	0.037	0.037
18097	IN	Marion	33	1	0.0056	0.003	0.003	0.003	0.003	0.004	0.005	0.007	0.009	0.010	0.011	0.011
18163	IN	Vanderburgh	31	2	0.0047	0.002	0.002	0.003	0.003	0.004	0.005	0.005	0.006	0.007	0.007	0.007
25025	MA	Suffolk	24	2	0.0093	0.005	0.005	0.006	0.006	0.008	0.009	0.011	0.013	0.015	0.016	0.016
26081	MI	Kent	10	1	0.0055	0.004	0.004	0.004	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006
26163	MI	Wayne	32	2	0.0119	0.004	0.004	0.004	0.005	0.005	0.012	0.017	0.021	0.023	0.024	0.024
27017	MN	Carlton	10	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
27037	MN	Dakota	112	5	0.0036	0.000	0.000	0.001	0.001	0.001	0.003	0.005	0.007	0.012	0.013	0.015
27053	MN	Hennepin	124	4	0.0033	0.000	0.001	0.001	0.001	0.002	0.003	0.004	0.006	0.006	0.015	0.016
27075	MN	Lake	8	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
27123	MN	Ramsey	65	3	0.0061	0.001	0.001	0.001	0.001	0.002	0.005	0.008	0.014	0.016	0.017	0.017
27137	MN	Saint Louis	70	2	0.0016	0.000	0.000	0.000	0.000	0.001	0.001	0.002	0.004	0.004	0.005	0.005
27163	MN	Washington	70	3	0.0017	0.000	0.000	0.000	0.000	0.001	0.001	0.003	0.004	0.004	0.005	0.005
29097	MO	Jasper	10	1	0.0135	0.009	0.009	0.009	0.011	0.012	0.014	0.015	0.016	0.017	0.017	0.017
29187	MO	Saint Francois	21	2	0.0337	0.011	0.011	0.012	0.012	0.027	0.035	0.042	0.048	0.053	0.054	0.054
29189	MO	Saint Louis	33	1	0.0243	0.005	0.005	0.005	0.006	0.007	0.008	0.050	0.050	0.050	0.055	0.055
36047	NY	Kings	24	1	0.0131	0.011	0.011	0.011	0.011	0.012	0.013	0.014	0.016	0.018	0.019	0.019
39017	OH	Butler	30	1	0.0055	0.003	0.003	0.004	0.004	0.005	0.006	0.006	0.007	0.007	0.008	0.008
39029	OH	Columbiana	105	3	0.0148	0.005	0.005	0.007	0.008	0.010	0.013	0.017	0.021	0.028	0.054	0.057
39035	OH	Cuyahoga	105	3	0.0144	0.005	0.006	0.006	0.008	0.010	0.013	0.018	0.023	0.027	0.033	0.035
39049	OH	Franklin	36	1	0.0092	0.005	0.005	0.005	0.005	0.008	0.010	0.011	0.011	0.012	0.012	0.012
39143	OH	Sandusky	10	1	0.0052	0.004	0.004	0.004	0.004	0.005	0.005	0.006	0.006	0.006	0.006	0.006
39167	OH	Washington	48	2	0.0047	0.002	0.002	0.002	0.003	0.004	0.004	0.006	0.007	0.007	0.008	0.008
40115	OK	Ottawa	12	2	0.0128	0.005	0.005	0.005	0.006	0.010	0.014	0.016	0.018	0.019	0.019	0.019
42003	PA	Allegheny	36	1	0.0101	0.000	0.000	0.000	0.000	0.007	0.012	0.014	0.016	0.018	0.025	0.025
42021	PA	Cambria	23	1	0.0459	0.040	0.040	0.040	0.040	0.040	0.041	0.046	0.069	0.070	0.073	0.073
42045	PA	Delaware	14	1	0.0427	0.040	0.040	0.040	0.040	0.040	0.042	0.045	0.046	0.047	0.047	0.047
42101	PA	Philadelphia	22	1	0.0214	0.013	0.013	0.014	0.014	0.018	0.022	0.025	0.029	0.029	0.030	0.030
42129	PA	Westmoreland	24	1	0.0417	0.037	0.037	0.040	0.040	0.040	0.041	0.043	0.046	0.047	0.048	0.048

Stcou code	State	County name	N monthly means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
48061	TX	Cameron	33	1	0.0042	0.002	0.002	0.003	0.003	0.004	0.004	0.005	0.005	0.006	0.006	0.006
48141	TX	El Paso	56	3	0.0212	0.014	0.014	0.014	0.015	0.016	0.018	0.023	0.038	0.040	0.040	0.040
48201	TX	Harris	30	1	0.0051	0.004	0.004	0.004	0.004	0.005	0.005	0.006	0.006	0.007	0.007	0.007
48479	TX	Webb	23	1	0.0121	0.006	0.006	0.007	0.007	0.008	0.010	0.016	0.021	0.022	0.026	0.026
49035	UT	Salt Lake	10	1	0.0145	0.007	0.007	0.007	0.007	0.008	0.011	0.016	0.032	0.036	0.036	0.036

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.

3.8.2 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 2008-2010 were used to generate the box plots, which are stratified by season as follows: 1 = winter (December-February), 2 = spring (March-May), 3 = summer (June-August), and 4 = fall (September-November). The comparison tables include the Pearson correlation coefficient (R), Spearman rank-ordered correlation coefficient (ρ), the 90th percentile of the absolute difference in concentrations (P90) in μg/m^3, the coefficient of divergence (*COD*) and the straight-line distance between monitor pairs (d) in km. The *COD* provides an indication of the variability across the monitoring sites within each county and is defined as follows:

$$COD_{jk} = \sqrt{\frac{1}{p}\sum_{i=1}^{p}\left(\frac{X_{ij} - X_{ik}}{X_{ij} + X_{ik}}\right)^2}$$

Equation 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 2008-2010 are near

or below the analysis method's stated method detection limit (MDL). The MDL is generally taken as 0.01 because it is the upper value of the range of MDLs reported for 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.

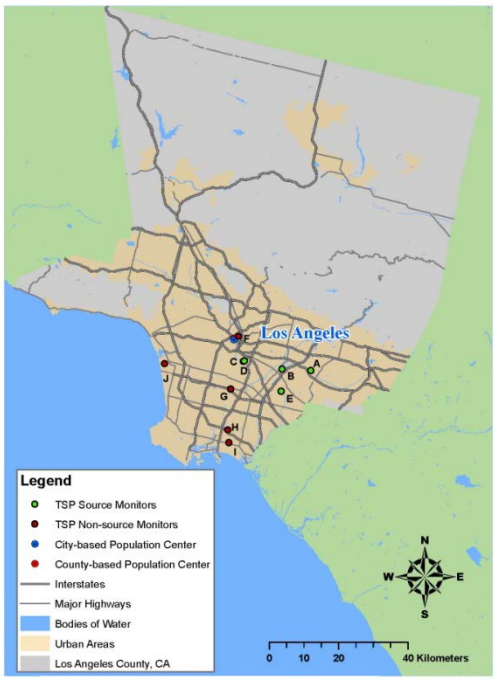
Figure 3-42 illustrates Pb monitor locations within Los Angeles County, CA. Ten monitors are located within Los Angeles County, five of which were source-oriented and the other five were non-source-oriented monitors. Monitor A was located immediately downwind of the Quemetco battery recycling facility in the City of Industry, CA. This source was estimated to produce 0.32 tons of Pb/yr (U.S. EPA, 2008c). Monitor C was sited in a street canyon just upwind of the Exide Pb recycling facility, which was estimated to produce 2.0 tons of Pb/yr (U.S. EPA, 2008c). Monitor D was situated slightly northwest of the same Pb recycling facility. It is still in relatively close proximity but not downwind on most occasions. Monitor B was located 12 km downwind of the Exide facility. Monitor E was located nearby the Trojan Battery recycling facility, which emitted 0.79 tons Pb/yr (U.S. EPA, 2008c). Location of the non-source-oriented monitors varied. Monitor F was positioned on a roof top 60 meters away from a 4-lane arterial road and 100 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 3-43 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 3-42 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 3-44. 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 $\mu g/m^3$.

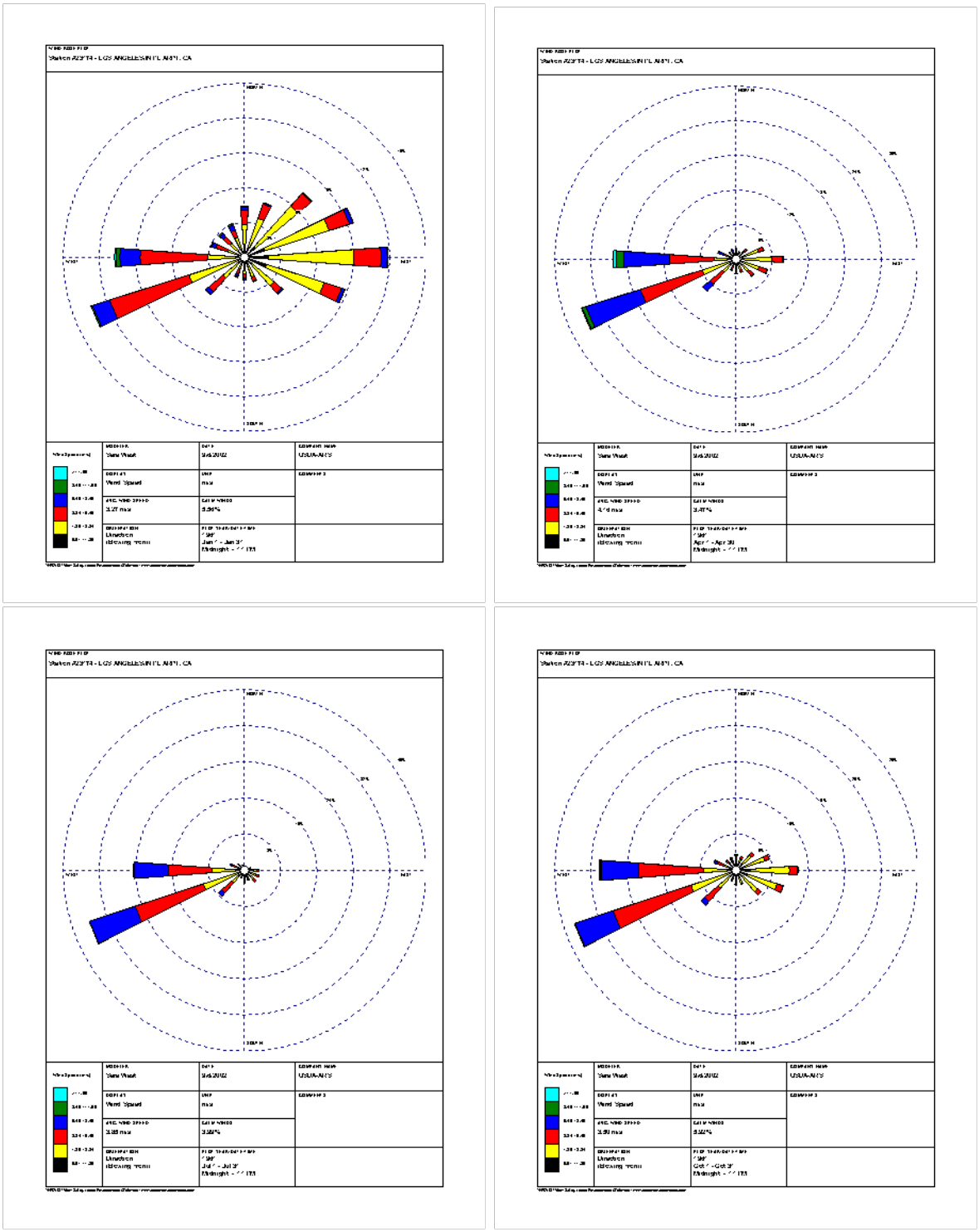
The non-source-oriented monitors located at sites F-J all recorded low concentrations, with average values ranging from 0.004 to 0.018 $\mu g/m^3$ (Figure 3-44). The highest average year-round concentrations were recorded at site F. The 95th percentiles at these sites ranged from 0.01 to 0.04 $\mu g/m^3$. 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 3-25), illustrate that Pb has high intra-urban spatial variability. For the source-oriented monitors, the highest correlation ($R = 0.59$, $\rho = 0.57$) occurred for monitors C and D, which covered the same site. Because monitor D was slightly farther from the Exide source and slightly upstream of the predominant wind direction, the signal it received from the source site was correspondingly lower. Hence, the correlation between these sites was moderate despite their relatively close proximity. In general, low or even negative correlations were observed between the source-oriented and non-source-oriented monitors. The exception to this was the Spearman-ranked correlation between source-oriented monitor B and non-source-oriented monitor F, with $\rho = 0.74$. Pearson correlation was much lower for this pair ($R = 0.33$). Monitors B and F are roughly 16 km apart, whereas monitor B is only 12 km from monitors D and C, 8 km from monitor E, and 6 km from monitor A. It is possible that monitors B and F both captured a source that was either longer in range or more ubiquitous and so would have been obscured by the stronger source signals at sites A, C, D, and E. Comparisons between the non-source-oriented monitors revealed moderate correlation between sites (G to J [$R = 0.29$ to 0.71, $\rho = 0.37$ to 0.65]). Sites G, H, I and J are all located in the southwestern quadrant of Los Angeles. It is possible that they are also exposed to a ubiquitous source that produces a common signal at these four sites.



Note: Monitor locations are denoted by green markers, and source locations are denoted by red markers. Top: view of all Pb FRM monitors in Los Angeles County. Bottom left: Close up of the industrial site near monitors C and D. Bottom right: Close up of the populated area captured by monitor F.

Figure 3-42 Pb TSP monitor and source locations within Los Angeles County, CA (06-037), 2007-2009.



Source: NRCS (2011).

Note: Clockwise from top left: January, April, July, and October. Note that the wind percentages vary from month to month.

Figure 3-43 Wind roses for Los Angeles County, CA, from meteorological data at the Los Angeles International Airport, 1961-1990.

Site	A	B	C	D	E	F	G	H	I	J
SITE ID	06-037-1404	06-037-1602	06-037-1405	06-037-1406	06-037-1403	06-037-1103	06-037-1301	06-037-4002	06-037-4004	06-037-5005
MEAN	0.074	0.013	0.68	0.12	0.068	0.018	0.015	0.0083	0.0087	0.0040
SD	0.040	0.017	1.0	0.092	0.052	0.011	0.012	0.0068	0.0069	0.0064
OBS	66	112	617	242	128	121	108	120	117	109
% BELOW MDL	0	75	0	0	0	45	65	85	85	95
Source orientation	Source	Source	Source	Source	Source	Non-source	Non-source	Non-source	Non-source	Non-source

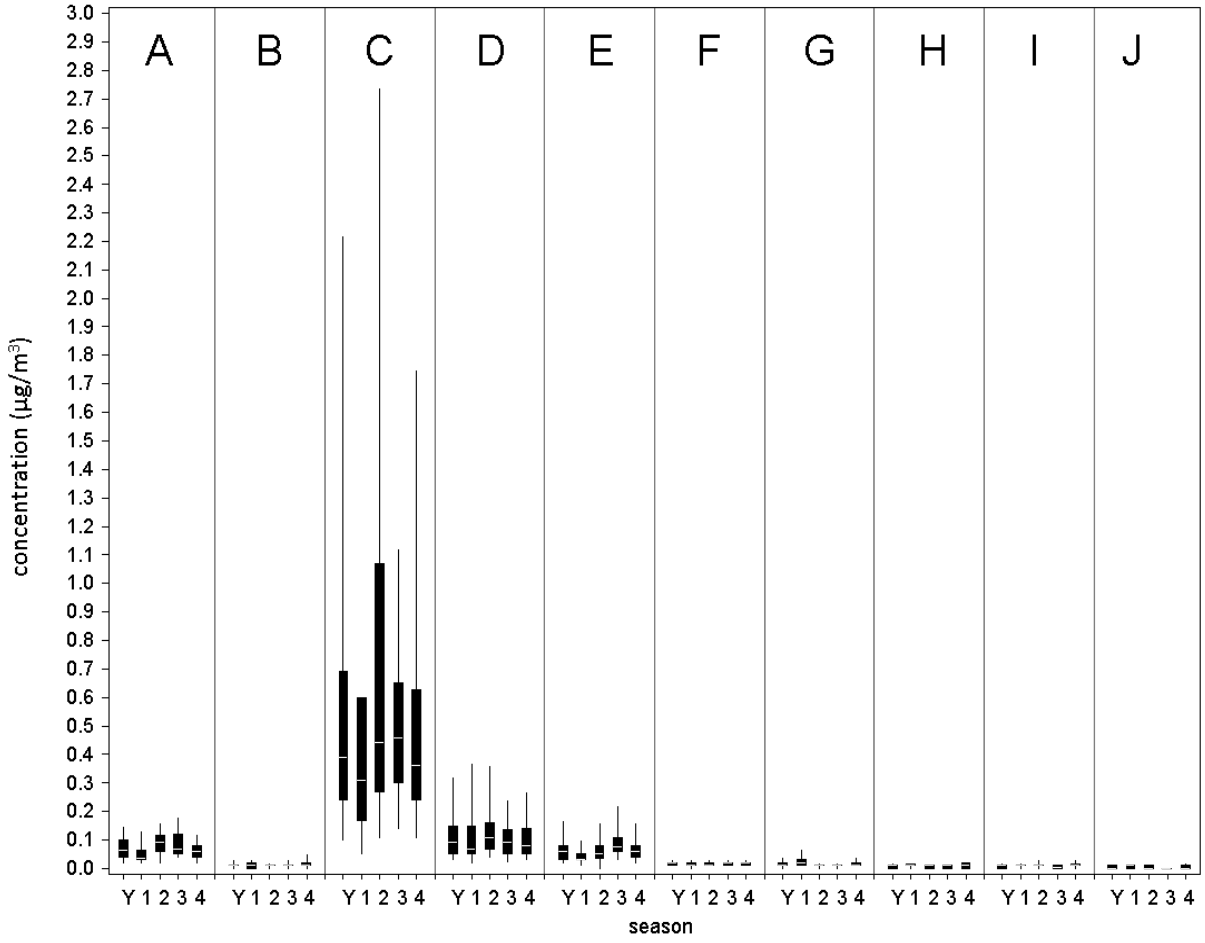


Figure 3-44 Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Los Angeles County, CA (06-037), 2007-2009.

Table 3-25 Comparisons between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Los Angeles County, CA (06-037), 2007-2009

			A	B	C	D	E	F	G	H	I	J
			Source	Source	Source	Source	Source	Non-Source	Non-Source	Non-Source	Non-Source	Non-Source
A	Source	R	1.00	-0.04	0.14	0.10	0.17	0.03	0.00	-0.08	-0.07	-0.27
		ρ	1.00	0.16	0.10	0.08	0.27	-0.15	0.00	0.14	-0.02	-0.09
		P90	0.00	0.08	0.49	0.10	0.10	0.08	0.06	0.08	0.08	0.08
		COD	0.00	0.63	0.64	0.31	0.34	0.57	0.57	0.79	0.77	0.85
B	Source	R		1.00	0.06	0.17	-0.06	0.33	0.29	0.40	0.22	0.20
		ρ		1.00	0.05	0.05	0.07	0.74	0.12	0.28	0.11	0.10
		P90		0.00	3.59	0.25	0.10	0.02	0.02	0.01	0.02	0.02
		COD		0.00	0.96	0.84	0.71	0.46	0.48	0.61	0.60	0.81
C	Source	R			1.00	0.59	0.08	0.12	0.24	0.28	0.18	0.08
		ρ			1.00	0.57	0.03	-0.08	0.26	0.28	0.20	0.13
		P90			0.00	1.76	2.14	3.59	4.22	3.59	3.59	3.92
		COD			0.00	0.68	0.77	0.95	0.96	0.98	0.98	0.99
D	Source	R				1.00	0.18	0.33	0.09	0.32	0.20	0.03
		ρ				1.00	0.12	0.17	0.11	0.24	0.21	0.07
		P90				0.00	0.17	0.24	0.25	0.25	0.25	0.25
		COD				0.00	0.42	0.78	0.80	0.89	0.89	0.95
E	Source	R					1.00	0.05	0.07	0.00	0.09	-0.07
		ρ					1.00	0.13	0.06	0.24	0.07	0.18
		P90					0.00	0.10	0.10	0.11	0.11	0.11
		COD					0.00	0.61	0.64	0.78	0.79	0.90
F	Non-Source	R						1.00	0.10	0.43	0.34	0.21
		ρ						1.00	0.02	0.19	0.09	0.09
		P90						0.00	0.02	0.02	0.02	0.02
		COD						0.00	0.39	0.61	0.58	0.82
G	Non-Source	R							1.00	0.71	0.55	0.54
		ρ							1.00	0.65	0.39	0.38
		P90							0.00	0.01	0.02	0.02
		COD							0.00	0.54	0.61	0.85
H	Non-Source	R								1.00	0.60	0.51
		ρ								1.00	0.51	0.40
		P90								0.00	0.01	0.01
		COD								0.00	0.55	0.77
I	Non-Source	R									1.00	0.29
		ρ									1.00	0.37
		P90									0.00	0.01
		COD									0.00	0.78
J	Non-Source	R										1.00
		ρ										1.00
		P90										0.00
		COD										0.00

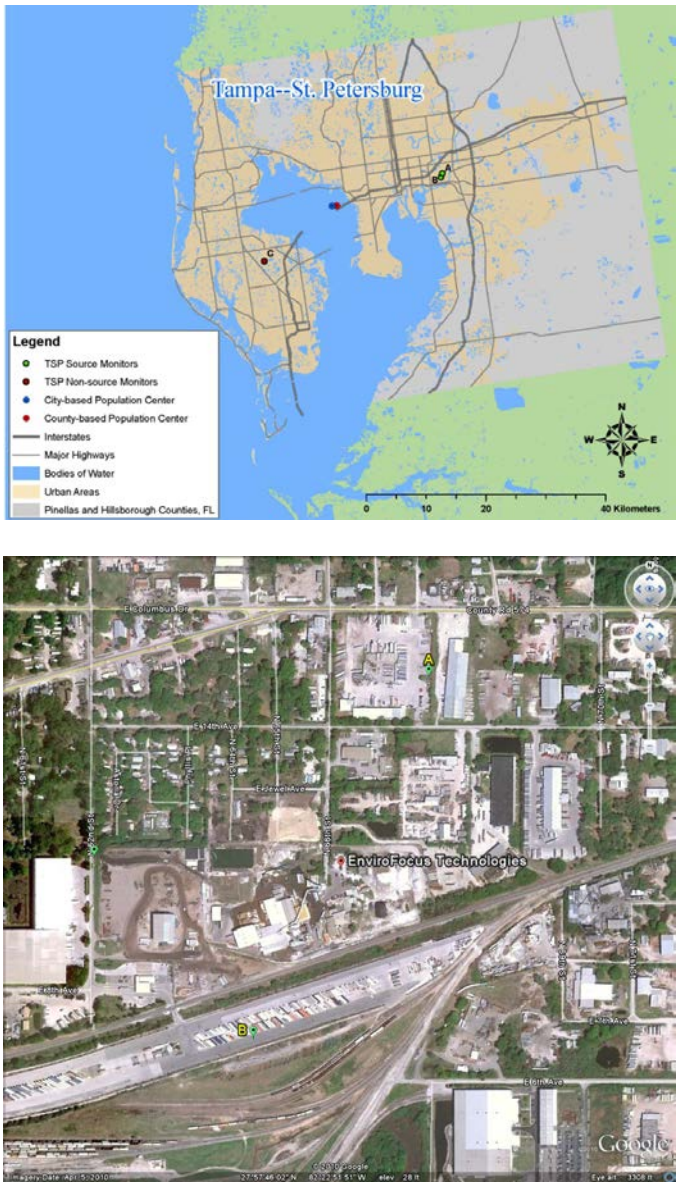
Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (ρ), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

Figure 3-45 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/year (U.S. EPA, 2008d), 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 3-46 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.

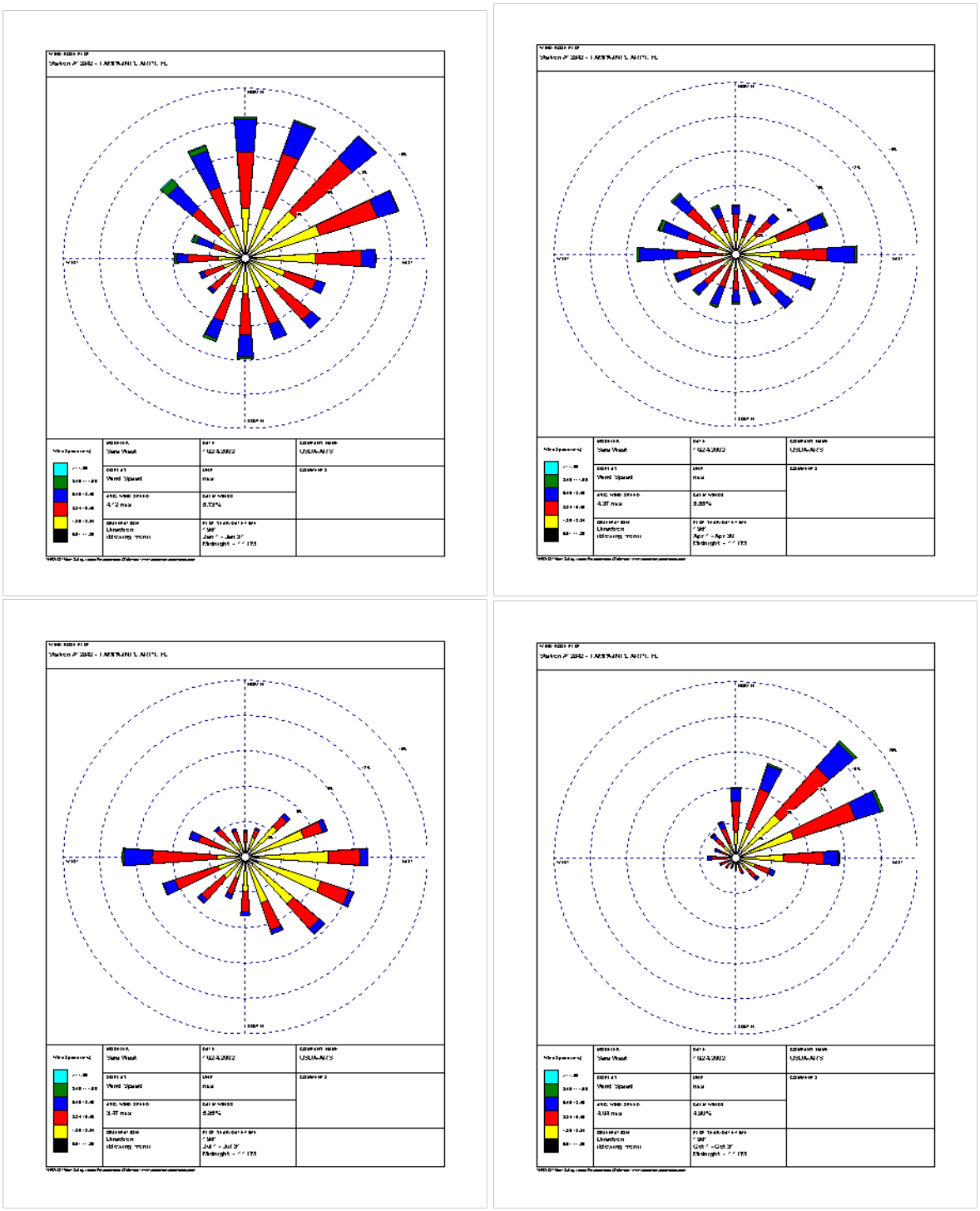
Seasonal and year-round concentrations are reported for Hillsborough and Pinellas Counties in Figure 3-47. The average annual concentration at monitor A was 0.15 $\mu g/m^3$, and the 95th percentile was 0.70 $\mu g/m^3$. During winter, the 60th percentile of the data met the level of the NAAQS. At this 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 3-26, illustrate that Pb has high intra-urban spatial variability. The source-oriented monitors were anticorrelated ($R = -0.09$, $\rho = -0.08$). This was likely related to the fact that they were designated to monitor the same source and were downwind of the source at different times.



Top: view of all Pb FRM monitors in Hillsborough and Pinellas Counties.
Bottom: Close up of industrial site around monitors A and B.

Figure 3-45 Pb TSP monitor locations within Hillsborough and Pinellas Counties, FL (12-057 and 12-103), 2007-2009.



Source: NRCS (2011).

Note: Clockwise from top left: January, April, July, and October. Note that wind percentages vary from month to month.

Figure 3-46 Wind roses for Hillsborough/Pinellas Counties, FL, obtained from meteorological data at Tampa International Airport, 1961-1990.

Site	A	B	C
SITE ID	12-057-1073	12-057-1066	12-103-3005
MEAN	0.15	0.45	0.00
SD	0.27	1.08	0.00
OBS	154	155	58
% BELOW MDL	20	5	95
Source orientation	Source	Source	Non-source

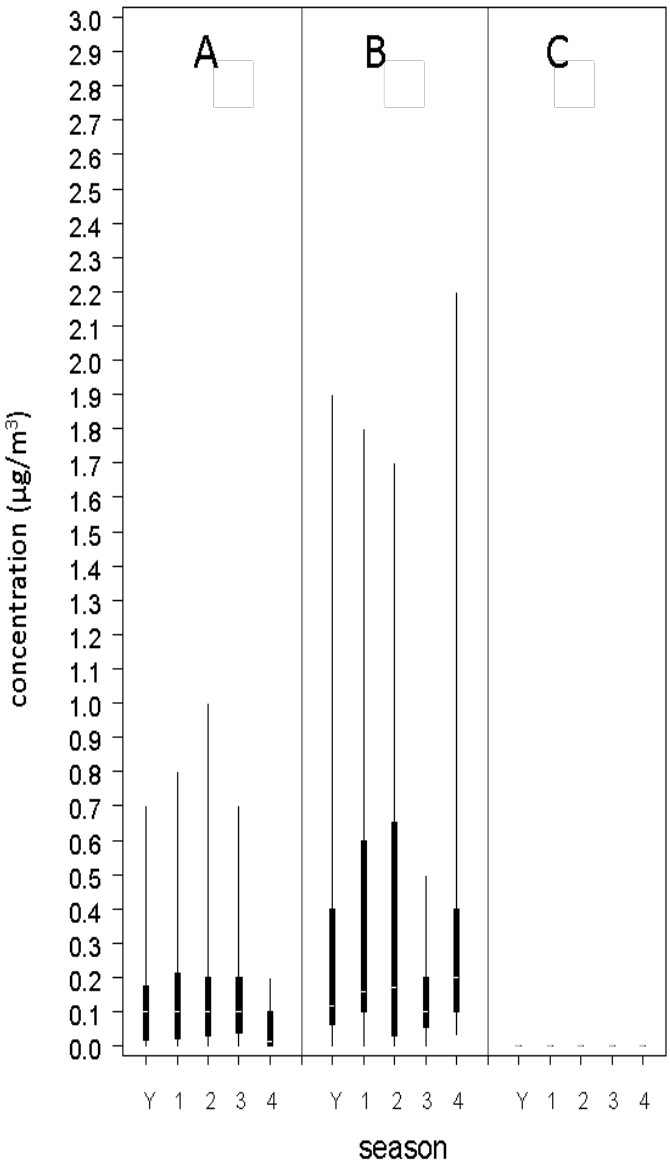


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Figure 3-47 Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Hillsborough and Pinellas Counties, FL (12-057 and 12-103), 2007-2009.

Table 3-26 Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Hillsborough and Pinellas Counties, FL (12-057 and 12-103), 2007-2009

			A	B	C
			Source	Source	Non-source
A	Source	R	1.00	-0.09	
		ρ	1.00	-0.08	
		P90	0.00	1.20	0.50
		COD	0.00	0.71	1.00
B	Source	R		1.00	
		ρ		1.00	
		P90		0.00	2.20
		COD		0.00	1.00
C	Non-source	R			1.00
		ρ			1.00
		P90			0.00
		COD			0.00

Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (*ρ*), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

Figure 3-48 illustrates Pb monitor locations within Cook County, IL. Eight monitors were located within Cook County, four of which were designated by the Illinois Environmental Protection Agency (IEPA) in data reported to the AQS as source-oriented and the other four were non-source-oriented monitors. Monitor A was situated within 10 km of 6 sources ranging in emissions from 0.14 to 1.08 tons/year (U.S. EPA, 2008a). Monitor A was also sited in the median of I-90/I-94. Monitor B was located on the northern roadside of I-290 5 meters from the closest lane of traffic and was within 10 km of 2 Pb sources (0.41 and 1.08 tons/year) (U.S. EPA, 2008a). Monitor C was also located within 10 km of 6 sources in Cook County and Lake County, IN; the largest of those sources was 2.99 tons/year and was located 8 km southeast of monitor C (U.S. EPA, 2008a). Monitor C was placed on the roof of a high school. Monitor D was located roughly 60 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/year 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/year source (U.S. EPA, 2008a). Monitor G was situated atop an elementary school in a residential neighborhood on the south side of Chicago, roughly 100 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/year (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 3-49 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 3-50 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.

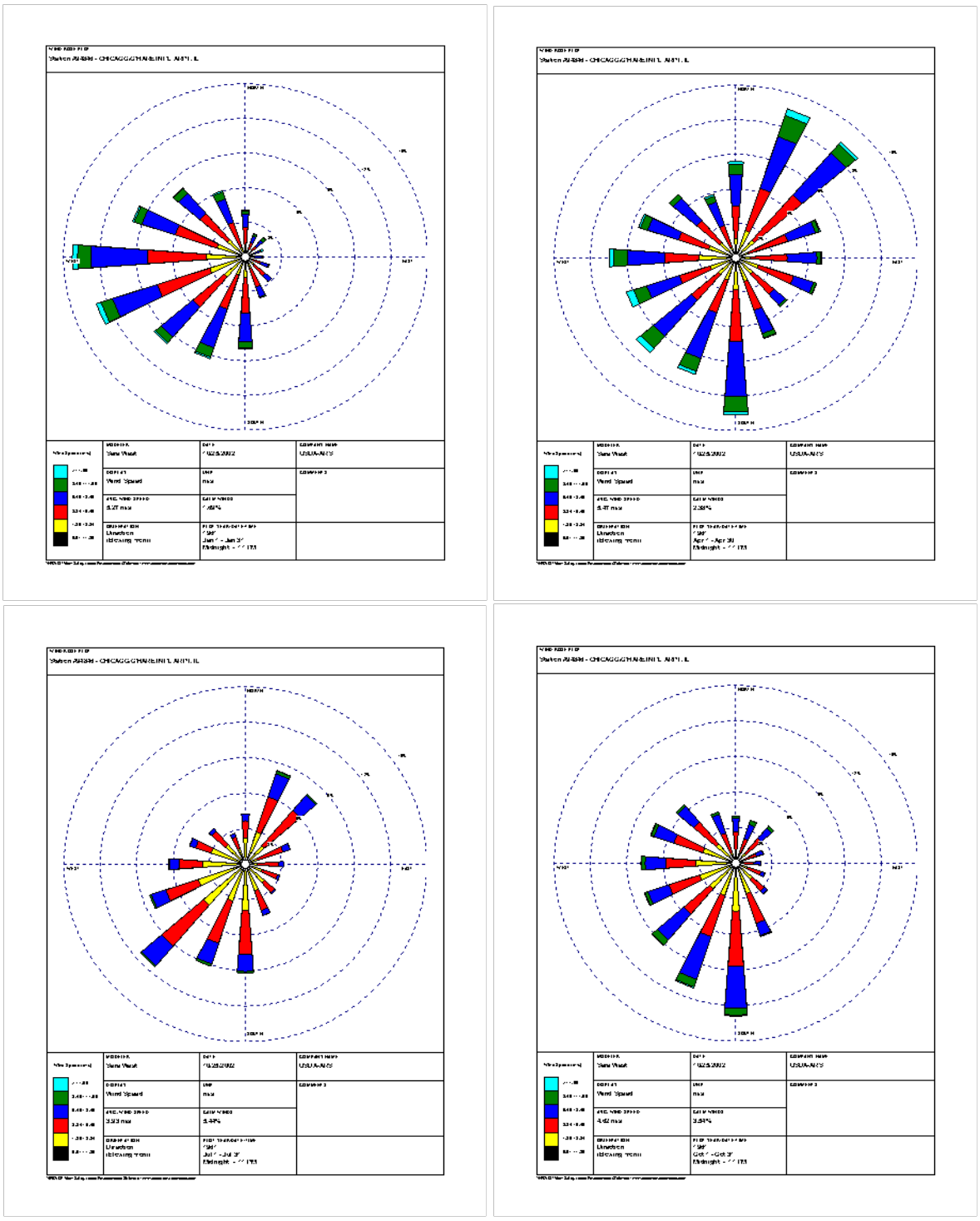
Three “near-road” monitors, A, B, and D can be compared with the other monitors to consider the possibility of roadside resuspension of Pb dust from contemporaneous sources, as discussed in Section 3.2.2.5. It would be expected that resuspension would diminish with distance from the road. The 2 roadside 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. The average concentration at this site was 0.012 $\mu g/m^3$, and 85% of data were below the MDL. Non-source monitors, E, F, G, and H had average concentrations of 0.011-0.017 $\mu g/m^3$. It is possible that the difference between Pb concentrations at monitors A and B and Pb concentrations at the other monitors was related to proximity to the roadway, although this cannot be stated with certainty without

source apportionment data to confirm or refute the influence of industrial plumes from Lake County, IN or local sources at each of the monitors.

Comparison among the monitor data demonstrates a high degree of spatial variability (Table 3-27). None of the source-oriented monitors were well correlated with each other. The highest correlation between source-oriented monitors occurred for monitors (A and B [$R = 0.32$, $\rho = 0.26$]). 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 Spearman correlation occurred between monitors D and H ($\rho = 0.53$), but Pearson correlation was much lower for this pair ($R = 0.19$). Both were located on the north side of Cook County, but monitor H was roughly 20 km northeast of monitor D. Winds blew from the southwest roughly 20-30% of the time throughout the year and from the northeast 20-25% of the time between the months of March and July, so the correlation may have been related to a common signal transported across both sites. Monitors B and F ($R = 0.52$, $\rho = 0.46$) 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 ($R = 0.42$ to 0.54, $\rho = 0.36$ to 0.45), and the correlation between monitors (E and G) was moderate ($R = 0.65$, $\rho = 0.40$). 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.

Top: view of all Pb FRM monitors in Cook County.
Bottom left: Close up of the high traffic site around monitor A.
Bottom right: Close up of O'Hare International Airport adjacent to monitor D.

Figure 3-48 Pb TSP Monitor locations within Cook County, IL (17-031), 2007-2009.



Source: NRCS (2011)

Note: Clockwise from the top left: January, April, July, and October. Note that the wind percentages vary from month to month.

Figure 3-49 Wind roses for Cook County, IL, obtained from meteorological data at O'Hare International Airport, 1961-1990.

Site	A	B	C	D	E	F	G	H
SITE ID	17-031-0026	17-031-6003	17-031-0022	17-031-3103	17-031-0001	17-031-0052	17-031-3301	17-031-4201
MEAN	0.030	0.024	0.031	0.012	0.013	0.017	0.017	0.011
SD	0.020	0.013	0.036	0.0062	0.0078	0.0098	0.0097	0.0031
OBS	179	175	177	168	177	175	171	168
% BELOW MDL	15	25	25	85	75	55	50	95
Source orientation	Source	Source	Source	Source	Non-source	Non-source	Non-source	Non-source

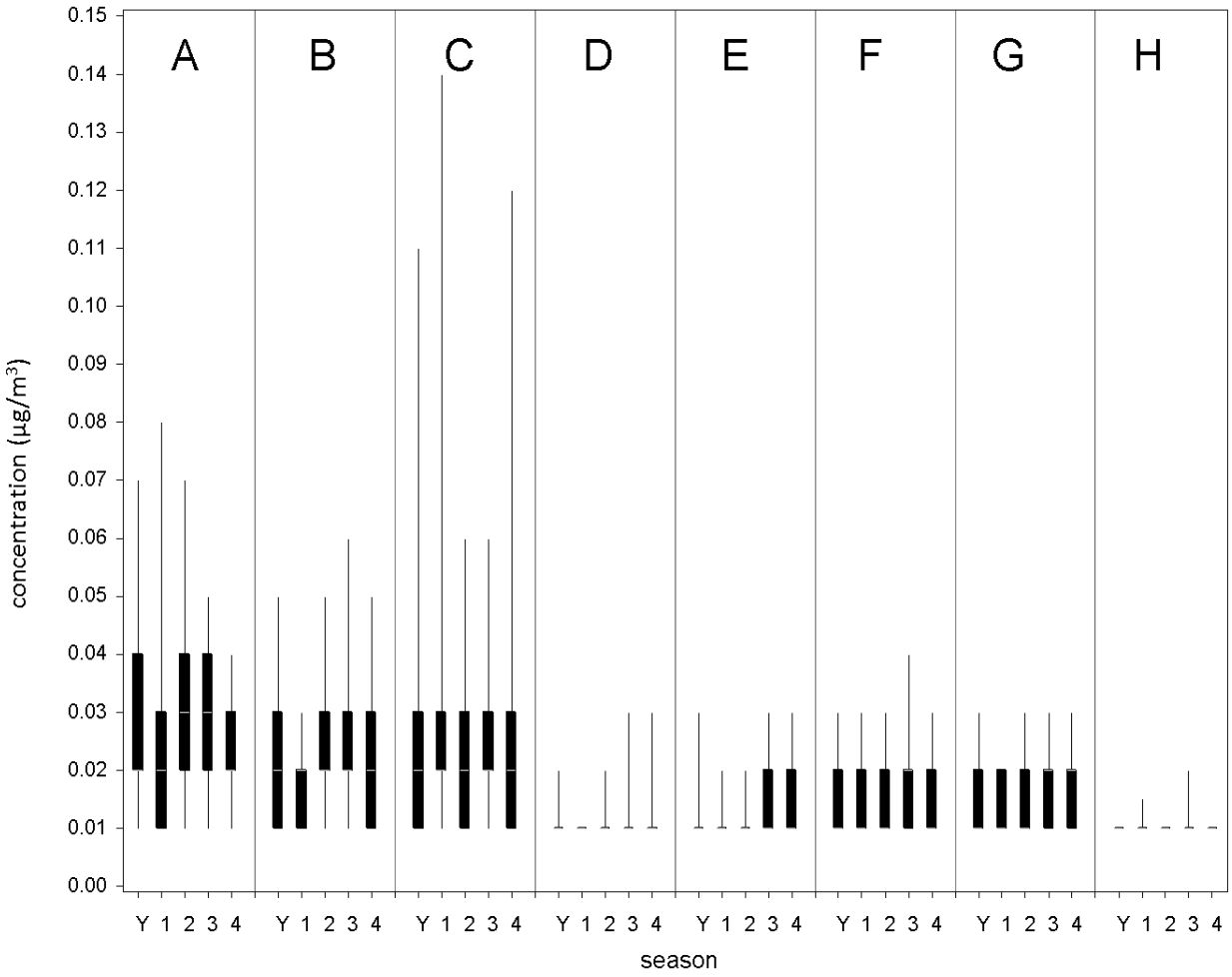


Figure 3-50 Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Cook County, IL (17-031), 2007-2009.

Table 3-27 Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Cook County, IL (17-031), 2007-2009

			A	B	C	D	E	F	G	H
			Source	Source	Source	Source	Non-Source	Non-Source	Non-Source	Non-Source
A	**Source**	R	1.00	0.32	0.00	0.05	0.17	0.39	0.34	0.06
		ρ	1.00	0.26	-0.01	0.08	0.06	0.32	0.18	0.06
		P90	0.00	0.03	0.06	0.04	0.04	0.03	0.03	0.04
		COD	0.00	0.29	0.38	0.43	0.41	0.36	0.36	0.45
B	**Source**	R		1.00	0.14	0.07	0.54	0.52	0.60	0.06
		ρ		1.00	0.05	0.10	0.32	0.46	0.35	-0.01
		P90		0.00	0.04	0.03	0.03	0.02	0.02	0.03
		COD		0.00	0.33	0.36	0.34	0.29	0.30	0.40
C	**Source**	R			1.00	0.01	0.24	0.05	0.19	-0.04
		ρ			1.00	0.04	0.16	0.10	0.17	0.06
		P90			0.00	0.05	0.05	0.04	0.05	0.05
		COD			0.00	0.40	0.39	0.35	0.35	0.42
D	**Source**	R				1.00	0.18	0.12	0.08	0.19
		ρ				1.00	0.21	0.37	0.07	0.53
		P90				0.00	0.01	0.01	0.02	0.01
		COD				0.00	0.19	0.24	0.28	0.15
E	**Non-Source**	R					1.00	0.42	0.65	-0.01
		ρ					1.00	0.36	0.40	0.07
		P90					0.00	0.02	0.01	0.01
		COD					0.00	0.24	0.24	0.20
F	**Non-Source**	R						1.00	0.54	0.42
		ρ						1.00	0.41	0.45
		P90						0.00	0.01	0.02
		COD						0.00	0.24	0.26
G	**Non-Source**	R							1.00	0.01
		ρ							1.00	0.05
		P90							0.00	0.02
		COD							0.00	0.27
H	**Non-Source**	R								1.00
		ρ								1.00
		P90								0.00
		COD								0.00

Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (ρ), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

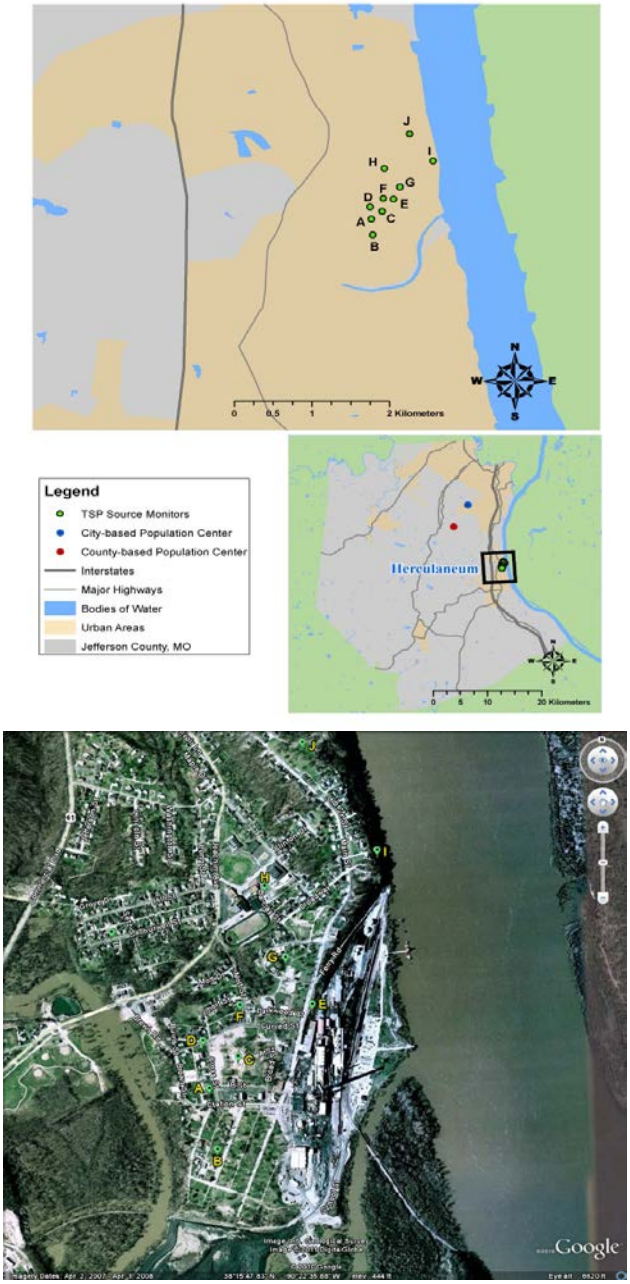
Figure 3-51 illustrates Pb monitor locations with Jefferson County, MO. Ten source-oriented monitors surrounded the Doe Run primary Pb smelter in Herculaneum, MO on

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, and the facility was estimated to have emitted 41.1 tons Pb/yr (U.S. EPA, 2008f). Figure 3-52 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 3-53 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.

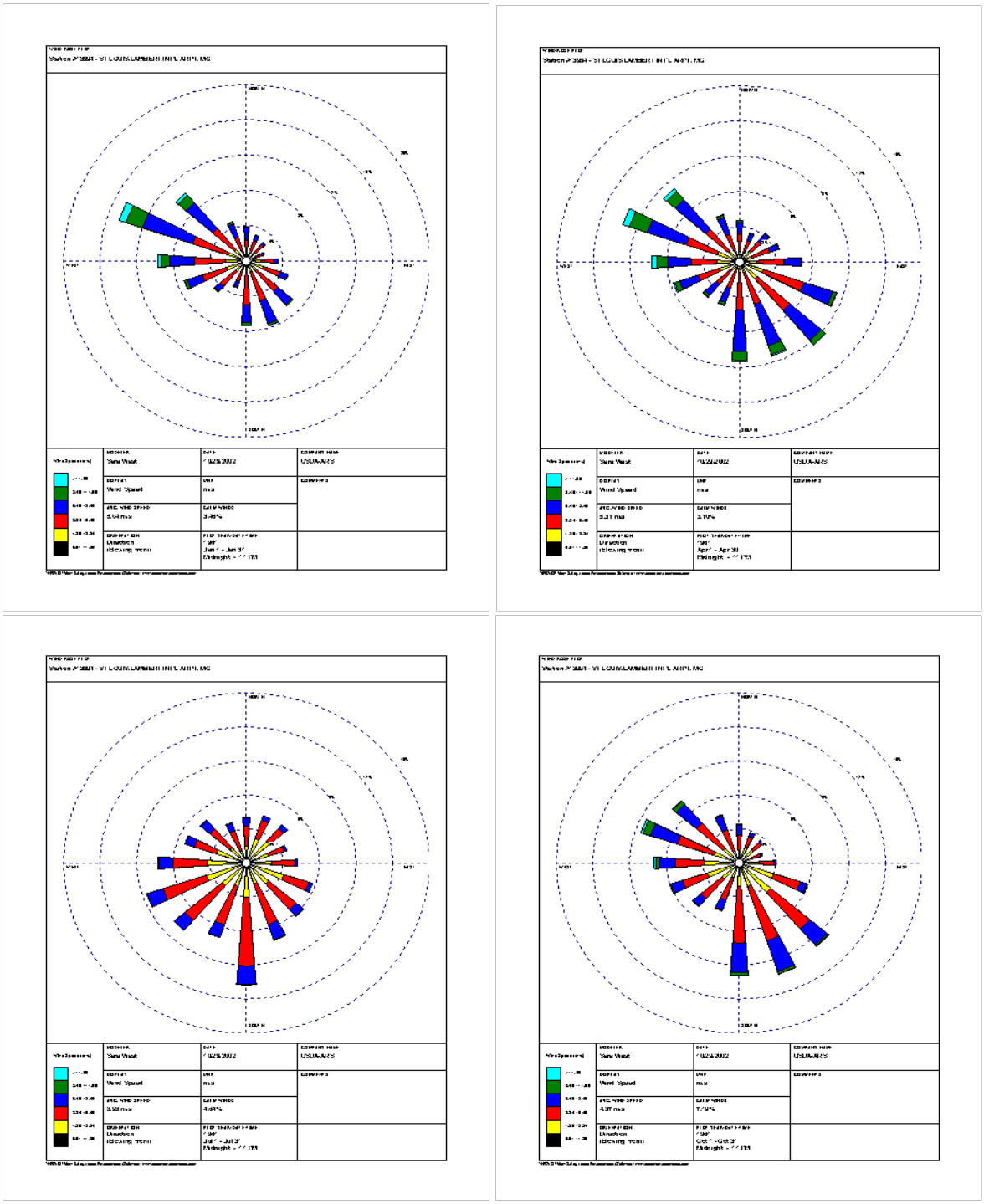
Spatial variability among the monitors is lower than at many sites, because the monitors are relatively close together and are located on one side of the same source (Table 3-28). Correlations range substantially ($R = -0.03$ to 0.96, $\rho = -0.04$ to 0.96). High correlations ($R \geq 0.75$, $\rho \geq 0.75$) occurred for monitors (A and C), (A and D), (C and D), (D and F), (E and F), (G and H), and (I and J). Monitors (A and C), (A and D), (C and D), (D and F), (E and F), and (G and H) are all within 250 m of each other. For the highest correlation ($R = 0.96$, $\rho = 0.96$, [for monitors E and F]), monitor F is 250 m directly east of monitor E. Low correlation ($R \leq 0.25$, $\rho \leq 0.25$) generally occurred when monitors B, I, and J were compared with monitors A, C, D, E, F, G, and H. Monitors B, I, and J were on the outskirts of the measurement area and so were likely oriented such that the southeasterly

winds did not carry pollutants to these sites concurrently with the signal recorded by the other monitors.



Note: All monitors surround the Doe Run industrial facility. Top: Map view of all monitors in Jefferson County. Bottom: Satellite view of the monitors and the Doe Run facility.

Figure 3-51 Pb TSP Monitor locations within Jefferson County, MO (29-099), 2007-2009.



Source: NRCS (2011)

Note: Clockwise from top left: January, April, July, and October. Note wind percentages vary from month to month.

Figure 3-52 Wind roses for Jefferson County, MO, obtained from meteorological data at St. Louis/Lambert International Airport, 1961-1990.

Site	A	B	C	D	E	F	G	H	I	J
SITE ID	29-099-0022	29-099-0024	29-099-0015	29-099-0023	29-099-0004	29-099-0020	29-099-0021	29-099-0005	29-099-0011	29-099-0013
MEAN	0.43	0.36	1.36	0.39	1.12	0.69	0.75	0.29	0.34	0.18
SD	0.54	0.49	1.97	0.54	1.67	1.01	1.25	0.59	0.85	0.33
OBS	622	209	1E3	632	1E3	575	953	351	366	177
% BELOW MDL	0	5	0	0	5	0	5	25	5	15
Source orientation	Source	Source	Source	Source	Source	Source	Source	Source	Source	Source

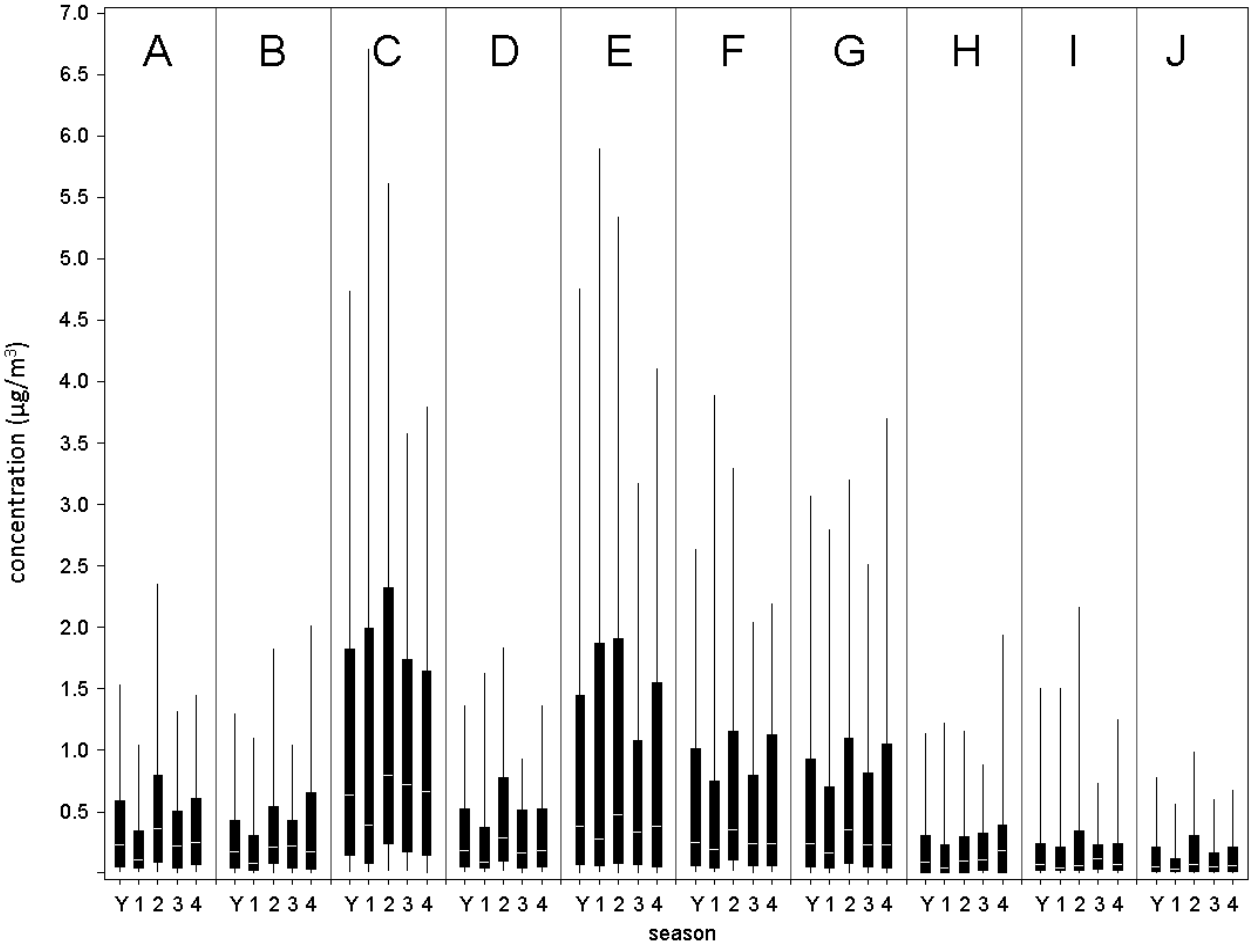


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Figure 3-53 Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Jefferson County, MO (29-099), 2007-2009.

Table 3-28 Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Jefferson County, MO (29-099), 2007-2009

			A	B	C	D	E	F	G	H	I	J
			Source	Source	Source	Source	Source	Source	Source	Source	Source	Source
A	**Source**	R	1.00	0.66	0.80	0.84	0.60	0.65	0.33	0.32	0.07	0.05
		ρ	1.00	0.59	0.80	0.83	0.57	0.64	0.33	0.35	0.07	0.05
		P90	0.00	0.71	1.55	0.42	1.93	1.14	1.41	0.74	0.92	0.78
		COD	0.00	0.46	0.48	0.30	0.55	0.45	0.57	0.64	0.67	0.69
B	**Source**	R		1.00	0.54	0.40	0.15	0.15	0.08	0.16	0.11	0.01
		ρ		1.00	0.53	0.43	0.10	0.14	0.07	0.22	0.10	0.09
		P90		0.00	1.86	0.87	2.77	1.96	2.08	0.94	1.04	0.91
		COD		0.00	0.58	0.51	0.69	0.62	0.68	0.68	0.65	0.65
C	**Source**	R			1.00	0.86	0.56	0.72	0.28	0.32	-0.03	-0.03
		ρ			1.00	0.86	0.59	0.72	0.26	0.27	-0.04	0.04
		P90			0.00	1.56	2.26	1.26	2.94	2.65	3.18	2.60
		COD			0.00	0.50	0.50	0.46	0.60	0.74	0.73	0.73
D	**Source**	R				1.00	0.70	0.80	0.41	0.48	0.17	0.10
		ρ				1.00	0.71	0.80	0.41	0.56	0.14	0.18
		P90				0.00	1.83	1.02	1.38	0.76	0.88	0.70
		COD				0.00	0.50	0.36	0.53	0.61	0.63	0.66
E	**Source**	R					1.00	0.96	0.57	0.53	0.09	0.14
		ρ					1.00	0.96	0.54	0.46	0.06	0.16
		P90					0.00	0.86	2.16	2.50	3.09	2.57
		COD					0.00	0.35	0.49	0.66	0.70	0.72
F	**Source**	R						1.00	0.56	0.56	0.12	0.20
		ρ						1.00	0.56	0.54	0.10	0.19
		P90						0.00	1.13	1.51	1.74	1.40
		COD						0.00	0.47	0.63	0.65	0.70
G	**Source**	R							1.00	0.85	0.36	0.34
		ρ							1.00	0.87	0.28	0.38
		P90							0.00	1.53	2.10	2.08
		COD							0.00	0.61	0.63	0.66
H	**Source**	R								1.00	0.24	0.33
		ρ								1.00	0.20	0.30
		P90								0.00	0.89	0.56
		COD								0.00	0.67	0.65
I	**Source**	R									1.00	0.87
		ρ									1.00	0.79
		P90									0.00	0.62

			A	B	C	D	E	F	G	H	I	J
		COD									0.00	0.48
J	**Source**	R										1.00
		ρ										1.00
		P90										0.00
		COD										0.00

Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (p), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

Figure 3-54 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/year source facilities and one 0.2 tons/year source (U.S. EPA, 2008g). 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, 2008g). 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 tons/year 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 3-55 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.

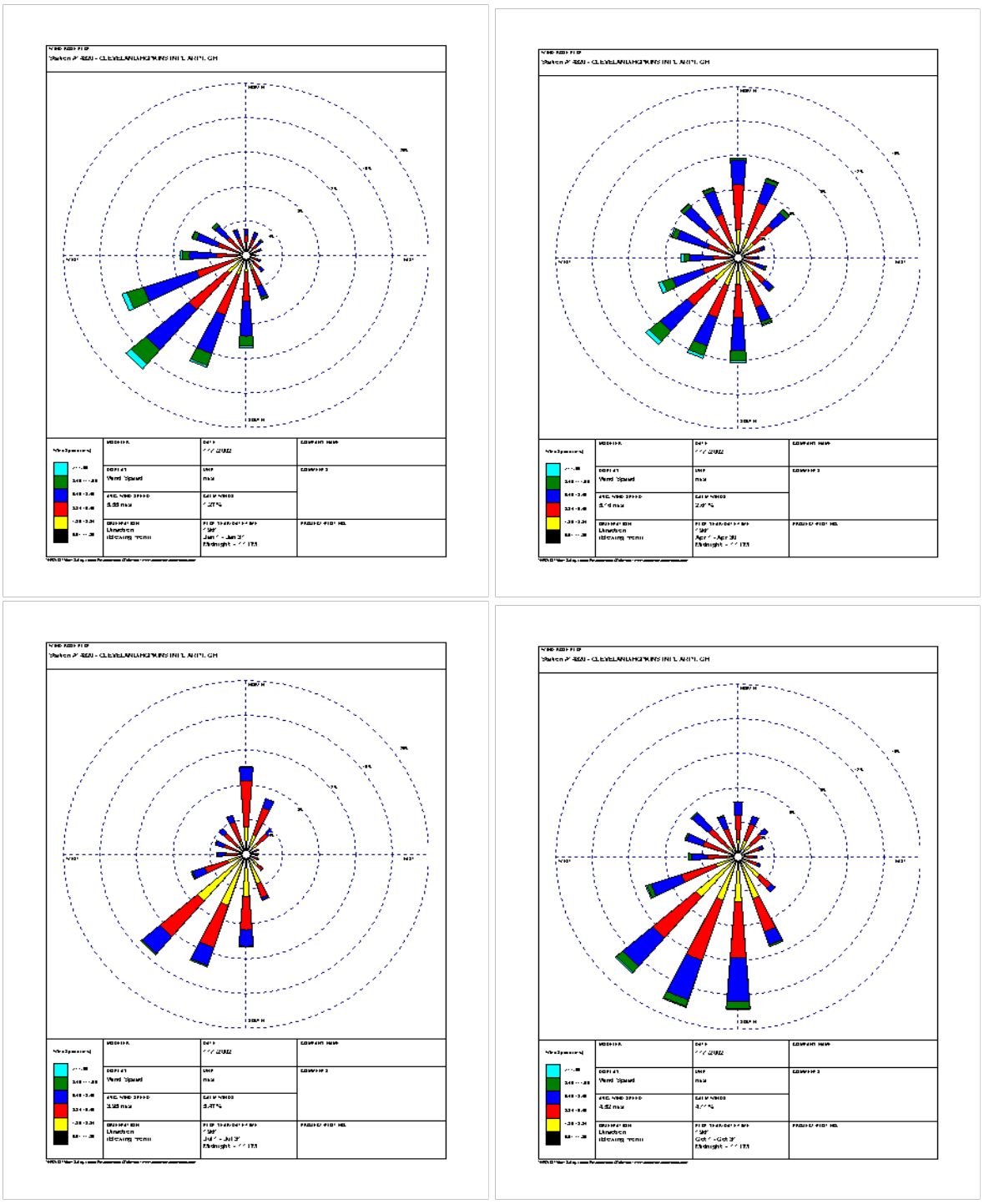
Figure 3-56 illustrates the seasonal distribution of Pb concentration data at the five monitoring sites. The influence of southern winds, along with close proximity to a potentially-emitting facility, could have caused the elevated concentrations observed at monitor B (average: 0.10 $\mu g/m^3$). The 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 μg/m^3 and 0.013 μg/m^3, respectively. Maximum concentrations reached 0.04 μg/m^3 at all three monitors.

The level of spatial variability is illustrated by the intersampler correlations presented in Table 3-29. Monitors A and B appear to be anticorrelated (R = -0.06, ρ = -0.13). If the Ferro site was the dominant source in this area, then the anticorrelation was likely caused by the positioning of monitors A and B on opposite sides of that facility. At any given time, potential emissions from the Ferro plant may have affected monitors A and B at distinct times. Monitors C, D, and E correlated moderately to well with each other (R = 0.37 to 0.74, ρ = 0.67 to 0.77). Given that all 3 monitors are separated by roughly 2.8 km, it is possible that the relatively high correlations related to common sources, as suggested in the previous paragraph. Little correlation was observed between the source-oriented and non-source-oriented monitors.

Note: Top: view of all Pb FRM monitors in Cuyahoga County. Bottom left: Close up of industrial site around monitors A and B. Bottom right: Close up of monitor D north of I-490.

Figure 3-54 Pb TSP Monitor locations within Cuyahoga County, OH (39-035), 2007-2009.



Source: NRCS (2011)

Note: Clockwise from top left: Jan, April, July, and October. Note wind percentages vary from month to month.

Figure 3-55 Wind roses for Cuyahoga County, OH, obtained from meteorological data at Cleveland/Hopkins International Airport, 1961-90.

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

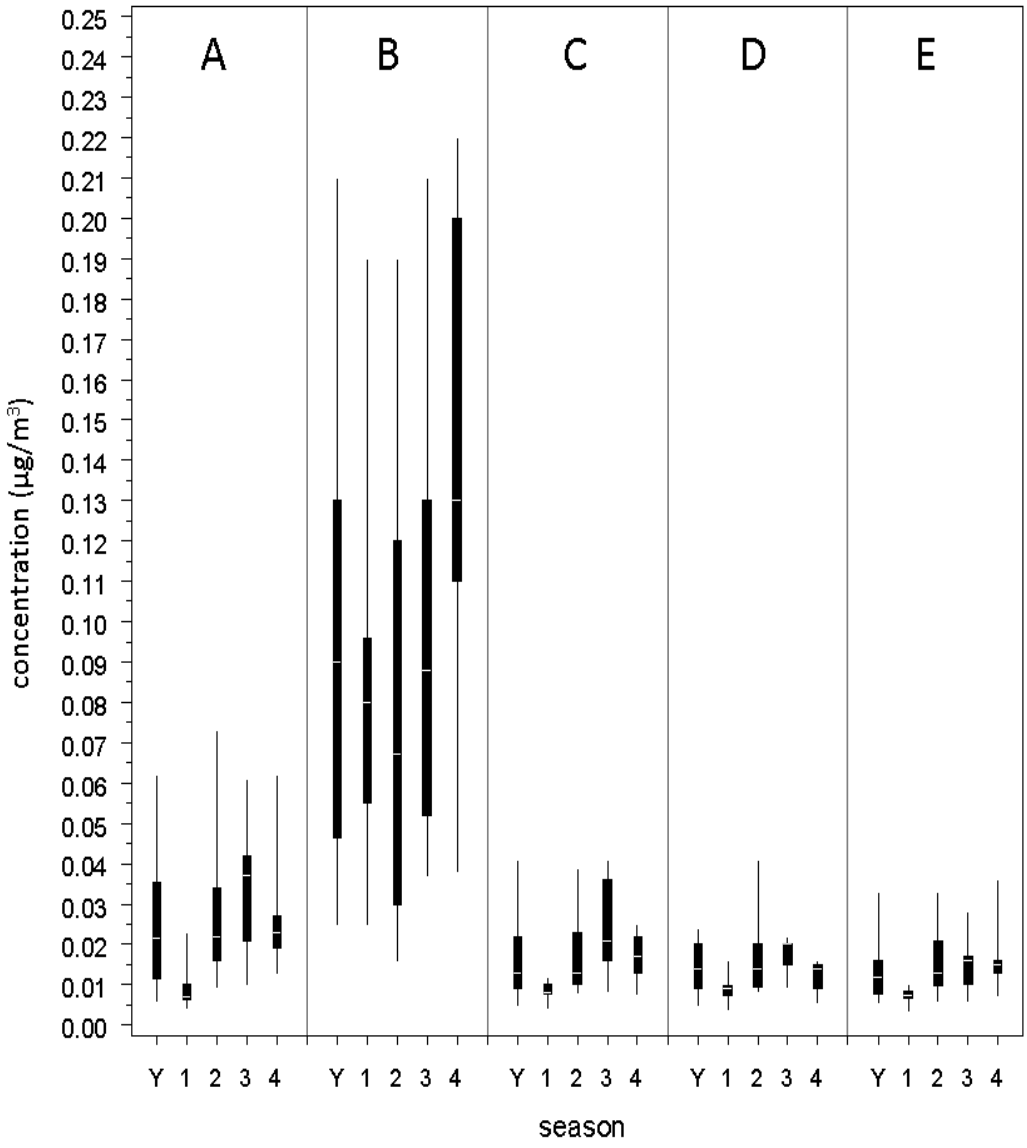


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Figure 3-56 Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Cuyahoga County, OH (39-035), 2007-2009.

Table 3-29 Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Cuyahoga County, OH (39-035), 2007-2009

			A	B	C	D	E
			Source	Source	Source	Non-Source	Non-Source
A	Source	R	1.00	-0.06	0.21	0.17	0.24
		ρ	1.00	-0.13	0.24	0.19	0.21
		P90	0.00	0.18	0.05	0.04	0.05
		COD	0.00	0.64	0.33	0.35	0.37
B	Source	R		1.00	0.26	0.43	0.11
		ρ		1.00	0.31	0.24	0.34
		P90		0.00	0.18	0.19	0.19
		COD		0.00	0.69	0.71	0.73
C	Source	R			1.00	0.74	0.51
		ρ			1.00	0.77	0.67
		P90			0.00	0.01	0.01
		COD			0.00	0.17	0.18
D	Non-Source	R				1.00	0.37
		ρ				1.00	0.67
		P90				0.00	0.01
		COD				0.00	0.17
E	Non-Source	R					1.00
		ρ					1.00
		P90					0.00
		COD					0.00

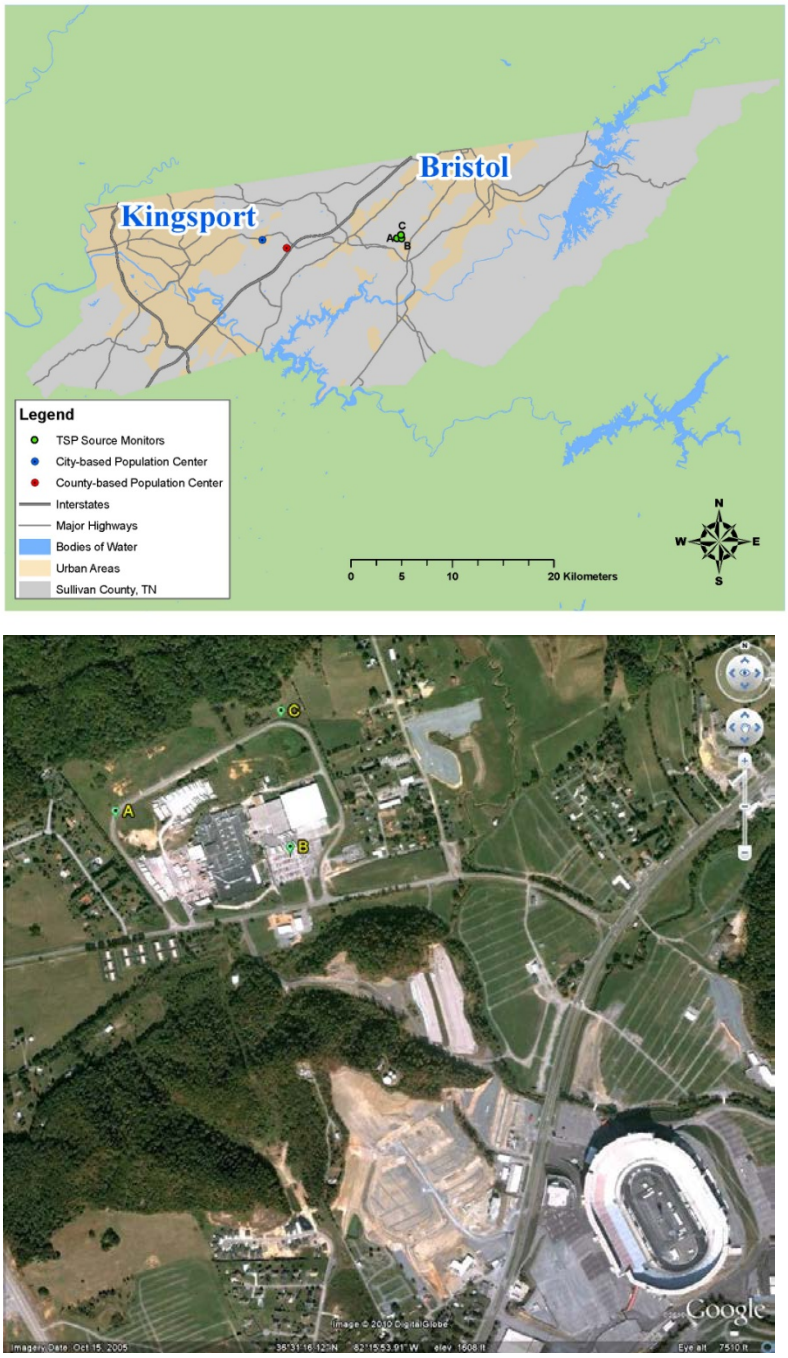
Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (p), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

Figure 3-57 illustrates Pb monitor locations within Sullivan County, TN. Three source-oriented monitors were situated around an Exide Pb recycling facility emitting 0.78 tons/year (U.S. EPA, 2008h). Monitors A and C are positioned along the facility's service road and are approximately 100 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 3-58 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.

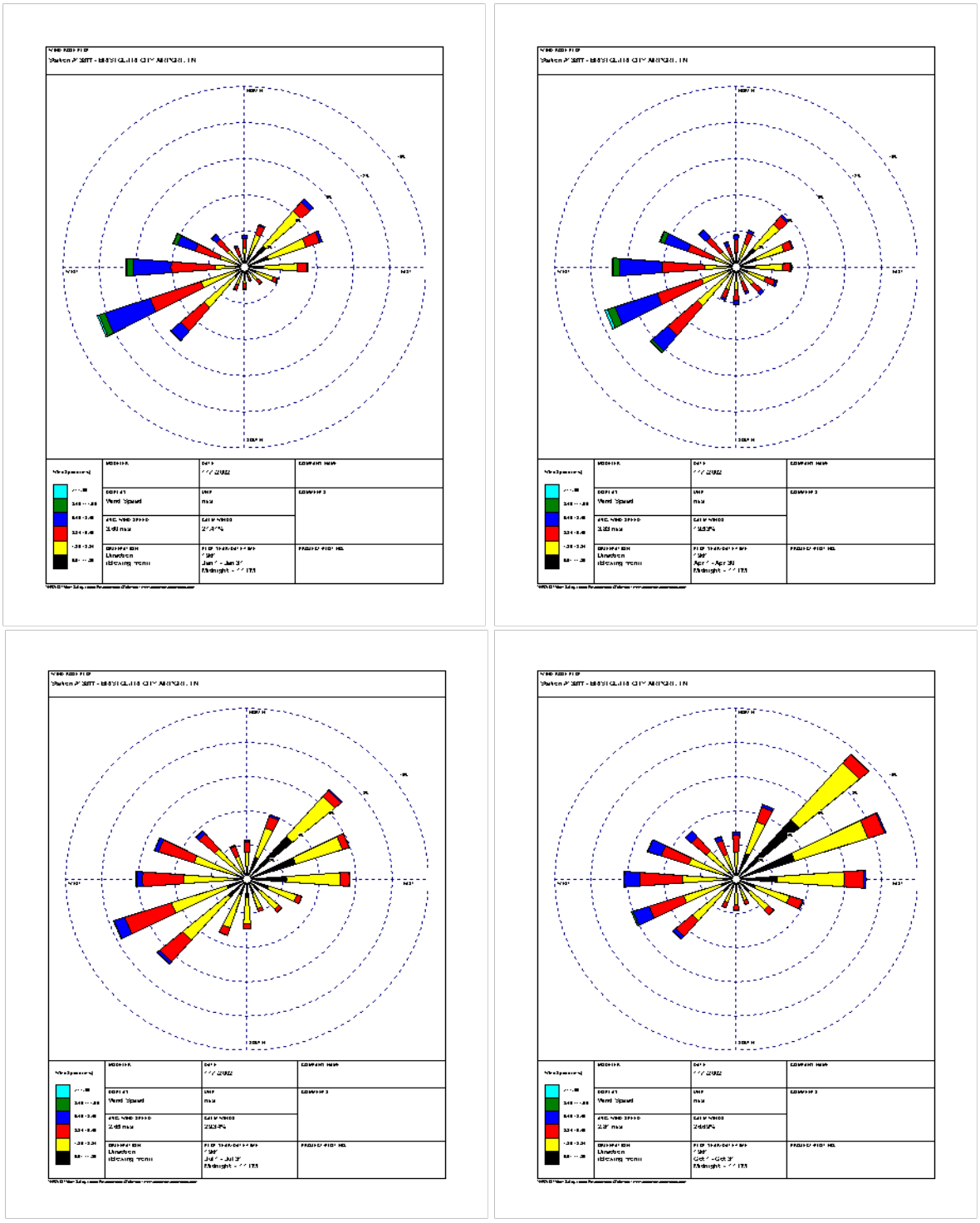
The data presented in Figure 3-59 illustrates that concentrations above the level of the NAAQS occurred frequently at the monitors. The average concentrations at monitors A, B, and C were 0.11 $\mu g/m^3$, 0.051 $\mu g/m^3$, and 0.059 $\mu g/m^3$, respectively. Median concentrations were 0.08 $\mu g/m^3$, 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 ($R = 0.06$ to 0.14, $\rho = -0.04$ to 0.13) were quite low (Table 3-30). The correlation between monitors B and C was moderate ($R = 0.31$, $\rho = 0.45$). It makes sense that the correlation for these monitors would be somewhat higher because they are both oriented to the east of the Pb recycling facility, although monitor C is to the northeast and monitor B to the east-southeast.



Note: Top: Map, bottom: Satellite image. Monitors A, B, and C surround the Exide Pb recycling facility. Just to the southeast is the Bristol motor speedway.

Figure 3-57 Pb TSP Monitor locations within Sullivan County, TN (47-163), 2008-20102007-2009.



Source: NRCS (2011)

Note: Clockwise from top left: January, April, July, and October. Note that the wind percentages vary from month to month.

Figure 3-58 Wind roses for Sullivan County, TN, obtained from meteorological data at Bristol/Tri City Airport, 1961-90.

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

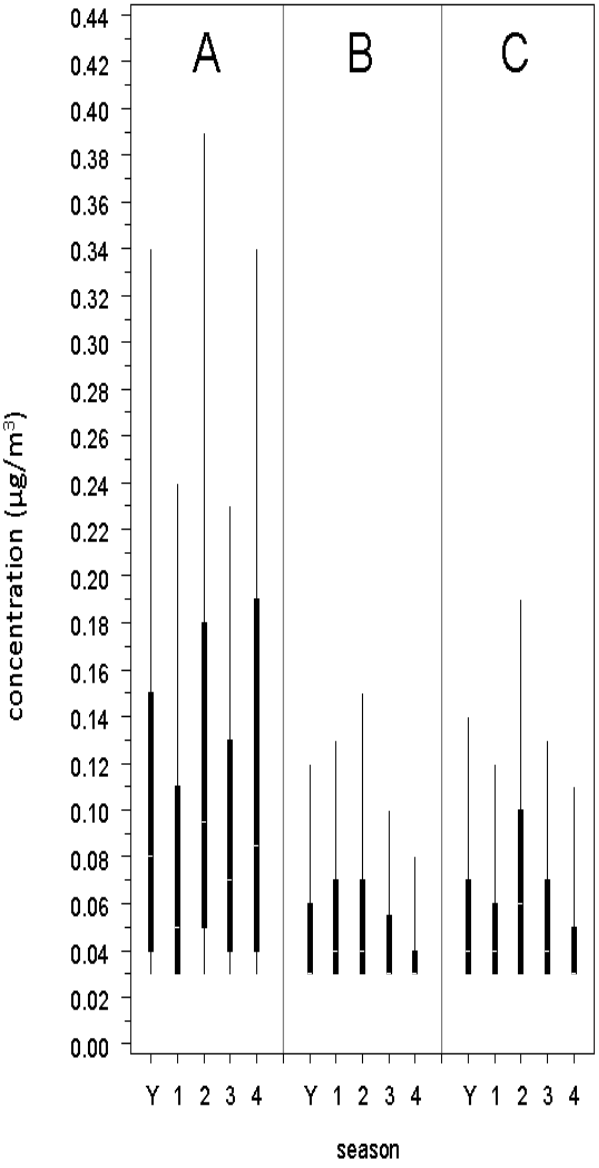


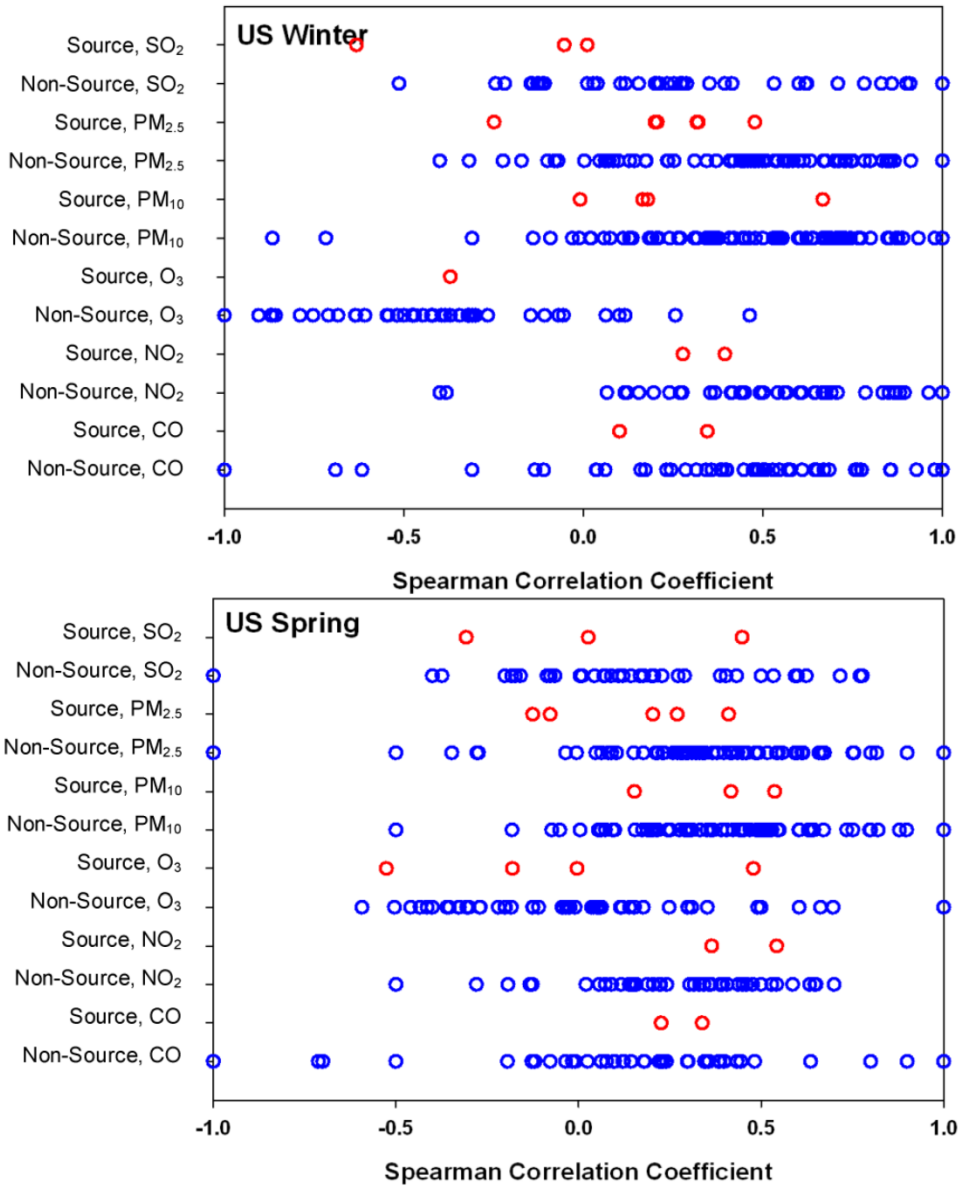
Figure 3-59 Box plots of annual and seasonal Pb TSP concentrations (µg/m^3) from source-oriented monitors within Sullivan County, TN (47-163), 2007-2009.

Table 3-30 Correlations between Pb TSP concentrations from source-oriented monitors within Sullivan County, TN (47-163), 2007-2009

			A	B	C
			Source	**Source**	**Source**
A	**Source**	R	1.00	0.06	0.14
		ρ	1.00	-0.04	0.13
		P90	0.00	0.21	0.19
		COD	0.00	0.47	0.43
B	**Source**	R		1.00	0.31
		ρ		1.00	0.45
		P90		0.00	0.06
		COD		0.00	0.23
C	**Source**	R			1.00
		ρ			1.00
		P90			0.00
		COD			0.00

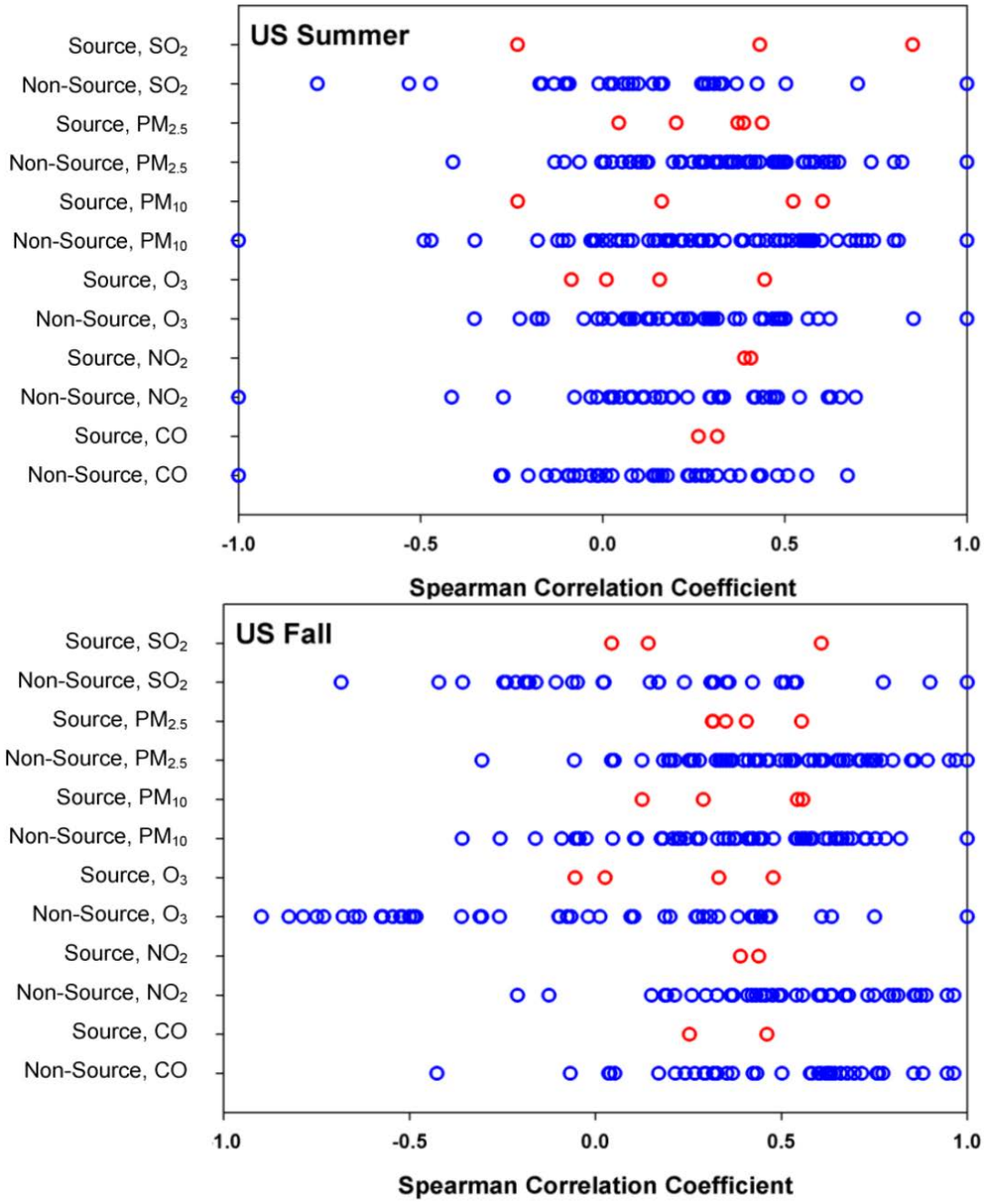
Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (*ρ*), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

3.8.3 Lead Concentration in a Multipollutant Context



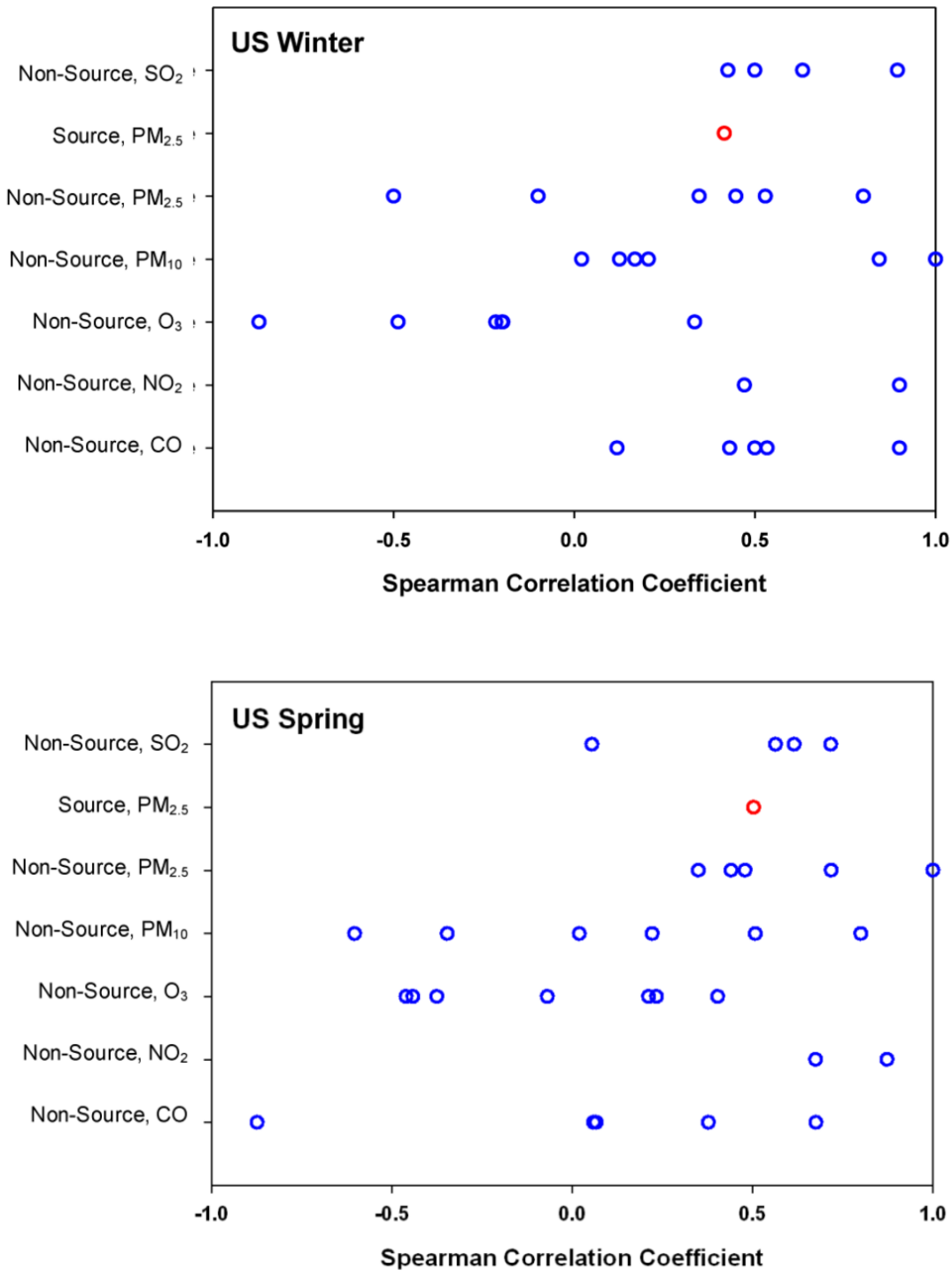
Note: Top: winter; Bottom: spring.

Figure 3-60 Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2007-2008.



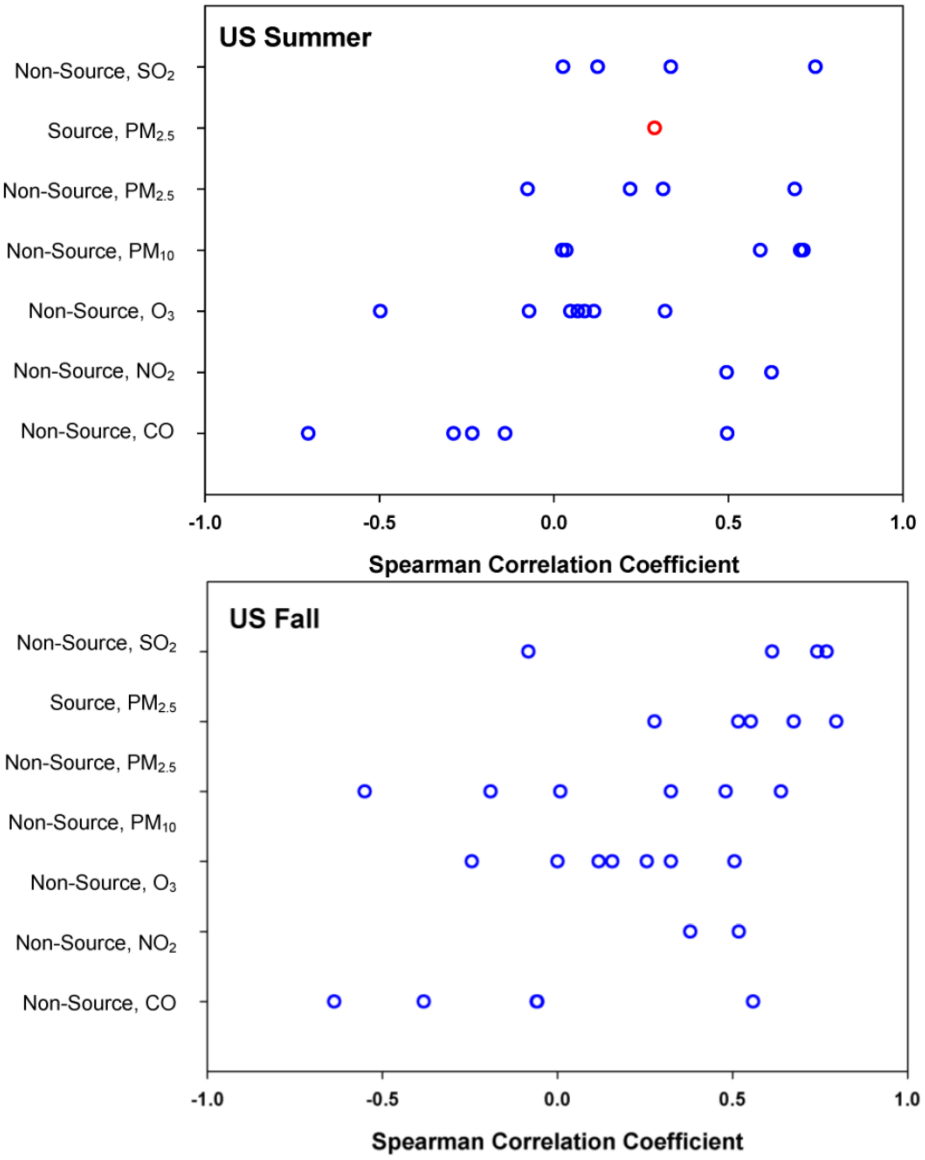
Note: Top: summer; Bottom: fall.

Figure 3-61 Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2007-2008.



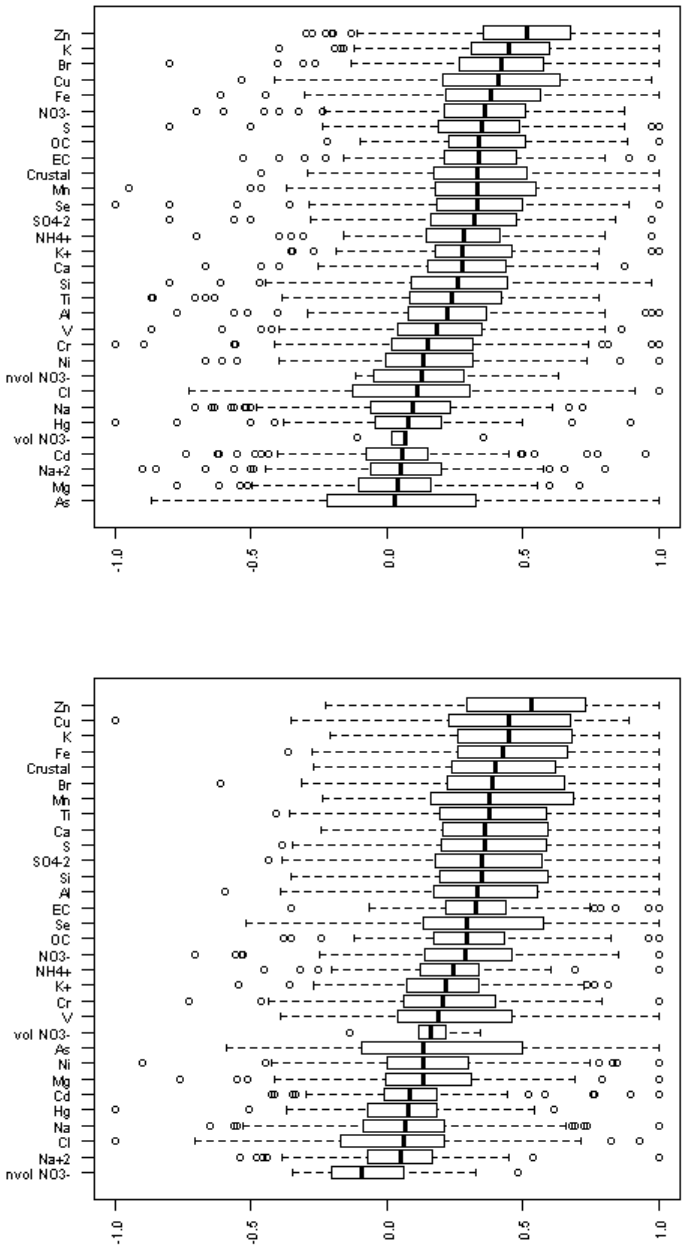
Note: Top: winter; Bottom: spring.

Figure 3-62 Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2009.



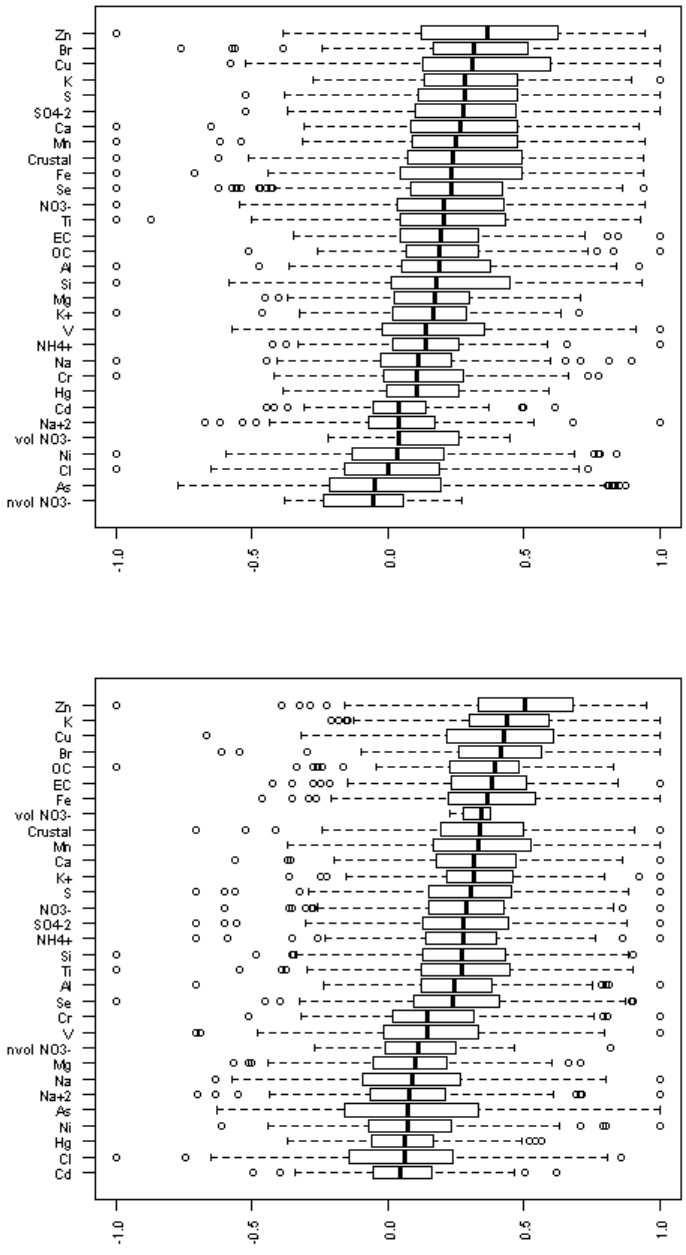
Note: Top: summer; Bottom: fall.

Figure 3-63 Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2009.



Top: winter; bottom: spring. Note: "nvol" = non-volatile, "vol" = volatile, and organic carbon (OC) samples were blank-adjusted.

Figure 3-64 Seasonal correlations of monitored Pb-$PM_{2.5}$ concentration with copollutant concentrations, 2007-2009.



Top: summer; bottom: fall. Note: “nvol” = non-volatile, “vol” = volatile, and organic carbon (OC) samples were blank-adjusted.

Figure 3-65 Seasonal correlations of monitored Pb-$PM_{2.5}$ concentration with copollutant concentrations, 2007-2009.

Table 3-31 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. Pb is considered to be a multimedia contaminant with multiple pathways of exposure. The relative importance of various media in affecting Pb exposure changes with source strength and location, location and time activity of the exposed individuals, behavior of the exposed individuals, and risk factors such as age and socioeconomic factors (risk factors are discussed in detail in Chapter 6). Blood Pb and bone Pb biomarkers (discussed in Section 4.3, Section 4.4, Section 4.5, and Section 4.6), are often used to indicate composite Pb exposure resulting from multiple media and pathways of exposure.

The recent information provided here builds upon the conclusions of the 2006 Pb AQCD (2006b), which found that air Pb concentrations and blood Pb levels have decreased substantially following the restrictions on Pb in on-road vehicle gasoline, Pb in household paints, the use of Pb solder, and reductions in industrial Pb emissions that have occurred since the late 1970s. Nevertheless, detectable quantities of Pb have still been observed to be bioaccessible in various media types. It was reported in the 2006 Pb AQCD (U.S. EPA, 2006b) that airborne maximum quarterly Pb concentrations in the U.S. were in the range of 0.03-0.05 $\mu g/m^3$ for non-source-oriented monitors for the years 2000-2004 and were 0.10-0.22 $\mu g/m^3$ for source-oriented monitors during that time period, while blood Pb levels reached a median of 1.70 μg/dL among children (1-5 years of age) in 2001-2002. It was also observed that Pb exposures were associated with nearby industrial Pb sources, presence of Pb-based paint, and Pb deposited onto food in several of the studies described in the 2006 Pb AQCD.

4.1.1 Pathways for Lead Exposure

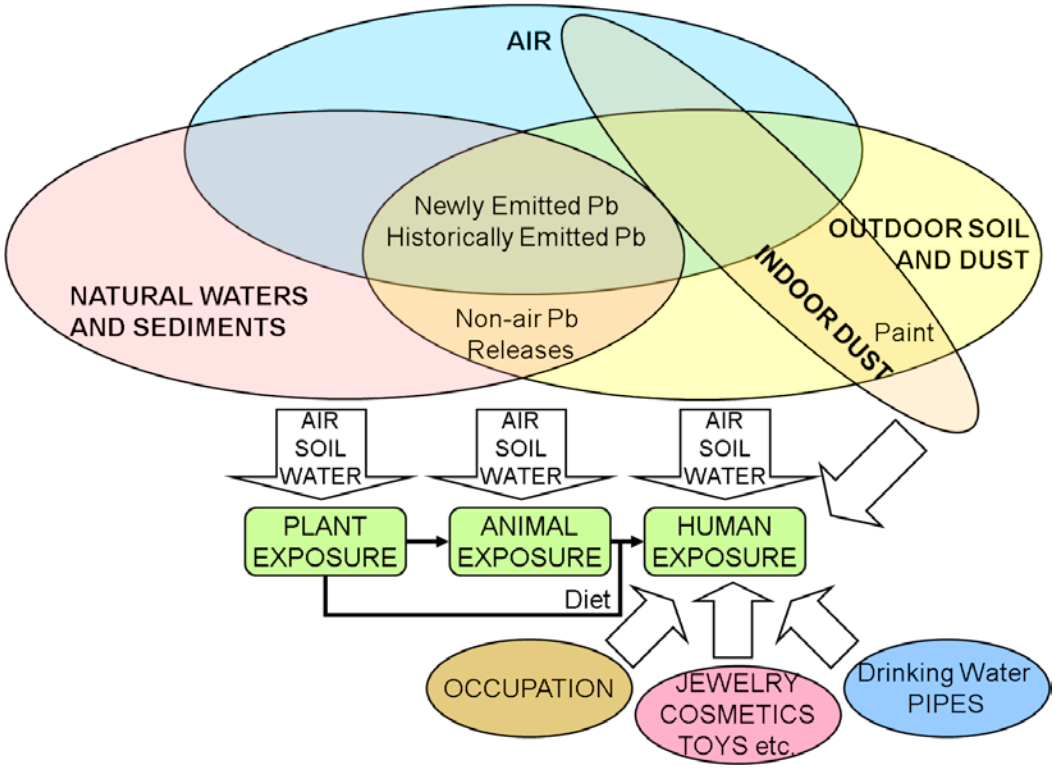
Pathways of Pb exposure are difficult to assess because Pb has multiple sources in the environment and passes through various environmental media. These issues are described in detail in Sections 3.2 and 3.3. Air-related pathways of Pb exposure are the focus of this ISA. Pb can be emitted to air, soil, or water and then cycle through any or all of these media. In addition to primary emission of particle-bound or gaseous Pb to the atmosphere, Pb can be resuspended to the air from soil or dust. Additionally, Pb-bearing PM can be deposited from the air to soil or water through wet and dry deposition. Air-

related Pb exposures also include inhalation and ingestion of Pb-contaminated food, water or other materials including dust and soil via hand-to-mouth contact. In general, air-related pathways include those pathways where Pb passes through ambient air on its path from a source to human exposure. Some non-air-related exposures of Pb include ingestion of indoor Pb paint, Pb in diet as a result of inadvertent additions during food processing, and Pb in drinking water attributable to Pb in distribution systems, as well as other generally less prevalent pathways.

Particle size of Pb-PM is relevant to transport through various media leading to exposure. The inhalability of airborne particles is a gradually decreasing function of particle size. Inhalability criteria established from experimental data obtained at wind speeds of 1-8 m/s describe PM inhalability of 77% for particles <10 μm (d_{ae}, aerodynamic diameter). Inhalability of particles ranging in size from 40 to 100 μm d_{ae} is 50%; above 100 μm, inhalability data are lacking (Soderholm, 1989; ACGIH, 1985). Of the particles that are not inhaled, their settling to surfaces makes them available for subsequent ingestion. The main pathway for Pb ingestion by children is by hand to mouth (Lanphear et al., 1998). In a playground environment in London, U.K., Duggan et al. (1985) reported that hand to mouth transfer was effectively limited to particles smaller than 10 μm, even when the soil itself exhibited a much larger particle size distribution. More recently, Yamamoto et al. (2006) reported for a cohort of children in Kanagawa Prefecture, Japan (greater Tokyo area) that the mode of size distributions of particles adhering to children's hands was 39 ± 26 μm, with the upper tail ranging from 200-300 μm. Differences in the size distribution results may be related to differences in the soil between the two locations and/or to differences between the analytical methods used to measure size distribution; Duggan and Inskip (1985) used optical microscopy of the dust wipes, while Yamamoto et al. (2006) used a laser scattering device measuring sampled particles suspended in an aqueous solution. Similar studies focusing on particle size distributions of ingestion of house dust are lacking. Ingestion of house dust has been reported to be the major source of lead intake during early childhood (Lanphear et al., 2002). If a similar particle size distribution holds for household dust, then ingestion of indoor Pb of atmospheric origin could also be strongly dependent on dust particle size. Therefore, larger particles of atmospheric origin, which may not be considered relevant for exposure by inhalation exposure, are still relevant for Pb exposure by ingestion. However, no studies in the literature have presented information on the relative contributions of Pb from different PM size fractions to blood Pb concentrations.

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.



Note: The Venn diagram is used to illustrate the passage of Pb through multiple environmental media compartments through which exposure can occur.

Figure 4-1 Conceptual model of multimedia Pb exposure.

The relative importance of different sources or pathways of potential exposure to Pb in the environment is often difficult to discern. Individual factors such as home

environment, location, and risk factors (described in more detail in Chapter 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). 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: Reprinted with permission of Nature Publishing Group, Clayton et al. (1999)

Several studies have used a combination of measured values and default model values to represent exposures and determine their relative contributions to blood Pb. Cornelis et al. (2006) used the Integrated Exposure Uptake Biokinetic model (IEUBK), described in detail in the 2006 Pb AQCD (U.S. EPA, 2006b) to model children's exposures to Pb emissions from a non-ferrous smelter in Hoboken, Belgium. They point out that ambient air Pb concentrations decrease with distance from the smelter while IEUBK is fairly insensitive to changes in ambient air concentration. They input the average ambient air Pb concentration of 0.81µg/m3 and assumed that the indoor air Pb concentration was 30% of the outdoor Pb. In the absence of indoor air Pb samples, the default assumption was adopted for the Cornelis et al. (2006) study. Similarly, Carrizales et al. (2006) analyzed exposures to children living near a copper smelter in San Luis Potosi, Mexico. They employed the IEUBK default options for assignment of Pb dust concentration as

70% of the soil Pb concentration, while air Pb concentration was assigned based on measurements by the Mexican government. Based on these assumptions, they attributed 87% of blood Pb to soil and dust exposure. Input soil and dust Pb levels were obtained from an average of 28 sites. Using defaults for all IEUBK values, the soil/dust Pb ingestion pathway has been predicted to contribute 63-75% (depending of a child's age) of blood Pb, whereas the air Pb inhalation pathway contributed <1% (SRC, 2007). This analysis did not specifically estimated the portion of the soil/dust Pb ingestion pathway that derives from air Pb, such as recently airborne Pb deposited to soil and dust which remains available for inhalation and ingestion. In the 1986 Pb AQCD, blood Pb-air Pb slopes were examined that included both direct (inhalation) and indirect (via soil, dust, etc.) air Pb contributions, resulting in the conclusion that slopes for the total air Pb contribution were roughly twice that of the slope due to inhaled air Pb alone. This "recent air" Pb was described in the 2007 Pb Risk Assessment (U.S. EPA, 2007f) to include those pathways involving Pb that is or has recently been in the outdoor ambient air, including inhalation and ingestion of indoor dust Pb derived from recent ambient air (e.g., air Pb that has penetrated into the residence recently and loaded indoor dust). Beyond the direct inhalation pathway (outdoor and indoor ambient air Pb), Appendix I of the 2007 Pb Risk Assessment (U.S. EPA, 2007f) provides estimates of the contribution of various other pathways (e.g., diet, drinking water, outdoor soil ingestion) as well as those involving recent air Pb to blood Pb. Estimates of recent air Pb contributions were highly dependent on the exposure scenario evaluated. For the General Urban Case Study in that assessment, "current conditions" at the time of the risk assessment (0.14 µg/m3, mean of maximum quarterly average concentration of Pb in TSP) were modeled as remaining constant throughout the 7 years of the exposure modeled in the biokinetic model. A median concurrent blood Pb concentration of 1.8-2.8 µg/dL (depending on the exposure and risk model) was estimated as the average of the results at 75 and 81 months of age. The recent air Pb ingestion pathways contributed a median of 12-28% (depending on the exposure model) to blood Pb beyond the 0.5-0.6% attributable to inhalation of recent airborne Pb. In simulations for the 95th percentile of current conditions (0.87 µg/m3, 95th percentile of maximum quarterly average concentration of Pb in TSP), median children's blood Pb increased to 2.0-3.1 µg/dL and the contribution of recent air Pb ingestion pathways increased to 22-37% of blood Pb. Results of other exposure scenarios for which greater or lesser contributions of recent air Pb to blood Pb were predicted are available in Appendix I of the 2007 Pb Risk Assessment (U.S. EPA, 2007f).

4.1.2 Environmental Exposure Assessment Methodologies

A number of monitoring and modeling techniques have been employed for exposure assessment. These are detailed in either the 2006 Pb AQCD (U.S. EPA, 2006b) or in the subsequent Risk and Exposure Assessment performed as part of the same NAAQS review (U.S. EPA, 2007g). Some of these methods are briefly described here to provide a context for the exposure studies described in Section 4.1.3. Blood Pb sampling is described in detail in Section 4.3.2.

Data collection to assess Pb exposure pathways may involve air, soil, and dust samples. Methods used for digesting air Pb samples are described in Section 3.4, as are ambient air Pb monitoring techniques. Factors affecting collection of ambient air Pb samples are described in detail in Section 3.4. For the monitors in the FRM network, the primary role is compliance assessment. Accordingly, this network includes monitors in locations near sources of air Pb emissions which are expected to or have been shown to contribute to ambient air Pb concentrations in excess of the Pb NAAQS. In such locations, Pb may be associated with relatively larger size particles, contributing to air Pb concentration gradients with distance from the source and greater deposition in the near-source locations. The FRM network also includes non-source-oriented monitors for which the main objective is to gather information on neighborhood-scale lead concentrations that are typical in urban areas so to better understand ambient air-related Pb exposures for populations in these areas. This part of the Pb NAAQS network, is required to be operational as of December 27, 2011. These monitor locations are distributed across a broad geographic area, representing approximately 63 large urban areas which contain approximately half of the total U.S. population (based on recently published 2010 Census Bureau data). In lieu of more detailed analysis of population proximity for these newly established monitors, population counts were calculated near previously existing monitors for which data are presented in Section 3.5. For the monitors in that limited dataset, among the total population of 311,127,619 people in the 2010 Census (ESRI, 2011), 181,100 (0.06%) lived within 1 km of a source-oriented monitor, while 918,351 (0.30%) lived within 1 km of a non-source-oriented monitor.

Dust sampling has not changed drastically since it was first proposed by Sayre et al. (1974), in which a disposable paper towel was soaked in 20% denatured alcohol and 1:750 benzalkonium chloride and then used to wipe a 1 ft^2 sampling area in a systematic fashion. Que Hee et al. (1985) and Sterling et al. (1999) compared wipe testing with vacuum methods. Sampling efficiency for the first attempt varied between 53-76% with vacuum pump flow rate and tube type and was 52% for the wipe method for the Que Hee et al. (1985) study, with 100% efficiency after five consecutive samples were obtained. Sterling et al. (1999) observed that two of three vacuuming methods had significantly

higher geometric mean collection (vacuum 1: 94.3 µg/ft^2; vacuum 2: 23.5 µg/ft^2) compared with dust wipes (5.6 µg/ft^2).

Models may also be used in exposure assessment. For example, two dispersion models, the American Meteorological Society/Environmental Protection Agency Regulatory Model (AERMOD), and Industrial Source Complex-Plume Rise Model Enhancements (ISC-PRIME) were employed to model dispersion of Pb emissions from specific industrial facilities (Cimorelli et al., 2005; Perry et al., 2005; EPRI, 1997), and to estimate ambient air Pb concentrations at some of the case studies included in the 2007 Risk and Exposure Assessment (U.S. EPA, 2007g). These models assume plume dispersion follows a Gaussian distribution from a point source. For the two point source case studies included in the 2007 risk assessment, the plume models were used to track emissions to ambient air near homes located within a few miles of emitting facilities. However, dispersion models can also be used to track long distance transport of Pb emissions, as performed by Krell and Roeckner (1988) to model the dispersion and deposition of Pb and Cd from European nations into the North Sea.

Several models estimate blood Pb levels resulting from estimated exposure to Pb in environmental media. These models, which are described in detail in the 2006 Pb AQCD (U.S. EPA, 2006b) include the IEUBK model, and the EPA All Ages Lead Model (AALM), which combines and expands the thorough exposure and absorption modules of the IEUBK model with the comprehensive biokinetic model of Leggett (1993).

The Stochastic Human Exposure and Dose (SHEDS) and NORMTOX models also are capable of modeling metals exposures through various routes including inhalation, ingestion, and dermal exposure (Loos et al., 2010; Burke et al., 2002). Pb exposure modeling can also be accomplished using the Modeling Environment for Total Risk (MENTOR) framework, in which airborne Pb levels could be modeled using AQS, dispersion modeling, or chemical transport modeling, while human exposure is modeled with SHEDS or a similar exposure model (Georgopoulos and Lioy, 2006). Additionally, housing data and time-activity data from the Consolidated Human Activity Database (CHAD) are incorporated into MENTOR to develop refined estimates of Pb exposure and tissue burden. However, a literature search did not produce any Pb exposure studies using the SHEDS, NORMTOX, or MENTOR modeling systems. In general, these models take input for several environmental Pb exposure media including soil, dust, food and water, outdoor air, and indoor air. The models are designed to evaluate different exposure scenarios based on specification of particular conditions.

4.1.3 Exposure Studies

4.1.3.1 Airborne Lead Exposure

Limited personal exposure monitoring data for airborne Pb were available for the 2006 Pb AQCD (U.S. EPA, 2006b). As described above, the NHEXAS study showed personal air Pb concentrations to be significantly higher than indoor or outdoor air Pb concentrations (Clayton et al., 1999). Indoor air Pb concentration was moderately correlated with floor dust and residential yard soil Pb concentration (Rabinowitz et al., 1985). Egeghy et al. (2005) performed multivariate fixed effects analysis of the NHEXAS-Maryland data and found that Pb levels measured in indoor air were significantly associated with log-transformed outdoor air Pb levels, ambient temperature, number of hours in which windows were open, 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: Reprinted with permission of 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 air samples of $PM_{2.5}$ and found that average personal Pb-$PM_{2.5}$ concentration was roughly three times higher than outdoor air Pb-$PM_{2.5}$ concentration and two times higher than indoor Pb-$PM_{2.5}$ concentration (Table 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. Pekey et al. (2010) measured indoor and outdoor trace element composition of $PM_{2.5}$ and PM_{10} in Kocaeli, an industrial region of Turkey, and found that average airborne Pb concentrations were higher outdoors than indoors for both $PM_{2.5}$ and PM_{10} during summer and for PM_{10} during winter, but that indoor Pb concentration was higher than outdoor Pb concentration for $PM_{2.5}$ during winter. The indoor-to-outdoor ratio of airborne Pb varied by environment; it tended to be less than one, but the ratio varied from one microenvironment to another. In a pilot study in Windsor, Ontario, Rasmussen et al. (2007) observed that the concentration of Pb in $PM_{2.5}$ from a personal exposure sample was roughly 40% higher than the concentration of Pb in outdoor $PM_{2.5}$ and 150% higher than Pb in indoor $PM_{2.5}$. The three studies that included personal samples recorded measurements that were consistently higher than indoor or outdoor levels.

Domestic wood burning is a potential source of Pb compounds (Section 3.2.2.5). Molnar et al. (2005) measured trace element concentration in indoor and personal exposure $PM_{2.5}$ samples for homes in which wood is burned and in a reference group where no wood burning occurs in the home. For both indoor and personal samples, Molnar et al. (2005) observed that Pb concentrations were higher for the wood burning group, but that the differences were not significant (indoor concentration: 6.0 $\mu g/m^3$ vs. 4.3 $\mu g/m^3$, $p = 0.26$; personal exposure: 4.6 $\mu g/m^3$ vs. 3.0 $\mu g/m^3$, $p = 0.06$).

Indoor activity has been associated with resuspension of settled dust, which could cause airborne contact with particle-bound Pb. Qian et al. (2008) estimated a PM_{10} resuspension rate of 1.4×10^{-4} hr^{-1} for one person walking across a carpeted floor. Measurements of submicron particles illustrated a roughly two-fold increase of airborne particle concentration for particles smaller than 1.8 µm for activity vs. low activity periods, with maximum concentrations reaching 4-11 times the maximum value during low activity periods. For PM_{10}, average concentration was 2.5 times higher than background levels during activity periods, while peak concentration was 4.5 times higher. Qian and Ferro (2008) observed that resuspension rates depend on particle size, floor material, and ventilation position. Increases in walking speed and weight of the walker did not consistently produce increases in resuspension. 5-10 µm particles produced a higher resuspension rate compared with smaller particles. Newly carpeted areas produced significantly higher resuspension rates than vinyl floors. Zhang et al. (2008) modeled and conducted experiments of particle dispersion from walking and observed that human activity did affect resuspension. They found that larger particles were more readily

detached from the carpet by walking motion, but that smaller particles are more easily resuspended once detached. Hunt and Johnson (In Press) studied the duration and spatial extent of resuspension of 0.3-5.0 µm particles following walking by a soiled shoe. 0.3-0.5 µm particle concentration remained increased over a time period of 23 min, while 1-5 µm particles declined in concentration over the same time period. Experiments and computational fluid dynamics simulations by Eisner et al. (2010) for a mechanical foot moving on carpeting suggested that the rotating motion of the moving foot on the carpet induced rotating air movement beneath the foot that re-entrained the particles. If Pb adsorption onto particles varies with sources, size selectivity of resuspension processes could lead to enhanced Pb exposure from one source relative to another.

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 3-26 in the Appendix to Chapter 3 illustrates that Pb concentrations in the four studies summarized there are typically well below the level of the NAAQS. The higher personal air concentratoins occurred in a heavily industrialized area of Kocaeli, Turkey with an incinerator and several industrial facilities including metal processing, cement, petroleum refining, agriculture processing. Otherwise, concentrations were all between 0.002 and 0.006 µg/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-PM measurements from several studies

Study	Location	Pb Metric	Sampling Period	Personal Pb	Indoor Pb	Outdoor Pb
Clayton et al. (1999)	IL, IN, MI, MN, OH, WI	Med. Pb-PM_{50} (ng/m^3)	July, 1995-May, 1997	13	6.6	8.5
Adgate et al. (2007)	Minneapolis-St. Paul, MN	Avg. Pb-$PM_{2.5}$ (ng/m^3)	Spring, Summer, Fall, 1999	6.2	3.4	2.0
Molnar et al. (2007)	Stockholm, Sweden	Avg. Pb-$PM_{2.5}$ (ng/m^3)	December, 2003-July, 2004		Homes: 3.4 Schools: 2.5 Preschools: 1.8	Homes: 4.5 Schools: 4.6 Preschools: 2.6
Tovalin-Ahumada et al. (2007)	Mexico City, Mexico	Med. Pb-$PM_{2.5}$ (ng/m^3)	April-May, 2002		26	56
	Puebla, Mexico	Med. Pb-$PM_{2.5}$ (ng/m^3)	April-May, 2002		4	4
Pekey et al. (2010)	Kocaeli, Turkey	Avg. Pb-$PM_{2.5}$ (ng/m^3)	May-June, 2006, December, 2006-January 2007		Summer: 34 Winter: 85	Summer: 47 Winter: 72
		Avg. Pb-PM_{10} (ng/m^3)	May-June, 2006, December, 2006-January 2007		Summer: 57 Winter: 125	Summer: 78 Winter: 159
Rasmussen et al. (2007)	Windsor, Ontario, Canada	Med. Pb-$PM_{2.5}$ (mg/kg)	April, 2004	311	124	221

4.1.3.2 Exposure to Lead in Soil and Dust

The 2006 Pb AQCD (U.S. EPA, 2006b) lists indoor Pb dust infiltrated from outdoors as a potential source of exposure to Pb soil and dust. Thus, outdoor soil Pb may present an inhalation exposure if resuspended indoors or an ingestion exposure during hand-to-mouth contact. A detailed description of studies of outdoor soil Pb concentration is provided in Section 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 dust Pb concentrations for 2006-2011 observational studies in which indoor dust Pb was measured.

Table 4-4 Measurements of indoor dust Pb concentration from 2006-2011 studies

Reference	Study Location	Metric (units)	Sample Site	Indoor Pb Concentration
Caravanos et al. (2006a)	New York City, New York	Weekly dust loading ($\mu g/m^2$)	Glass plate next to open window of academic building	Median: 52
Khoder et al. (2010)	Giza, Egypt (extensive leaded gasoline use; industrial area)	Weekly dust loading ($\mu g/m^2$)	Glass plate in second-floor living room of apartments	Median: 408
Brattin and Griffin (2011)	Eureka, Utah near Eureka Mills Superfund Site	Dust concentration (mg/kg)	Indoor home site (not specified)	160-2000
	Denver, CO, near VBI70 Superfund Site		Indoor home site (not specified)	11-660
	East Helena, MT, near East Helena Superfund Site		Indoor home site (not specified)	68-1000
Yu et al. (2006)	Syracuse, New York	Dust concentration range (mg/kg)	Floor	Range: 209-1770
Turner and Simmonds (2006)	Birmingham, Plymouth, and 2 rural sites, UK	Dust concentration (mg/kg)	Floor	Median: 178
Gaitens et al. (2009)	U.S. (nationwide)	Dust loading ($\mu g/m^2$)	Smooth floor	Median: 1.7 Avg.: 4.4
			Rough floor	Median: 5.6 Avg.: 16
			Smooth windowsill	Median: 2.5 Avg.: 190
			Rough windowsill	Median: 55 Avg.: 480
Wilson et al. (2007)	Milwaukee, Wisconsin	Dust concentration ($\mu g/m^2$)	Central perimeter	Avg.: 107
			Entry	Avg.: 140
			Window	Avg.: 151
Zota et al. (2011)	Ottawa County, Oklahoma (area surrounding the Tar Creek Superfund Site)	Dust concentration (mg/kg)	Indoor (site not specified)	Avg.: 109 Median: 63 Max.: 881
Spalinger et al. (2007)	Rural towns, Idaho	Dust concentration (mg/kg)	Vacuum	Median: 120 Max: 830
			Floor	Median: 95 Max: 1,300
	Bunker Hill, Idaho Superfund site	Dust concentration (mg/kg)	Vacuum	Median: 470 Max: 2,000
			Floor	Median: 290 Max: 4,600

Several studies suggested the infiltration of Pb dust into buildings. For example, Caravanos et al. (2006a) 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 $\mu g/m^2$ for the indoor site, 153 $\mu g/m^2$ for the unsheltered outdoor site, and 347 $\mu g/m^2$ for the sheltered outdoor site. This paper demonstrated the likely role of outdoor Pb in influencing indoor dust Pb loading and indicated that under quiescent conditions (e.g., no cleaning) near an

open second-story window, the indoor dust Pb level might exceed EPA's hazard level for interior floor dust of 430 µg/m^2 (40 µg/ft^2). Khoder et al. (2010) used the same methodology to study Pb dust deposition in residential households in the town of Giza, Egypt, located between two industrial areas and where leaded gasoline is still in use; the investigators reported a median weekly deposition rate of 408 µg/m^2 and an exterior median deposition rate of 2,600 µg/m^2. In the latter study, Pb deposition rate correlated with total dust deposition rate (R=0.92), Cd deposition rate (R=0.95), and Ni deposition rate (R=0.90). Statistically significant differences in Pb deposition rates were observed between old and new homes ($p < 0.01$) in the Khoder et al. (2010) study, although the only quantitative information provided regarding home age stated that the oldest home was 22 years old when the study was performed in 2007. Khoder et al. (2010) found no statistically significant difference between Pb loadings when segregating the data by proximity to roadways. Recently, Brattin and Griffin (2011) performed linear regressions of dust Pb on soil Pb based on data collected previously for outdoor soil Pb and indoor dust Pb near mining and/or smelting Superfund sites in Utah, Colorado, and Montana (U.S. EPA, 2005f, 2002a, 2001). They observed that the dust Pb concentration was 4-35% of outdoor soil Pb. Excluding outliers on the regression, dust Pb concentration ranged from 160-2,000 mg/kg, 11-660 mg/kg, and 68-1,000 mg/kg at three sites.

Correlations between indoor and outdoor Pb content in dust can be partially explained with speciation. Beauchemin et al. (2011) used XANES to speciate in-home paint samples to assess the contributions of indoor paint and outdoor material to indoor dust Pb concentrations. In indoor dust samples of particles <150 µm in size, Pb oxide, Pb sulfate, and Pb carbonate were measured. These materials commonly were used in white paint. In the size fraction of particles <36 µm, half of the measured Pb was associated with Fe-oxyhydroxides such as ferrihydrite and goethite and presumably adsorbed onto these species. This finding suggested that a mix of indoor and outdoor sources may affect the composition of dust in this size fraction.

Residual Pb dust contamination following cleaning activities has been documented. For instance, Hunt et al. (2008) performed tests where a test soil prepared by drying, grinding, and sieving Pb-contaminated yard soil samples from Herculaneum, MO 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. After the first walk, tile Pb dust loading was 2,670 µg/m^2; after the first vacuuming, it decreased to 398 µg/m^2. After multiple walks, tile Pb dust loading was 7,100 µg/m^2, and it decreased to 1,400 µg/m^2 after multiple vacuuming. 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 result indicates

that Pb-bearing fine particles are not completely captured by home cleaning. Yu et al. (2006) analyzed dust samples from 50 homes in northern New Jersey (typically of older housing stock, although the study does not specify housing age). The investigators obtained dust from vacuuming carpet samples and found that total 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 in dust in homes of children ages 12-60 months. The median value of Pb dust loading on floors was reported to be 1.7 $\mu g/m^2$ (mean: 4.4 $\mu g/m^2$), with floors that were not smooth and cleanable having a median Pb dust loading value of 5.6 $\mu g/m^2$ (mean: 16 $\mu g/m^2$). Floor Pb dust loading value 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 loading on smooth windowsills was 25 $\mu g/m^2$ (mean: 190 $\mu g/m^2$). When windowsills were not smooth, the median Pb dust loading was 55 $\mu g/m^2$ (mean: 480 $\mu g/m^2$). Windowsill Pb dust level was also 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. Sill surface was nearly significantly associated ($p = 0.076$) with deterioration of outdoor paint when homes were built prior to 1950. Dust Pb loading was found by Egeghy et al. (2005) to be significantly associated with the log-transform of soil Pb concentration, 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). Wilson et al. (2007) studied Pb dust samples from homes in Milwaukee, WI children with and without elevated blood Pb $\geq$ 10 $\mu g/dL$. They found that Pb dust samples obtained from the floor were always significantly higher in residences of children with elevated blood Pb, with the exception of samples from the bathroom floor. Windowsill dust was not significantly higher in residences of children with elevated blood Pb.

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 historic and active metals mining and smelting sources. Near an active smelter in Port Pirie, Australia, median hand dust Pb loadings increased with age among a cohort of fourteen children followed over age 0-36 months (2-5 months: 54 $\mu g/m^2$, 6-9 months: 173 $\mu g/m^2$, 10-15 months: 424 $\mu g/m^2$, > 15 months: 336 $\mu g/m^2$) (Simon et al., 2007). Zota et al. (2011) studied Pb dust and indoor Pb-$PM_{2.5}$ concentration in Ottawa County, OK near the Tar Creek Superfund Site, in which a metals mine had closed. Statistically significant correlations among outdoor soil Pb concentration, indoor dust Pb concentration, indoor dust Pb loading, and indoor air Pb-$PM_{2.5}$ concentrations were observed (r = 0.25-0.65), with an average dust Pb concentration of 109 mg/kg, dust Pb loading of 54 $\mu g/m^2$, soil Pb concentration of 201 mg/kg, and indoor Pb-$PM_{2.5}$ concentration of 1 ng/m^3. Dust Pb concentrations were found to increase significantly with proximity to two chat (i.e., dry mining waste) sources and to decrease with distance to the street and presence of central air conditioning. Spalinger et al. (2007) measured Pb in dust in homes in a 34 km^2 area surrounding a designated Superfund site where formerly a Pb and Zn smelter operated at Bunker Hill, ID. During spring of 1999, vacuum and floor mat samples were taken from homes in three towns within the 34 km^2 area and five “background” towns further from the Superfund site. For the background towns, Pb concentration in vacuum dust had a median of 120 mg/kg and 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 $\mu g/m^2$ 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 Pb concentration 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 indoor dust in houses in these towns was 300 $\mu g/m^2$ per day, and the maximum Pb dust loading rate was 51,000 $\mu g/m^2$ per day. These results suggest that those living in close proximity to large Pb and Zn smelters or mines that are now Superfund sites are at much greater risk of exposure to Pb dust compared to the general population.

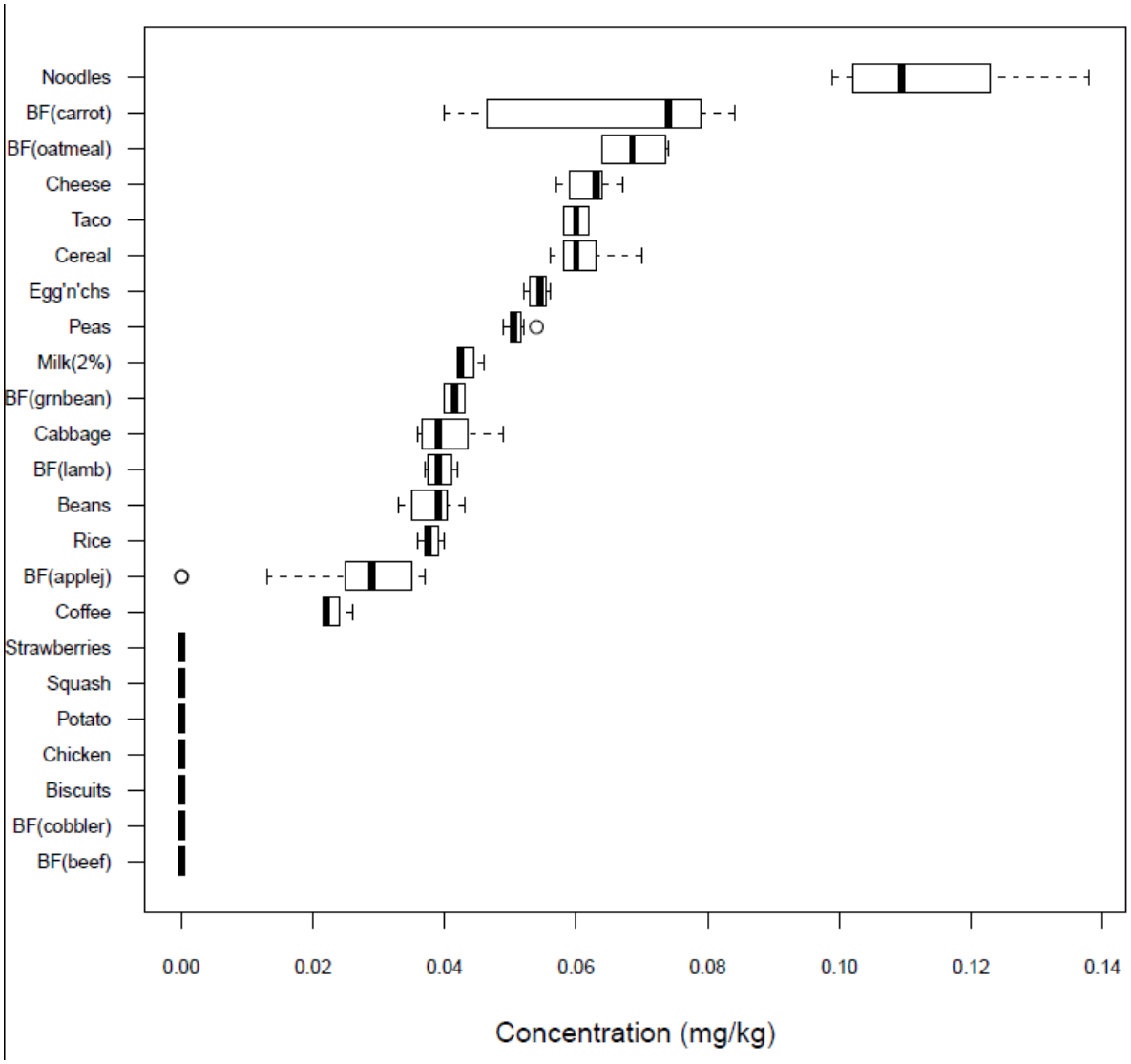
Pb exposure has been reported on children’s playgrounds. Mielke et al. (2011b) reported median soil Pb concentration of 558 mg/kg on playground soils at eleven New Orleans daycare or community centers. Following remediation efforts to cover playground soil with clean soil, median concentration dropped to 4.1 mg/kg. Duggan et al. (1985)

reported on the concentration and size distribution of wipe samples on the hands of 368 pre-school children from eleven schools in London, UK. Hand Pb residue (PbH) values were modeled as linear and power functions of Pb dust (PbD) from this study to obtain $PbH = 16.1 + 0.0064PbD$ or $PbH = 0.393PbD^{0.533}$. Given that the Duggan et al. (1985) study was performed when Pb additives were used in gasoline, dust Pb concentration values are not reported here. Measurements of the size distribution produced geometric mean diameters of 4.5 µm and 1.5 µm depending on the analysis method. The hand wipe samples were effectively limited to particles smaller than 10 µm, even when the soil itself exhibited a much larger particle size distribution. However, these studies focused on playground lead exposure. Que Hee et al. (1985) performed repeated wipe sampling of hands after rubbing in a reference dust sample and in reporting results for a "small adult hand" observed that loose particles up to 246 µm in diameter adhered, which is similar to the upper limit of 200-300 µm observed by Yamamoto et al. (2006). If a similar relationship holds for household dust, then exposure to Pb in dust by hand-to-mouth activity may be influenced by the dust particle size distribution and the relationship of Pb content to particle size fraction.

4.1.3.3 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, agriculture, urban gardening, dietary supplements, tobacco, cultural food sources, and breastfeeding. The breadth of dietary Pb exposures is illustrated in Figure 4-2, which illustrates the data obtained in the 2008 FDA Total Diet Study market basket survey (FDA, 2008). Among the highest Pb concentrations were those for noodles, baby food carrots, baby food oatmeal, Swiss cheese, beef tacos from a Mexican restaurant, and fruit-flavored cereal. The source of the Pb in each case is unclear. Possible sources of Pb in food samples include introduction during processing or preparation with drinking water contaminated with Pb, deposition of Pb onto raw materials for each food, and Pb exposure in livestock that produce dairy or meat ingredients. Manton et al. (2005) used Pb isotope ratios to estimate sources of dietary Pb among a cohort of mothers and children from Omaha, NE using a combination of food samples, hand wipes, house dust wipes, and aerosol samples collected between 1990 and 1997 and speciated for Pb isotopes. Drinking water Pb was not included in this study. The authors cited results from Egan et al. (2002) that imported vegetables contributed 55% of Pb dietary intake for infants, 30% for 2-6 y old children, and 20% for 25-30 y old women. Imported candy contributed 10% of Pb dietary intake for 2-6 y old children and 9% for 25-30 y old women. Isotopic data from Manton et al. (2005) suggested that, with the exception of children age 0-12 mos, house dust is a large

contributor to dietary Pb. The pattern of certain Pb-isotope ratios observed in the diet of children 0-12 mos are suggested to derive from Ca salts in limestone that may have been used in dietary supplements in baby formula. The contribution of ambient Pb aerosols to dietary Pb samples was not statistically significant for this urban exposure study.



Source Data: (FDA, 2008)

Note: from the 2008 FDA Total Diet Study. "BF" denotes baby food.

Figure 4-2 Market basket survey results for Pb concentration in foods.

Drinking Water

Differences in sources and transport of drinking water may cause variation in water 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 because Pb can be leached from the glass, the median Pb concentration in the bottled water samples was 8.5 ng/L (range ≤ 1 to 761 ng/L). This level (i.e., 8.5 ng/L) is significantly less than the Maximum Contaminant Level for Pb of 15 µg/L. Pb in drinking water supplies can derive from atmospheric deposition onto surface waters, as described in Section 3.3.1.2, or via corrosion of Pb in the distribution network exacerbated by contact with acidic disinfection byproducts, as described in the following paragraphs.

It is now recognized that environmental nanoparticles (NPs) (~1-100 nm) can play a key role in determining the chemical characteristics of 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 influence Pb concentrations in drinking water. Schock et al. (2008) characterized Pb pipe scales from 91 pipes made available from 26 different municipal water systems from across the northern U.S. They found a wide range of elements including Cu, Zn and V as well as Al, Fe and Mn. Interestingly, V was present at nearly one percent levels in pipes from many geographically diverse systems. In a separate study, Gerke et al. (2009) identified the corrosion product, vanadinite ($Pb_5(VO_4)_3Cl$) in Pb pipe corrosion byproducts collected from 15 Pb or Pb-lined pipes representing 8 different municipal drinking water distribution systems in the Northeastern and Midwest regions of the U.S. Vanadinite was most frequently found in the surface layers of the corrosion products. The vanadate ion, VO_4^{3-}, essentially replaces the phosphate ion in pyromorphite and hydroxyapatite structures. It is not known whether the application of orthophosphate as a corrosion inhibitor would destabilize vanadinite, but this substitution would have implications for V release into drinking water. The stability of vanadinite in the presence of monochloramine is also not known, and its stability might have implications for both Pb and V release into drinking water.

In recent years, drinking water treatment plants in many municipalities have switched from using chlorine to other disinfecting agents because their disinfection byproducts may be less carcinogenic. However, chloramines are more acidic than chlorine and can increase Pb solubility (Raab et al., 1991) and increase Pb concentrations in tap water. For example, after observing elevated Pb concentrations in drinking water samples, Kim and Herrera (2010) observed Pb oxide corrosion scales occurring after using acidic alum as a disinfection agent. 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 and Dudi, 2004). Maas et al. (2007) tested the effect of fluoridation and chlorine-based (chlorine and chloramines) disinfection agents on Pb leaching from plumbing soldered with Pb. When using chlorine disinfection agents alone, the Pb concentration in water samples doubled during the first week of application (from 100 to 200 ppb) but then decreased over time. When adding fluorosilicic acid and ammonia, the Pb concentration spiked to 900 ppb and increased further over time. 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. (2007a) modeled blood Pb levels among children living in Wayne County, NC as a function of household age, use of chloramines and other covariates. Blood Pb levels were 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. (2007a) found that significance increased with the age of the home. However, the study did not control for the presence of Pb paint in the dwellings, so it is difficult to distinguish the influence of Pb pipes from Pb in paint on blood Pb levels.

Several chemical mechanisms may contribute to release of Pb during use of chloramine disinfection agents. Edwards and Dudi (2004) hypothesized that Pb leaching occurs when chloramines cause the breakdown of brass alloys and solder containing Pb. After observing that nitrification also leads to increased Pb concentrations in water, they also proposed that chloramines may trigger nitrification and hence cause decreasing pH, alkalinity and dissolved oxygen that lead to corrosion after observing that nitrification also leads to increased Pb concentrations in water. However, Zhang et al. (2009b) found no evidence that nitrification brought about significant leaching of Pb from Pb pipes. Lytle et al. (2009) suggested that a lack of increased Pb(II) concentrations in drinking water following a change from free chlorine to 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

The 2006 Pb AQCD (2006b) states that surface deposition "represents a significant contribution to the total Pb in and on the plant", while uptake through a plants roots can also contribute to a plant's Pb concentration. Consequently, Pb content in plants may contribute to human dietary exposure. Uptake of Pb by plants growing in contaminated soil has been repeatedly demonstrated in some species during controlled potted plant experiments (Del Río-Celestino et al., 2006). In this study, most species retained Pb in the roots with little mobilization to the shoots of the plants. However, certain species *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 had high bioaccumulation, measured as the percent of Pb concentration measured in the plant compared with the Pb concentration in the soil, with little translocation of the Pb to the shoots, 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. This is consistent with the 2006 Pb AQCD (2006b), which reported that Pb deposition onto leafy vegetables accounted for most Pb.

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. Pb contamination of crops can occur through Pb emissions during aerial application of fertilizers and pesticides. In 2009, the U.S. Federal Aviation Administration (FAA) recorded 960,000 hours of flight time for aerial application. This term encompasses crop and timber production including seeding cropland and fertilizer and pesticide application. It is estimated that 42% of these flight-hours involved piston engine aircraft utilizing leaded fuel (FAA, 2009).

Fernandez et al. (2010; 2008; 2007) measured Pb from atmospheric deposition in two adjacent plots of land having the same soil composition but different uses: one was pasture land and one was agricultural. In the arable land, size distributions of soil particle-bound Pb, were uniformly distributed. In pasture land, size distributions of soil particle-bound Pb were bimodal with peaks around 2-20 μm and 50-100 μm (Fernandez et al., 2010). For the agricultural plot, Pb concentration was constant around 70 mg/kg in samples taken over the first 30 cm of soil, at which time it dropped below 10 mg/kg at soil depths between 35 and 100 cm. In contrast, Pb concentration in pasture land peaked at a depth of 10 cm at a concentration of roughly 70 mg/kg and then dropped off gradually to approach zero concentration at a depth of approximately 50 cm. The sharp change in concentration for the arable land was attributed to a combination of plowing the soil and use of fertilizers to 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 in 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. (2008a) observed that the median concentration of Pb in California crop soil samples was 16.2 mg/kg (range: 6.0-62.2 mg/kg). Chen et al. (2008b) further observed that in three of the seven California agricultural regions sampled, concentrations of Pb increased following addition of fertilizer, but the increase was less than that for P and Zn indicators of fertilizer. In four

regions, there was no increase of Pb at all. Furthermore, Tu et al. (2000) observed a decrease in Pb fraction with increasing P application. Nziguheba and Smolders (2008) also surveyed phosphate-based fertilizers sold in European markets to determine the contribution of these fertilizers to heavy metal concentrations in agricultural products. They reported a median fertilizer Pb concentration of 2.1 mg/kg based on total weight of the fertilizer, with a 95th percentile concentration of 7.5 mg/kg. Across Europe, Nziguheba and Smolders (2008) estimated that the amount of Pb applied via fertilizers to be only 2.6% of that resulting from atmospheric deposition.

Although Pb in on-road vehicle gasoline has been phased out in the U.S., some imported crops 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, during the time when leaded gasoline was still legally sold. Rankin et al. (2005) observed that the ratios of ^{207}Pb to ^{206}Pb and ^{208}Pb to ^{207}Pb were similar to those of Pb in gasoline. Although this study showed that Pb concentration in the shelled cocoa beans was low (~1 ng/g), manufactured cocoa powder and baking chocolate had Pb concentrations similar to those of the cocoa bean shells, on the order of 200 ng/g, and Pb concentration in chocolate products was roughly 50 ng/g (Rankin et al., 2005). It is possible that the increases were attributed to contamination of the cocoa by the shells during storage or manufacture, but the authors note that more research is needed to verify the source of contamination. Likewise, it is possible that resuspended Pb that originated from legacy mobile and industrial sources could deposit on crops.

The extent to which soil Pb contributes Pb to agricultural crops varies with soil characteristics. 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.

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%	2,500%
Cereals	Grain	All	9	1.0%	3.6	1.8%	1.6%	0.19%	4.8%
	Straw	All	4	2.3%	3.5	3.8%	4.0%	0.51%	9.6%
Maize	Grain	All	9	0.12%	2.3	0.17%	0.14%	0.052%	0.38%
	Straw	All	3	0.28%	6.6	0.85%	1.3%	0.060%	2.3%
Rice	Grain	All	2			2.2%	1.4%	1.2%	3.2%
Leafy Vegetables		All	31	8.0%	13	210%	610%	0.32%	2,500%
		Sand	4	7.3%	1.5	7.8%	3.3%	4.9%	11%
		Loam	3	82%	1.0	82%	3.5%	79%	86%
		Clay	7	2.8%	4.1	5.1%	4.8%	0.41%	12%
Non-Leafy Vegetables	Fruits	All	5	1.5%	26	78%	170%	0.15%	390%
	Shoots	All	2			0.88%	0.42%	0.58%	1.17%
Legumes	Pods	All	17	0.53%	12	34%	120%	0.046%	490%
		Sand	3	0.27%	3.2	0.42%	0.34%	0.065%	0.89%
		Loam	5	0.14%	4.4	0.42%	0.34%	0.065%	0.89%
		Clay	4	0.080%	1.0	0.33%	0.47%	0.046%	1.0%
	Shoots	All	1			0.080%			
Root Crops	Roots	All	27	1.5%	16	41%	98%	0.024%	330%
		Sand	5	6.4%	1.6	7.0%	3.4%	4.2%	12%
		Loam	5	2.3%	4.7	0.50%	0.68%	0.024%	1.7%
	Shoots	All	12	6.3%	15	250%	570%	0.30%	16%
Tubers	Tubers	All	30	0.15%	7.4	9.1%	48%	0.015%	260%
		Sand	5	0.64%	3.5	1.2%	1.6%	0.16%	3.9%
		Loam	17	0.052%	2.4	0.073%	0.062%	0.015%	0.23%
Fruits	Fruits	All	5	0.77%	2.6	1.0%	0.60%	0.15%	1.7%
	Leaves	All	1			25%			
Grasses		All	17	31%	1.8	36%	22%	11%	100%
Natural Pastures		All	34	92%	4.8	23%	29%	0.22%	100%
Leguminous Fodder		All	1			1.6%			
All Cereals		All	20	0.43%	4.7	1.1%	1.4%	0.052%	4.8%
		Sand	5	0.61%	5.3	1.3%	1.3%	0.052%	3.2%
		Loam	8	0.17%	3.9	0.53%	1.1%	0.059%	3.2%
		Clay	6	0.90%	4.0	1.8%	1.8%	0.22%	4.8%
Pastures/Grasses		All	51	14%	4.2	27%	27%	0.22%	100%
Fodder		All	24	2.5%	12	130%	420%	0.060%	1,600%
		Sand	4	4.5%	2.3	5.6%	4.0%	1.6%	11%
		Clay	4	0.82%	5.7	2.7%	4.6%	0.16%	9.6%

Source: Reprinted with permission of Elsevier Publishers, Vandenhove et al. (2009).

Findings from Pb uptake studies have implications for urban gardening if urban soils may be contaminated with Pb, as described in Section 4.1.3.2. 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 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 equivalent to 10-25% of the exposure to Pb from drinking water for children living in the Boston neighborhoods studied. Because soil Pb levels in urban areas will depend on surrounding sources (Pruvot et al., 2006), Pb exposures in urban garden vegetables will vary.

Game

Game meat consumption also may pose a risk of Pb exposure. In Pb mining or smelting areas, several studies have documented Pb concentrations in game [e.g., (Nwude et al., 2010; Reglero et al., 2009a)]. Jankovská et al. (2010) studied Pb accumulation in sheep infected with tapeworm and found that sheep harboring the parasites contained significantly less Pb compared with their uninfected counterparts ($p \leq 0.05$).

Potential Pb exposure through consumption of animals exposed to or killed with Pb shot has also been well documented (Hunt et al., 2009; Tsuji et al., 2009; Tsuji et al., 2008; Hunt et al., 2006). For example, Martínez-Haro et al. (2010) observed Pb in the feces of mallards that ingested gunshot of 34-13,930 mg/kg with a median of 1,104 mg/kg, while mallards that did not ingest gunshot had feces Pb levels < 12.5 mg/kg. Mateo et al. (2011) studied Pb bioaccessibility as a function of cooking method for breast meat from partridges killed with gunshot. They observed that preparation in cold or hot vinegar increased bioaccessibility compared with total Pb in the samples.

Fish

Accumulation in fish could also lead to human exposure to Pb (U.S. EPA, 2006b, 1986a). Ghosh et al. (2007) demonstrated in laboratory experiments that exposure to Pb in water can lead to linearly increasing 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 sediment Pb concentrations ranged from 580 to 6,500 mg/kg. In total, 65% of the surveyed individuals fished for food from the Bayou, with 86% consuming fish from the Bayou each week. In a study of the effect of coal mining on levels of metals in fish (measured as blood Pb) in northeastern Oklahoma, Schmitt et al. (2005) found that Pb concentrations in blood varied with respect to species of fish, but Pb concentrations were higher in fish 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., 2007a), 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.

Breast Milk

Studies breastfeeding women suggest that infants may be exposed to Pb in breast milk. Ettinger et al. (2004a) observed among Mexico City women studied in 1994-1995 that at 1 month postpartum, 88 women breastfeeding exclusively (with mean blood Pb level of 9.4 µg/dL) had breast milk Pb concentrations of 1.4 ± 1.1 µg/L, and 165 women breastfeeding partially (with mean blood Pb level of 9.5 µg/dL) had breast milk Pb concentrations of 1.5 ± 1.2 µg/L. During the same time period, Ettinger et al. (2006) studied breastfeeding women in Mexico City over a child's first year of life and sampled Pb concentration in breast milk at 1, 4, and 7 mo post-partum. They observed that mean breast milk concentrations dropped from 1.4 µg/L at 1 mo (mean maternal blood Pb = 9.3 µg/dL) to a mean of 1.2 µg/L at 4 mo (mean maternal blood Pb = 9.0 µg/dL) to 0.9 µg/L at 7 mo (mean maternal blood Pb = 8.1 µg/dL); this reduction was statistically significant ($p < 0.00001$). Among the 310 women included in the study, 181 had previous pregnancies. In one study of nursing mothers living in Pb contaminated city in Australia, 10 of the 11 mothers had breast milk concentrations <5 µg/L (Simon et al., 2007). The authors hypothesized that breast milk concentration was too low to be a major contributor to blood Pb level in these infants relative to other factors such as hand loading of lead. However, one mother with a blood Pb level of 25 µg/dL had a breast milk Pb level of 28 µg/L (Simon et al., 2007).

4.1.3.4 Occupational

Occupational environments have the potential to expose individuals to Pb. Some modern day occupational exposures are briefly discussed below in the context of understanding potential exposures that are not attributed to ambient air. For example, Miller et al. (2010) obtained personal and area samples of particle-borne Pb in a precious metals refinery; year of the study was not reported. It was not stated explicitly, but it is likely that Miller et al. (2010) measured the PM as TSP because the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for Pb is based on TSP rather than a smaller size cut, and the OSHA PEL was used for comparison. Concentrations measured by personal samples ranged from 2 to 6 µg/m^3, and concentrations from area samples ranged from 4 to 14 µg/m^3. The OSHA PEL is 5 µg/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% of particulate mass below 2.5 µm diameter, was not reported; year of the study was also 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.

4.1.3.5 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 (Miodovnik and Landrigan, 2009; Levin et al., 2008).

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)

Product Category	Product	Location of Purchase	Pb Content (units)	Reference
	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. (2009a)
	Lemongrass, licorice, holy basil, cloves, ginger	India	Average: Lemongrass & Holy Basil Leaves: 6.1 mg/kg; Licorice Stolons: 6.1 mg/kg, Clove Dried Flower Buds: 7.8 mg/kg, Ginger Rhizome: 5.8 mg/kg	Naithani and Kakkar (2006)
	B-Success 28, Operation Sweep, Aloe Vera Plus Bitter Aloes, Zarausmacine, Virgy-Virgy Computer Worm-Expeller, Dorasine Powder, Sexual Energy, U&DEE Infection Cleansing Powder, U&DEE Sweet Bitter, Natural Power Stone, Chama Black Stone, Portugal Antiseptic Soap, Edysol Antiseptic Soap, H-Nal, M-Reg, Veins Flocher, Diabor, C-Candi, C-Cysta, Firas, D-Diab, P-Pile, Infecta, Ribacin Forte, Aloe Vera Cure Formula	Nigeria	925-27,000 µg	Obi et al. (2006)
	Shell of Hen's Egg	India	14 mg/kg	Sharma et al. (2009)
	Berberis (*B. aristata, B. chitria, B. asiatica, B. lyceum*), Daruharidra	India	Berberis: Roots: 3.1-24.7 mg/kg Stems: 8.0-23.8 mg/kg Daruharidra: 16.9-49.8 mg/kg	Srivastava et al. (2006)
	Greta powder	U.S. (California)	770,000 ppm	CDC (2002)
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	U.K.	0.15-1.56 mg/kg	McNeill et al. (2006)
	Cigarette Tobacco (^{210}Pb concentrations)	Pakistan	Activity conc.: 7-20 Bq/kg	Tahir and Alaamer (2008)
Toys	Red and yellow painted toy vehicles and tracks	Brazil	500-6,000 mg/kg	Godoi et al. (2009)
	535 PVC and non-PVC toys from day care centers	U.S. (Nevada)	PVC: avg. 325 mg/kg Non-PVC: avg. 89 mg/kg Yellow: 216 mg/kg Non-yellow: 94 mg/kg	Greenway and Gerstenberger (2010)
	Soft plastic toys	India	Average (by city): 21-280 mg/kg	Kumar and Pastore (2007)
	Toy necklace	U.S.	388,000 mg/kg	Meyer et al. (2008)
	Soft plastic toys	Nigeria	2.5-1,445 mg/kg	Omolaoye et al. (2010b)
Vitamins	Vitamins for young children, older children, and pregnant or lactating women	U.S.	Average: Young children: 2.9 µg/day Older children: 1.8 µg/day Pregnant and lactating women: 4.9 µg/day	Mindak et al. (2008)

[a]*Hoodia gordonii*, from Eastern Cape, South Africa *Euterpe oleracea* from Ninole Orchard, Ninole, Hawaii
Note that the country of origin is not provided because it was not published in the references cited.

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, 2006b). These models support predictions about the kinetics of Pb in blood and other selected tissues based on the empirically-based information about Pb biokinetics. In Section 4.3 (and Section 4.2.2.1), 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 Pb AQCD also presented dermal absorption of inorganic and organic Pb compounds, which is generally considered to be much less than by inhalation or ingestion. A study published subsequent to the 2006 Pb AQCD measured rates of absorption of Pb in skin patches harvested from nude mice (Pan et al., 2010). Following application of 12 mg Pb as Pb-acetate or Pb nitrate, the absorption rate (measured over a 10-hour observation period) was approximately 0.02 µg Pb/cm^2 per hour. Absorbed Pb was detected in liver and kidney of nude mice following a 120-hr occluded dermal application of approximately 14 mg Pb as either Pb-acetate or Pb nitrate. Uptake of Pb into the skin at the site of application was greater when Pb-acetate was applied to the skin compared to lead nitrate; however, liver and kidney Pb concentrations observed at the conclusion of the study (120 hours following the application of Pb) were not different for the two Pb compound. No additional information provides evidence of dermal absorption being a major exposure route of environmental Pb.

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+}). Bioaccessibility is typically assessed by measuring the fraction of Pb in a sample that can

be extracted into a physiological or physiological-like solution (e.g., gastric juice or solution similar to gastric juice).

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 and Roy, 1994). Larger particles (>2.5 µm) that are primarily deposited in the ciliated airways (nasopharyngeal and tracheobronchial regions) can be transferred by mucociliary transport into the esophagus and swallowed, thus being absorbed via the gut.

Inhaled Pb lodging deep in the respiratory tract seems to be absorbed equally and totally, regardless of chemical form (Morrow et al., 1980; Chamberlain et al., 1978; Rabinowitz et al., 1977). Absorption half-times ($_{t1/2}$) have been estimated for radon decay progeny in adults who inhaled aerosols of Pb and bismuth isotopes generated from decay of ^{220}Rn or ^{222}Rn. The absorption half-time for Pb from the respiratory tract to blood was estimated to be approximately 10 hours in subjects who inhaled aerosols having an activity median particle diameter of approximately 160 nm (range 50-500 nm) (Marsh and Birchall, 1999), and approximately 68 min for aerosols having diameters of approximately 0.3–3 nm (Butterweck et al., 2002). Given the submicron particle size of the exposure, these rates are thought to represent, primarily, absorption from the bronchiolar and alveolar regions of the respiratory tract.

Several studies have quantified the bioaccessibility of Pb in atmospheric PM, based on various in vitro extraction methods. In a study of PM_{10} and $PM_{2.5}$ samples from downtown Vienna, Austria, Falta et al. (2008) used synthetic gastric juice to investigate the bioaccessibility of metals including Pb. The rationale was that inhaled particles in the 2.5-10 µm size range are mostly deposited in the tracheal and bronchial regions of the lung from where they are transported within hours by mucociliary clearance, i.e., they are mainly swallowed. In contrast, the <2.5 µm particles are deposited in the pulmonary alveoli where they can stay for months to years. The study aimed to determine the bioaccessibility of the 2.5-10 µm PM. It is important to note that they do not isolate the 2.5-10 µm size range; instead, they infer the characteristics from the difference between the $PM_{2.5}$ and PM_{10} fractions. The Pb concentrations associated with the two fractions were almost identical, as was the percentage extracted by synthetic gastric juice (86% and 83% Pb for $PM_{2.5}$ and PM_{10} fractions, respectively). The mean daily bioavailable mass

was calculated to be 16 ng for the $PM_{2.5-10}$ size range. Since the quantitative clearance of these particles to the stomach was assumed, this value represents an upper estimate for the amount of bioavailable Pb. Niu et al. (2010) determined the bioaccessibility of Pb in fine (100-1,000 nm) and ultrafine-sized (<100 nm) urban airborne PM from two sites within the city of Ottawa, Canada. For all size fractions, the median Pb concentrations for particles smaller than 10 µm were 8,800 and 7,800 mg/kg for the two different locations. The bioaccessibility was based on ammonium acetate extractability and it was found that, within the fine and ultrafine-size ranges, 13-28% Pb was extracted. The Falta et al. (2008) and Niu et al. (2010) results illustrate that different extraction techniques result in different 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 particles, with percent bioaccessibility decreasing with increasing particle size. This result indicated that Pb was potentially most bioavailable in the ultrafine-size range.

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

The above considerations indicate that the relationship between air Pb exposure and blood Pb will depend on numerous exposure variables (e.g., particle size, solubility, exposure frequency and duration) and physiological variables (age, activity level, transport and absorption in the respiratory tract, blood Pb kinetics). Mechanistic models provide one means for integrating these variable into predictions of blood Pb – air Pb relationships; although, predictions are subject to simplifications and generalizations made in constructing the models. As an example, the ICRP (Pounds and Leggett, 1998; ICRP, 1994; Leggett, 1993) model (Section 4.3 for a brief description) can be used to predict blood Pb – air Pb slopes for specific direct Pb inhalation exposure scenarios. For a long-term continuous (24 hours/day) exposure of a typical adult male engaged in light exercise (ventilation rate 20-22 m^3/day) to Pb-bearing particles having a 1 µm uniform particle size, the predicted blood Pb – air Pb slopes range from 0.7 µg/dL per µg/m^3 (for low solubility particles; e.g., Pb oxide) to 3 µg/dL per µg/m^3 (for highly soluble Pb; e.g., Pb salts). Empirical estimates of blood Pb – air Pb slopes for various populations, derived from epidemiological studies, are summarized in Section 4.5.1.

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 Pb 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 (e.g., persons exposed to leaded gasoline used piston-engine aircraft). Mahaffey (1977) estimated that 40% of inhaled Pb in urban air (largely attributed to combustion of gasoline containing tetraethyllead) 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 in engine exhaust from fuel containing tetraethyllead at a concentration of 0.001 μg/m^3 for a period of months 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 (Sherlock and Quinn, 1986; Sherlock et al., 1984; Pocock et al., 1983; Sherlock et al., 1982). While evidence for capacity-limited processes at the level of the intestinal epithelium is compelling, the dose at which absorption becomes appreciably limited in humans is not known.

In adults, estimates of absorption of ingested water-soluble Pb compounds (e.g., Pb chloride, Pb nitrate, Pb-acetate) range from 3 to 10% in fed subjects (Maddaloni et al., 1998; Watson et al., 1986; James et al., 1985; Heard and Chamberlain, 1982; Rabinowitz et al., 1980). The absence of food in the GI tract increases absorption of water-soluble Pb

in adults. Reported estimates of soluble Pb absorption range from 26 to 70% in fasted adults (Maddaloni et al., 1998; James et al., 1985; Blake et al., 1983; Heard and Chamberlain, 1982; Rabinowitz et al., 1980). Reported fed:fasted ratios for soluble Pb absorption in adults range from 0.04 to 0.2 (James et al., 1985; Blake et al., 1983; Heard and Chamberlain, 1982; Rabinowitz et al., 1980).

Limited evidence demonstrates that GI absorption of water-soluble Pb is higher in children than in adults. Estimates derived from dietary balance studies conducted in infants and children (ages 2 weeks to 8 years) indicate that ~ 40-50% of ingested Pb is absorbed (Ziegler et al., 1978; Alexander et al., 1974). Experimental studies provide further evidence for greater absorption of Pb from the gut in young animals compared to adult animals (Aungst et al., 1981; Kostial et al., 1978; Pounds et al., 1978; Forbes and Reina, 1972). The mechanisms for an apparent age difference in GI absorption of Pb have not been completely elucidated and may include both physiological and dietary factors (Mushak, 1991). Eating breakfast was shown to be significant predictor of blood Pb concentrations in 1,344 children 3-5 year s old (Liu et al., 2011b). Blood Pb concentrations were lower in children who regularly ate breakfast compared to children who did not eat breakfast, and the difference persisted after controlling for nutritional variables (blood iron, calcium, copper, magnesium, zinc). This observation may be explained by lower GI absorption of Pb ingested with or in close temporal proximity to meals. Direct evidence for meals lowering GI absorption of Pb has been reported for adults (Maddaloni et al., 1998; James et al., 1985).

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 (Schell et al., 2004; Marcus and Schwartz, 1987; Mahaffey and Annest, 1986). 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; Morrison and Quarterman, 1987; Barton et al., 1978b). An analysis of data from a sample 448 woman (age 20-55 years) did not find a significant association between iron body stores (indicated from serum ferritin concentration) and blood Pb concentrations, although depleted irons stores (serum ferritin of <12 µg/L) was associated with higher blood concentrations of cadmium, cobalt and manganese higher (Meltzer et al., 2010).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 and Mann, 1983; Heard and Chamberlain, 1982). A similar effect of calcium occurs in rats (Barton et al., 1978a). 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; Schell et al., 2004; Mahaffey et al., 1986; 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 (Fullmer and Rosen, 1990; Bronner et al., 1986). 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 and 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.

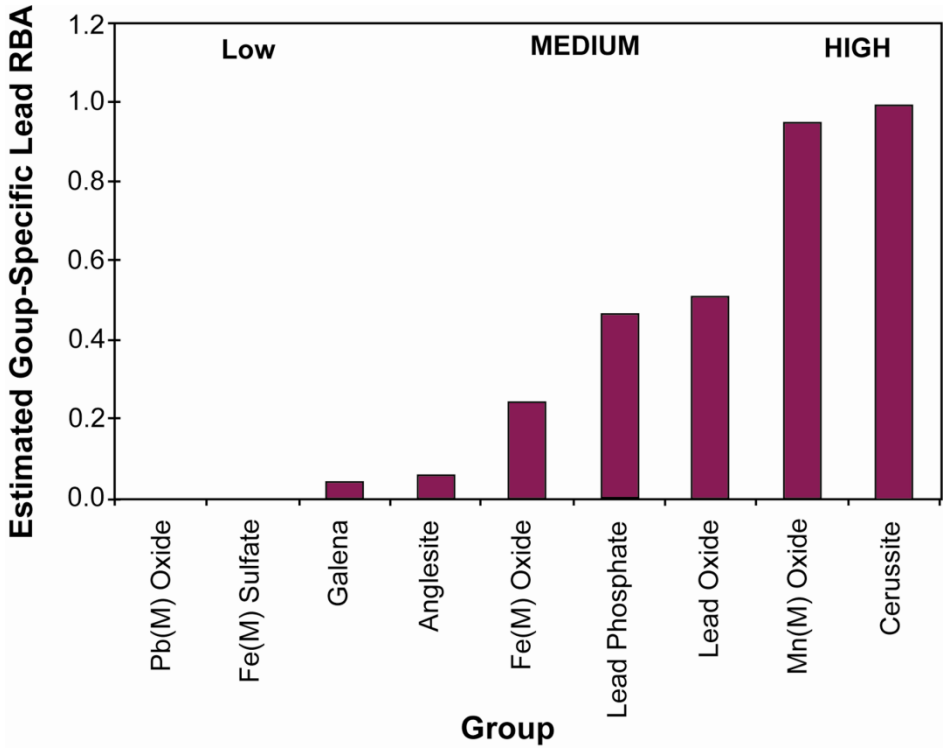
Blood Pb concentrations in young children have also been shown to increase in association with lower dietary Zn levels (Schell et al., 2004). Mechanisms for how Zn affects blood Pb concentration, i.e., whether it involves changes in absorption or changes in distribution and/or elimination of Pb, have 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. Absorption of Pb has been shown to vary depending upon the Pb mineralogy and physical characteristics of the Pb in the soil (e.g., encapsulated or exposed) and size of the Pb-bearing grains. GI absorption of larger Pb-containing particles (>100 µm) tends to be lower than smaller particles (Healy et al., 1992; Barltrop and Meek, 1979). Absorption of Pb in soils and dust has been most extensively studied in the in vivo swine model. Gastric function of swine is thought to be sufficiently similar to that of humans to justify use of swine as a model for assessing factors that may affect GI absorption of Pb from soils in

humans (Juhasz et al., 2009; U.S. EPA, 2007b; Casteel et al., 2006; Casteel et al., 1997; Weis and Lavelle, 1991). Other practical advantages of the swine model over rodent models have been described, and include: absence of coprophagia; ease with which Pb dosing can be administered and controlled; and higher absorption fraction of soluble Pb (e.g., Pb-acetate) in swine, which is more similar to humans than rats (Smith et al., 2009a). The swine studies measure blood and/or tissue Pb (e.g., kidney, liver, bone) concentrations following oral dosing of swine with either soil or with a highly water soluble and fully bioaccessible form of Pb (e.g., Pb-acetate). A comparison of the internal concentrations of Pb under these two conditions provides a measure of the bioavailability (i.e., absorption) of Pb in soil relative to that of Pb-acetate, which is typically referred to as relative bioavailability (RBA). Relative bioavailability measured in the swine assay is equivalent to the ratio of the absorbed fraction (AF) of ingested dose of soil Pb to that of water-soluble Pb-acetate (e.g., RBA = $AF_{Soil\ Pb}/AF_{Pb\text{-}acetate}$).

Collectively, published studies conducted in swine have provided 39 estimates of Pb RBA for 38 different soil or "soil-like" test materials (Bannon et al., 2009; Smith et al., 2009a; Casteel et al., 2006; Marschner et al., 2006). The mean of RBA estimates from 25 soils is 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 4-3 shows some of the materials that fall into these three categories. Mineral phases observed in mineralogical wastes can be expected to change over time (i.e., weathering), which could change the RBA over time. The above observations in swine are supported by various studies conducted in rats that have found RBA of Pb in soils to vary considerably and to be less that 100% (Smith et al., 2009a, 2008; Freeman et al., 1996; Freeman et al., 1994; Freeman et al., 1992).



Source: Casteel et al. (2006).
Note: based on results from juvenile swine assays.

Figure 4-3 Estimated relative bioavailability (RBA, compared to Pb-acetate) of ingested Pb in mineral groups.

Drexler and Brattin (2007) developed an in vitro bioaccessibility (IVBA) assay for soil Pb that utilizes extraction fluid comprised of glycine, deionized water, and hydrochloric acid at a pH of 1.50 that is combined with sieved test material (<250 µm) for 1 hour. The assay was tested for predicting in vivo RBA of 18 soil-like test materials that were assayed in a juvenile swine assay (Casteel et al., 2006). A regression model relating IVBA and RBA was derived based on these data (Equation 4-1):

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

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, 2007e). 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 among different in vitro assays applied to the same soil test materials have found considerable variability in IVBA estimates (Juhasz et al., 2011; Smith et al., 2011; Saikat et al., 2007; Van de Wiele et al., 2007). This variability has been attributed to differences in assay conditions, including pH, liquid:soil ratios, inclusion or absence of food material, and differences in methods used to separate dissolved and particle-bound Pb (e.g., centrifugation versus filtration). Smith et al. (2011) found that algorithms for predicting RBA based on two different IVBA assays did not yield similar predictions of RBA when applied to the same material. Given the dependence of IVBA outcomes on assay conditions, in vitro assays used to predict in vivo RBA should be evaluated against in vivo RBA estimates to quantitatively assess uncertainty in RBA predictions (U.S. EPA, 2007e).

Absorption of Pb in house dusts has not been rigorously evaluated quantitatively in humans or in experimental animal models. The RBA for paint Pb mixed with soil was reported to be approximately 72% (95% CI: 44, 98) in juvenile swine, suggesting that paint Pb dust reaching the gastrointestinal tract maybe highly bioavailable (Casteel et al., 2006). The same material yielded a bioaccessibility value (based on IVBA assay) of 75% (Drexler and 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 are that a validated IVBA methodology for soil would perform well for predicting RBA of interior dust, this validation has not actually been experimentally confirmed. Factors that may affect in 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 bioaccessibility of Pb in house dusts have been reported, based on results from in vitro extraction assays that have not been validated for predicting in vivo bioavailability. Bioaccessibility assays that sequentially extract soil at gastric pH followed by intestinal pH tend to show higher bioaccessibility of soil and dust Pb when incubated at gastric conditions (Juhasz et al., 2011; Lu et al., 2011; Smith et al., 2011; Roussel et al., 2010; Yu et al., 2006). Yu et al. (2006) dissolved Pb dust, obtained from vacuuming carpet samples into simulated gastric and intestinal acids (also Section 4.1.3.2). The carpet samples were obtained from homes located in northern New Jersey. Pb concentration in carpet ranged from 209 to 1,770 mg/kg dust, with 52-77% of Pb dissolving in simulated gastric acid and 5-32% dissolving in simulated intestinal acids. In a similar test in the U.K., Turner and Simmonds (2006) observed median Pb dust concentrations of 178 mg/kg with approximately 80% bioaccessibility in simulated gastric acid. Jin et al. (2005) observed that bioaccessibility of Pb in soil was proportional to the soil acidity and organic matter content of the soil.

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 (Rabinowitz et al., 1976). The highest soft tissue concentrations in adults occur in liver and kidney cortex (Gerhardsson et al., 1995; Oldereid et al., 1993; Gerhardsson et al., 1986; Barry, 1975; Gross et al., 1975). Pb in blood (i.e., plasma) exchanges with both of these compartments.

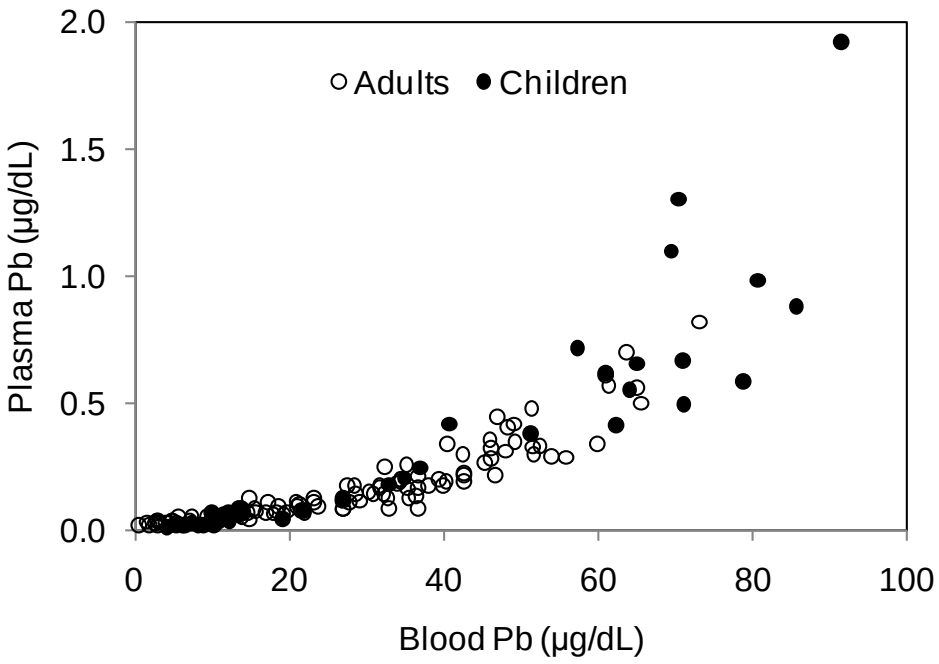
4.2.2.1 Blood

Blood comprises ~1% of total Pb body burden. Pb in blood is found primarily (>99%) in the RBCs (Smith et al., 2002; Manton et al., 2001; Bergdahl et al., 1999; Bergdahl et al., 1998; Hernandez-Avila et al., 1998; Bergdahl et al., 1997a; Schutz et al., 1996). δ-aminolevulinic acid dehydratase (ALAD) is the primary binding ligand for Pb in erythrocytes (Bergdahl et al., 1998; Xie et al., 1998; Bergdahl et al., 1997a; Sakai et al., 1982). Two other Pb-binding proteins have been identified in the RBC, a 45 kDa protein (K_{max} 700 µg/dL; K_d 5.5 µg/L) and a smaller protein(s) having a molecular weight <10 kDa (Bergdahl et al., 1998; Bergdahl et al., 1997a; Bergdahl et al., 1996). Of the three principal Pb-binding proteins identified in RBCs, ALAD has the strongest affinity

for Pb (Bergdahl et al., 1998) and appears to dominate the ligand distribution of Pb (35 to 84% of total erythrocyte Pb) at blood Pb levels below 40 µg/dL (Bergdahl et al., 1998; Bergdahl et al., 1996; Sakai et al., 1982). Pb binding to ALAD is saturable; the binding capacity was estimated to be ~850 µg/dL RBCs (or ~40 µg/dL whole blood) and the apparent dissociation constant has been estimated to be ~1.5 µg/L (Bergdahl et al., 1998). Binding to fetal hemoglobin within the RBC has a higher affinity for Pb than adult hemoglobin. This suggests that erythrocytes of neonates should be able to store more Pb than infants (Simon et al., 2007). Hematocrit is somewhat higher in the neonate at birth (51%) than in later infancy (35% at 6 months), which may lead to a decrease in the total binding capacity of blood over the first 6 months of life that results in a redistribution of Pb among other tissues (Simon et al., 2007).

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

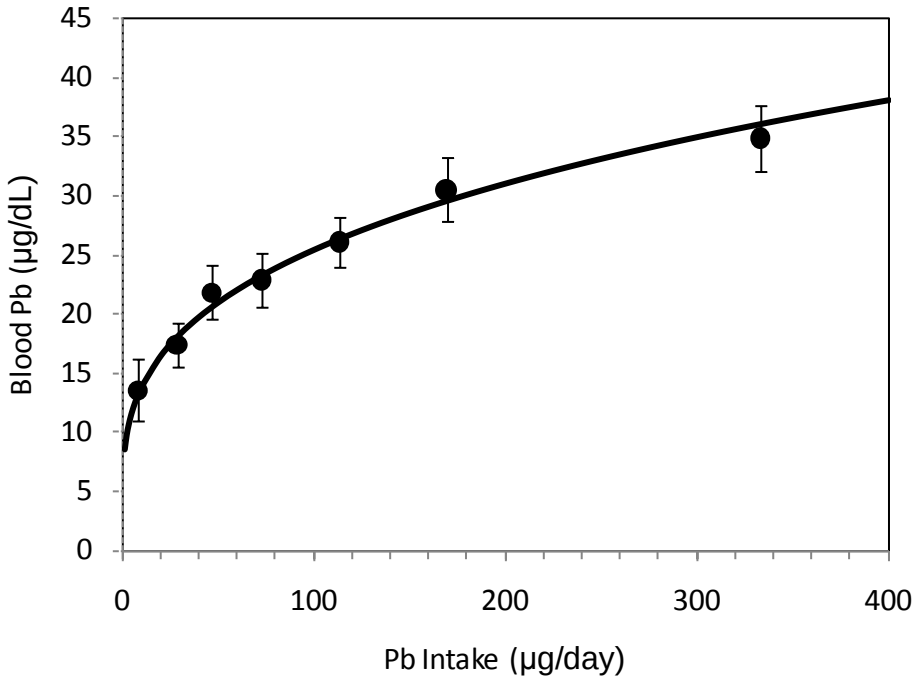
Typically, at blood Pb concentrations <100 µg/dL, only a small fraction (<1%) of blood Pb is found in plasma (Marcus, 1985; Manton and Cook, 1984; DeSilva, 1981). However, as previously noted, plasma Pb may be the more biologically labile and toxicologically active fraction of the circulating Pb. Approximately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



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

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

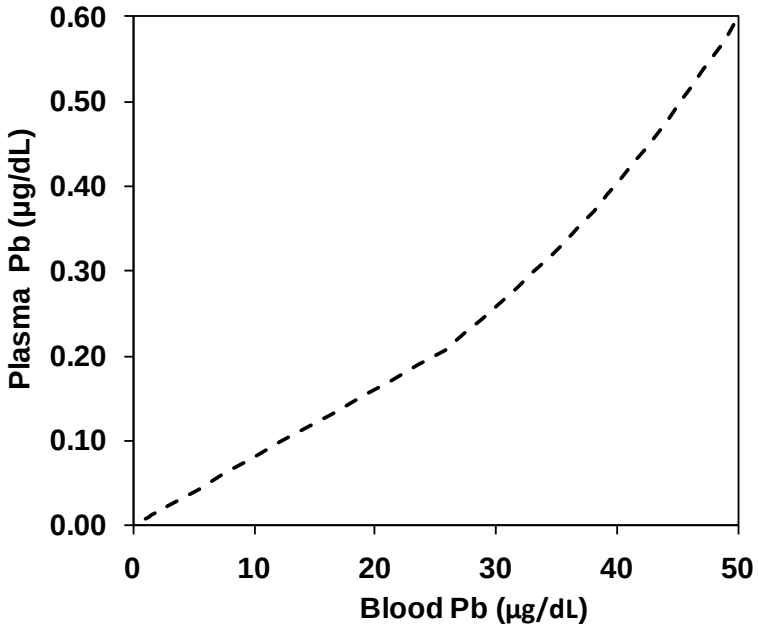
As shown in Figure 4-4, the limited binding capacity of Pb binding proteins in RBCs produces a curvilinear relationship between blood and plasma Pb concentration. The limited binding capacity of RBC binding proteins also confers, or at least contributes, to a curvilinear relationship between Pb intake and blood Pb concentration. A curvilinear relationship between Pb intake and blood Pb concentration has been observed in children (Sherlock and Quinn, 1986; Lacey et al., 1985; Ryu et al., 1983). As shown in Figure 4-5, 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.

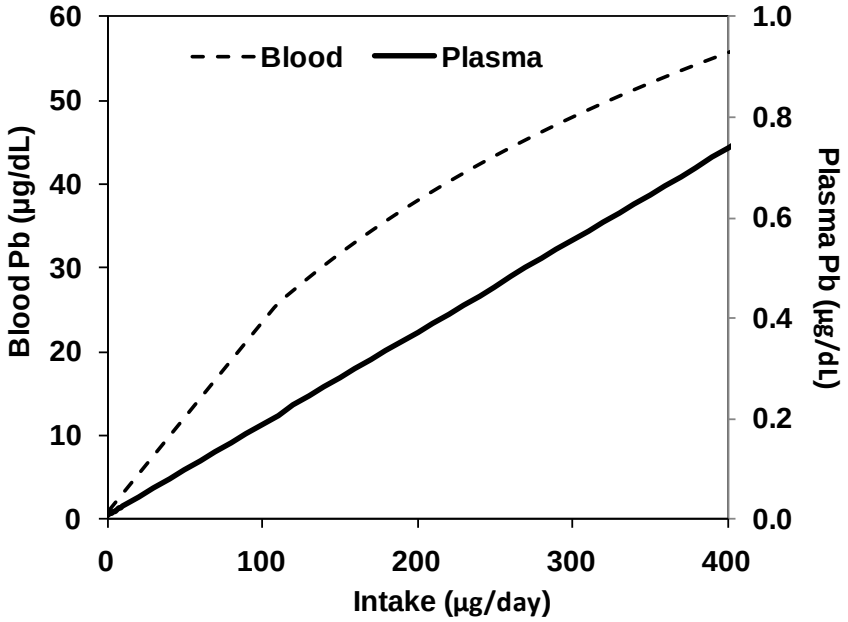


Source: Adapted with permission of Taylor & Francis Publishing, Sherlock and Quinn (1986).
Data represent mean and standard errors for intake; the line is the regression model (blood Pb = 3.9 + 2.43 (Pb intake [µg/week]$^{1/3}$).

Figure 4-5 Relationship between Pb intake and blood Pb concentration in infants (n = 105, age 13 weeks, formula-fed).

Figure 4-6 shows the predicted relationship between quasi-steady state blood and plasma Pb concentrations in a 4-year old child using the ICRP model (Pounds and Leggett, 1998; ICRP, 1994; Leggett, 1993), see Section 4.3 for a brief description of the ICRP model]. The abrupt inflection point that occurs at approximately 25 µg/dL blood Pb is an artifact of the numerical approach to simulate the saturation of binding using discontinuous first-order rate constants for uptake and exit of Pb from the RBC. A continuous function of binding sites and affinity, using empirical estimates of both parameters, yield a similar but continuous curvature in the relationship (Bergdahl et al., 1998; O'Flaherty, 1995). Nevertheless, either approach predicts a pseudo-linear relationship at blood Pb concentrations below approximately 25 µg/dL which, in this model, corresponds to an intake of approximately 100 µg/day (absorption rate ≈ 30 µg/day) (upper panel). An important consequence of the limited Pb binding capacity of RBC proteins is that the plasma Pb concentration will continue to grow at a linear rate above the saturation point for RBC protein binding. One implication of 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.





Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

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

Studies conducted in swine provide additional evidence in support of RBC binding kinetics influencing distribution of Pb to tissues. In these studies, the relationship between the ingested dose of Pb and tissue Pb concentrations (e.g., liver, kidney, bone) was linear, whereas, the relationship between dose and blood Pb was curvilinear with the

slope decreasing as the dose increased (Casteel et al., 2006). Saturable binding of Pb to RBC proteins also contributes to a curvilinear relationship between urinary Pb excretion and plasma Pb concentration (Section 4.2.3) (Besser et al., 2008; Bergdahl et al., 1997b).

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 cancellous). The proportion of cortical to trabecular bone in the human body varies by age, but on average is about 80 to 20 percent (O'Flaherty, 1998; Leggett, 1993; ICRP, 1973).

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

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

Pb accumulates in bone regions having the most active calcification at the time of exposure. Pb accumulation is thought to occur predominantly in trabecular bone during childhood and in both cortical and trabecular bone in adulthood (Aufderheide and Wittmers, 1992). Early Pb uptake in children is greater in trabecular bone due to its larger surface area and higher metabolic rate. With continued exposure, Pb concentrations in bone may increase with age throughout the lifetime beginning in childhood, indicative of a relatively slow turnover of Pb in adult bone (Park et al., 2009a; Barry and Connolly, 1981; Barry, 1975; Gross et al., 1975; Schroeder and Tipton, 1968). The cortical and

trabecular bones have distinct rates of turnover and Pb release. For example, tibia has a turnover rate of about 2% per year whereas trabecular bone has a turnover rate of more than 8% per year in adults (Rabinowitz, 1991).

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

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 (Gerhardsson et al., 1995; Oldereid et al., 1993; Gerhardsson et al., 1986; Barry, 1975; Gross et al., 1975). Presumably, the Pb in these soft tissues (i.e., kidney, liver, and brain) exists predominantly bound to protein. High affinity cytosolic Pb-binding proteins have been identified in rat kidney and brain (DuVal and Fowler, 1989; Fowler, 1989). The Pb-binding proteins in rat are cleavage products of α2μ globulin, a member of the protein superfamily known as retinol-binding proteins that are generally observed only in male rats (Fowler and DuVal, 1991). Other high-affinity Pb-binding proteins (K_d ~14 nM) have been isolated in human kidney, two of which have been identified as a 5 kDa peptide, thymosin 4 and a 9 kDa peptide, acyl-CoA binding protein (Smith et al., 1998). Pb also binds to metallothionein, but does not appear to be a significant inducer of the protein in comparison with the inducers Cd and Zn (Waalkes and Klaassen, 1985; Eaton et al., 1980).

The liver and kidneys rapidly accumulate systemic Pb ($t_{1/2}$=0.21 and 0.41 hours, respectively), which amounts to 10-15% and 15-20% of intravenously injected Pb, respectively (Leggett, 1993). A linear relationship in dose-tissue Pb concentrations for kidney and liver has been demonstrated in swine, dogs, and rats (Smith et al., 2008;

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

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. Group mean ratios range from about 0.7 to 1.0 at the time of delivery for mean maternal blood Pb levels ranging from 1.7 to 8.6 µg/dL (Amaral et al., 2010; Kordas et al., 2009; Patel and Prabhu, 2009; Carbone et al., 1998; Goyer, 1990; Graziano et al., 1990). In a study of mothers having blood Pb levels of <14 µg/dL, the ratio of cord blood Pb to maternal blood Pb decreased with decreasing maternal blood Pb (r=0.82) (Carbone et al., 1998). 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 (Gulson et al., 1999).

Transplacental transfer of Pb may be facilitated by an increase in the plasma/blood Pb concentration ratio during pregnancy (Montenegro et al., 2008; Lamadrid-Figueroa et al., 2006). Maternal-to-fetal transfer of Pb appears to be related partly to the mobilization of Pb from the maternal skeleton. Evidence for transfer of maternal bone Pb to the fetus has been provided by stable Pb isotope studies in cynomolgus monkeys exposed during pregnancy. Approximately 7-39% of the maternal Pb burden transferred to the fetus was derived from the maternal skeleton, with the remainder derived from contemporaneous exposure (O'Flaherty, 1998; Franklin et al., 1997).

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 Pb 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; Rabinowitz et al., 1976; Kehoe, 1961a, b, c). Absorbed Pb is excreted primarily in urine and feces, with sweat, saliva, hair, nails, and breast milk being minor routes of excretion (Kehoe, 1987; Chamberlain et al., 1978; Rabinowitz et al., 1976; Griffin et al., 1975; Hursh et al., 1969; Hursh and Suomela, 1968).

Approximately 30% of intravenously injected Pb in humans (40-50% in beagles and baboons) is excreted via urine and feces during the first 20 days following administration (Leggett, 1993). The kinetics of urinary excretion following a single dose of Pb is similar to that of blood (Chamberlain et al., 1978), likely due to the fact that Pb in urine derives largely from Pb in plasma. Evidence for this is the observation that urinary Pb excretion is strongly correlated with the rate of glomerular filtration of Pb (Araki et al., 1986) and plasma Pb concentration (Bergdahl et al., 1997b; Rentschler et al., In Press) (i.e., glomerular filtration rate × plasma Pb concentration), and both relationships are linear. While the relationship between urinary Pb excretion and plasma Pb concentration is linear, the plasma Pb relationship to blood Pb concentration is curvilinear (as described in Section 4.2.2.1 and demonstrated in Figure 4-6). This relationship contributes to an increase in the renal clearance of Pb from blood with increasing blood Pb concentrations (Chamberlain, 1983). Similarly, a linear relationship between plasma Pb concentration and urinary excretion rate predicts a linear relationship between Pb intake (at constant absorption fraction) and urinary Pb excretion rate, whereas the relationship with blood Pb concentration would be expected to be curvilinear (Section 4.3.7).

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; Manton and Cook, 1984; Manton and Malloy, 1983; Chamberlain et al., 1978), which corresponds to half-time for transfer of

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

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

Organic 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 exposed to tetraethyl Pb showed that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb (Vural and Duydu, 1995; Zhang et al., 1994; Turlakiewicz and Chmielnicka, 1985).

4.3 Lead Biomarkers

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

For any health endpoint of interest, the most useful biomarker of exposure is one that provides information about the Pb dose at the critical target organ and, moreover, reflects the exposure averaging time that is appropriate to the underlying pathogenetic processes

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

As an exposure biomarker, blood Pb concentration has other limitations. Only about 5% of an individual's total body Pb burden resides in blood. Furthermore, blood consists of several subcompartments. More than 90% of Pb in whole blood is bound to red cell proteins such as hemoglobin, with the balance in plasma. From a toxicological perspective, the unbound fraction is likely to be the most important subcompartment of blood Pb because it distributes into soft tissues. The concentration of Pb in plasma is much lower than in whole blood (<1%). The greater relative abundance of Pb in whole blood makes its measurement much easier (and more affordable) than measurement of Pb in plasma. The use of whole blood Pb as a surrogate for plasma Pb could be justified if the ratio of whole blood Pb to plasma Pb were well characterized, but this is not so. At least some studies suggest that it varies several-fold among individuals with the same blood Pb level. Moreover, binding Pb in red blood cells is limited, so the ratio of blood Pb to plasma Pb would be expected to be nonlinear. Thus, interpreting whole blood Pb level as a proxy for plasma Pb level, which, itself, is a proxy for brain Pb level, will result in some exposure misclassification.

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

The development of X-ray-fluorescence (XRF) methods for measuring Pb in mineralized tissues offers another approach for characterization and reconstruction of exposure history. Such tissues are long-term Pb storage sites, with a half-life measured in decades

and contain ~90% of the total body Pb burden in adults and 70% in children. Thus, bone Pb reflects a long exposure averaging time.

Mechanistic models are used throughout the section as a means to describe basic concepts that derive from the wealth of information on Pb toxicokinetics. Although predictions from models are inherently uncertain, models can serve to illustrate expected interrelationships between Pb intake and tissue distribution that are important in interpreting human clinical and epidemiologic studies. Thus, models serve as the only means 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.

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

Numerous mechanistic models of Pb biokinetics in humans have been proposed, and these are described in the 2006 Pb AQCD (U.S. EPA, 2006b) and in the supporting literature cited in that report. In this section, for simplicity and for internal consistency, we have limited the discussion to predictions from a single model, the ICRP Pb biokinetics model (Pounds and Leggett, 1998; ICRP, 1994; Leggett, 1993). The ICRP model consists of a systemic biokinetics model (Leggett, 1993) and a human respiratory tract model (ICRP, 1994). The Leggett model simulates age-dependent kinetics of tissue distribution and excretion of lead ingestion and inhalation intakes. This model was originally developed for the purpose of supporting radiation dosimetry predictions and it has been used to develop cancer risk coefficients for internal radiation exposures to lead and other alkaline earth elements that have biokinetics similar to those of calcium (ICRP, 1993). Although the ICRP model has not been validated by U.S. EPA as a regulatory model for lead risk assessment, it has been applied in Pb risk assessment (Abrahams et

al., 2006; Lorenzana et al., 2005; Khoury and Diamond, 2003). Portions of the model have been incorporated into an AALM that is being developed by EPA (2005a). In addition to the above considerations regarding previous applications of the ICRP model, the model was selected for use in the ISA because it has several useful features for predicting exposure-body burden relationships. The model simulates blood Pb and tissue Pb concentration dynamics associated with the uptake and elimination phases of exposures of ≥ 1 day in duration; and it simulates age-dependent and particle size-dependent deposition and clearance of inhaled lead in the respiratory tract. These types of simulations can only be approximated with the U.S. EPA IEUBK Model for lead in children because it simulates exposures in time steps of 1 year (i.e., age-year average exposures); lumps the simulation of deposition, mechanical clearance, and absorption of inhaled lead into a single absorption term representing the combined processes of gastrointestinal and respiratory tract absorption of inhaled lead; simulates steady state blood Pb concentrations and was does not allow access to the underlying simulations of tissue Pb concentrations which serve as intermediate variables in the model for predicting steady state blood Pb concentrations. Other models have been developed that allow simulations of tissue Pb concentrations (e.g., O'Flaherty, 1995; Leggett, 1993) and comparisons of these models have been previously described (Maddaloni et al., 2005).

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

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 for estimating trabecular bone Pb, because it has more bone mass and may afford better measurement precision than the calcaneus.

Bone Pb measurements are typically expressed in units of Pb/g bone mineral. This convention may potentially introduce variability into the bone Pb measurements related to variation in bone density. Typically, potential associations between bone density and bone Pb concentration are not evaluated in epidemiologic studies (Theppeang et al., 2008a; Hu et al., 2007a). An important consequence of expressing bone Pb measures relative to bone mineral content is that lower bone mineral density is associated with greater measurement uncertainty in bone Pb. This can have important implications for studies in older women for whom low bone mineral density is more common than in other populations including men and younger adults.

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

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

Since 1986, several investigators have reported refinements to hardware and software to improve the precision and accuracy of XRF measurements and there have been a number of investigations into the precision, accuracy and variability in XRF measurements [e.g., (Todd et al., 2002; Todd et al., 2001; Aro et al., 2000; Todd et al., 2000)]. Todd

et al. (2000) provided a detailed discussion of factors that influence the variability and measurement uncertainty, including repositioning, sample measurement duration, overlying tissue, operator expertise, detector resolution, and changes to measurement process over time. Some of these aspects were also discussed by Hu et al. (1995). From their cadaver and in vivo measurements, Todd et al. (2000) concluded that the uncertainty in an individual measurement was an underestimate of the standard deviation of replicate measurements, suggesting a methodological deficiency probably shared by most current ^{109}Cd-based K-shell XRF Pb measurement systems. In examining the reproducibility of the bone Pb measurements over a 4½ month period, Todd et al. found the average difference between the XRF results from short term and longer term measurements was 1.2 µg/g, indicating only a small amount of variability in the XRF results over a sustained period of time.

In the epidemiologic literature, XRF bone Pb data have typically been reported in two ways: one that involves a methodological approach to assessing the minimum detection limit and the other termed an epidemiologic approach by Rosen and Pounds (1998). In the former approach, a minimum detection limit is defined using various methods, including two or three times the square root of the background counts; one, two, or three times the SD of the background; or two times the observed median error. This approach relies upon the minimum detection limit to define a quantitative estimate that is of sufficient precision to be included in the statistical analysis, as demonstrated by Bellinger et al. (1994a), Gerhardsson et al. (1993), and Christoffersson et al. (1986).

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

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 and Smith, 1995). Limits of detection for Pb using AAS are on the order of 5-10 µg/dL for flame AAS measurements

and approximately 0.1 µg/dL for flameless AAS measurements (Flegal and Smith, 1995; NIOSH, 1994). A detection limit of 0.005 µg/dL has been achieved for Pb in blood samples analyzed by GFAAS.

For measurement of Pb in plasma, ICP-MS provides sufficient sensitivity (Schutz et al., 1996). While the technique has been applied to assessing Pb exposures in adults, ICP-MS has not received widespread use in epidemiologic studies.

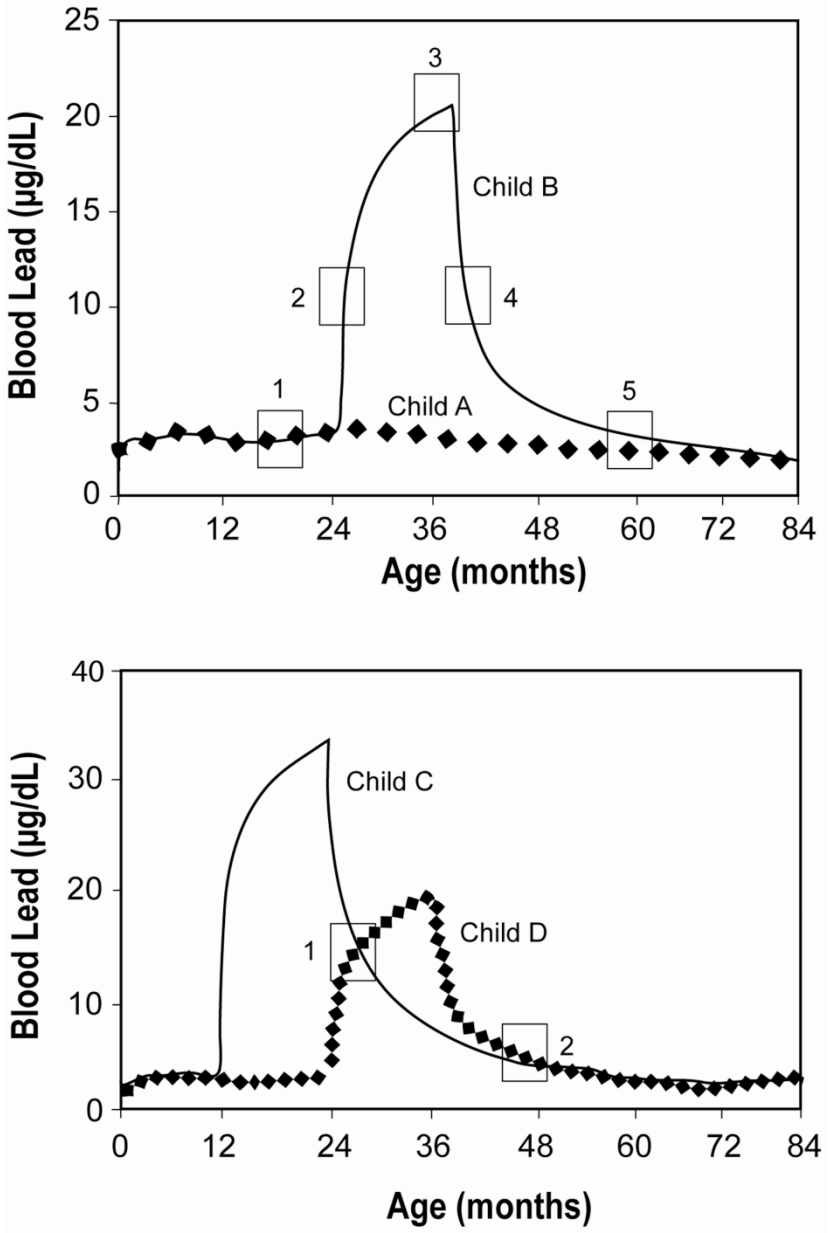
The primary binding ligand for Pb in RBC, ALAD, is encoded by a single gene in humans that is polymorphic in two alleles (ALAD1 and ALAD2) (Scinicariello et al., 2007). Since the ALAD1 and ALAD 2 alleles can be co-dominantly expressed, 3 different genotypes (ALAD 1-1, ALAD 1-2, and ALAD 2-2) are possible.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

The 2006 Pb AQCD 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 (Kim et al., 2004; Pérez-Bravo et al., 2004; Bergdahl et al., 1997b; Smith et al., 1995a; Wetmur, 1994; Wetmur et al., 1991a; Astrin et al., 1987). More recent meta analyses provide further support for ALAD2 carriers having higher blood Pb levels than ALAD1-1 homozygotes (Scinicariello et al., 2007; Zhao et al., 2007). The mechanism for this association may be higher Pb binding affinity of ALAD2. Although, this interpretation would be consistent with the structural differences that result in greater electronegativity of ALAD1 compared to ALAD2 (Wetmur, 1994; Wetmur et al., 1991b), measurements of Pb binding affinity to ALAD1 and ALAD2 (i.e., Pb^{2+} displacement of Zn^{2+} binding to recombinant ALAD1 and ALAD2) have not revealed differences in Pb binding affinity (Jaffe et al., 2000). In a meta-analysis of 24 studies, Scinicariello et al. (2007), observed the greatest differences for ALAD2 compared to ALAD1 in highly exposed adults with little difference among environmentally-exposed adults; large differences were also observed for children at low exposures. However, there are few studies that evaluated children and the largest study contributing to the meta analysis may have been influenced by selection bias (Scinicariello et al., 2007). Individual studies find similar results in occupationally-exposed adults, with blood Pb levels being higher in individuals with ALAD2 alleles (Miyaki et al., 2009; Shaik and Jamil, 2009). A subsequent meta analysis of adult data from NHANES III did not find any differences in blood Pb level between all carriers of either the ALAD 1-1 or ALAD 1-2/2-2 allele (Scinicariello et al., 2010). Other studies provide further support for no blood Pb differences among ALAD1 and ALAD2 carriers (Sobin et al., 2009; Rabstein et

al., 2008; Montenegro et al., 2006; Wananukul et al., 2006) or lower blood Pb levels for individuals with ALAD 1-2/2-2 (Krieg et al., 2009; Chia et al., 2006).

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

Analyses of serial blood Pb concentrations measured in longitudinal epidemiologic studies found relatively strong correlations (e.g., r = 0.5-0.8) between individual blood Pb concentrations measured after 6-12 months of age (Schnaas et al., 2000; Dietrich et al., 1993b; McMichael et al., 1988; Otto et al., 1985a; Rabinowitz et al., 1984). These observations suggest that, in general, exposure characteristics of an individual child (e.g., exposure levels and/or exposure behaviors) tend to be relatively constant across age. However, a single blood Pb measurement may not distinguish between a history of long-term lower-level Pb exposure from a history that includes higher acute exposures (Mushak, 1998). This concept is illustrated in Figure 4-7. Two hypothetical children are simulated. Child A has a relatively constant Pb intake from birth, whereas Child B has the same Pb intake as Child A for the first two years of life, then a 1-year elevated intake beginning at age 24 months (Figure 4-7, upper panel) that returns to the same intake as Child A at 36 months. The absorption fraction is assumed to be the same for both children. Blood Pb samples 1 and 5 for Child A and B, or 2 and 4 for Child B, will yield similar blood Pb concentrations (~3 or 10 µg/dL, respectively), yet the exposure contexts for these samples are very different. Two samples (e.g., 1 and 2, or 4 and 5), at a minimum, are needed to ascertain if the blood Pb concentration is changing over time. The rate of change can provide information about the magnitude of change in exposure, but not necessarily about the time history of the change (Figure 4-7, lower panel). Time-integrated measurements of Pb concentration may provide a means for accounting for some of these factors and, thereby, provide a better measure of long-term Pb exposure.



Note: Child A and Child B 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).

Figure 4-7 Simulation of temporal relationships between Pb exposure and blood Pb concentration in children.

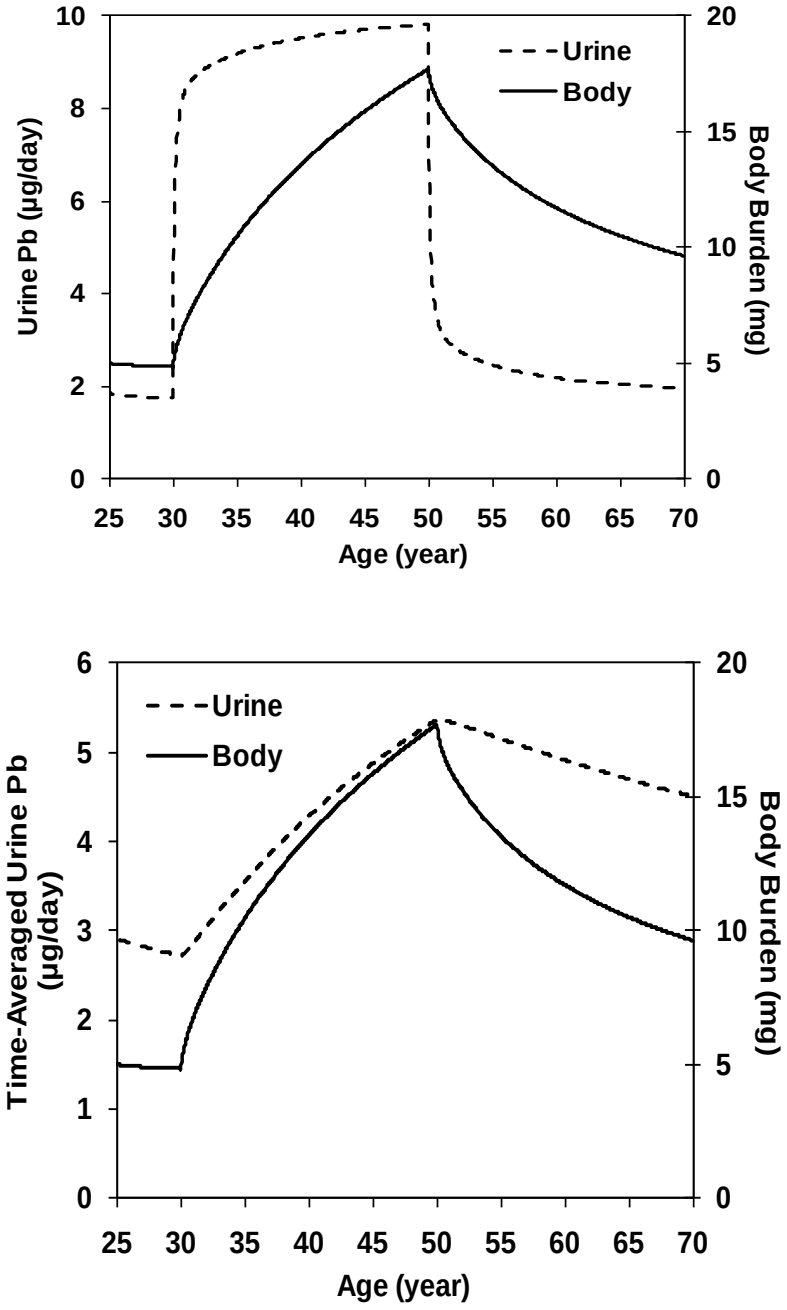
4.3.3 Urine Lead Measurements

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 (Fukui et al., 1999; Araki et al., 1990). Urine flow rate can vary by a factor or more than 10, depending on the state of hydration and other factors that affect glomerular filtration rate and renal tubular reabsorption of the glomerular filtrate. All of these factors can be affected by Pb exposure at levels that produce nephrotoxicity (i.e., decreased glomerular filtration rate, impaired renal tubular transport function). Therefore, urine Pb concentration measurements provide little reliable information about exposure (or Pb body burden), unless they can be adjusted to account for unmeasured variability in urine flow rate (Araki et al., 1990).

Urinary Pb concentration reflects, mainly, the concentration of Pb in the blood. As such, urinary concentrations by reflect both recent and past exposures to Pb (see Section 4.3.5). A single urinary Pb measurement cannot distinguish between a long-term low level of exposure or a higher acute exposure. Urinary Pb measurements would be expected to correlate with concurrent blood Pb (see Section 4.3.6 for additional discussion of the relationship between blood and urine Pb). Chiang et al. (2008) reported a significant, but relatively weak correlation between urinary Pb levels (µg/dg creatinine) and individual Pb intakes (µg/day) estimated in a group of 10- to 12-year-old children (β: 0.053, R = 0.320, p = 0.02, n = 57). A contributing factor to the relatively weak correlation may have been the temporal displacement between the urine sampling and measurements used to estimate intake, which may have been as long as 6 months for some children.

Thus, a single urine Pb measurement, or a series of measurements taken over short-time span, is likely a relatively poor index of Pb body burden for the same reasons that blood Pb is not a good indicator of body burden. On the other hand, long-term average measurements of urinary Pb can be expected to be a better index of body burden (Figure 4-8).



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

Figure 4-8 Simulation of relationship between urinary Pb excretion and body burden in adults.

4.3.4 Lead in Other 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, 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 (Gulson et al., 1997; Rabinowitz et al., 1993). Limited studies have demonstrated moderate-to-high correlations between tooth Pb levels and blood Pb levels (Rabinowitz, 1995; Rabinowitz et al., 1989).

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

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

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 Pb AQCD (U.S. EPA, 2006b). Earlier reports suggested a relatively strong correlation between salivary Pb concentration and blood Pb concentration (Omokhodion and Crockford, 1991; Brodeur et al., 1983; P'an, 1981); however, more recent assessments have shown relatively weak or inconsistent associations (2011; 2010; Costa de Almeida et al., 2009; Barbosa et al., 2006c; 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. (2006c) 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-aminolevulinic acid (δ-ALA) levels was recognized decades ago as having potential use as a biomarker of Pb exposure (Mitchell et al., 1977; Hernberg et al., 1970). More recently reference values for blood ALAD activity ratio (the ratio of ALAD activity in the blood sample to that measured after fully activating the enzyme in the sample) have been reported (Gultepe et al., 2009). Inhibition of erythrocyte ALAD by Pb results in a rise in the plasma concentration of the ALAD substrate δ-ALA. The δ-ALA biomarker can be measured in serum and has been used as a surrogate for Pb measurements in studies in which whole blood samples or adequately prepared plasma or serum samples were not available for Pb measurements (Opler et al., 2008; Opler et al., 2004).

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; Rabinowitz et al., 1976). Studies of a limited number of adults (four individuals with hip or knee replacement, a married couple, and 10 female Australian immigrants) in which the Pb exposure was from historical environmental sources have found that bone Pb stores can contribute 40-70% to blood Pb (Smith et al., 1996; Gulson et al., 1995; Manton, 1985). 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 are dependent on age and intensity of exposure. Bone compartments are much more labile in infants and children than in adults as reflected by half-times for movement of Pb from bone into the plasma (e.g., cortical $t_{1/2}$ = 0.23 years at birth, 1.2 years at 5 years of age, 3.7 years at 15 years of age, and 23 years in adults; trabecular $t_{1/2}$ = 0.23 years at

birth, 1.0 years at 5 years of age, 2.0 years at 15 years of age, and 3.9 years in adults) (Leggett, 1993). Slow transfer rates for the movement of Pb from nonexchangeable bone pools to the plasma are the dominant transfer process determining long-term accumulation and elimination of bone Pb burden.

The apparent slow clearance of Pb from the blood over months and years following the cessation or reduction in exposures reflects elimination of Pb stores in bone. Longer fast-phase elimination half-times (60-120 days) were reported for five adults with lead poisoning following roughly a month to 12 years of exposure and relatively high blood Pb concentrations of 70-110 µg/dL (Rentschler et al., In Press). A slower phase becomes evident with longer observation periods following a decrease in exposure. Nilsson et al. (1991) reported in tri-exponential decay in the blood Pb concentrations of 14 individuals having a median occupational exposure period of 26 years. Representing 22% of blood Pb, the fast compartment had a clearance half time of 34 day. The intermediate compartment, 27% of blood Pb, had a clearance half time of 1.12 year. And, the slow compartment, 50% of blood Pb, had a clearance half time of 13 years. The authors attributed the fast, intermediate, and slow compartment clearance to elimination of Pb from blood and some soft tissues, from trabecular bone, and cortical bone, respectively. Children who have been removed from a relatively brief exposure to elevated environmental Pb also exhibit faster slow-phase kinetics than children removed from exposures that lasted several years, with half-times of 10 and 20-38 months, respectively (Manton et al., 2000). Rothenberg et al. (1998) also showed that exposures in the first 6 months of life could contribute to elevated blood lead through at least 3 years relative to children with lower early life exposures, despite similar environmental exposures at later time points. In both adults and children, the longer half-times measured under the latter conditions reflect the contribution of bone Pb stores to blood Pb following a change in exposure.

The longer half-life of Pb in bone compared to blood Pb, allows a more cumulative measure of 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; Smith et al., 1996; Kehoe, 1987; O'Flaherty et al., 1982), even for exposures that occurred during childhood (McNeill et al., 2000). The more widespread use of in vivo XRF Pb measurements in bone and indirect measurements of bone processes with stable Pb isotopes have enhanced the use of bone Pb as a biomarker of Pb body burden.

Several studies have found a stronger relationship between patella Pb and blood Pb than tibia Pb and blood Pb (Park et al., 2009a; Hu et al., 1998; Hernandez-Avila et al., 1996; Hu et al., 1996b). Hu et al. (1998) suggest that trabecular bone is the predominant bone type providing Pb back into circulation under steady-state and pathologic conditions. The

stronger relationship between blood Pb and trabecular Pb compared with cortical bone is probably associated with the larger surface area of trabecular bone allowing for more Pb to bind via ion exchange mechanisms and more rapid turnover making it more sensitive to changing patterns of exposure.

4.3.5.1 Children

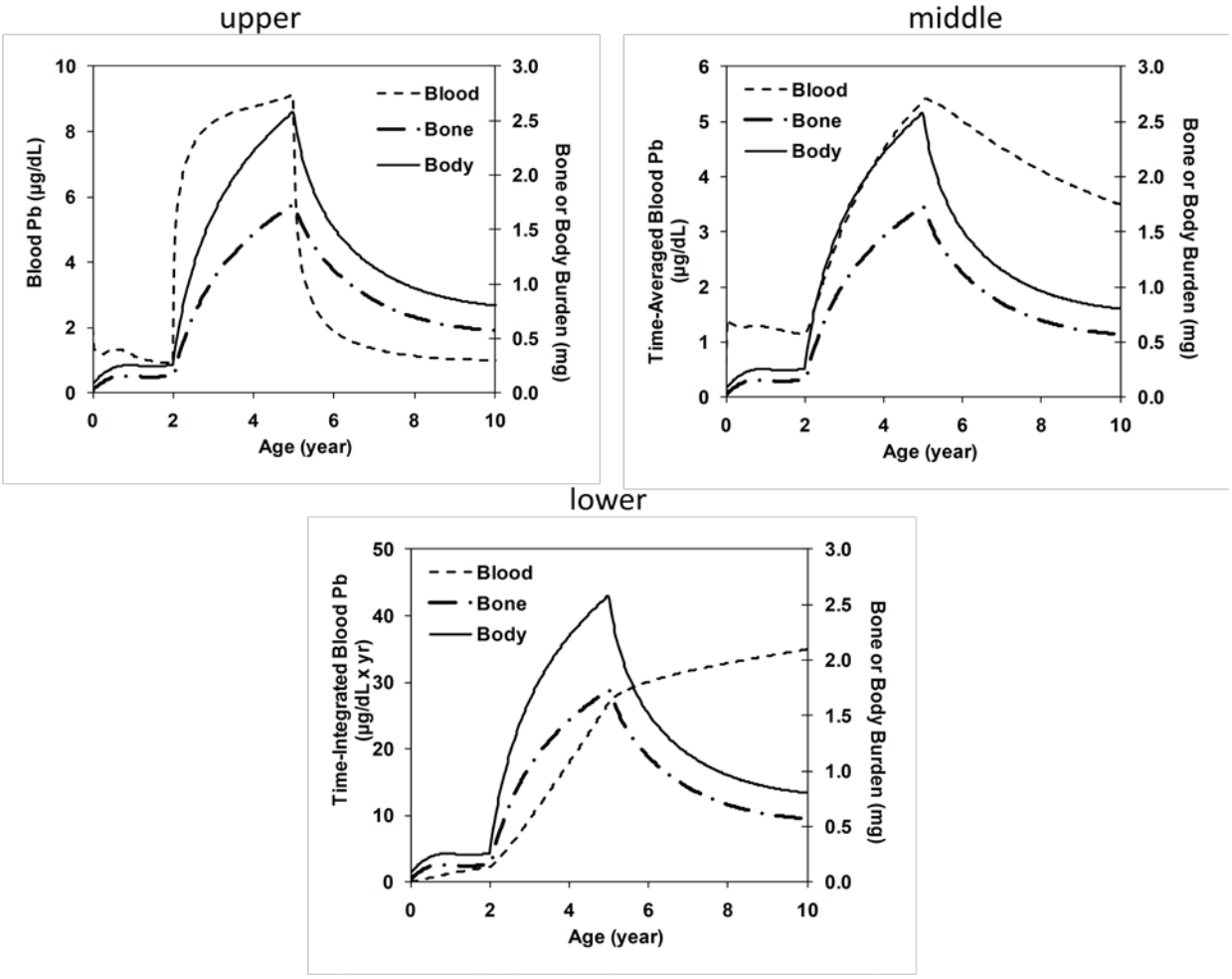
As mentioned in Section 4.2.2.2, bone growth in children will contribute to accumulation of Pb in bone, which will comprise most of the Pb body burden. As a result, Pb in bone will more closely reflect Pb body burden than blood Pb. However, changes in blood Pb concentration in children (i.e., associated with changing exposures) are thought to more closely parallel changes in total body burden. Figure 4-9 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 into the bone mineral matrix.

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

Two alternative blood Pb metrics depicted in Figure 4-9 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

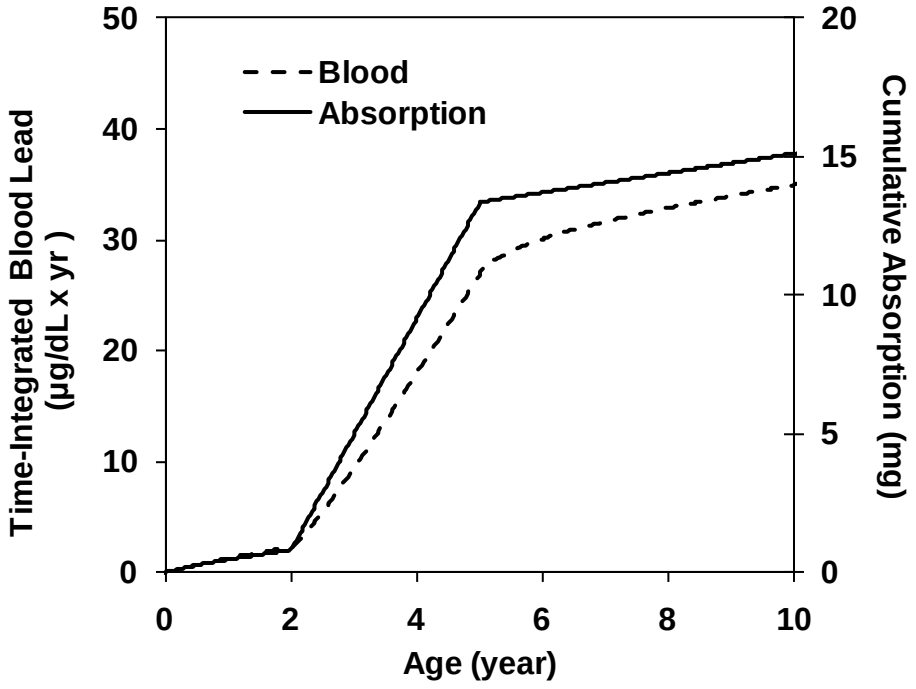
(conceptually identical to cumulative blood lead index [CBLI] used in epidemiologic studies) is a cumulative function and increases throughout childhood; however, the slope of the increase is higher during the exposure event than prior to or following the event. Following cessation of exposure, the time-integrated blood Pb and body burden diverge. This result is expected, as the time-integrated blood Pb curve is a cumulative function which cannot decrease over time and bone Pb levels will decrease with 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 4-10). This pattern is similar for adults.



Note: Blood Pb concentration is thought to parallel body burden more closely in children than in adults, due to more rapid turnover of bone and bone-Pb stores in children (upper panel). 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 blood Pb concentration and body burden in children, with a constant Pb intake from age 2 to 5.



Note: 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).

Figure 4-10 Simulation of relationship between time-integrated blood Pb concentration and cumulative Pb absorption in children.

4.3.5.2 Adults

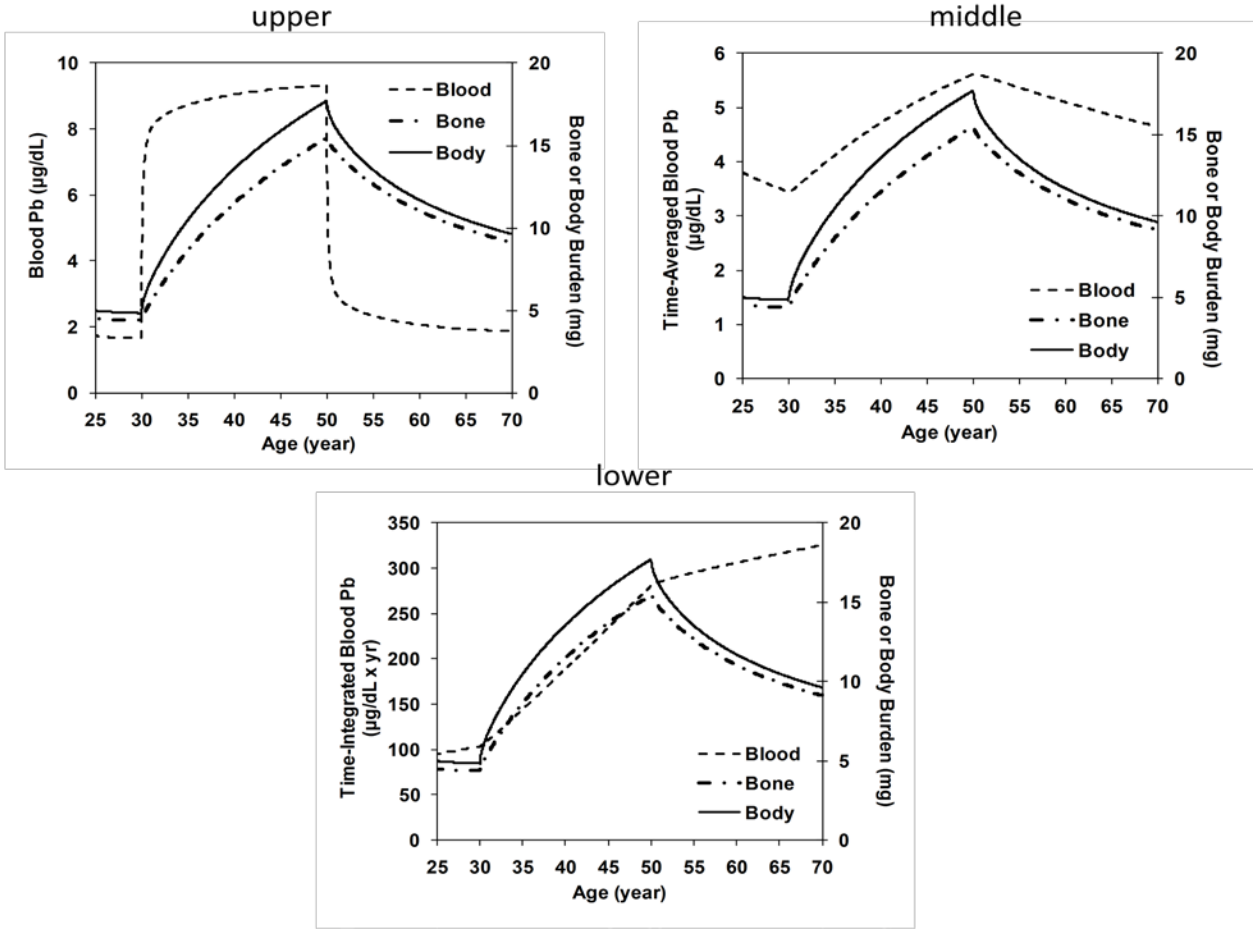
In adults, where a relatively large fraction of the body burden residing in bone has a slower turnover compared to blood, a constant Pb uptake (or constant intake and fractional absorption) gives rise to a quasi-steady state blood Pb concentration, while the body burden continues to increase over a much longer period, largely as a consequence of continued accumulation of Pb in bone. This pattern is illustrated in Figure 4-11 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 4-11 reveals that the accumulation and elimination phases of blood Pb kinetics are not

symmetrical; elimination is slower than accumulation as a result of the gradual release of bone Pb stores to blood. This response, known as the prolonged terminal elimination phase of Pb from blood, has been observed in retired Pb workers and in workers who continued to work after improved industrial hygiene standards reduced their exposures. In the adult simulation shown in Figure 4-11, 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 (Wilker et al., 2011; 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. These simulations in Figure 4-11 illustrate how a single blood Pb concentration measurement, or a series of measurements taken over a short-time span, could be a relatively poor index of Pb body burden.

One important potential implication of the profoundly different kinetics of Pb in blood and bone is that, for a constant Pb exposure, Pb in bone will increase with increasing duration of exposure and, therefore, with age. In contrast, blood Pb concentration will achieve a quasi-steady state. As a result, the relationship between blood Pb and bone Pb will diverge with increasing exposure duration and age. This divergence can impart different degrees of age-confounding when either blood Pb or bone Pb is used as an internal dose metric in dose-response models. In a review of epidemiologic studies that evaluated the associations between blood Pb, bone Pb and cognitive function, the 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).

The aforementioned expectation for an increase in bone Pb and body burden with age applies to scenarios of constant exposure but not necessarily to real world populations in which individual and population exposures have changed over time. Longitudinal studies of blood and bone Pb trends have not always found strong dependence on age (Nie et al., 2009; Kim et al., 1997). Kim et al. (1997) found that bone Pb levels increased with increasing age in elderly adults (age 52-83) years), only when the data were analyzed cross-sectionally. When analyzed longitudinally, the trend for individual patella Pb was a 23% decrease over a 3-year period (approximate $t_{1/2}$ of 8 years), whereas tibia Pb levels

did not change with over the same period. Therefore, although older individuals tended to have higher bone Pb levels, the 3-year temporal trend for individuals was a loss of Pb from the more labile Pb stores in trabecular bone. Nie et al. (2011) observed that longitudinal observations of blood and bone Pb in elderly adults did not show a significant age effect on the association between blood Pb and bone Pb (patella and tibia), when the sample population (n=776) was stratified into age tertiles (mean age 62, 69 or 77 years). Nie et al. (2009) did find that regressed function bone Pb and appeared to level off at bone Pb levels >20 µg/g bone mineral.



Note: A constant baseline intake 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).

Figure 4-11 Simulation of relationship between blood Pb concentration, bone Pb and body burden in adults.

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

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

Mobilization of Lead from Bone in Adulthood

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

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

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

Gulson et al. (2004a) observed that calcium supplementation was found to delay increased mobilization of Pb from bone during pregnancy and halved the flux of Pb release from bone during late pregnancy and postpartum. In another study, women whose daily 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 (1,200 mg/day) in pregnant Mexican women resulted in an 11% reduction in blood Pb level compared to placebo and a 24% average reduction for the most compliant women (Ettinger et al., 2009). When considering baseline blood Pb levels in women who were more compliant in taking calcium supplementation, the reductions were similar for those <5 µg/dL and those ≥ 5 µg/dL (14% and 17%, respectively). This result is in contrast to a study of women who had blood Pb concentrations <5 µg/dL, where calcium supplementation had no effect on blood Pb concentrations (Gulson et al., 2006b). These investigators attributed their results to changes in bone resorption with decoupling of trabecular and cortical bone sites.

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

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

Blood Pb levels increase during lactation due to alterations in the endogenous bone Pb release rate. After adjusting for patella Pb concentration, an increase in blood Pb levels of 12.7% (95% CI: 6.2, 19.6) was observed for women who practiced partial lactation and an increase of 18.6% (95% CI: 7.1, 31.4) for women who practiced exclusive lactation compared to those who stopped lactation (Tellez-Rojo et al., 2002). In another Mexico City study, Ettinger et al. (2006; 2004b) concluded that an interquartile increase in patella Pb was associated with a 14% increase in breast milk Pb, whereas for tibia Pb the increase was ~5%. Breast milk:maternal blood Pb concentration ratios are generally <0.1, although values of 0.9 have been reported (Koyashiki et al., 2010; Ettinger et al., 2006; Gulson et al., 1998b). 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).

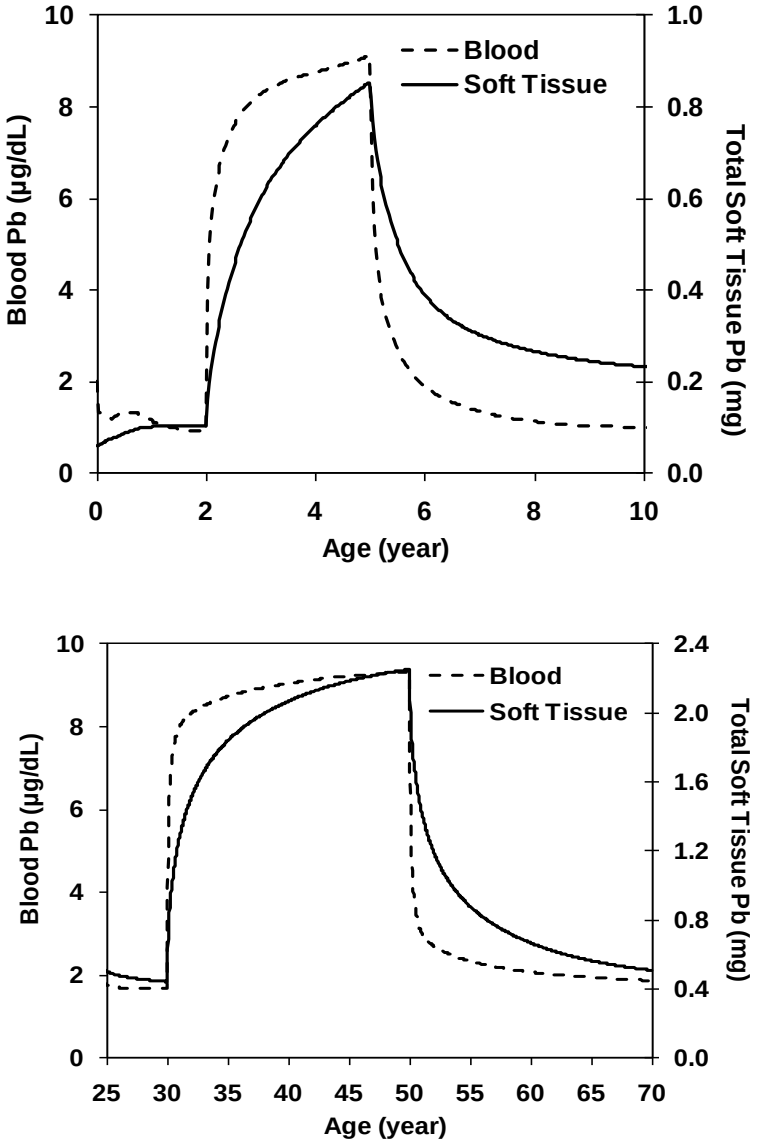
The Pb content in some bones (i.e., mid femur and pelvic bone) plateau at middle age and then decreases at older ages (Drasch et al., 1987). This decrease is most pronounced in females and may be due to osteoporosis and release of Pb from resorbed bone to blood (Gulson et al., 2002). Two studies indicate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

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

et al., 2003; Korrick et al., 2002; Webber et al., 1995). In women with severe weight loss (28% of BMI in 6 months) sufficient to increase bone turnover, increased blood Pb levels of approximately 2.1 µg/dL (250%) were reported, and these blood Pb increases were associated with biomarkers of increased bone turnover (e.g., urinary pyridinoline cross-links) (Riedt et al., 2009).

4.3.6 Relationship between Lead in Blood and Lead in Soft Tissues

Figure 4-12 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.

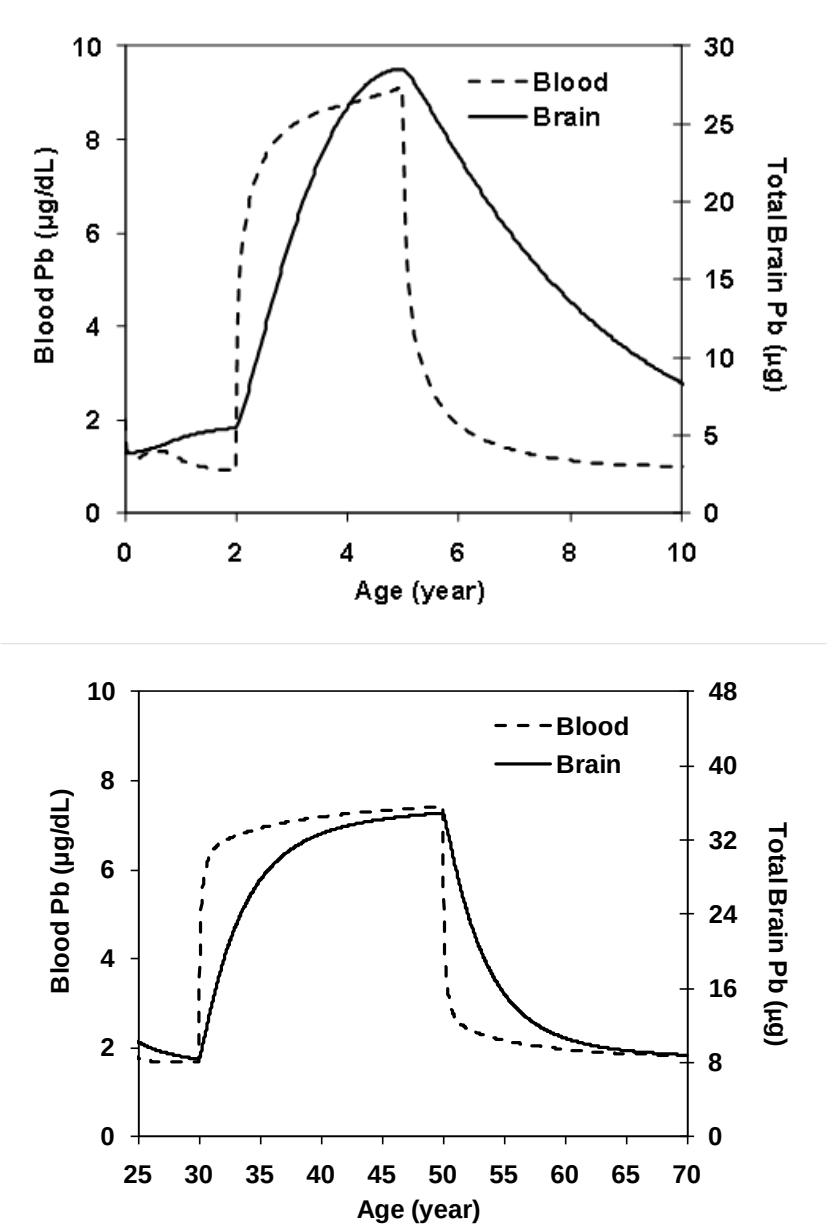


Note: Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

Figure 4-12 Simulation of blood and soft tissue (including brain) Pb in children and adults who experience a period of increased Pb intake.

Information on Pb levels in human brain are limited to autopsy data and the simulation of brain Pb shown in Figure 4-13 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 4-13. Although in this model, brain Pb to blood Pb transfer half-times are assumed to be the same in children and adults, uptake kinetics are assumed to be faster during infancy and childhood, which achieves a higher fraction of the soft tissue burden in brain, consistent with higher brain/body mass relationships. The uptake half times predicted by Leggett (1993) vary from 0.9 to 3.7 days, depending on age. Brain Pb kinetics represented in the simulations are simple outcomes of modeling assumptions and cannot currently be verified with available observations in humans.

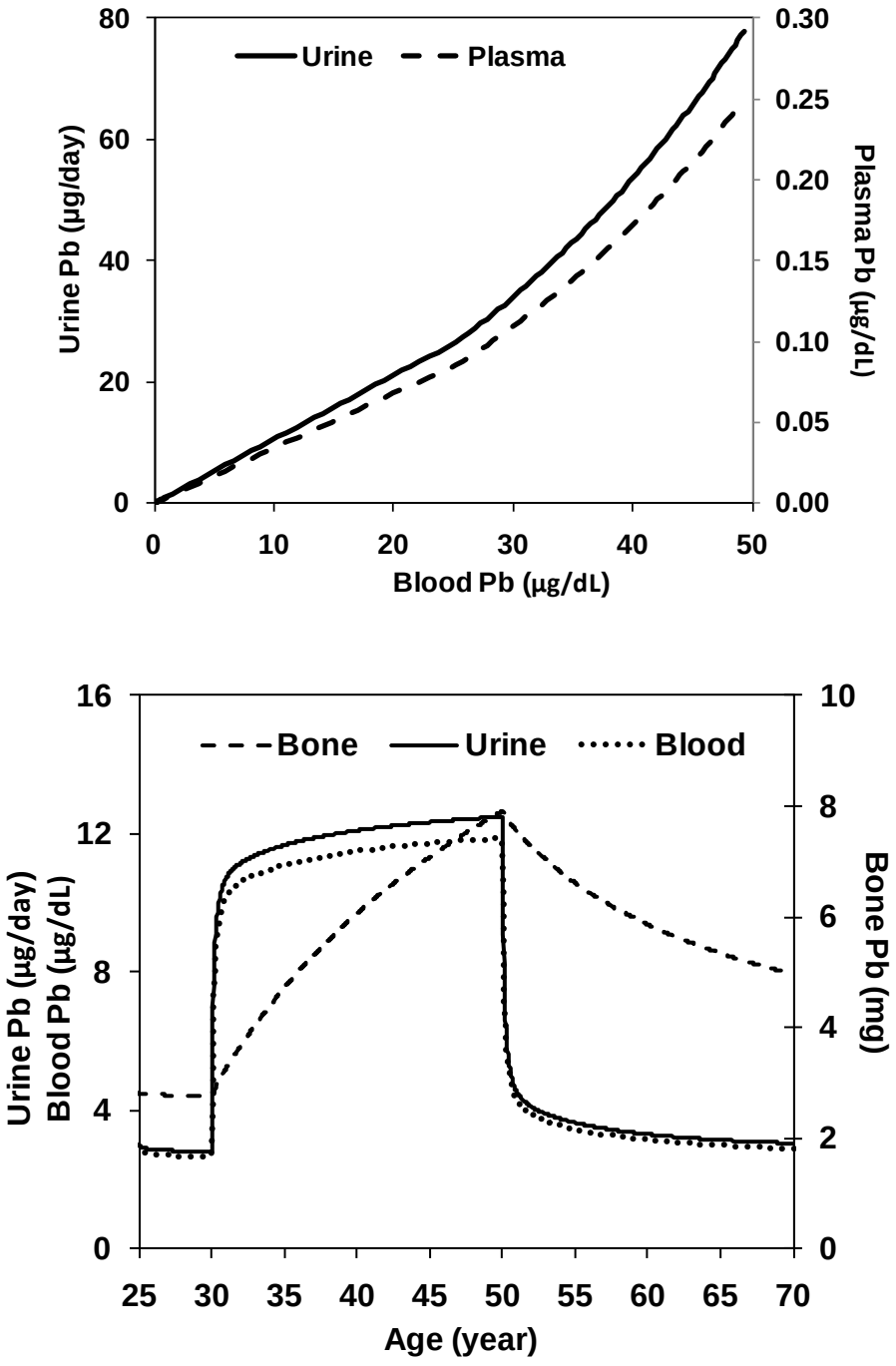


Note: Simulation based on ICRP Pb biokinetics model (Leggett, 1993).

Figure 4-13 Simulation of blood and brain Pb in children and adults who experience a period of increased Pb intake.

Urinary filtering and excretion of Pb is associated with plasma Pb concentrations. Given the curvilinear relationship between blood Pb and plasma Pb, a secondary expectation is for a curvilinear relationship between blood Pb and urinary Pb excretion that may become evident only at relatively high blood Pb concentrations (e.g., >25 µg/dL). Figure 4-14 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 lower blood Pb concentrations (<25 µg/dL), urinary Pb excretion is predicted to closely parallel plasma Pb concentration for any given blood Pb level (Figure 4-14, top panel). It follows from this that, similar to blood Pb, urinary Pb will respond much more rapidly to an abrupt change in Pb exposure than will bone Pb. One important implication of this relationship is that, as described previously for blood Pb, the relationships between urinary Pb and bone Pb will diverge with increasing exposure duration and age, even if exposure remains constant. Furthermore, following an abrupt cessation of exposure, urine Pb will quickly decrease while bone Pb will remain elevated (Figure 4-14, lower panel).



Note: 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).

Figure 4-14 Relationship between Pb in urine and Pb in blood.

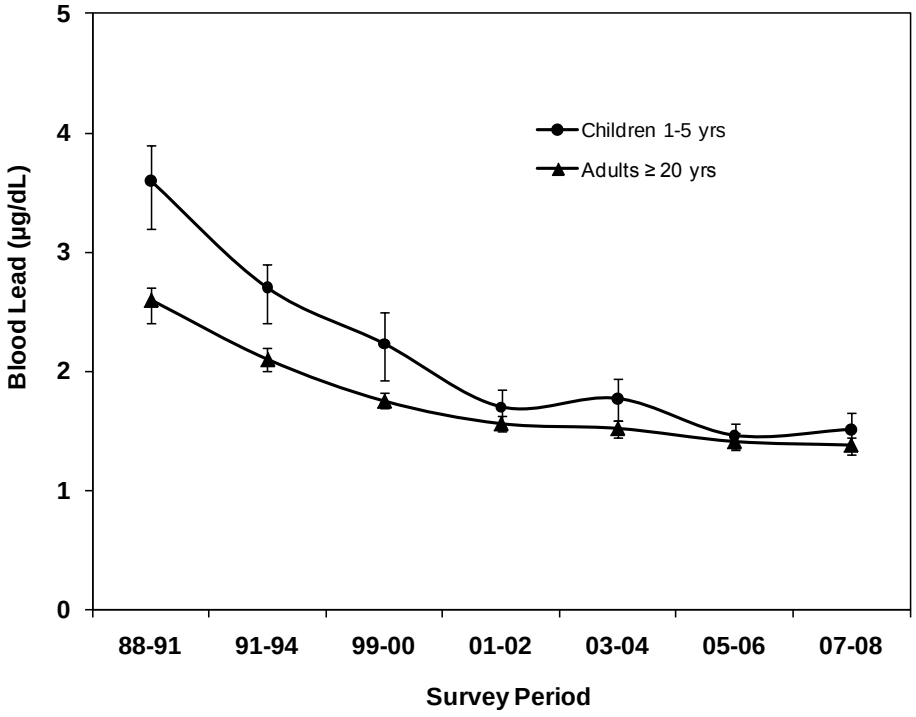
4.4 Observational Studies of Lead Biomarker Levels

4.4.1 Lead in Blood

Overall, trends in blood Pb levels have been decreasing among U.S. residents over the past 20 years. Blood Pb concentrations in the U.S. general population have been monitored in the NHANES. Analyses of these data show a progressive downward trend in blood Pb concentrations during the period 1976-2008, with the most dramatic declines coincident with the phase out of leaded gasoline (Pirkle et al., 1998; Brody et al., 1994; Pirkle et al., 1994; Schwartz and Pitcher, 1989). The temporal trend for the period 1988-2008 is shown in Figure 4-15. 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 4-16 uses NHANES data to illustrate temporal trends in 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 in 2007-2008 (NCHS, 2010). For 2005-2008, 95% of childhood blood Pb levels were less than 5 µg/dL. The geometric mean blood Pb concentration among adults ≥ 20 years of age was 1.38 µg/dL (95% CI: 1.31, 1.46) for the sample collected during the period 2007-2008 (CDC, 2011). Based on these same data, the geometric mean for all males (aged ≥ 1 y) was 1.47 µg/dL (95% CI: 1.39, 1.56), and for females (aged ≥ 1 y) was 1.11 µg/dL (95% CI: 1.06, 1.16).

There has been a steep decline in mean blood Pb levels from 1975 through 2010 among all birth cohorts from 1975 to 2010; Figure 4-17. For all cohorts, blood Pb generally decreases with age during childhood until adolescence; following adolescence (in the early 20s), blood Pb generally levels off or even increases with age. It is possible that bone growth in young people and occupational exposure for adults influences the shape of these curves. For the 1960 to 1970 birth cohort, the mean blood Pb is the highest of the cohorts in the 1970s, but beginning in 1993 the mean blood Pb is one of the lowest of the cohorts. This interaction between time and cohort may be due to the faster release of Pb from bone in younger people (Rabinowitz, 1991). This interaction is also apparent for some of the other more recently born cohorts. In comparison, the slopes of blood Pb over time are nearly parallel among the cohorts born before 1930. This suggests that the time-cohort-interaction diminishes among older people. Also, the leveling of the blood Pb in the 2000s could be due to aging of the birth cohort and consequent slowing of their Pb release from bone.

When race/ethnicity groups were compared for years 1999-2004, blood Pb levels in children were highest in the ethnicity category non-Hispanic black (GM 2.8, 95% CI: 2.5, 3.0) compared to the categories Mexican-American (GM 1.9, 95% CI: 1.7, 2.0) and non-Hispanic white (GM 1.7, 95% CI: 1.6, 1.8) (Jones et al., 2009a). Figure 4-18 demonstrates the change in percent of children (aged 1-5 years) with various blood Pb levels by race/ethnicity between the survey during 1988-1991 and that during 1999-2004. When these data for children aged 1-5 years were aggregated for all survey years from 1988 to 2004, residence in older housing, poverty, age, and being non-Hispanic black were significant predictors of higher Pb levels (Jones et al., 2009a).



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

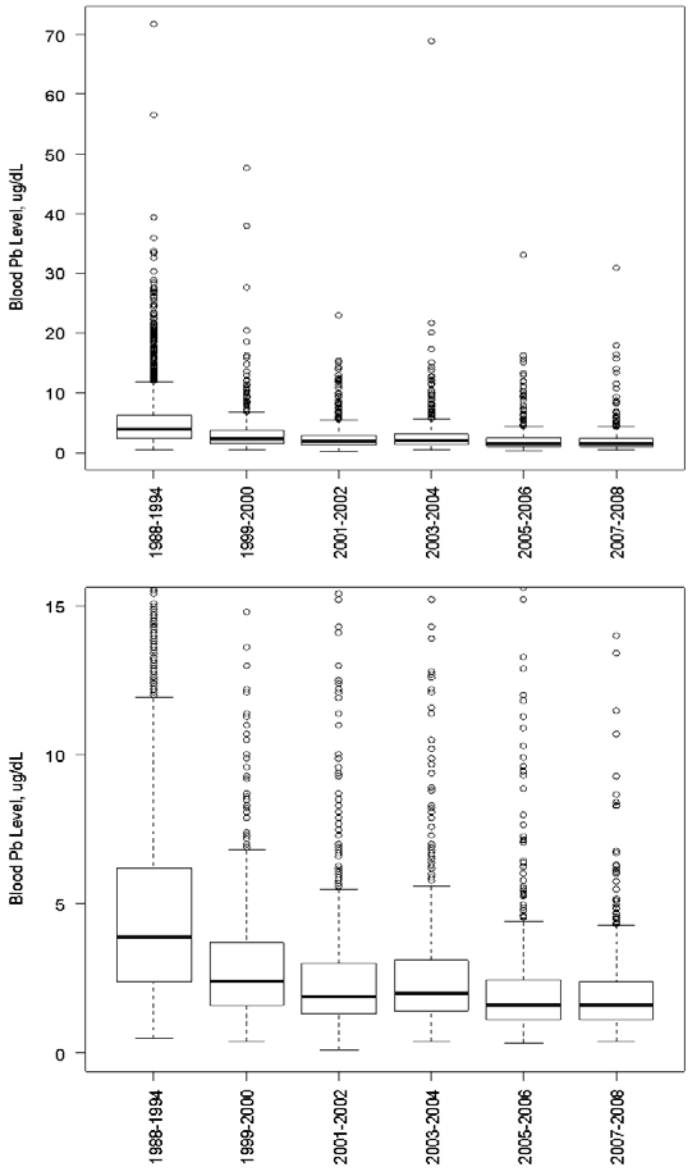
Figure 4-15 Temporal trend in blood Pb concentration.

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	8,945
	2003-2004	1.43	1.36, 1.50	8,373
	2005-2006	1.29	1.23, 1.36	8,407
	2007-2008	1.27	1.21, 1.34	8,266
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	1,044
	2003-2004	1.25	1.12, 1.39	856
	2005-2006	1.02	0.95, 1.01	934
	2007-2008	0.99	0.91, 1.07	1,011
12-19 yr	1999-2000	1.10	1.04, 1.17	2,135
	2001-2002	0.94	0.90, 0.99	2,231
	2003-2004	0.95	0.88, 1.02	2,081
	2005-2006	0.80	0.75, 0.85	1,996
	2007-2008	0.80	0.74, 0.86	1,074
≥ 20 yr	1999-2000	1.75	1.68, 1.81	4,207
	2001-2002	1.56	1.49, 1.62	4,772
	2003-2004	1.52	1.45, 1.60	4,525
	2005-2006	1.41	1.34, 1.48	4,509
	2007-2008	1.38	1.31, 1.46	5,364
Males	1999-2000	2.01	1.93, 2.09	3,913
	2001-2002	1.78	1.71, 1.86	4,339
	2003-2004	1.69	1.62, 1.75	4,132
	2005-2006	1.52	1.42, 1.62	4,092
	2007-2008	1.47	1.39, 1.56	4,147
Females	1999-2000	1.37	1.32, 1.43	4,057
	2001-2002	1.19	1.14, 1.25	4,606
	2003-2004	1.22	1.14, 1.31	4,241
	2005-2006	1.11	1.05, 1.17	4,315
	2007-2008	1.11	1.06, 1.16	4,119
Mexican - Americans	1999-2000	1.83	1.75, 1.91	2,742
	2001-2002	1.46	1.34, 1.60	2,268
	2003-2004	1.55	1.43, 1.69	2,085
	2005-2006	1.29	1.21, 1.38	2,236
	2007-2008	1.25	1.15, 1.36	1,712
Non-Hispanic blacks	1999-2000	1.87	1.75, 2.00	1,842
	2001-2002	1.65	1.52, 1.80	2,219
	2003-2004	1.69	1.52, 1.89	2,293
	2005-2006	1.39	1.26, 1.53	2,193
	2007-2008	1.39	1.30, 1.48	1,746

Survey Stratum	Period	Geometric Mean (µg/dL)	95% Confidence Interval	Number of Subjects
Non-Hispanic whites	1999-2000	1.62	1.55, 1.69	2,716
	2001-2002	1.43	1.37, 1.48	3,806
	2003-2004	1.37	1.32, 1.43	3,478
	2005-2006	1.28	1.19, 1.37	3,310
	2007-2008	1.24	1.16, 1.33	3,461

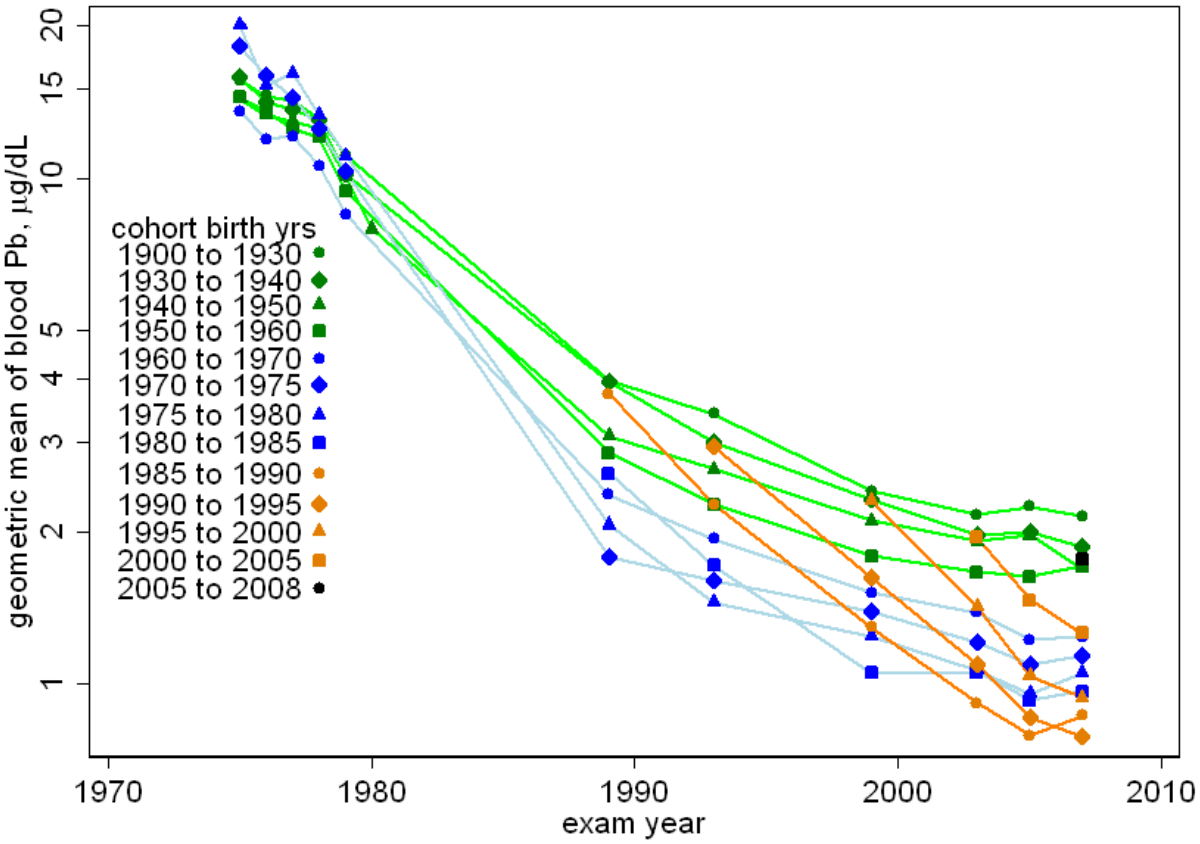
Source: Adapted from data from the NHANES (CDC, 2011).
Age strata correspond to the NHANES study design.



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

Note: Top: all data. Bottom: data for subjects having blood Pb levels less than 15 µg/dL.

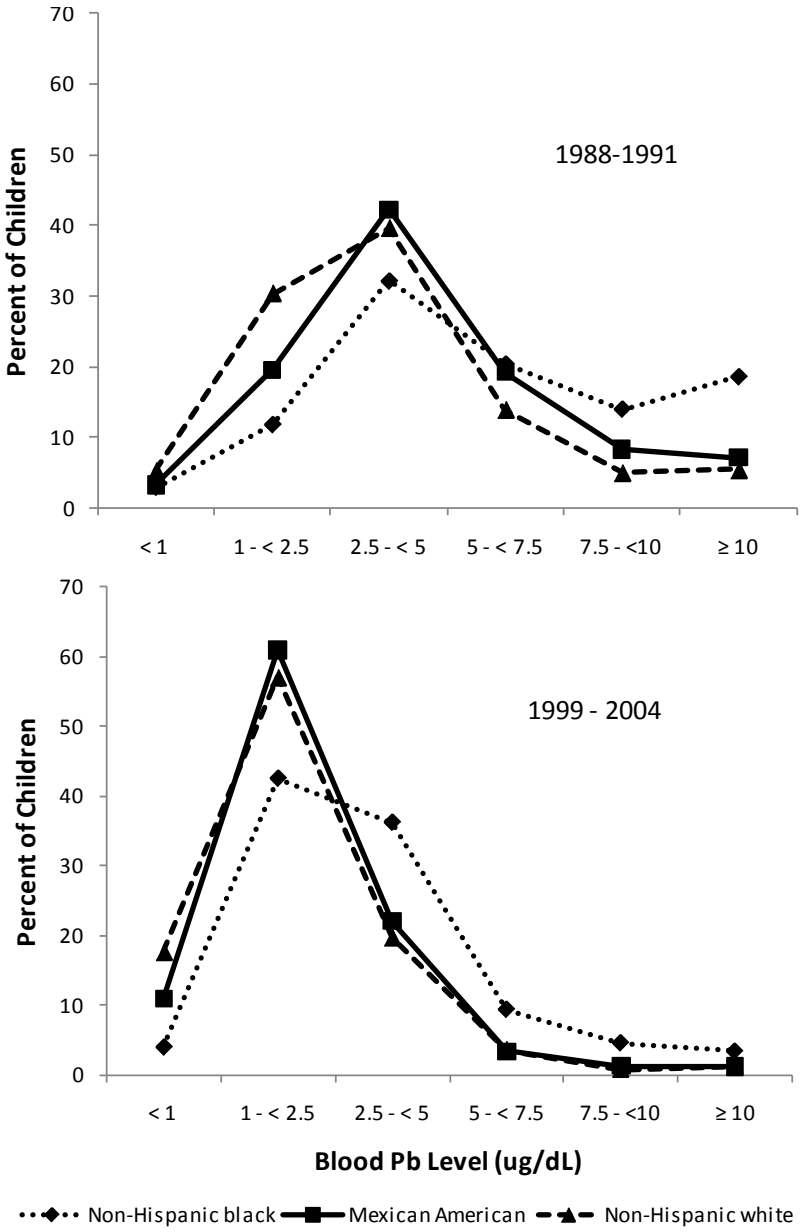
Figure 4-16 Box plots of blood Pb levels among U.S. children (1-5 years old) from the NHANES survey, 1988-2008.



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

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

Figure 4-17 Blood Pb cohort means versus year of exam.



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

Figure 4-18 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 [e.g., (Gulson et al., 2008; Laidlaw et al., 2005; Haley and Talbot, 2004; Johnson et al., 1996)]. Seasonal variation in blood Pb concentration was evident in Australian children (n=107) with a group mean blood Pb level of 2.57 µg/dL when repeated blood Pb measurements were made over a 5-year period, with lower levels in summer compared

with winter (Gulson et al., 2008). A cross-sectional study conducted in New York State from 1995-1998 demonstrated seasonality, with the greatest percent of one and two year old children with blood Pb ≥ 10 µg/dL (n=262,687) occurring in August and the lowest percent in March and April (Haley and Talbot, 2004). 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: 1999-2002; Syracuse, NY: 1994-1998; New Orleans, LA: 1998-2003). Blood Pb data was obtained from public health screening programs conducted in the three cities. Blood Pb samples were dominated by children <5 years of age and age distribution varied across the three cities. The number of blood Pb measurements included in the analyses were as follows: Indianapolis, 15,969; Syracuse, 14,457 (Johnson and Bretsch, 2002; Johnson et al., 1996); New Orleans, 2,295 (Mielke et al., 2007a). 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 ($p < 0.00001$).

Studies have examined the change in blood Pb with changes in potential Pb sources. Gulson et al. (2004b) observed that children living near a Zn-Pb smelter in Australia had blood Pb levels ranging from 10 to 42 µg/dL, with 55-100% of Pb attributed to the smelter based on isotope ratio analysis. Rubio-Andrade et al. (2011) followed a cohort of 6-8 y old children living within 3.5 km of a Mexican smelter at 0, 6, 12, and 60 months after environmental intervention took place. Soil Pb was concurrently obtained but not reported at 6, 12, or 60 months. Median blood Pb level at initiation of the study was 10.1 µg/dL for the 598 initial participants, and median soil Pb was 3,300 mg/kg at the start of the study. After 60 months, median blood Pb level was 4.4 µg/dL for the remaining 232 participants, and median soil Pb concentration was 370 mg/kg at that time. Bonnard and McKone (2009) modeled blood Pb of French children ages 21-74 months living within a village containing a Pb smelter and estimated blood Pb levels of 3.2-10.9 µg/dL. It should be noted that these studies are suggestive but not conclusive in showing that exposure to sources elevates blood Pb because these studies do not control for factors such as non-ambient in-home exposures. For this reason, Newhook et al. (2003) point out that they utilize World Health Organization guidelines on fenceline concentrations in lieu of blood Pb levels in the vicinity of industrial sources to quantify exposures related to the sources. Lanphear et al. (1998) noted that the probability of children having blood Pb ≥ 10 µg/dL increases both with exterior soil Pb content and interior Pb dust loading. Mielke et al. (2011a) noted significant increases in percentages

of children younger than 7 y old with blood Pb level ≥ 10 µg/dL for those living in inner city New Orleans housing developments (22.9%) compared with children living in communities located on the city outskirts (9.1%). At the same time, median soil Pb was significantly higher in the inner city (438 mg/kg) compared with the city outskirts (117 mg/kg).

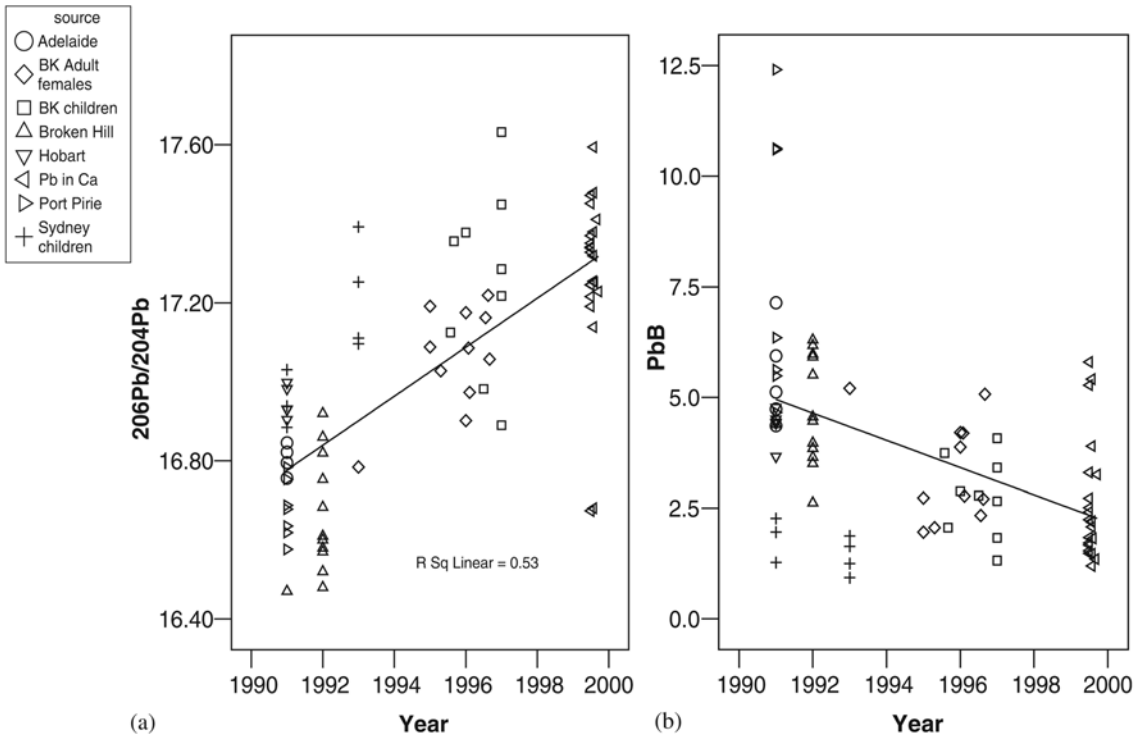
For infants <1 year old, very little data are available on blood Pb levels. Simon et al. (2007) followed a cohort of 13 children living near an Australian smelter from birth through 36 months. In general, immediately after birth blood Pb levels fell for 1-2 months to approximately 47% of birth blood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

Pb body burden has been reported among individuals known to consume wild game hunted with Pb shot. For example, fifty men from Nuuk, Greenland participated in a study in which they recorded their diet and produced blood samples (Johansen et al., 2006). Men who regularly ate hunted game had an average blood Pb concentration of 12.8 µg/dL, in contrast with those who did not and had an average blood Pb concentration of 1.5 µg/dL. Umbilical cord blood was collected from a cohort of Inuit newborns from northern Quebec, where the Inuit population consumes game killed with Pb shot (Lévesque et al., 2003). The geometric mean cord blood Pb level was 0.19 µmol/L, with a range of 0.01-1.31 µmol/L; the Canadian level of concern for cord blood Pb is 0.48 µmol/L. The authors contrasted the finding that 7% of Inuit newborns had cord blood Pb concentration ≥ 0.48 µmol/L in contrast with 0.16% of the Caucasian population in southern Quebec.

Recent studies have sought to characterize human exposure to Pb from piston-engine aircraft emissions. Section 3.2.2.1 describes a study by Carr et al. (2011) in which Pb concentrations, both modeled and monitored, extended beyond airport property. Miranda et al. (2011) used GIS to study the association between blood Pb level and distance from airports in six North Carolina Counties. They observed that the trend in blood Pb level decreases monotonically with distance class from the airports, with subjects within 500 m

of the airports having significantly increased blood Pb levels with residential proximity to airports ($\beta = 0.043$, 95% CI (0.006,0.080), $p < 0.05$) compared with the general population for a given county after controlling for proportion of black, Hispanic, percent receiving public assistance, and household median income at the census block group level and including dummy variables for season during which the children were screened for blood Pb.

Trends in blood Pb levels have been accompanied by changes in Pb isotope ratios for 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 (Gulson et al., 2008; Ranft et al., 2008; Gulson et al., 2006a; Ranft et al., 2006). Gulson et al. (2006a) illustrated how a linear increase in the isotopic ratio $^{206}Pb/^{204}Pb$ occurred in concert with a decrease in blood Pb levels among selected study populations in Australia during the period 1990-2000 (Figure 4-19). Gulson et al. (2006a) point out that the isotopic signature of $^{206}Pb/^{204}Pb$ derived from Australian mines (median ~16.8) differs from that of European and Asian mines, where $^{206}Pb/^{204}Pb$ varies between ~17.4 and ~18.1. Liang et al. (2010) also examined the trends in blood Pb level over the period 1990 to 2006 in Shanghai and saw a reduction corresponding to the phase out of Pb in gasoline. A plot of $^{208}Pb/^{206}Pb$ to $^{207}Pb/^{206}Pb$ for blood and environmental samples showed overlap between the isotopic signature for coal combustion ash and that measured in blood. This result suggests a growing influence of Pb from coal ash in Shanghai in the absence of Pb in automobile emissions. Oulhote et al. (2011) examined Pb isotope ratios in blood Pb samples of 125 French children aged 6 m-6 y. The study found that Pb isotope ratios could be used to attribute Pb exposure to one source for 32% of children and to eliminate an unlikely source of Pb exposure in 30% of children.



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

Figure 4-19 Trends in $^{206}Pb/^{204}Pb$ isotope ratio in blood Pb (a) and trends in blood Pb levels (b) 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. Table 4-8 and Table 4-9 are compilations of data from epidemiologic studies that provided bone Pb concentrations by K-XRF and/or variability in concentrations among individuals without reported occupational exposure and those with occupational exposures, respectively. In non-occupationally exposed individuals, typical group mean tibia bone Pb concentrations ranged from 10 to 30 µg/g. Patella bone Pb levels are typically higher than tibia bone Pb levels in the studies considered (Table 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	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Bandeen-Roche et al. (2009)	**Cohort:** Baltimore Memory Study cohort **Age (yrs):**50-70 **N:**1140 **Location:** Baltimore, MD **Study Period**:2001-2005	Cumulative	Tibia	Mean±SD Tibia: 18.8 ± 11.6	Not reported
Bellinger et al. (1994a)	**Cohort:** Not reported **Age (yrs):** 5-8 (recruited); 19-20 (follow-up) **N:** 79 **Location:** Boston, MA **Study Period:** 1989-1990	Cumulative	Tibia Patella	Mean (Range): Tibia: 5.4 (3-16) Patella: 9.2 (4-18)	High exposure: >24 Low exposure: <8.7
Cheng et al. (2001)	**Cohort:** Normative Aging Study cohort **Age (yrs):** Mean±SD: Normotensive: 65.49 ± 7.17 Borderline hypertension: 68.3 ± 7.79 Definite hypertension: 67.93 ± 6.79 **N:** 833 males **Location:** Boston, MA **Study Period:** 8/1/1991-12/31/1997	Cumulative	Tibia Patella	Mean±SD Tibia: Normotensive: 20.27 ± 11.55 Borderline hypertension: 23.46 ± 15.02 Definite hypertension: 22.69 ± 14.71 Patella: Normotensive: 28.95 ± 18.01 Borderline hypertension: 33.73 ± 21.76 Definite hypertension: 32.72 ± 19.55	Lowest quintile: Tibia: 8.5 Patella: 12.0 Highest quintile: Tibia: 36.0 Patella: 53.0
Coon et al. (2006)	**Cohort:** Participants from Henry Ford Health System (HFHS) **Age (yrs):** ≥ 50; Mean: 69.9 **N:** 121 cases; 414 controls **Location:** Southeastern Michigan **Study Period:** 1995-1999 (participants received primary health care services)	Cumulative	Tibia Calcaneus	Mean±SD: Tibia: 12.5 ± 7.8 Calcaneus: 20.5 ± 10.2	Tibia Q1: 0-5.91 Q2: 5.92-10.40 Q3: 10.41-15.50 Q4: ≥ 15.51 Calcaneus Q1: 0-11.70 Q2: 11.71-19.07 Q3: 19.08-25.28 Q4: ≥ 25.29
Elmarsafawy et al. (2006)	**Cohort:**Normative Aging Study **Age (yrs):** Not reported **N:** 471 elderly males **Location:** Greater Boston area, MA **Study Period:** 6/1991-12/1994	Not reported	Tibia Patella	Mean±SD: Tibia: 21.6 ±1 2.0 Patella: 31.7 ± 18.3	Not reported
Glass et al. (2009)	**Cohort:**Baltimore Memory Study **Age (yrs):** Mean: 59.4; Range: 50-70 **N:** 1,001 **Location:** Baltimore, MD **Study Period:** 2001-2005	Cumulative (lifetime)	Tibia	Mean±SD: Tibia: 18.8 ± 11.1	NPH Scale: Lowest tertile: Mean Tibia level: 16.3 ± 11.0 Middle tertile: Mean Tibia level: 19.3 ± 10.7 Highest tertile: Mean Tibia level: 20.3 ± 11.4
Hsieh et al. (2009b)	**Cohort:**Not reported **Age (yrs):** Mean: Control: 46.06 **N:** 18 controls **Location:** Not reported **Study Period:** Not reported	Control group for occupational exposure group	Tibia Patella	Mean±SD Tibia Control: 18.51 ± 22.40 Patella Control: 7.14 ± 9.81	Not reported
Hu et al. (1996b) [As reported in Navas-Acien et al., (2008)]	**Cohort:**Normative Aging Study **Age (yrs):** 48-92; Mean ± SD: 66.6 ± 7.2 **N:** 590 males **Location:** Boston, MA **Study Period:** 8/1991-12/1994	Cumulative	Tibia Patella	Mean±SD: Tibia: 21.8 ± 12.1 Patella: 32.1 ± 18.7 Range: Tibia: <1-96 Patella: 1-142	Figures 1 and 2 show both types of bone Pb levels increasing with age

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Jain et al. (2007)	**Cohort:**VA-Normative Aging Study **Age (yrs):** Not reported **N:** 837 males **Location:** Greater Boston, MA **Study Period:** 9/1/1991-12/31/2001	Not reported	Tibia Patella	Mean ± SD Tibia: Non-Cases: 21.4 ± 13.6 Cases: 24.2 ± 15.9 Patella: Non-cases: 30.6±19.7 Cases: 36.8 ± 20.8 Range: Tibia: 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)	**Cohort:**Not reported **Age (yrs):** 30-80 **N:** 256 controls (Bone samples collected from 41 controls) **Location:** New England (Boston, MA) **Study Period:** 1993-1996	Cumulative Control group for occupational exposure group	Tibia Patella	Mean±SE Tibia Controls: 11.1 ± 1.6 Patella Controls: 16.7 ± 2.0	Controls Tibia: N (%) -7-7: 14 (34) 8-14: 12 (29) 15-61: 15 (37) Patella: N (%) -4-9: 14 (34) 10-20: 14 (34) 21-107: 13 (32)
Khalil et al. (2009b)	**Cohort:**1982 Pb Occupational Study **Age (yrs):** Control mean: 55 **N:** 51 controls **Location:** Eastern Pennsylvania **Study Period:** 1982-2004	Control group for occupational exposure group	Tibia	Median (IQR) Tibia Control: 12 (-8-32)	Not reported
Korrick et al. (1999) (As reported in from Navas-Acien et al., (2008)	**Cohort:** Nurses' Health Study **Age (yrs):** Combined: 47-74; Mean±SD: Combined: 58.7 ± 7.2; Cases: 61.1 ± 7.1; High controls: 61.1 ± 7.2; Low controls: 58.7 ± 7.1 **N:** 284 females; (89 cases; 195 controls) **Location:** Boston, MA **Study Period:** 7/1993-7/1995	Nonoccupationally exposed	Tibia Patella	Mean ± SD Tibia: Combined: 13.3 ± 9.0 Cases: 13.0 ± 9.4 High controls: 14.7 ± 10 Low controls: 12.7 ± 8.1 Patella: Combined: 17.3 ± 11.1 Cases: 19.5 ± 12.9 High controls: 17.2 ± 9 Low controls: 15.8 ± 10.6 Range Tibia Combined: -5-69 Patella Combined: -5-87	Patella: 10th percentile: 6 90th percentile: 31
Lee et al. (2001a) [As reported in Navas-Acien et al., (2008)]	**Cohort:** Not reported **Age (yrs):** 22.0-60.2 Mean ± SD: Controls: 34.5 ± 9.1 **N:** 135 controls **Location:** South Korea **Study Period:** 10/24/1997-8/19/1999	Control group for occupational exposure group	Tibia	Mean ± SD Tibia Controls: 5.8 ± 7.0 Range Tibia Controls: -11-27	Not reported

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Martin et al. (2006)	**Cohort:** Baltimore Memory Study **Age (yrs):** 50-70; Mean: 59.4 **N:** 964 **Location:** Baltimore, MD **Study Period:** 5/2001-9/2002 (1st study visit) 8/2002-3/2004 (2nd study visit – tibia Pb measured)	Cumulative (lifetime)	Tibia	Mean ± SD Tibia: 18.8 ± 12.4	Tibia IQR: 11.9-24.8
Needleman et al. (2002)	**Cohort:** Not reported **Age (yrs):** 12-18; Mean age ± SD: African American cases: 15.8 ± 1.4 African American controls: 15.5 ± 1.1; White cases: 15.7 ± 1.3; White controls: 15.8 ± 1.1 **N:** 194 male youth cases; 146 male youth controls **Location:** Allegheny County, PA (cases); Pittsburgh, PA (controls) **Study Period:** 4/1996-8/1998	Not reported	Tibia	Mean ± SD Tibia Cases (ppm): All subjects: 11.0 ± 32.7 African American: 9.0 ± 33.6 White: 20 ± 27.5 Tibia Controls (ppm): All subjects: 1.5 ± 32.1 African American: -1.4 ± 31.9 White: 3.5 ± 32.6	Table 4 of paper distributes bone Pb by ≥ 25 or <25 for race, two parental figures, and parent occupation
Osterberg et al. (1997) [As reported in Shih et al., (2007)]	**Cohort:** Not reported **Age (yrs):** Median: 41.5 **N:** 19 male controls **Location:** Not reported **Study Period:** Not reported	Control group for occupational exposure group	Finger bone	Median (range) Finger Bone Controls: 4 (-19-18)	Not reported
Park et al. (2006)	**Cohort:** Normative Aging Study **Age (yrs):** Mean: 72.9 ± 6.5 **N:** 413 males **Location:** Greater Boston, MA **Study Period:** 11/14/2000-12/22/2004; (HRV measurements taken); 1991-2002 (bone Pb measurements taken)	Not reported	Tibia Patella	Median (IQR) Tibia: 19.0 (11-28) Patella: 23.0 (15-34) Estimated Patella[a]: 16.3 (10.4-25.8)	Median (IQR) for No. of metabolic abnormalities: Tibia: 0: 18.5 (10.5-23) 1: 19 (11-28) 2: 19 (12-26) Patella: 0: 22 (13.5-32) 1: 25 (16-36) 2: 20 (15-32) Estimated Patella: 0: 16.3 (10.8-24.8) 1: 17.1 (11-29.3) 2: 15.1 (9.4-22.1)
Park et al. (2009b)	**Cohort:** Normative Aging Study **Age (yrs):** Mean: 67.3 ± 7.2 **N:** 613 males **Location:** Greater Boston, MA **Study Period:** 8/1991 - 12/1995	Not reported	Tibia Patella	Median (IQR) Tibia: 19 (14-27) Patella: 26 (18-37)	Table 1 of paper distributes tibia and patella Pb by genotype; Table 2 of paper distributes tibia and patella Pb by number of gene variants
Park et al. (2010)	**Cohort:** VA Normative Aging Study cohort **Age (yrs):** Mean: 64.9 (at bone Pb measurement) **N:** 448 males **Location:** Eastern Massachusetts **Study Period:** 1991-1996	Cumulative (chronic exposure)	Tibia Patella	Mean±SD Tibia: 22.5 ± 14.2 Patella: 32.5 ± 20.4	Tibia IQR: 15 Patella IQR: 21 Table 2 of paper provides age-adjusted mean bone Pb levels (age, race, education, smoking [pack-yr], occupational noise, noise notch, BMI, hypertension, diabetes)
Payton et al. (1998)	**Cohort:** VA Normative Aging Study cohort **Age (yrs):** Mean: 66.8 **N:** 141 males **Location:** Boston, MA **Study Period:** 4/1993-3/1994	Not reported	Tibia Patella	Mean ± SD Tibia: 22.5 ± 12.2 Patella: 31.7 ± 19.2	Not reported

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Peters et al. (2007)	**Cohort:** Normative Aging Study cohort **Age (yrs):** Mean: 66.9 **N:** 513 male cases **Location:** Boston, MA **Study Period:** 1991-1996	Cumulative	Tibia Patella	Mean ± SD Tibia: 21.5 ± 13.4 Patella: 31.5 ± 19.3	Not reported
Rajan et al. (2007)	**Cohort:** VA Normative Aging Study Cohort **Age (yrs):** Mean: 67.5 (at bone scan) **N:** 1075 males **Location:** Boston, MA **Study Period:** 1991-2002	Not reported	Tibia Patella	Mean ± SD Tibia: 22.1 ± 13.8 Patella: 31.4 ± 19.6	Not reported
Rajan et al. (2008)	**Cohort:** VA Normative Aging Study Cohort **Age (yrs):** ≥ 45 **N:** 720 males **Location:** Boston, MA **Study Period:** 1993-2001	Current and cumulative	Tibia Patella	Mean ± SD ALAD 1-1 Tibia: 21.9 ± 13.8 Patella: 29.3 ± 19.1 ALAD 1-2/2-2 Tibia: 21.2 ± 11.6 Patella: 27.9 ± 17.3	Not reported
Rhodes et al. (2003)	**Cohort:** VA Normative Aging Study Cohort **Age (yrs):** Mean: 67.1 **N:** 526 males **Location:** Boston, MA **Study Period:** 1/1/1991-12/31/1995	Not reported	Tibia Patella	Mean ± SD Tibia: 21.9 ± 13.5 Patella: 32.1 ± 19.8	No. of participants (%) Tibia: <1-15: 173 (33) 16-24: 186 (35) 25-126: 167 (32) Patella: <1-22: 189 (36) 23-35: 165 (31) 36-165: 172 (33)
Roels et al. (1994)	**Cohort:** Not reported **Age (yrs):** 30-60 **N:** 68 males **Location:** Belgium **Study Period:** Not reported	Control group for occupational exposure group	Tibia	Geometric Mean (Range) Tibia Controls: Normotensive: 21.7 (<15.2-69.3) Hypertensive: 20.2 (<15.2-52.9) Total: 21.4 (<15.2-69.3)	Not reported
Rothenberg et al. (2002b), as reported in Navas-Acien et al. (2008)	**Cohort:** Not reported **Age (yrs):** 15-44; Mean ± SD: 31.0 ± 7.7 **N:** 720 females **Location:** Los Angeles, CA **Study Period:** 6/1995-5/2001	Not reported	Tibia Calcaneus	Mean ± SD Tibia: 8.0 ± 11.4 Calcaneus: 10.7 ± 11.9	Tibia quartiles: Q1: -33.7-0.9 Q2: 1.0-8.0 Q3: 8.1-16.1 Q4: 16.2-42.5 Calcaneus quartiles: Q1: -30.6-3.0 Q2: 3.1-10.0 Q3: 10.1-18.7 Q4: 18.8-49.0
Shih et al., (2006)	**Cohort:** Baltimore Memory Study cohort **Age (yrs):** Mean: 59.39 **N:** 985 **Location:** Baltimore, MD **Study Period:** Not reported	Not reported	Tibia	Mean ± SD: Tibia: 18.7 ± 11.2	Not reported

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Stokes et al. (1998), as reported in Shih et al. (2007)	**Cohort:** Not reported **Age (yrs):** 19-29 (in 1994); Mean ± SD: Cases: 24.3 ± 3.18 Control: 24.2 ± 3.02 Cases: 9 months-9 yr (during 1/1/1974-12/31/1975) **N:** 257 cases; 276 controls **Location:** Silver Valley, ID; Spokane, WA **Study Period:** 7/10/1994-8/7/1994	Cumulative (lifelong) Environmental (resided near Pb smelter during childhood)	Tibia	Mean (Range): Tibia Cases: 4.6 (-28.9-37) Tibia Controls: 0.6 (-46.4-17.4)	Tibia No. of Cases: <1 µg/g: 31.5% 1-5 µg/g: 24.4% 5-10 µg/g: 22.3% >10 µg/g: 21.8% No. of Controls: <1 µg/g: 50.4% 1-5 µg/g: 25.6% 5-10 µg/g: 19.4% >10 µg/g: 4.7% Mean ± SD Tibia concentration by age group: Cases: 19-21: 1.47 ± 8.35 22-24: 4.48 ± 7.45 25-27: 4.82 ± 8.92 28-30: 6.64 ± 9.53 Controls: 19-21: 1.27 ± 6.60 22-24: -0.61 ± 6.19 25-27: 0.60 ± 8.60 28-30: 1.74 ± 6.42
Van Wijngaarden et al. (2009)	**Cohort:** Not reported **Age (yrs):** Mean: 61.5 **N:** 47 **Location:** Rochester, NY **Study Period:** Not reported	Cumulative	Tibia Calcaneus	Mean ± SD Tibia: 2.0 ± 5.2 Calcaneus: 6.1 ± 8.5	Not reported
Wasserman et al. (2003)	**Cohort:** Yugoslavia Prospective Study of Environmental Pb Exposure **Age (yrs):** 10-12 **N:** 167 children **Location:** Kosovska, Mitrovica, Kosovo, Yugoslavia; Pristina, Kosovo, Yugoslavia **Study Period:** 5/1985-12/1986 (mother's enrollment); 1986-1999 (follow-up through age 12 yr); Tibia Pb measured 11-13 yr old	Cumulative (lifetime) Environmental (Pb smelter, refinery, battery plant)	Tibia	Mean ± SD: Tibia Pristina: 1.36 ± 6.5 Mitrovica: 39.09 ± 24.55	Tibia quartiles: Q1: -14.4-1.85 Q2: 1.85-10.5 Q3: 10.5-35 Q4: 35-193.5 Table 3 of paper distributes tibia Pb by sex, ethnicity, address at birth relative to factory, and maternal education
Weisskopf et al. (2004), as reported in Shih et al. (2007)	**Cohort:** Normative Aging Study **Age (yrs):** Mean ± SD: 67.4 ± 6.6 **N:** 466 males **Location:** Boston, MA **Study Period:** 1991-2002	Environmental	Tibia Patella	Median (IQR) Tibia: 19 (12,26) Patella: 23 (15, 35)	Tibia IQR: 14 Patella IQR: 20 Table 3 of paper shows mean Pb levels across categorical variables (yr of education, smoking status, computer experience, first language English)
Weisskopf et al. (2007b)	**Cohort:** VA Normative Aging Study cohort **Age (yrs):** Mean: Lowest Patella quintile: 73.2; Highest Patella quintile: 80.7 **N:** 31 males **Location:** Boston, MA **Study Period:** Bone Pb measured: 1994-1999 Scans performed: 2002-2004	Not reported	Tibia Patella	Median (IQR) Tibia Lowest quintile: 13 (9-17) Highest quintile: 41 (38-59) Patella Lowest quintile: 9 (5-15) Highest quintile: 63 (43-86)	Not reported
Weisskopf et al. (2007a)	**Cohort:** VA Normative Aging Study cohort **Age (yrs):** Mean: 68.7 **N:** 1,089 males **Location:** Boston, MA **Study Period:** 1993-2001	Concurrent and cumulative	Tibia Patella	Median (IQR) Tibia: 20 (13-28) Patella: 25 (17-37)	Table 1 of paper shows distribution of Pb biomarkers by categories of covariates (age, education, smoking status, alcohol intake, physical activity, computer experience, first language English)

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Weisskopf et al. (2009)	**Cohort:** Normative Aging Study; (95% white) **Age (yrs):** Mean ± SD (at Patella baseline); Tertile 1: 65.2 ± 7.1; Tertile 2: 66.5 ± 6.5 Tertile 3: 70.2 ± 7.2 **N:** 868 males **Location:** Greater Boston area, MA **Study Period:** 1991-1999	Cumulative	Tibia Patella	Mean ± SD Tibia: 21.8 ± 13.6 Patella: 31.2 ± 19.4	Patella tertiles: 1: <22 2: 22-35 3: >35
Weisskopf et al. (2010)	**Cohort:** BUMC, BWH, BIDMC, HVMA, Normative Aging Study (NAS), Harvard Cooperative Program on Aging (HCPOA) **Age (yrs):** Mean: Cases: 66.5; Controls: 69.4 **N:** 330 cases; 308 controls **Location:** Boston, MA **Study Period:** 2003-2007 1991-1999 (NAS patients bone Pb measured)	Cumulative	Tibia Patella	Mean ± SD: Tibia: 10.7 ± 12.1 Patella: 13.6 ± 15.9	Tibia quartiles: Q1: <3.1 Q2: 3.5-9.6 Q3: 10.0-17.0 Q4: >17.3 Patella quartiles: Q1: <2.7 Q2: 3.5-11.0 Q3: 11.3-20.9 Q4: >20.9
Weuve et al. (2006)	**Cohort:** VA Normative Aging Study cohort **Age (yrs):** ≥ 45 **N:** 720 males **Location:** Boston, MA **Study Period:** 1991 (measuring bone Pb levels) End date not reported	Cumulative	Tibia Patella	Median (1st-3rd quartile): Tibia: 19 (13-28) Patella: 27 (18-39)	Table 1 of paper shows distribution of mean Pb biomarker levels by characteristics of participants (age, education, computer experience, smoking status, alcohol consumption, tertile of calcium intake, tertile of physical activity, diabetes)
Weuve et al. (2009)	**Cohort:** Nurses' Health Study cohort **Age (yrs):** 47-74 **N:** 587 females **Location:** Boston, MA **Study Period:** 1995-2005	Recent and cumulative	Tibia Patella	Mean ± SD: Tibia: 10.5 ± 9.7 Patella: 12.6 ± 11.6	Not reported
Wright et al. (2003b), as reported in Shih et al. (2007)	**Cohort:** Normative Aging Study **Age (yrs):** Mean ± SD: 68.2 ± 6.9 **N:** 736 males **Location:** Boston, MA **Study Period:** 1991-1997	Environmental	Tibia Patella	Mean ± SD: Tibia: 22.4 ± 15.3 Patella: 29.5 ± 21.2	Tibia: Difference in mean from Lowest-highest quartile: 34.2 Patella: Difference in mean from lowest-highest quartile: 47

Table 4-9 Epidemiologic studies that provide bone Pb measurements for occupationally exposed populations

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Bleecker et al. (1997), as reported in Shih et al. (2007)	**Cohort:** Canada Lead Study **Age (yrs):** Cumulative: 24-64 Younger: 24-43 Older: 44-64 Mean ± SD: Cumulative: 44.1 ± 8.36 Younger: 37.2 ± 4.57 Older: 50.9 ± 4.86 **N:** 80 males **Location:** Canada **Study Period:** Not Reported	Occupational (Pb smelter workers)	Tibia	Mean ± SD (Tibia): Cumulative: 41.0 ± 24.44 Younger: 35 ± 24.11 Older: 46.9 ± 23.59 Range (Tibia): Cumulative: -12-90 Younger: -12-80 Older: 3-90	Not reported
Bleecker et al. (2007a)	**Cohort:** Not reported **Age (yrs):** Mean: 39.7 **N:** 61 **Location:** Northern Canada **Study Period:** Not Reported	Occupational (primary Pb smelter workers)	Tibia	Mean: Tibia: 38.6	Not reported
Caffo et al. (2008)	**Cohort:** Not reported **Age (yrs):** Mean: 60.39 **N:** 513 males **Location:** Delaware and New Jersey, U.S. **Study Period:** 1994-1997 (Phase 1 recruitment); 2001-2003 (Phase 2 recruitment)	Cumulative Occupational (Former organolead manufacturing workers)	Tibia	Mean ± SD: Peak Tibia: 23.99 ± 18.46	Not reported
Dorsey et al. (2006)	**Cohort:** Not reported **Age (yrs):** Mean: 43.4 **N:** 652 **Location:** Korea **Study Period:** 10/24/1997-8/19/1999 (enrolled)	Occupational (Pb workers)	Tibia Patella	Mean ± SD: Tibia: 33.5 ± 43.4 Patella: 75.1 ± 101.1	Not reported
Glenn et al. (2003), as reported in Navas-Acien et al. (2008)	**Cohort:** Not reported **Age (yrs):** 40-70; Mean: 55.8 (baseline) **N:** 496 males **Location:** Eastern U.S. **Study Period:** 6/1994-6/1996 (enrolled); 6/1998 (follow-up period ended)	Occupational (Chemical manufacturing facility; inorganic and organic Pb)	Tibia	Mean ± SD: Tibia: 14.7 ± 9.4 (at yr 3) Peak Tibia: 24.3 ± 18.1 Range: Tibia: -1.6-52 (at year 3) Peak Tibia: -2.2-118.8	Not reported
Glenn et al. (2006)	**Cohort:** Not reported **Age (yrs):** 0-36.2 (baseline); Mean ± SD: 41.4 ± 9.5 (baseline) **N:** 575; (76% male; 24% female) **Location:** South Korea **Study Period:** 10/1997-6/2001	Cumulative and recent Occupational (Pb-using facilities)	Tibia	Mean ± SD: Tibia: 38.4 ± 42.9 Tibia-Women: Visit 1: 28.2±19.7 Visit 2: 22.8±20.9 Tibia-Men: Visit 1: 41.7±47.6 Visit 2: 37.1±48.1	Not reported
Hanninen et al. (1998), as reported in Shih et al. (2007)	**Cohort:** Not reported **Age (yrs):** Mean±SD: Male: 43; Female: 48 BPb (max) ≤ 2.4 µmol/L: 41.7 ± 9.3 BPb (max) >2.4 µmol/L: 46.6 ± 6.2 **N:** 54; (43 males, 11 females) **Location:** Helsinki, Finland **Study Period:** Not reported	Occupational (Pb acid battery factory workers)	Tibia Calcaneus	Mean±SD: Tibia: 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

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Hsieh et al. 2009 (2009b)	**Cohort:** Not reported **Age (yrs):** Mean: Cases: 45.71 Controls: 46.06 **N:** 22 cases; 18 controls **Location:** Not Reported **Study Period:** Not reported	Occupational (Pb paint factory workers)	Tibia Patella	Mean ± SD Tibia Case: 61.55 ± 30.21 Control: 18.51 ± 22.40 Patella Case: 66.29 ± 19.48 Control: 7.14 ± 9.81	Not reported
Kamel et al. (2002); Kamel et al. (2005); Kamel et al. (2008)	**Cohort:** Not reported **Age (yrs):** 30-80 **N:** 109 cases; 256 controls; (Bone samples collected from 104 cases and 41 controls) **Location:** New England (Boston, MA) **Study Period:** 1993-1996	Cumulative Occupational (Pb fumes, dust, or particles)	Tibia Patella	Mean ± SE Tibia Cases: 14.9 ± 1.6 Controls: 11.1 ± 1.6 Patella Cases: 20.5 ± 2.1 Controls: 16.7 ± 2.0	Cases Tibia Pb: N (%) -7-7: 21 (20) 8-14: 35 (34) 15-61: 48 (46) Patella Pb: N (%) -4-9: 27 (26) 10-20: 40 (38) 21-107: 37 (36) Controls Tibia Pb: N (%) -7-7: 14 (34) 8-14: 12 (29) 15-61: 15 (37) Patella Pb: N (%) -4-9: 14 (34) 10-20: 14 (34) 21-107: 13 (32)
Khalil et al. (2009b)	**Cohort:** 1982 Pb Occupational Study cohort **Age (yrs):** Mean: Cases: 54 Controls: 55 **N:** 83 cases; 51 controls **Location:** Eastern Pennsylvania **Study Period:** 1982-2004	Occupational (Pb battery plant workers)	Tibia	Median (IQR) Tibia Cases: 57 (20-86) Controls: 12 (-8-32)	Not reported
Osterberg et al. (1997), as reported in Shih et al. (2007)	**Cohort:** Not reported **Age (yrs):** Median: 41.5 **N:** 38 male cases; 19 male controls **Location:** Not reported **Study Period:** Not Reported	Occupational (secondary Pb smelter – inorganic Pb)	Finger bone	Median Finger Bone: High Cases: 32 Low cases: 16 Control: 4 Range Finger Bone: High Cases: 17-101 Low cases: -7-49 Control: -19-18	Not reported
Roels et al. (1994)	**Cohort:** Not reported **Age (yrs):** 30-60 **N:** 76 male cases; 68 male controls **Location:** Belgium **Study Period:** Not Reported	Occupational (Pb smelter workers) Mean case exposure: 18 yr (range: 6 to 36 yr)	Tibia	Geometric Mean (Range) Tibia Cases: Normotensive: 64.0 (19.6-167.1) Hypertensive: 69.0 (21.7-162.3) Total: 65.8 (19.6-167.1) Tibia Controls: Normotensive: 21.7 (<15.2-69.3) Hypertensive: 20.2 (<15.2-52.9) Total: 21.4 (<15.2-69.3)	Not reported

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Schwartz (2000c) et al., as reported in Shih et al., (2007)	**Cohort:** U.S. Organolead Study **Age (yrs):** Mean ± SD: Cases: 55.6 ± 7.4 Controls: 58.6 ± 7.0 **N:** 535 male cases 118 male controls **Location:** Eastern U.S. **Study Period:** 6/1994-10/1997 (enrolled); Completed 2-4 annual follow-up visits; Tibia Pb taken in 3rd year	Occupational (tetraethyl and tetramethyl Pb manufacturing facility)	Tibia	Mean ± SD Current Tibia: Cases: 14.4 ± 9.3 Peak Tibia: Cases: 22.6 ± 16.5	Not reported
Schwartz et al. (2000b), as reported in Navas-Acien et al. (2008)	**Cohort:** Not reported **Age (yrs):** 41.7-73.7 (Combined) Mean ± SD: Combined: 57.6 ± 7.6 Hypertensive: 60.2 ± 6.9 Nonhypertensive: 56.6 ± 7.5 **N:** 543 males **Location:** Eastern U.S. **Study Period:** 1995 (recruited); 1996-1997 (Tibia Pb taken during the 3rd yr)	Occupational (former organolead manufacturing workers)	Tibia	Mean ± SD Tibia: Combined: 14.4 ± 9.3 Hypertensive: 15.4 ± 9.1 Nonhypertensive: 14.0 ± 9.3 Range Tibia: Combined: -1.6-52	Not reported
Schwartz et al. (2001); Lee et al. (2001a)	**Cohort:** Not reported **Age (yrs):** Mean: Exposed: 40.4 Control: 34.5 **N:** 803 cases; 135 controls **Location:** South Korea **Study Period:** 10/24/1997-8/19/1999	Occupational (battery manufacturing, secondary smelting, Pb oxide manufacturing, car radiator manufacturing)	Tibia	Mean ± SD Tibia Cases: 37.1 ± 40.3 Control: 5.8 ± 7.0 Range: Tibia Cases: -7-338 Controls: -11-27	Not reported
Schwartz et al. (2005)	**Cohort:** Not reported **Age (yrs):** Mean at 1st visit: 41.4 **N:** 576 **Location:** South Korea **Study Period:** 10/1997-6/2001	Occupational (current and former Pb workers)	Tibia	Mean ± SD Tibia: 38.4 ± 43	Tibia: 25th percentile at V1: 14.4 75th percentile at V1: 47.1
Stewart et al. (1999), as reported in Shih et al., (2007)	**Cohort:** U.S. Organolead Study **Age (yrs):** 40-70 (in 1995) 38% ≥ 60 yrs Mean: 58 **N:** 534 males **Location:** Eastern U.S. **Study Period:** Not Reported	Occupational (tetraethyl and tetramethyl Pb manufacturing facility)	Tibia	Mean ± SD Tibia: Current: 14.4 ± 9.3 Peak: 23.7 ± 17.4 Range: Tibia Current: -1.6-52 Peak: -2.2-105.9	Current Tibia Pb: N (%) <5: 77 (14.2) 5-9.99: 113 (20.8) 10-14.99: 119 (21.9) 15-19.99: 117 (21.5) ≥ 20: 118 (21.7) Peak Tibia Pb: N (%) <5: 49 (9.1) 5-9.99: 64 (11.8) 10-14.99: 70 (12.9) 15-19.99: 87 (16.1) 20-24.99: 79 (14.6) 25-29.99: 55 (10.2) ≥ 30: 137 (26.1)
Stewart et al. (2006)	**Cohort:** Not reported **Age (yrs):** Mean: 56.1 **N:** 532 males **Location:** Eastern U.S. **Study Period:** 1994-1997; 2001-2003	Cumulative Occupational (Organolead workers - not occupationally exposed to Pb at time of enrollment)	Tibia	Mean ± SD Current Tibia: 14.5 ± 9.6 Peak Tibia: 23.9 ± 18.3	Not reported
Weaver et al. (2008)	**Cohort:** Not reported **Age (yrs):** Mean ± SD: 43.3 ± 9.8 **N:** 652 **Location:** South Korea **Study Period:** 12/1999-6/2001	Occupational (Current and former Pb workers; plants produced Pb batteries, Pb oxide, Pb crystal, or radiators, or were secondary Pb smelters)	Patella	Mean±SD Patella: 37.5 ± 41.8	Not reported

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)[a]	95% Confidence Interval	Number of Subjects
All	1999-2000	0.721	0.700, 0.742	2,465
	2001-2002	0.639	0.603, 0.677	2,689
	2003-2004	0.632	0.603, 0.662	2,558
	2005-2006	0.546	0.502, 0.573	2,576
	2007-2008	0.515	0.483, 0.549	2,627
6-11 yr	1999-2000	1.170	0.975, 1.41	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	1,406
	2001-2002	0.658	0.617, 0.703	1,559
	2003-2004	0.641	0.606, 0.679	1,543
	2005-2006	0.573	0.548, 0.600	1,520
	2007-2008	0.546	0.513, 0.580	1,857
Males	1999-2000	0.720	0.679, 0.763	1,227
	2001-2002	0.639	0.607, 0.673	1,334
	2003-2004	0.615	0.588, 0.644	1,281
	2005-2006	0.551	0.522, 0.582	1,271
	2007-2008	0.502	0.471, 0.534	1,327
Females	1999-2000	0.722	0.681, 0.765	1,238
	2001-2002	0.639	0.594, 0.688	1,355
	2003-2004	0.648	0.601, 0.698	1,277
	2005-2006	0.541	0.507, 0.577	1,305
	2007-2008	0.527	0.489, 0.568	1,300
Mexican - Americans	1999-2000	0.940	0.876, 1.01	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

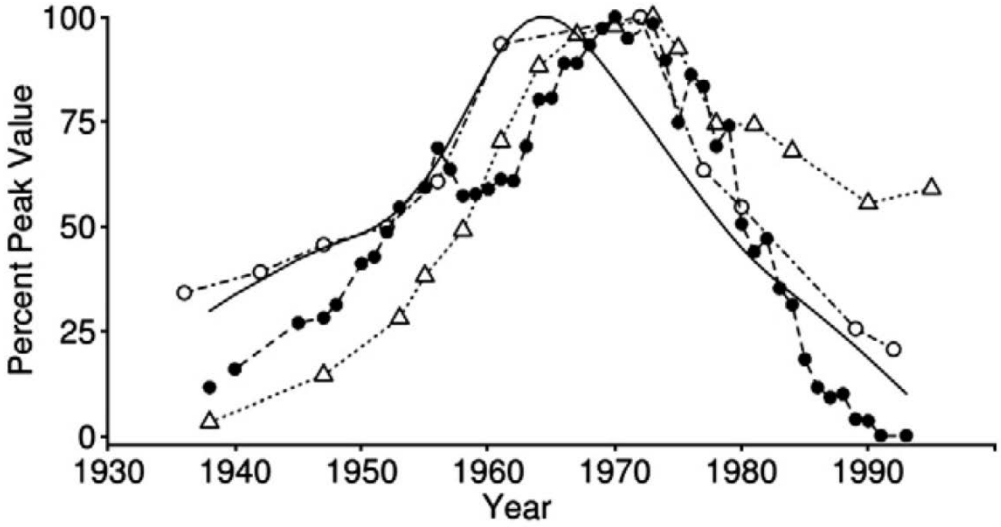
Survey Stratum	Period	Geometric Mean (µg/g CR)[a]	95% Confidence Interval	Number of Subjects
	2005-2006	0.483	0.459, 0.508	692
	2007-2008	0.452	0.414, 0.492	589
Non-Hispanic whites	1999-2000	0.696	0.668, 0.725	822
	2001-2002	0.615	0.579, 0.654	1,132
	2003-2004	0.623	0.592, 0.655	1,074
	2005-2006	0.541	0.500, 0.585	1,041
	2007-2008	0.506	0.466, 0.550	1,095

[a]Values are µg Pb/g creatinine (CR)

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 4-20. 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: Reprinted with permission of Elsevier Publishing, Robbins et al. (2010).

Note: (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.

Figure 4-20 Comparison of relative temporal changes in tooth enamel Pb concentration.

Several Brazillian studies have found increased levels of Pb in teeth in areas where Pb sources are present. For example, Costa de Almeida et al. (2007) reported Pb concentration in tooth enamel among 4-6 y old kindergarteners in São Paulo, Brazil to be significantly higher for children living near a Pb-acid battery processing plant in the Baruru neighborhood compared with 4-6 y old children in other parts of the city (non-exposed median: 206 mg/kg, n = 247; exposed median: 786 mg/kg, n = 26; $p < 0.0001$). Subsequent analysis revealed that 55% of 4-6 y old children from Baruru had tooth enamel Pb concentrations greater than 600 mg/kg, forming a significant comparison with other neighborhoods having 0-33% of 4-6 y old children with tooth enamel Pb greater than 600 mg/kg ($p < 0.0001$) (de Almeida et al., 2008). The authors did not describe controlling for additional factors, such as socioeconomics or housing conditions. Arruda-Neto et al. (2009) studied Pb in tooth samples among São Paulo children to compare exposures of children age 4-12 living near a dam with heavy metal sediments with those of children ages 4-13 living in a control area thought to have few exposures. They observed a significant comparison (near dam: avg 1.28 ± 0.11 mg/kg, n = 50; control region: avg 0.91 mg/kg, n = 24). In a related study of Pb measures in teeth among the general population ages 7-60 y, Arruda-Neto et al. (2009) observed peak Pb concentrations of 115% of age 7 y concentrations at age 10 y followed by a steep drop to 50% among 20 y old subjects. Tooth Pb concentrations stayed fairly constant throughout adulthood but then dropped to just above 30% among 65 y old subjects. Note that the

authors did not clarify if average or median values were presented, nor did they adjust for potentially confounding factors.

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 1986 Pb AQCD (U.S. EPA, 1986a) described epidemiological studies of relationships between air Pb and blood Pb. Of the studies examined, the total blood Pb-air Pb slope (when considering both direct and indirect exposures derived from air) was estimated to be approximately double the slope estimated from the direct contribution due to inhaled air alone (U.S. EPA, 1986a). Much of the pertinent earlier literature (e.g., prior to 1984) was summarized by Brunekreef (1984). Based on the studies available at that time that considered multiple air-related Pb exposure pathways in the aggregate, the 1986 Pb AQCD concluded that "the blood Pb versus air Pb slope β is much smaller at high blood and air levels." This is to say that the slope β was much smaller for occupational exposures where high blood Pb levels (>40 μg/dL) and high air Pb levels (much greater than 10 $\mu g/m^3$) prevailed relative to lower environmental exposures which showed lower blood Pb and air Pb concentrations (<30 μg/dL and <3 $\mu g/m^3$). For those environmental exposures, it was concluded that the relationship between blood Pb and air Pb "for direct

inhalation appears to be approximately linear in the range of normal ambient exposures (0.1-2.0 μg/m^3)" (pp 1–98 of the 1986 Pb AQCD). 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 4-21). This slope corresponds to an increase of 4.6 μg/dL blood Pb per μg/m^3 air Pb at an air Pb concentration of 1.5 μg/m^3 for all groups included in the study (n=96). The 1.5 μg/m^3 value is the median of the air Pb concentrations that match the 96 blood Pb concentrations in Figure 3 of Brunekreef et al. (1984), taken from the Appendix to the same paper. When the analysis was limited to children whose blood Pb concentrations were <20 μg/dL, the slope was 0.2159 (R^2=0.33), which corresponds to an increase of 4.8 μg/dL blood Pb per μg/m^3 air Pb at the median air concentration (0.54 μg/m^3, n=43).

Newer studies that provide estimates for the blood Pb-air Pb slope are described below. In some studies, the blood Pb-air Pb relationship was described with a non-linear regression function, in which the blood Pb-air Pb slope varied with air Pb concentration. In Table 4-11, slopes corresponding to the central estimate of the air Pb concentrations are provided, which are considered to be the best estimate of the slope from each study. These were calculated by evaluating each regression function at ± 0.01 μg/m^3 from the central estimate of the air Pb concentration. Air Pb concentration ranges and central estimates varied across studies, making it difficult to interpret comparisons based solely on the central estimates of the slopes; therefore, Figure 4-21 depicts the relationship between the blood Pb-air Pb slope as a function of air Pb concentration for the range of air Pb concentrations evaluated in each study (the central estimate is also shown). Figure 4-21 provides a more informative picture of the extent to which slope estimates vary (and overlap) within and between studies. In the Schnaas et al. (2004) analysis, the effect of air Pb on blood Pb may have been underestimated due to inclusion of location and SES terms in their regression model. It was specifically noted by the authors that air Pb differed significantly between the locations and the poorer residential areas were usually the more industrialized areas with higher pollution. Hence, the inclusion of these terms may have accounted for some of the variance in blood Pb attributable to air Pb. With the exception of Ranft et al. (2008), all studies included in Table 4-11 and Figure 4-22 include a blood Pb-air Pb relationship that reflects all air-related pathways of exposure. The Ranft et al. (2008)study includes a separate term for soil Pb, so the blood Pb-air Pb slope presented for that study underestimates the slope that would reflect all air-related pathways, since soil Pb encompasses deposited ambient air Pb. 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. 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.

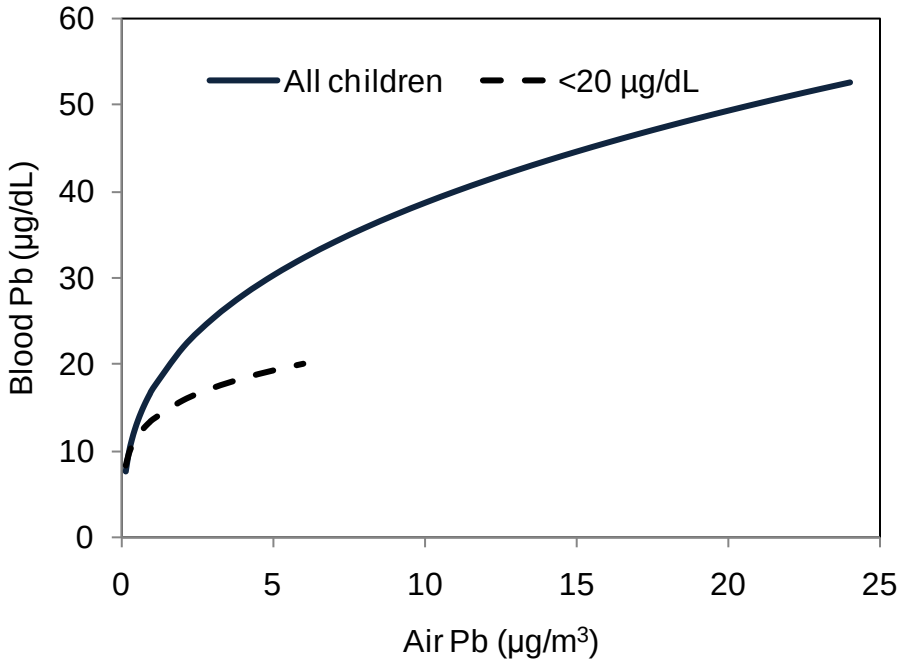
Table 4-11 Summary of estimated slopes for blood Pb to air Pb relationships in humans

Reference	Study Methods	Model Description	Blood Pb–Air Pb Slopea
Children Populations			
Brunekreef et al. (1984)	**Location:** Various countries **Years:** 1974-1983 **Subjects**: Children (varying age ranges; n>190,000) **Analysis:** Meta analysis of 18 studies	**Model:** Log-Log **Blood Pb:** 5-41 µg/dL (mean range for studies) **Air Pb:** 0.1-24 µg/m^3 (mean range for studies)	**All children:** 4.6 (1.5)[c] **Children <20 µg/dL:** 4.8 (0.54)[d]
Hayes et al. (1994)	**Location:** Chicago, IL **Years:** 1974-1988 **Subjects:** 0.5-6 yr (n = 9,604) **Analysis:** Regression of quarterly median blood Pb and quarterly mean air Pb	**Model:** Log-Log **Blood Pb:** 12-30 µg/dL (annual median range) **Air Pb:** 0.05-1.2 µg/m^3 (annual mean range)	8.2 (0.62)[e]
Hilts et al. (2003)	**Location:** Trail, BC **Years:** 1989-2001 **Subjects:** 0.5-6 yr (Estimated n = 220-460, based on 292-536 blood Pb measurements/yr with 75-85% participation) **Analysis:** Regression of blood Pb screening and community air Pb following upgrading of a local smelter	**Model:** Linear **Blood Pb:** 4.7-11.5 µg/dL (annual geometric mean range) **Air Pb:** 0.03-1.1 µg/m^3 (annual geometric mean range)	6.5 (0.48)[f]
Ranft et al. (2008)	**Location:** Germany **Years:** 1983-2000 **Subjects:** 6-11 yr (n = 843) **Analysis:** Pooled regression 5 cross-sectional studies	**Model:** multivariate 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 (0.1)[g]
Schnaas et al. (2004)	**Location:** Mexico City **Years**: 1987-2002 **Subjects**: 0.5-10 yr (n = 321) **Analysis:** Regression of lifetime blood Pb from longitudinal blood Pb measurements and annual average air Pb data	**Model:** Log-Log **Blood Pb:** 5-12 µg/dL (annual GM range) **Air Pb:** 0.07-2.8 µg/m^3 (annual mean range in yr of birth)	2.2 (0.4)[h]
Schwartz and Pitcher (1989), U.S. EPA (1986a)	**Location:** Chicago, IL **Years:** 1976-1980 **Subjects**: Black children, 0-5 yr (n = 5,476) **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)[b]	8.6 (0.75)[i]
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 (0.45)[j]
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)	0.01 (58)[k]

Mixed Child-Adult Populations

Schwartz and Pitcher (1989), U.S. EPA (1986a)	**Location:** U.S. **Years:** 1976-1980 **Subjects**: 0.5-74 yr (n = 9,987) **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 (0.75)[l]

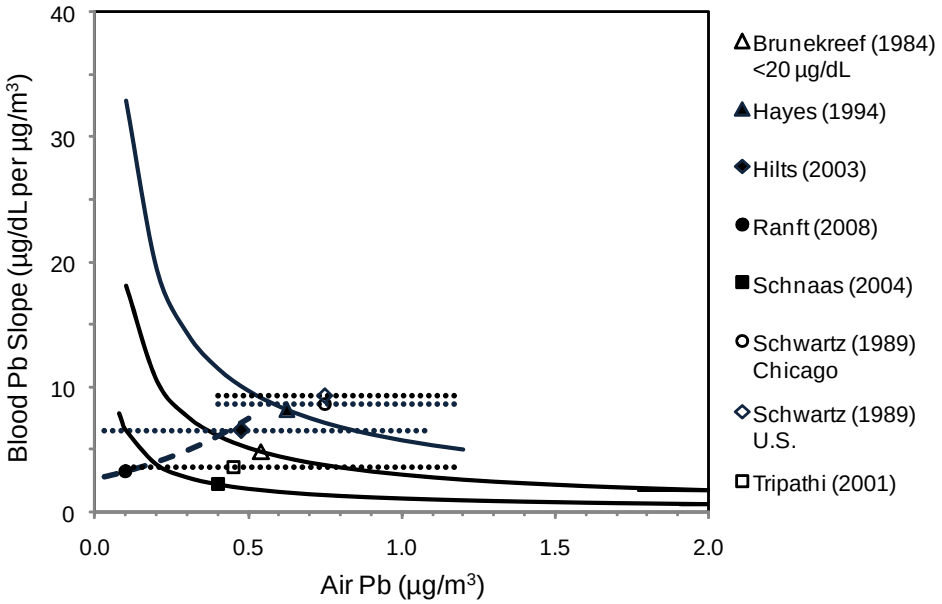
[a]Slope is predicted change in blood Pb (µg/dL per µg/m3) evaluated at ± 0.01 µg/m3 from central estimate of air Pb for the study (shown in parentheses)
[b]Based on data for U.S. (1986 Pb AQCD)
[c] ln(PbB) = ln(PbA) x 0.3485 +2.853
[d] ln(PbB) = ln(PbA) x 0.2159 + 2.620
[e] ln(PbB) = ln(PbA) x 0.24 +3.17
[f] PbB = PbA x 6.5
[g] PbB =1.5 x EXP(0.9361 x (PbA-0.1)/0.44), where 1.5 µg/dL is the background PbB, and 0.1 µg/m^3 is the median PbA for the study; model also adjusted for soil Pb concentration, which may reduce estimated slope
[h] ln(PbB) = Ln(PbA) x 0.213 +1.615 for the 1987 cohort, see text for more study details.
[i] PbB = PbA x 8.6
[j] PbB =Pb A x 3.6
[k] ln(PbB) = ln(PbA) x 0.05 +2.12
[l] PbB = PbA x 9.63
GM, geometric mean; GSD, geometric standard deviation; PbB, blood Pb concentration (µg/dL); PbA, air lead concentration (µg/m^3)



Data provided from Brunekreef (1984).

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

Figure 4-21 Predicted relationship between air Pb and blood Pb based on a meta analysis of 18 studies.



Note: Slopes are calculated for a change in air Pb (±0.01 µg/m^3) over ranges of air Pb concentrations reported in each study (lines). The air Pb axis is truncated at 2 µg/m^3; the actual range for the Brunkreefe et al. (1984) study was 0.1-6.4 µg/dL per µg/m^3 and for the Schnaas et al. (2004) study was 0.08-2.8 µg/dL per µg/m^3. The slope axis has been truncated at 40; the actual range for the Hayes et al. (1994) study was 5-56 µg/dL per µg/m^3 (the high end of the range was estimated for the minimum annual average air Pb of 0.05 µg/m^3). The two estimates for Schwartz and Pitcher (1989) represent data for U.S. and Chicago. Models are log-log (solid lines), log-linear (dashed line), and linear (dotted lines). Symbols show the slope at the central estimate of air Pb (e.g., mean or median reported for each study).

Figure 4-22 Blood Pb – air Pb slope (µg/dL per µg/m^3) predicted from various epidemiologic studies (links available in Table 4-11).

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 who lived within 4 km from the smelter (n: 292-536 eligible per year, 75-85% participation). Air Pb concentrations were obtained from high volume suspended particulate samplers placed within 2 km of the smelter that operated 24 hours every 6th day. Data on Pb levels in air, residential soil, interior dust, and blood for three sampling periods are summarized in Table 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 factors include aggressive public education and exposure intervention programs (Hilts et al., 1998; Hilts, 1996). 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/m3)
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	0.069 ± 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 geometric means; 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 (from the year 2000) yielding a value of 1.63 per 800 mg/kg increase in soil Pb. The GMR values can be converted to regression slopes (slope = [starting blood Pb×ln(GMR)]/[95th − 5th percentile air or soil Pb]) for calculating equivalent air:blood Pb ratios. The model predicts an increase of 3.2 µg/dL blood Pb per 1 µg/m^3 increase in air Pb at the median air Pb concentration for the study (0.1 µg/m^3) and assuming a background blood Pb concentration of 1.5 µg/dL. Based on the GMR estimate of 1.63 for soil Pb, a 1,000 mg/kg increase in soil Pb would be associated with an increase in blood Pb of 0.9 µg/dL per mg/kg soil at the median soil Pb concentration of 206 mg/kg and assuming a background blood Pb concentration of 1.5 µg/dL. The degree of confounding of the GMR and estimates resulting from the air and soil Pb correlation was not reported, although the correlation coefficient for the two variables was 0.136 for the whole data set and 0.703 when data collected in 1983 was omitted. Because the model also included Pb levels in soil, the blood Pb-air Pb ratio may be underestimated since some of the Pb in soil was likely derived from air. The blood Pb-air Pb slope does not include the portion of the soil/dust Pb ingestion pathway that derives from air Pb, such as recently airborne Pb deposited to soil and dust which remains available for inhalation and ingestion. To estimate the blood Pb-air Pb ratio that included all air-related pathways, data for geometric mean of blood Pb and air Pb among the cohort of children studied were extracted from Figure 1 in Ranft et al. (2008) for each of the five study years. The extracted values of the geometric mean of blood Pb and air Pb were used in regressions employing linear and log-log fits. The linear model obtained was: PbB = (13.65×PbA) + 2.96 (R^2 = 0.92); i.e., the linear regression produced a constant slope of 14 µg/dL per µg/m^3. The log-log model was: ln(PbB) = (0.48×ln(PbA) + 2.61 (R^2 = 0.91), resulting in an inverse curve for dPbB/dPbA vs. PbA with a slope of 22 µg/dL per µg/m^3 at PbA = 0.1 µg/m^3.

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 their year of birth and appropriate air monitoring zones. Associations between lifetime (across the first 10 years of life) blood Pb concentration, air Pb concentration for year of birth and other variables (e.g., age, year of birth, family use of glazed pottery) were evaluated using multivariate regression models. 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 2.2 µg/dL blood Pb per µg/m^3 at the median annual air Pb concentration of 0.4 µg/m^3 estimated over the years of the study. Slopes for other birth cohorts ranged from -0.003 (1992) to 0.166 (1988) with a median of 0.153 for all six cohorts. This median slope corresponds to an increase of 1.7 µg/dL blood Pb per µg/m^3 at the median annual air Pb concentration of 0.4 µg/m^3 estimated over the years of the study. Considering all cohorts simultaneously, data for annual geometric mean of blood Pb and air Pb were extracted from Figure 1 in Schnaas et al. (2004). However, in employing this approach, blood Pb is confounded by age and year because in the early years of the study, only younger children were available and in the later years of the study, only older children contributed data. The extracted values of the geometric mean of blood Pb and mean air Pb were used in regressions employing linear and log-log models for comparison to other studies and a log-linear model as employeed by the authors. The linear model obtained was: PbB = (2.50×PbA) + 5.61 (R^2 = 0.84), i.e., the linear model produced a constant slope of 2.50 µg/dL per µg/m^3. However, inspection of the graph (not shown here) suggested a bi-linear fit. Regression of the data over the interval 0.1-0.4 µg/m^3 produced a slope of 9.0 µg/dL per µg/m^3 (R^2 = 0.83), and regression of the data over the interval 0.4-2.8 µg/m^3 produced a slope of 1.52 µg/dL per µg/m^3 (R^2 = 0.83). The log-log model was: ln(PbB) = (0.26×ln(PbA)) + 2.20 (R^2 = 0.94), resulting in an inverse curve for dPbB/dPbA vs. PbA, with a slope of 4.5 µg/dL per µg/m^3 at PbA = 0.4 µg/m^3. The log-linear model as employed by the authors was: ln(PbB) = (0.32×PbA) + 1.73 (R^2 = 0.77); and described the data least well. The log-linear model produced an exponential curve of dPbB/dPbA vs. PbA, with a slope of 2.04 µg/dL per µg/m^3 at PbA = 0.4 µg/m^3.

Schwartz and Pitcher (1989) reported a multivariate regression analysis of associations between U.S. gasoline Pb consumption (i.e., sales) and blood Pb concentrations in the U.S. population during the period 1976-1980 when use of Pb in gasoline was being phased out. Although this analysis did not directly derive a slope for the air Pb-blood Pb relationships, other analyses have shown a strong correlation between U.S. gasoline Pb consumption and ambient air Pb levels during this same period (U.S. EPA, 1986a). Therefore, it is possible to infer an air Pb-blood Pb relationship from these data. Two sources of blood Pb data were used in Schwartz and Pitcher (1989): NHANES II provided measurements for U.S. children 6 months to 74 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 (the value cited in Schwartz and Pitcher (1989) is 1.97 µg/dL blood per 100 metric tons of gasoline Pb/day). U.S. EPA (1986a) 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 8.6 µg/dL per µg/m^3, based on the NHANES and Chicago data, respectively (e.g., 2.14/0.23 = 9.3 and 1.97/0.23=8.6).

Table 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 (1986a).

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.

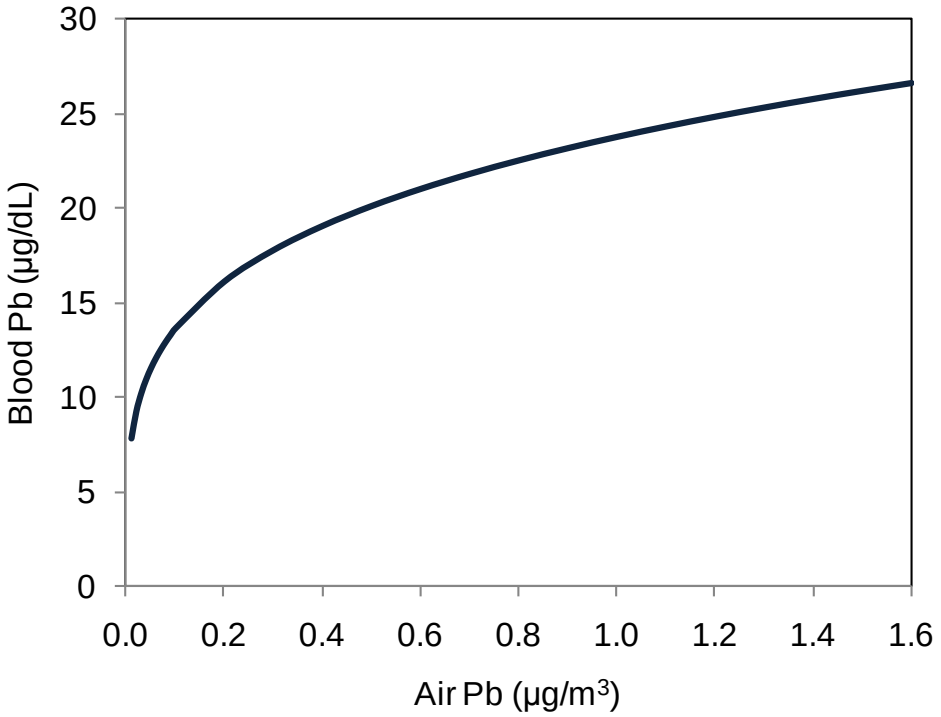
Table 4-14 Air Pb levels and blood Pb levels in children in Mumbai, India

	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 4-23. This slope corresponds to an increase of 8.2 µg/dL blood Pb per µg/m^3 at the average annual mean air Pb concentration of 0.62 µg/m^3.



Modified from Hayes et al. (1994).

Note: The regression model is: (ln[µg/dL blood Pb] = 0.24·ln[µg/m^3 air Pb] + 3.17).

Figure 4-23 Predicted relationship between air Pb and blood Pb based on data from Chicago, IL in children age 0-5 y (1974–1988).

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. These Pb concentrations were reported to have been 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 slope corresponds to a 1.3-fold increase in blood Pb concentration for a 10-fold increase in air Pb concentration and a slope of 0.009 µg/dL per µg/m^3, at the average occupational air Pb concentration for the study (58 µg/m^3); non-occupational exposures were not included in the slope calculation. 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 and a slope of 0.02 µg/dL per µg/m^3, at the average occupational air Pb concentration for the study (58 µg/m^3).

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) Reference	0.14	0.15 (0.10)	0.14
Yes			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 (Maddaloni et al., 2005; U.S. EPA, 2003c; Abadin and Wheeler, 1997; Stern, 1996; Bowers et al., 1994; Stern, 1994; Carlisle and Wade, 1992). In slope factor models, Pb biokinetics are represented as a linear function between the blood Pb concentration and either Pb uptake (uptake slope factor, USF) or Pb intake (intake slope factor, ISF). The models take the general mathematical forms:

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

Equation 4-2

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

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

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

Dixon et al. (2009) reported a multivariate analysis of associations between environmental Pb concentrations and blood Pb concentrations, based on data collected in the NHANES (1999-2004). The analyses included 2,155 children, age 12-60 months. The population-weighted geometric mean blood Pb concentration was 2.03 µg/dL (GSD 1.03). A linear model applied to these data yielded an R^2 of 40% (Table 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

Variables	Overall p-value	Levelsa	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
		Age 2	–1.353 (0.354)	< 0.001
		Age 3	0.273 (0.083)	0.002
		Age 4	–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
		U.S.[b]	0.000	—
		Mexico	0.353 (0.097)	< 0.001
		Elsewhere	0.154 (0.121	0.209
Floor surface/condition × log floor PbD	< 0.001	Intercept for missing	0.178 (0.094)	0.065
		Not smooth and cleanable	0.386 (0.089)	< 0.001
		Smooth and cleanable or carpeted	0.205 (0.032)	< 0.001
Floor surface/condition × log floor PbD)^2		Not smooth and cleanable	0.023 (0.015)	0.124
		Smooth and cleanable or carpeted	0.027 (0.008)	0.001
Floor surface/condition × (log floor PbD)^3		Uncarpeted not smooth and cleanable	–0.020 (0.014)	0.159
		Smooth and cleanable or carpeted	–0.009 (0.004)	0.012
Log windowsill PbD	0.002	Intercept for missing	0.053 (0.040	0.186
		Slope	0.041 (0.011	< 0.001
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	—

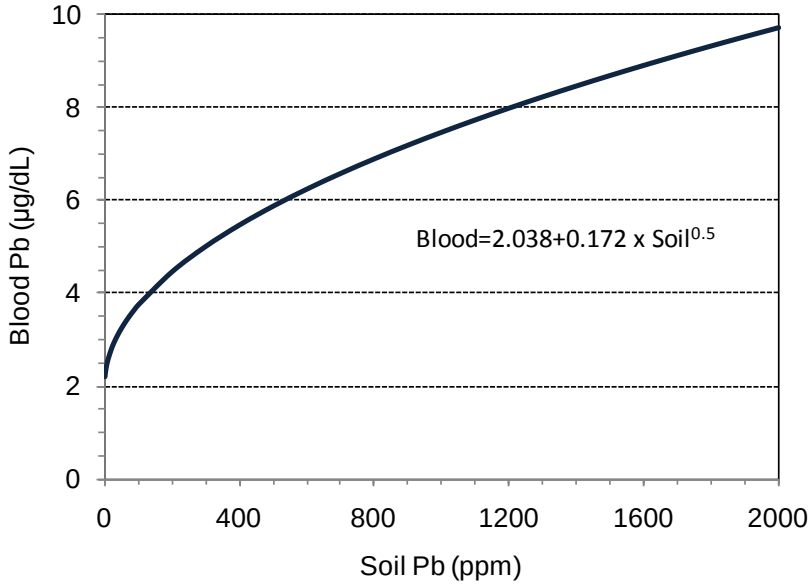
[a]Children: n = 2,155 (age 10-60 months); R^2 = 40%
[b]Includes the 50 states and the District of Columbia
Source: Dixon et al. (2009).

Mielke et al. (2007a) analyzed blood Pb and soil Pb concentration data collected as part of a blood Pb screening program in New Orleans (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:

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

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 1,000 ppm (Figure 4-24).



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

Figure 4-24 Predicted relationship between soil Pb concentration and blood Pb concentration in children based on data collected in the New

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

Several studies have linked elevated blood Pb levels to residential soil exposures for populations living nearby industrial or mining facilities. Gulson et al. (2009) studied the

blood Pb and isotopic Pb ratios of children younger than 5 years old and adults older than 18 years old living in the vicinity of a mine producing Magellan Pb ore in western Australia. They observed a median blood Pb level of 6.6 µg/dL for the children, with isotopic ratios indicating contributions from the mine ranging from 27 to 93%. A weak but significant linear association between blood Pb level and percent Magellan Pb was observed ($R^2 = 0.12$, $p = 0.018$). Among children with blood Pb levels over 9 µg/dL and among adults, the isotopic ratios revealed Pb exposures from a variety of sources. Garavan et al. (2008) measured soil Pb and blood Pb levels among children aged 1 month to 17.7 years old in an Irish town near a coal mine. The blood Pb measurements were instituted as part of a screening and community education program given that the presence of Pb had been documented in the environment. Garavan et al. (2008) found that over 3 years of the screening period, median blood Pb levels reduced by roughly 22% from 2.7 to 2.1 µg/dL.

An extensive discussion of the relationships between environmental Pb levels and blood Pb concentrations in children at the Bunker Hill Superfund Site, a former Pb mining and smelting site, was provided in the 2006 Pb 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. Note that the soil Pb component of the model was similar to that derived by Lewin et al. (1999), in which a model of blood Pb as a function of soil Pb among 0-6 y old children living near one of four industrial sites was given as PbB = 0.2438ln(PbS} + 0.2758.

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). Biokinetic models include parameters that represent processes of Pb transfer (a) from portals of entry into the body and (b) from blood to tissues and excreta. Linked together, exposure-intake and intake-biokinetics models (i.e., integrated exposure-intake-biokinetics models) provide an approach for predicting blood Pb concentrations (or Pb concentrations in other tissues) that corresponds to a specified exposure (medium, concentration, and duration). Detailed information on exposure and internal dose can be obtained from controlled experiments, but almost never from epidemiological observations or from public health monitoring programs. Exposure intake-biokinetics models can provide these predictions in the absence of complete information on the exposure history and blood Pb concentrations for an individual (or population) of interest. Therefore, these models are critical to applying epidemiologic-based information on blood Pb-response relationships to the quantification and characterization of human health risk. 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 (O'Flaherty, 1995; Leggett, 1993;

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

4.7 Summary and Conclusions

4.7.1 Exposure

Exposure data considered in this assessment build upon the conclusions of the 2006 Pb AQCD (2006b), 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. The atmosphere is the main environmental transport pathway for Pb, and, on a global scale, atmospheric Pb is primarily associated with fine particulate matter, which can deposit to soil and water. In addition to primary emission of particle-bearing or gaseous Pb to the atmosphere, Pb can be suspended to the air from soil or dust. Air-related pathways of Pb exposure are the focus of this assessment. In addition to inhalation of Pb from ambient air, air-related Pb exposure pathways include inhalation and ingestion of Pb from indoor dust and/or outdoor soil that originated from recent or historic ambient air (e.g., air Pb that has penetrated into the residence either via the air or tracking of soil). Non-air-related Pb exposures may include occupational exposures, hand-to-mouth contact with Pb-containing consumer goods, hand-to-mouth contact with dust or chips of peeling Pb-containing paint, or ingestion of Pb in drinking water conveyed through Pb pipes. Pb can cycle through multiple media prior to human exposure. Given the multitude of possible air-related exposure scenarios and the related difficulty of constructing Pb exposure histories, most studies of Pb exposure through air, water, and soil can be informative to this review. Other exposures, such as occupational exposures, contact with consumer goods in which Pb has been used, or ingestion of Pb in drinking water conveyed through Pb pipes may also contribute to Pb body burden.

A number of monitoring and modeling techniques have been employed for ambient Pb exposure assessment. Environmental Pb concentration data can be collected from ambient air Pb monitors, soil Pb samples, dust Pb samples, and dietary Pb samples to estimate human exposure. Exposure estimation error depends in part on the collection efficiency of these methods; collection efficiency for ambient air Pb FRM samplers is described in Section 3.4. Additionally, high spatial variability of the Pb concentrations in various media also can contribute to exposure error, as described in the 2009 PM ISA (U.S. EPA, 2009). Models, such as the Integrated Exposure Uptake Biokinetic (IEUBK)

model, simulate human exposure to Pb from multiple sources and through various routes including inhalation, ingestion, and dermal exposure. IEUBK model inputs include soil Pb concentration, air Pb concentration, dietary Pb intake including drinking water, Pb dust ingestion, human activity, and biokinetic factors. Measurements and/or assumptions can be utilized when formulating the model inputs; errors in measurements and assumptions thus have the potential to propagate through the exposure models.

Section 4.1 presents data illustrating potential exposure pathways. Soil can act as a reservoir for deposited Pb emissions, and exposure to soil contaminated with deposited Pb can occur through resuspended PM as well as shoe tracking and hand-to-mouth contact, which is the main pathway of childhood exposure to Pb. In general, soil Pb concentrations tended to be higher within inner-city communities compared with neighborhoods surrounding the city. Recent data by Yamamoto et al. (2006) have shown that the size distribution of particles collected on children's hands have a mode around 40 µm with the upper tail of the distribution extending to 200-300 µm. Infiltration of Pb dust into indoor environments 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 measurements for Pb concentration are typically higher than indoor or outdoor ambient Pb concentrations. These findings may be related to local resuspension with body movement.

4.7.2 Toxicokinetics

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

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

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

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

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

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

4.7.3 Lead Biomarkers

Overall, trends in blood Pb levels have been decreasing among U.S. children and adults over the past 20 years (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.

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

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

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

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

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 1986 Pb AQCD was drawn from a meta-analysis by Brunekreef (1984). Based on the studies available at that time that considered multiple air-related Pb exposure pathways in the aggregate, the 1986 Pb AQCD concluded that "the blood Pb versus air Pb slope β is much smaller at high blood and air levels." This is to say that the slope β was much smaller for occupational exposures where high blood Pb levels (>40 μg/dL) and high air Pb levels (much greater than 10 $\mu g/m^3$) prevailed relative to lower environmental exposures which showed lower blood Pb and air Pb concentrations (<30 μg/dL and <3 $\mu g/m^3$). For those environmental exposures, it was concluded that the relationship between blood Pb and air Pb "…for direct inhalation appears to be approximately linear in the range of normal ambient exposures (0.1-2.0 $\mu g/m^3$)" (pp 1-98 of the 1986 Pb AQCD). In addition to the meta-analysis of Brunekreef (1984), more recent studies have provided data from which estimates of the blood Pb-air Pb slope can be derived for children (Table 2-7). The range of estimates from these studies is 2-9 μg/dL per $\mu g/m^3$, which encompasses the estimate from the Brunekreef (1984) meta-analysis of (3-6 μg/dL per $\mu g/m^3$). Most studies have described the blood Pb-air Pb relationship as either log-log (Schnaas et al., 2004; Hayes et al., 1994; Brunekreef, 1984), which predicts an increase in the blood Pb-air Pb slope with decreasing air Pb concentration or linear (Hilts, 2003; Tripathi et al., 2001; Schwartz and Pitcher, 1989), which predicts a constant blood Pb-air Pb slope across all air Pb concentrations. These differences may simply reflect model selection by the investigators; alternative models are not reported in these studies. The blood Pb-air Pb slope may also be affected in some studies by the inclusion of parameters (e.g., soil Pb) that may account for some of the variance in blood Pb attributable to air Pb. Other factors that likely contribute to the derived blood Pb-air Pb slope include differences in the populations examined and Pb sources, which varied among individual studies.

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CHAPTER 5 INTEGRATED HEALTH EFFECTS OF LEAD EXPOSURE

5.1 Introduction

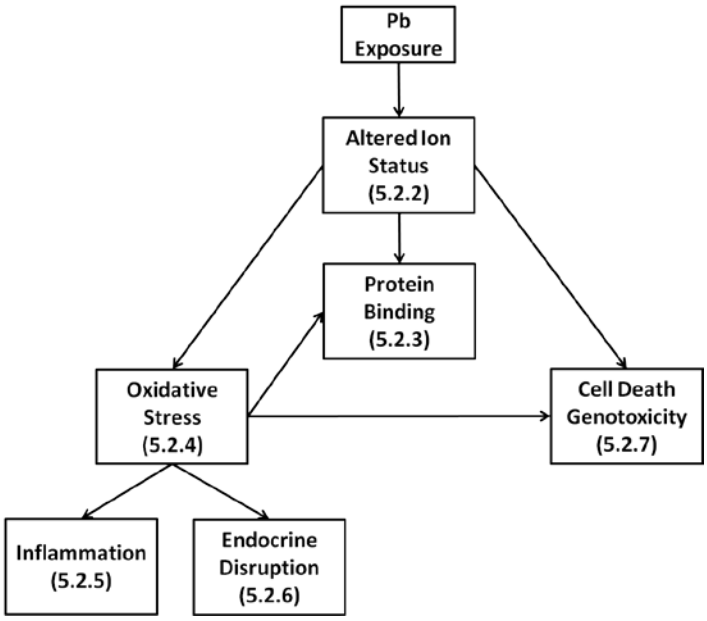
This chapter reviews, summarizes, and integrates the evidence for the broad spectrum of health effects associated with exposure to Pb. The chapter begins (Section 5.2) with a discussion of the evidence for the modes of action that mediate the health effects of Pb, including those modes of action that are shared by all of the health effects evaluated in this ISA and those modes of action that are specific for particular endpoints. Subsequent sections comprise evaluations of the epidemiologic and toxicological evidence for the health effects of Pb exposure on major outcome categories such as nervous system 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 and developmental effects (Section 5.8). Section 5.9 provides reviews of the evidence for the effects of Pb on other noncancer health outcomes, for which the cumulative bodies of evidence are smaller, including those related to the hepatic system (Section 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 outcome categories (e.g., nervous system, cardiovascular, renal) begin with a brief summary of conclusions from the 2006 Pb AQCD followed by 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). Sections for each of the major outcome categories (e.g., nervous system, cardiovascular, renal effects) conclude with an integrated summary of the evaluation of evidence and a conclusion regarding causality. Based upon the framework (described in the Preamble), a determination of causality was made for a broad outcome category (i.e., nervous system effects) by evaluating the coherence of evidence across disciplines and across a spectrum of related endpoints. Each discussion leading up to the causal determination characterizes the evidence on which the causal judgment was based, including the strength of evidence for the individual endpoints within the major outcome category.

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 affected. A mode of action (MOA) is the common set of biochemical, physiological, or behavioral responses (i.e., empirically observable precursor steps) that can cumulatively result in the formation of negative health outcomes. Although the effects of Pb 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). This section draws information from all of the subsequent health effects sections in Chapter 5, and identifies the major modes of action operating at the molecular, cellular, and tissue/organ level. In turn, the individual health effect sections bridge these effects to those observed on the organismal level. Each of the individual health effect sections includes a more detailed description of the mechanisms specific to the individual health effect. Accordingly, this section differs in structure and content from other health effects sections as it does not primarily focus on the literature published since the 2006 Pb AQCD, but rather incorporates recent information with older studies (which represent the current state of the science) on the possible modes of action of Pb.



Note: The subsections 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 health 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 negative actions, ultimately interfering with 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 (Ca^{2+}) is one of the most important carriers of cell signals and regulates virtually all aspects of cell function, including energy metabolism, signal transduction, hormonal regulation, cellular motility, and apoptosis (Carafoli, 2005). Ca^{2+} homeostasis is maintained through a tightly regulated balance of cellular transport and intracellular storage (Pentyala et al., 2010). Disruption of Ca^{2+} homeostasis by Pb has been observed in a number of different cell types and cell-free environments, indicating that this is a major mode of action for Pb-induced toxicity on a cellular level.

Ca^{2+} homeostasis is particularly important in bone cells, as the skeletal system serves as the major dynamic reservoir of Ca^{2+} in the body (Wiemann et al., 1999; Long et al., 1992). Bone cells also are unique in that they can 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., 1997; Schanne et al., 1989). 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 the ability of bone cells 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) plus Pb, versus Pb alone (Long and Rosen, 1992). Pb-induced increases in $[Ca^{2+}]_i$ were blocked by a protein kinase C (PKC) inhibitor, indicating that PKC activation may serve as 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 influx of Ca^{2+} independently of any inhibitory effect on Ca^{2+}-ATPases.

Ca^{2+} homeostasis has also been shown to be disturbed in erythrocytes exposed to Pb (Quintanar-Escorza et al., 2010; Quintanar-Escorza et al., 2007; Shin et al., 2007). In blood samples taken from Pb-exposed workers (mean [SD] blood Pb level: 74.4 [21.9] µg/dL), the $[Ca^{2+}]_i$ was approximately 2.5-fold higher than that seen in nonexposed workers (mean [SD] blood Pb level: 9.9 [2] µg/dL) (Quintanar-Escorza et al., 2007). The increase in $[Ca^{2+}]_i$ was associated with higher osmotic fragility and modifications in erythrocyte shape. In a separate investigation, erythrocytes from 10 healthy volunteers were exposed to Pb at concentrations of 0.2 to 6.0 µM for 24 or 120 hours, concentration-related increases in $[Ca^{2+}]_i$ were observed across all concentrations for both durations of exposure (Quintanar-Escorza et al., 2010). Subsequent exposures of erythrocytes to either 0.4 or 4.0 µM Pb [corresponding to 10 or 80 µg/dL in exposed workers (Quintanar-Escorza et al., 2007)] for 12-120 hours resulted in duration-related increases with durations >12 hours. Osmotic fragility (measured as percent hemolysis) was increased in erythrocytes exposed to 0.4 µM Pb for 24 hours. Co-incubation with a vitamin E analog mitigated these effects, indicating that the increase in $[Ca^{2+}]_i$ is dependent on the oxidative state of the erythrocytes. Shin et al. (2007) observed that incubation of human erythrocytes with 5 µM Pb for 1 hour resulted in a 30-fold increase in $[Ca^{2+}]_i$ in vitro,

inducing the pro-coagulant activity of exposed erythrocytes. Induction of pro-coagulant activity in erythrocytes could lead to thrombus formation and negatively contribute to overall cardiovascular health, whereas increased osmotic fragility could substantially reduce erythrocyte life span and ultimately lead to anemic conditions.

Similar to effects seen in erythrocytes, Pb has been observed to interfere with Ca^{2+} homeostasis in platelets and white blood cells. Dowd and Gupta (1991) observed that 1 µM Pb (for 3.5 hours) was the lowest exposure concentration to result in increases in $[Ca^{2+}]_i$ in human platelets. The observed increase in Ca^{2+} levels was attributed to the increased influx of external Ca^{2+}, possibly through receptor-operated Ca^{2+} channels. In mouse splenic lymphocytes, 1 µM Pb was the lowest exposure concentration found to increase $[Ca^{2+}]_{i,}$ with incubation periods of 10 minutes or greater (Li et al., 2008c). These increases in Ca^{2+} 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, possibly 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 0.1 µM Pb for 1-48 hours, although the observed decreases were not time-dependent. The decrease in $[Ca^{2+}]_i$ was shown to be due to increased efflux of Ca^{2+} out of the neuron via a calmodulin-regulated mechanism, possibly through stimulated Ca^{2+} efflux via Ca^{2+}-ATPase.

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 in the life cycle of most cells, cytosolic concentrations of free Ca^{2+} fluctuate around 100-200 nM and Ca^{2+} that has entered the cell must be removed in order to maintain normal homeostatic concentrations (Carafoli, 2005) . An important component 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 (mean [SD] blood Pb level: 55.6 [6.3] µg/dL) (Kharoubi et al., 2008a) and in erythrocytes of rats exposed to 0.2% Pb in drinking water for 5 weeks (mean [SD] blood Pb level: 97.56 [11.8] µg/dL) (Sivaprasad et al., 2003). Increases in lipid peroxidation were seen in both studies, and the decrements in ATPase activities may be explained by generation of free radicals in Pb-exposed animals. A decrease in the activity of Na^{+}-K^{+}-ATPase was observed in rabbit kidney membranes exposed to 0.01 to 10 µM Pb, possibly due to Pb inhibiting the hydrolytic cleavage of phosphorylated intermediates in the K-related branch of the pump (Gramigni et al., 2009). Similar decreases in Na^{+}-K^{+}-ATPase activity were observed in synaptosomes isolated from rats exposed to 200 ppm Pb in drinking water for 3 months (blood Pb level: 37.8 µg/dL) (Rafalowska et al., 1996) or 15 mg/kg Pb injected (i.p.) for 7 days (blood Pb level: 112.5 µg/dL) (Struzynska et al., 1997a). Inhibition of Na^{+}-K^{+}-ATPase activity was also observed in primary cerebellar granule neuronal cultures obtained from rats pre- and postnatally exposed to Pb (0.1 % Pb-acetate in dams drinking water, resulting in blood Pb level of 4 µg/dL) (Baranowska-Bosiacka et al., 2011a). The activity of Ca^{2+}-ATPase in the sarcoplasmic reticulum of rabbits exposed to 0.01 µM Pb was similarly decreased (Hechtenberg and Beyersmann, 1991). The inhibitory effect of Pb was diminished in the presence of high MgATP concentrations. The activity of generic ATPase was reported to be altered in the testes of rats exposed to 300 ppm Pb-acetate gestationally, and in drinking water after weaning to the age of 6, 8, 10, or 12 weeks (Liu et al., 2008). In pregnant rats fed a Pb-depleted (20 ± 5 µg/kg) or control (1 mg/kg) diet during gestation and lactation, no difference was observed in the activity of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase in the parental generation (Eder et al., 1990). However, the offspring (exposed via placental and lactational transfer of Pb) of Pb-depleted rats displayed decreased activities in both enzymes compared with offspring of rats with higher Pb exposures. A similar increase in the Na^{+}-K^{+}-ATPase activity was observed in rats exposed (i.p.) to 20 mg/kg Pb for 14 consecutive days (Jehan and Motlag, 1995). Co-exposure of Pb with zinc and copper greatly attenuated the increase in ATPase activity. Although the precise mechanism was not investigated, Navarro-Moreno et al. (2009) reported that Ca^{2+} uptake was diminished in proximal renal tubule cells in rats chronically exposed to 500 ppm Pb in drinking water for 7 months (mean [SD] blood Pb level: 43.0 [7.6] µg/dL).

In vitro studies of ATPase activities in human erythrocyte ghosts have also shown that Pb affects the transport of metal ions across membranes. Calderon-Salinas et al. (1999b) observed that 1-5x10^4 µM Pb and Ca^{2+} were capable of inhibiting the passive transport of each other in human erythrocyte ghosts incubated with both cations. Subsequent inhibition experiments indicated that both cations share the same electrogenic transport pathway (Sakuma et al., 1984). Further study by this group (Calderon-Salinas et al., 1999a) demonstrated that Pb can noncompetitively block the transport of Ca^{2+} by inhibiting the activity of Ca^{2+}-Mg^{2+}-ATPase at concentrations of 1-5x10^3 µM. Mas-Oliva (1989) demonstrated that the activity of Ca^{2+}-Mg^{2+}-ATPase in human erythrocyte ghosts was inhibited by incubation with 0.1-100 µM Pb. The inhibitory action was most likely due to direct reaction with sulfhydryl groups on the ATPase at Pb concentrations greater than 1 µM, but due to the action of Pb on calmodulin at lower concentrations. Grabowska and Guminska (1996) observed that 10 µg/dL Pb was the lowest concentration to decrease the activity of Na^+-K^+-ATPase in erythrocyte ghosts; activity of Ca^{2+}- Mg^{2+}-ATPase was less sensitive to Pb exposure, and Mg^{2+}-ATPase activity was not affected.

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 (mean [SD] blood Pb level: 12.34 [2.44] in males and 16.85 [6.01] µg/dL in females). Higher membrane concentrations of Ca^{2+} and 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. In a study of 247 mother-newborn pairs, Campagna et al. (2000) observed that newborn (cord) blood Pb (geometric mean [5th, 95th percentile]: 4.8 [2.8-9.2] µg/dL) was negatively and significantly associated with maternal blood Ca^{2+} pump activities; however, newborn (cord) blood Pb was not significantly associated with cord blood Ca^{2+} pump activities. Newborn hair Pb (geometric mean [5th, 95th percentile]: 1.1 [0.1-8.0] µg/g) was negatively and significantly associated with both maternal and cord blood Ca^{2+} pump activities. In a population of 81 newborns, Huel et al. (2008) found that newborn hair and cord blood Pb levels (mean [SD] newborn hair Pb and blood Pb levels: 1.22 [1.41] µg/g and 3.54 [1.72] µg/dL) were negatively associated with Ca^{2+}-ATPase activity in plasma membranes of erythrocytes isolated from cord blood; newborn hair Pb levels were more strongly associated with cord Ca^{2+} pump activity than were cord blood Pb levels.

Pb has also been shown to disrupt cation transport mechanisms through direct action on voltage-sensitive cation channels. Audesirk and Audesirk (1993, 1991) 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) corroborated 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 200 µM, possibly through blocking the intracellular Ca^{2+} binding domain, or through Ca^{2+} dependent dephosphorylation of the channel (Sun and Suszkiw, 1995). Recently, Pb has also been shown to enter cells (HEK293, HeLa, and PC12 cell lines) through store-operated Ca^{2+} channels (Chiu et al., 2009; Chang et al., 2008b). In particular, the Orai1-STIM1 complex was shown to be critical in the entry of Pb ions into cells, and increased Pb permeation was directly related to decreased $[Ca^{2+}]_i$ concentrations at exposure concentrations as low as 0.1 µM.

Pb also 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 concentration-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 channel 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 concentration-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 and Manalis, 1984; Suszkiw et al., 1984). However, in these same experiments, concentrations of Pb as

5 µM and higher were also observed to actually increase the spontaneous release of neurotransmitters. Subsequent research by other groups affirmed that Pb demonstrates Ca^{2+}-mimetic properties in enhancing neurotransmitter release from cells in the absence of Ca^{2+} and Ca^{2+}-induced depolarization. Tomsig and Suszkiw (1995, 1993) reported that Pb exposure induced the release of norepinephrine (NE) from bovine adrenal chromaffin cells, and was considerably more potent at doing so than was Ca^{2+} ($K_{0.5}$ of $4.6x10^3$ µM for Pb versus 2.4 µM for Ca^{2+}). Activation of PKC was observed to enhance the Pb-induced release of NE. Westerrink and Vijverberg (2002) observed that Pb acted as a high affinity substitute for Ca^{2+}, and triggered enhanced catecholamine release from PC12 cells at 10 µM in intact cells and $3x10^4$ µM 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 gestational day (GD)15-16 and continuing to 120 days postnatal, decreases in total potassium-stimulated hippocampal GABA release were seen at exposure levels of 0.1-0.5% (range of mean [SD] blood Pb levels: 26.8 [1.3] - 61.8 [2.9] µg/dL) (Lasley and Gilbert, 2002). Maximal effects were observed at 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 GABA release was increased with 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 and Gilbert, 2002). Suszkiw (2004) reports that augmentation of spontaneous release of neurotransmitters may involve Pb-induced activation of CaMKII-dependent phosphorylation of synapsin I or direct activation of synaptotagmin I. Further, Suszkiw (2004) suggests that unlike the intracellularly mediated effects of Pb on spontaneous release of neurotransmitters, Pb-induced inhibition of evoked transmitter releases is largely due to extracellular blockage of the voltage-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 and Yang, 2008). Metal binding sites on proteins are generally ion-specific and are influenced by multiple factors, including binding geometries, ligand preferences, ionic radius, and metal coordination numbers (Kirberger and Yang, 2008; Garza et al., 2006). The coordination chemistry that normally regulates metal-protein binding makes

many proteins particularly susceptible to perturbation from Pb, as it is able to function with flexible coordination numbers and can bind multiple ligands (Kirberger and Yang, 2008; Garza et al., 2006). However, due to differences in its physical properties, Pb induces abnormal conformational changes when it binds to proteins (Kirberger and Yang, 2008; Bitto et al., 2006; Garza et al., 2006; Magyar et al., 2005), and these structural changes elicit altered protein function. It is known that $[Ca^{2+}]_i$ is an important second messenger in cell signaling pathways, and operates by binding directly to and activating proteins such as calmodulin and PKC (Goldstein, 1993). Alterations in the functions of both of these proteins due to direct interaction with Pb have been well documented in the literature.

PKC is a family of serine/threonine protein kinases critical for cell signaling and important for cellular processes, including growth and differentiation (Goldstein, 1993). PKC contains a C2 Ca^{2+}-binding domain and requires 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 was Ca^{2+}-dependent activation by five orders of magnitude. Long et al. (1994) affirmed these findings, reporting that Pb had a K_{act} 4,800 times smaller than that of Ca^{2+} ($5.5x10^{-5}$ µM versus 25 µM, following a 3 minute exposure). However, Ca^{2+} had a higher maximal activation of PKC than did Pb. This possibly indicates the presence of multiple Ca^{2+}-binding sites on the protein, and that Pb may bind the first site more efficiently than does Ca^{2+}, but not subsequent sites. Tomsig and Suszkiw (1995) further demonstrated the ability of Pb to activate PKC 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 0.1 µM (Belloni-Olivi et al., 1996). The observation that neither Ca^{2+} nor diacylglycerol 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 (Watts et al., 1995; Chai and Webb, 1988) and human erythrocytes from Pb-exposed workers (range of blood Pb levels: 5.4 to 69.3 µg/dL) (Hwang et al., 2002), and decreased activity in mouse macrophages and the rat brain cortex at micromolar concentrations (Murakami et al., 1993; Lison et al., 1990).

Calmodulin is another important protein essential for proper Ca^{2+}-dependent cell signaling. Calmodulin contains an "EF-hand" Ca^{2+} binding domain, 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 (Vetter and Leclerc, 2003; Saimi and Kung, 2002). Habermann et al. (1983) observed that exposure to Pb altered numerous cellular functions of calmodulin, including activation of calmodulin-dependent phosphodiesterase activity after 10 minutes incubation (minimal activation at 0.1 µM, EC_{50} = 0.5-1.0 µM), stimulation of brain membrane phosphorylation at Pb concentrations greater than 0.4 µM after 1 minute incubation, and increased binding of calmodulin to brain membranes at Pb concentrations greater than 1 µM after 10 minutes incubation. Habermann et al. (1983) reported that the affinity of Pb for Ca^{2+}-binding sites on calmodulin was approximate to that of Ca^{2+} itself (K_d ~ 20 µM), whereas Richardt et al. (1986) observed that Pb was slightly more potent than was 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 was 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 4-5.5x10^{-4} µM (threshold = 1x10^{-4} µM) and 0.45-0.5 µM (threshold = 0.1 µM), respectively). Pb, in the absence of Ca^{2+}, was also observed to activate calmodulin-dependent cyclic nucleotide phosphodiesterase activity at much lower concentrations compared to Ca^{2+} (EC_{50} value 4.3x10^{-4} µM [threshold = 3x10^{-4} µM versus EC_{50} 1.2x10^{-3} µM (threshold = 0.2 µM; 50 minute incubation]). When incubated with physiological concentrations of Ca^{2+}, Pb induced phosphodiesterase activity at concentrations as low as 5x10^{-5} µM. Pb activated calcineurin, a phosphatase with widespread distribution in the brain and immune system, at threshold concentrations as low as 2x10^{-5} µM in the presence of Ca^{2+} (incubation time = 30 minutes), but inhibited its activity at concentrations greater than 2x10^{-4} µM (Kern and 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-exposure (<1 µM, 20 minute incubation) stimulatory effects of Pb exposure on the activity of Ca^{2+}-Mg^{2+}-ATPase was due to Pb binding to calmodulin and subsequent activation of the ion pore. Ferguson et al. (2000)

observed that exposure of rat hippocampal neurons to Pb for 1 to 48 hours resulted in increased activation of a calmodulin-dependent Ca^{2+} extrusion mechanism.

Pb has also been observed to alter the activity of other proteins that rely on Ca^{2+} binding for normal cellular function. Osteocalcin is a matrix protein important in bone resorption, osteoclast differentiation, and bone growth and has three Ca^{2+}-binding sites (Dowd et al., 2001). Incubation of osteocalcin in solution with Ca^{2+} and Pb resulted in the competitive displacement of Ca^{2+} by Pb (Dowd et al., 1994). Pb was found to bind to osteocalcin more than 1000-times more tightly than was Ca^{2+} ($K_d = 1.6x10^{-2}$ µM versus 7 µM, respectively), and analysis with nuclear magnetic resonance (NMR) indicated that Pb induced similar, though slightly different, secondary structures in osteocalcin, compared to Ca^{2+}. The authors hypothesized that the observed difference in Pb-bound osteocalcin structure may explain previous findings in the literature that Pb exposure reduced osteocalcin adsorption to hydroxyapatite (Dowd et al., 1994). Further research by this group also found that Pb bound osteocalcin approximately 10,000-times more tightly than did Ca^{2+} ($K_d = 0.085$ µM versus 1.25×10^3 µM, respectively) (Dowd et al., 2001). However, the authors reported that Pb exposure actually caused increased hydroxyapatite adsorption at concentrations 2-3 orders of magnitude lower than that seen with Ca^{2+}. Additionally, Pb can displace Ca^{2+} in numerous other Ca^{2+}-binding proteins important in muscle contractions, renal Ca^{2+} transport and neurotransmission, including troponin C, parvalbumin, CaBP I and II, phospholipase A_2, and synaptotagmin I, at concentrations as low as the nanomolar range (Bouton et al., 2001; Osterode and Ulberth, 2000; Richardt et al., 1986).

Pb can displace metal cations other than Ca^{2+} that are requisite for protein function. One of the most researched targets for molecular toxicity of Pb is the second enzyme in the heme synthetic pathway, aminolevulinic acid dehydratase (ALAD). ALAD contains four 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 et al., 1997a), and exposure to Pb results in inhibition of the enzyme in the erythrocytes of Pb-exposed workers and adolescents (blood Pb levels >10 µg/dL) (Ahamed et al., 2006; Ademuyiwa et al., 2005b), in human erythrocytes exposed to Pb for 60 minutes ($K_i = 7x10^4$ µM) (Simons, 1995), and in rats exposed to 25 mg/kg Pb once a week for 4 weeks (mean [SD] blood Pb level: 6.56 [0.98] µg/dL) (Lee et al., 2005). Additional experiments indicated that lower concentrations of 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; Huang et al., 2004; Reddy and Zawia, 2000; Hanas et al., 1999; Zawia et al., 1998). Pb was found to form tight complexes with the cysteine residues in GATA proteins (Pb stability constant (β^{Pb}) = 6.4×10^{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 ≥ 1.25 µM) and activate transcription (Pb concentration = $1x10^{6}$ µM). Pb at a minimum concentration of 10 µM also binds to the zinc domain of TFIIIA, inhibiting its ability to bind DNA at concentrations (Huang et al., 2004; Hanas et al., 1999). Huang et al. (2004) also reported that exposure to Pb caused the dissociation of TFIIIA-DNA adducts and using NMR spectroscopy, found that altered TFIIIA activity was the result of a Pb-induced abnormal protein conformation.

Pb exposure modulated the DNA-binding profiles of the transcription factors Sp1 and Erg-1 in rat pups exposed to 0.2% Pb-acetate via lactation, resulting in a shift in DNA-binding toward early development (i.e., the first week following birth) (Reddy and Zawia, 2000; Zawia et al., 1998). The shifts in Sp1 DNA-binding profiles were shown to be associated with abnormal expression of genes related to myelin formation (Section 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 and Zawia, 2000). However, in DNA-binding assays utilizing recombinant Sp1 (which has three zinc finger motifs, opposed to only one in the synthetic peptide), 37 µM Pb was the lowest concentration observed to abolish the DNA-binding capabilities of Sp1 (Razmiafshari and Zawia, 2000).

Pb has also been reported to competitively inhibit Mg binding and thus inhibit the activities of adenine and hypoxanthine/guanine phosphoribosyltransferase in erythrocyte lysates of rats exposed to 0.1% Pb in drinking water for 9 months (mean [SD] blood Pb level: 7.01 [1.64] µg/dL) and in human erythrocyte lysates exposed to 0.1 µM Pb for as little as 5 minutes (Baranowska-Bosiacka et al., 2009), and cGMP phosphodiesterase at picomolar concentrations in homogenized bovine retinas (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 in occupationally exposed humans with blood Pb levels of 5.4-69.3 µg/dL, in adult rodents with blood Pb

levels of 6.5 µg/dL (exposure 4 weeks), in suckling rats exposed to 0.2% Pb via lactation, and in cell-free and cellular in vitro experiments conducted at exposure concentrations ranging from micromoles to picomoles. 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[a]	Metal Cation; Reference
Enzymes	Aminolevulinic acid dehydratase	↓	Zn; Simons (1995)
	Ferrochelatase	↓	Fe (2Fe-2S Cluster); Crooks et al. (2010)
	Superoxide dismutase	↓↑	Mn, Cu, Zn, Fe; Antonyuk et al. (2009), Borgstahl et al. (1992)
	Catalase	↓↑	Fe (Heme); Putnam et al. (2000)
	Glutathione peroxidase	↓↑	Se; Rotruck et al. (1973)
	Guanylate cyclase	↓	Fe (Heme); Boerrigter and Burnett (2009)
	cGMP phosphodiesterase	↓	Mg, Zn; Ke (2004)
	NAD synthase	↓	Mg; Hara et al. (2003)
	NAD(P)H oxidase	↑	Ca; Leseney et al. (1999)
	Pyrimidine 5'-nucleotidase	↓	Mg, Ca; Bitto et al. (2006), Amici et al. (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)

[a]↑ 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 tubules and epididymal mitochondria characterized as a general swollen appearance with frequent rupture of the outer membrane, distorted cristae, loss of cristae, frequent inner compartment vacuolization, observation of small inclusion bodies, and fusion with adjacent mitochondria (Wang et al., 2010d; Marchlewicz et al., 2009; Navarro-Moreno et al., 2009; Goyer, 1968; Goyer et al., 1968).

Transmembrane mitochondrial ion transport mechanisms are perturbed by exposure to Pb. Pb inhibits the uptake of Ca^{2+} into mitochondria (Parr and Harris, 1976), while simultaneously stimulating the efflux of Ca^{2+} out of the organelle (Simons, 1993a), thus disrupting intracellular/mitochondrial Ca^{2+} homeostasis. Pb exposure has also been shown to decrease the mitochondrial transmembrane potential in primary cerebellar granule neuronal cultures from rats exposed to 0.1% Pb in drinking water throughout gestation and lactation (Baranowska-Bosiacka et al., 2011a), astroglia incubated with 0.1 or 1.0 µM Pb for 14 days (Legare et al., 1993), proximal tubule cells exposed to 0.25, 0.5, and 1.0 µM for 12 hours (Wang et al., 2009c), and retinal rod photoreceptor cells incubated with 0.01 to 10 µM for 15 minutes (He et al., 2000). Further research indicated that Pb-induced mitochondrial swelling and decreased membrane potential is the result of the opening of a mitochondrial transmembrane pore (MTP), possibly by directly binding to the metal (Ca^{2+})-binding site on the matrix side of the pore (Bragadin et al., 2007; He et al., 2000). Opening of the MTP is the first step of the mitochondrial-regulated apoptotic cascade pathway in many cells (Rana, 2008; Lidsky and Schneider, 2003). He et al. (2000) additionally observed 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). Pb-induced decreases in ATP and adenylate energy charge (AEC) 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). Similarly, ATP and AEC were decreased, and AMP increased, in primary cerebellar granule neuronal cultures from rats exposed to 0.1% Pb in drinking water throughout gestation and lactation (Baranowska-Bosiacka et al., 2011a). One µM Pb (48 hours) was the lowest concentration observed to decrease cellular ATP levels in differentiated PC-12 cells, and these changes were correlated with a Pb-induced decrease in the expression of the voltage-dependent anion channel, which maintains cellular ATP levels in neurons (Prins et al., 2010). Dowd et al. (1990) reported that oxidative phosphorylation was decreased up to 74% after exposure of osteoblasts to 10 µM Pb. Parr and Harris (1976) reported that Pb inhibited 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. Further, the enzymatic activities of complex I and IV of the respiratory chain have been shown to be decreased in the *peroneous longus* muscle of rats exposed to 250 ppm Pb or 5 ppm thallium in drinking water for 90 days (Méndez-Armenta et al., 2011). Contrary to the above findings, Rafalowska et al. (1996) reported that, although ATP levels did decrease, chronic exposure to Pb did not inhibit oxidative phosphorylation in the synaptosomes of rats exposed to 200 ppm 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. (1997a), although ATP levels were reported to increase after exposure to Pb.

Pb has also been shown to decrease glycolysis in osteoblasts exposed to 10 µM Pb and in human erythrocytes exposed to 30 µg/dL Pb (Grabowska and Guminska, 1996; Dowd et al., 1990). Contrary to these findings, Antonowicz et al. (1990) observed higher levels of glycolytic enzymes in erythrocytes obtained from Pb workers directly exposed to Pb, compared to controls exposed to lower concentrations of Pb (blood Pb levels: 82.1 versus 39.9 µg/dL), and suggested that Pb activated anaerobic glycolysis. In vitro exposure of human umbilical cord erythrocytes to 100-200 µg/dL Pb for 20 hours was observed to lower the cellular pools of adenine and guanine nucleotide pools, including NAD and NADPH (Baranowska-Bosiacka and Hlynczak, 2003). These decreases in nucleotide

pools were accompanied by an increase in purine degradation products (adenosine, etc.). Similar decreases in cellular nucleotide pools were observed when rats were exposed to 1% Pb in drinking water for four weeks (Baranowska-Bosiacka and Hlynczak, 2004). In erythrocytes, nucleotides are synthesized via salvage pathways such as the adenine pathway, which requires adenine phosphoribosyltransferase. The activity of this enzyme is inhibited by exposure to Pb in human and rat erythrocytes (see above for concentration and duration) (Baranowska-Bosiacka et al., 2009).

Disruptions in erythrocyte energy metabolism have been observed in 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 (mean [SD] blood Pb level: 34.6 [20.7] µg/dL) and observed an apparent concentration-dependent decrease in NAD synthetase activity with increased blood Pb level. The blood Pb level 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 (1990, 1987) observed that Pb inhibits the activity of porphobilinogen synthase in rabbit and human erythrocytes, and that the effect on the enzyme was dependent on the affinity for thiol groups at its active site. Taketani et al. (1985) examined the activity of Pb on ferrochelatase in rat liver mitochondria and observed that 10 µM Pb (30 minute incubation) reduced NAD(P)H-dependent heme synthesis by half when ferric, but not ferrous, iron was used. Pb inhibits the insertion of Fe^{2+} into the protoporphyrin ring and instead, Zn is inserted into the ring creating zinc protoporphyrin (ZPP). While not directly measuring the activity of ferrochelatase, numerous studies have shown that blood Pb levels are associated with increased erythrocyte ZPP levels in humans (average blood Pb levels ranging from 21.92 to 53.63 µg/dL) (Mohammad et al., 2008; Counter et al., 2007; Patil et al., 2006b; Ademuyiwa et al., 2005b) and animals (blood Pb level: 24.7 µg/dL) (Rendón-Ramirez et al., 2007).

5.2.3 Protein Binding

Evidence indicates that Pb binds 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 negative effects through sequestration. The ability of Pb to bind proteins was first reported by Blackman (1936): Pb intoxication in children autopsy cases was observed to induce the formation of intranuclear inclusion bodies in the liver and kidney. Since that time, further research has been conducted into characterizing the composition of intranuclear inclusion bodies and identifying specific Pb-binding proteins.

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 (Navarro-Moreno et al., 2009; Oskarsson and Fowler, 1985; Fowler et al., 1980; McLachlin et al., 1980; Choie and Richter, 1972; Goyer et al., 1970a; Goyer et al., 1970b). Inclusion bodies have also been observed in the mitochondria of kidneys and the perinuclear space in the neurons of rats exposed to 500 ppm Pb-acetate in drinking water for 60 days or 7 months (Navarro-Moreno et al., 2009; Deveci, 2006). Intranuclear and cytoplasmic inclusions have also been found in organs other than the kidney, including liver, lung, and glial cells (Singh et al., 1999; Goyer and Rhyne, 1973). Pb found within nuclei has also been shown to bind to the nuclear membrane and histone fractions (Sabbioni and Marafante, 1976).

Upon denaturing intranuclear inclusion bodies with strong denaturing agents, Moore et al. (1973) observed that proteins included in the bodies were rich in aspartic and glutamic acid, glycine, and cysteine. Further work by Moore and Goyer (1974) characterized the protein as a 27.5 kDa protein that migrates as a single band on acrylamide gel electrophoresis. In contrast with the findings of Moore and Goyer, Shelton and Egle (1982) identified a 32 kDa protein with an isoelectric point of 6.3 from the kidneys of rats exposed to 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 (McLachlin et al., 1980; Choie et

al., 1975). In addition to its presence in kidneys of Pb-exposed animals, p32/6.3 has been observed to be present and highly conserved in the brains of rats, mice, dogs, chickens, and humans (Egle and Shelton, 1986). Exposure of neuroblastoma cells to 50 or 100 µM Pb glutamate for 1 or 3 days increased the abundance of p32/6.3 (Klann and Shelton, 1989). Shelton et al. (1990) determined that p32/6.3 was enriched in the basal ganglia, diencephalon, hippocampus, cerebellum, brainstem, spinal cord, and cerebral cortex, and that it contained a high percentage of glycine, aspartic, and glutamic acid residues. Selvin-Testa et al. (1991) and Harry et al. (1996) reported that pre- and post-natal exposure of rats to 0.2-1.0% Pb in drinking water increased the levels of another brain protein, glial fibrillary acidic protein, in developing astrocytes and that this increase may be indicative of a demand for astrocytes to sequester Pb.

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) identified three proteins (MW = 11.5, 63, and >200 kDa) in rat kidney cytosol, two of which, the 11.5 and 63 kDa proteins, were able to translocate into the nucleus. The 11.5 kDa kidney protein was also able to reverse Pb binding to ALAD through chelation of Pb and donation of a zinc cation to ALAD (Goering and Fowler, 1985, 1984). Cadmium and zinc, but not Ca^{2+} or Fe, prevented the binding of Pb to the 63 and 11.5 kDa cytosolic proteins, which agrees with previous observations that cadmium is able to reduce total kidney Pb and prevent the formation of intranuclear inclusion bodies (Mistry et al., 1986; Mahaffey et al., 1981; Mahaffey and Fowler, 1977). Additional cytosolic Pb-binding proteins have been identified in the kidneys of Pb-exposed rats and humans, including the cleavage product of α2-microglobulin, acyl-CoA binding protein (MW = 9 kDa), and thymosin β4 (MW = 5 kDa) (Smith et al., 1998; Fowler and DuVal, 1991).

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

5.2.3.3 Erythrocytic Lead Binding Proteins

The majority (94%) of Pb in whole blood is found in erythrocytes (Ong and Lee, 1980a). Originally, the major Pb-binding protein in erythrocytes was identified as hemoglobin (Cohen et al., 2000; Lolin and O'Gorman, 1988; Ong and Lee, 1980a, b; Raghavan and Gonick, 1977). However, Bergdahl et al. (1997b) observed the principal Pb-binding protein to be 240 kDa and identified it as ALAD. Two smaller Pb-binding proteins were observed, but not identified (MW = 45 and <10 kDa). ALAD levels are inducible by Pb exposure; the total concentration of the enzyme, but not the activity, 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., 1982; Fujita et al., 1981).

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 (Scinicariello et al., 2007; Zhao et al., 2007; Kim et al., 2004; Pérez-Bravo et al., 2004; Smith et al., 1995a; Wetmur, 1994; Wetmur et al., 1991a; Astrin et al., 1987). 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 (Scinicariello et al., 2010; Krieg et al., 2009; Chen et al., 2008c; Chia et al., 2007; Chia et al., 2006; Wananukul et al., 2006).

The ALAD-2 protein binds Pb more tightly than the ALAD-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 et al., 1997a). This higher affinity for Pb in ALAD-2 carriers may sequester Pb and prevent its bioavailability for reaction with other enzymes or cellular components. This is supported by the observation that carriers of the ALAD-2 gene have higher levels of hemoglobin (Scinicariello et al., 2007), decreased plasma levulinic acid (Schwartz et al., 1997b), decreased levels of zinc protoporphyrin (Scinicariello et al., 2007; Kim et al., 2004), lower cortical bone Pb (Smith et al., 1995b), and lower amounts of DMSA-chelatable Pb (Scinicariello et al., 2007; Schwartz et al., 2000a; Schwartz et al., 1997a). However, the findings that ALAD-2 polymorphisms reduced the bioavailability of Pb are somewhat equivocal. Wu et al. (2003a) observed that ALAD-2 carriers had lower blood Pb level (5.8 ± 4.2 µg/dL) than carriers of the 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. (2003b) observed that ALAD-2 polymorphisms were associated with higher DMSA-chelatable Pb concentrations, when normalized to creatinine levels. Further, Montenegro et al. (2006) observed 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 potentially suggests that individuals with the ALAD 1-2/2-2 genotype are at increased risk of Pb-induced health effects due to lower amounts of Pb sequestration by erythrocyte ALAD, although this study did not specifically investigate the clinical implications of ALAD polymorphisms.

ALAD has the estimated capacity to bind Pb at 85 µg/dL in erythrocytes and 40 µg/dL in whole blood (Bergdahl et al., 1998). The 45 kiloDalton (kDa) and <10 kDa Pb-binding proteins bound approximately 12-26% and <1% of the blood Pb, respectively. At blood Pb concentrations greater than 40 µg/dL, greater binding to these components 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., 1981, 1980; Raghavan and 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., 1981, 1980). 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, b). Further Lolin and O'Gorman (1988) reported that the observed protein was only present when blood Pb levels were greater than 39 µg/dL, in agreement with the Pb-binding capacity of ALAD, identified by Bergdahl et al. (1998). Xie et al. (1998) confirmed this, observing the presence of a second low molecular weight protein with greater affinity than ALAD only at higher blood Pb levels. Church et al. (1993a, b) observed the presence of a 6-7 kDa protein in the blood of 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 weight metal-binding protein, most often zinc or copper, which is rich in cysteine residues and plays an important role in the protection against heavy metal toxicity, trace element homeostasis, and scavenging free radicals (Yu

et al., 2009). Exposure to Pb-acetate induces the production of Pb- and Zn-metallothionein in mice exposed via i.p. or intravenous (i.v.) injection at 30 mg/kg (Maitani et al., 1986), in mice exposed via i.p. injection at 300 µmol/kg (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 ppm) failed to induce metallothionein in the kidneys or intestines (Wang et al., 2009b; Jamieson et al., 2007). Goering and Fowler (1987a, b) observed that pretreatment of rats with 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 and Fowler, 1987a, b). 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 that rat brain Pb-binding proteins attenuated Pb-induced inhibition of ALAD.

Metallothionein has been reported to be important in the amelioration of Pb-induced toxicity effects. Liu et al. (1991) reported that 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 did 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. (2010a) observed that Pb-exposed workers with a mutant metallothionein allele had higher blood Pb levels than did carriers of the normal allele (24.17 and 21.27 versus 17.03 µg/dL), and were more susceptible to the effects of Pb on systolic BP and serum renal function parameters.

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 ($OH^\bullet$), and hydrogen peroxide (H_2O_2), which 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 health effects (Salawu et al., 2009; Shan et al., 2009; Vaziri, 2008b; Gonick et al., 1997; Sandhir and Gill, 1995; Khalil-Manesh et al., 1994; Khalil-Manesh et al., 1992b). 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). Oxidative stress is a common mode of action for a number of other metals (e.g., Cd, Mn, As, Co, Cr) that are often found with Pb and by which possible interactions with Pb have been suggested to occur (Jomova and Valko, 2011; Jomova et al., 2011; Matović et al., 2011; HaMai and Bondy, 2004). Not all of these co-occurring metals directly produce ROS or redox cycle, but instead may suppress the free radical scavenging ability of the organism thus leading to oxidative stress.

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-ALAD both identified as targets (Bergdahl et al., 1997a). Through its sulfhydryl and metal ion disrupting properties, Pb incorporates with and inhibits a number of enzymes in the heme biosynthetic process, including δ-ALA synthetase, δ-ALAD, and ferrochelatase. Pb has been shown to be able to disrupt the zinc 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 (mean: 7 µg/dL) have been found to inhibit the activity of δ-ALAD in humans, and the lowest blood Pb level observed to be associated with lower δ-ALAD activity in these

studies was 5 µg/dL (Ahamed et al., 2005; Sakai and Morita, 1996). A significant negative correlation (r = -0.6) was found between blood Pb levels in adolescents (range of blood Pb levels: 4-20 µg/dL) and blood δ-ALAD activity in adolescents (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^-, OH•, 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 (Oteiza et al., 1995; Bechara et al., 1993). Additionally, Pb has the capacity to stimulate ferrous ion initiated membrane lipid peroxidation serving as a catalyst for these events (Adonaylo and Oteiza, 1999; Quinlan et al., 1988). The extent of peroxidation of lipids varies based on the number of double bonds present in unsaturated fatty acids, since double bonds weaken the C-H bonds on the adjacent carbon, making H removal easier (Yiin and Lin, 1995). After Pb exposure (4-12 µg/dL in vitro, 24 hours), the production of malondialdehyde (MDA), a marker of oxidative stress and lipid oxidation end product, increased relative to the number of double bonds of the fatty acid. In the absence of Fe^{2+}, Pb has not been shown to promote lipid peroxidation; however, it may accelerate peroxidation by H_2O_2 (Quinlan et al., 1988). This could be due to altering membrane structure, restricting phospholipid movement, and facilitating the propagation of peroxidation.

Pb induces changes in the fatty acid composition of a membrane, which could lead to oxidative damage. Exposure to Pb (>62.5 ppm in drinking water, 3 weeks) in chicks promoted an increase in arachidonic acid (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 and Donaldson, 1991). It is possible that Pb depressed the desaturation of saturated fatty acids to the corresponding monoenoic fatty acids, while stimulating elongation and desaturation of linoleic acid to 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 and Knowles, 1993). Changes in membrane fluidity will disturb the conformation of the

active sites of membrane associated enzymes, disrupt metabolic regulation, and alter membrane permeability and function.

A number of recent studies report increased measures of lipid peroxidation in various organs, tissues, and species. Occupational Pb exposure resulting in elevated blood Pb levels (means >8 µg/dL) in various countries provides evidence of lipid peroxidation, including increased plasma MDA levels (Ergurhan-Ilhan et al., 2008; Khan et al., 2008; Mohammad et al., 2008; Quintanar-Escorza et al., 2007; Patil et al., 2006a; Patil et al., 2006b). One study found a correlation between the MDA levels and blood Pb levels even in the unexposed workers, although they had blood Pb levels higher than the mean blood Pb level of the current U.S. population (i.e., <12 µg/dL) (Quintanar-Escorza et al., 2007). Other studies found evidence of increased lipid peroxidation among the general population, including children, with elevated blood Pb levels (means >10 µg/dL) (Ahamed et al., 2008; Ahamed et al., 2006; Jin et al., 2006). In adolescents, Ahamed et al. (2006) found a blood MDA levels to be positively correlated (r = 0.7) with blood Pb levels ranging between 4-20 µg/dL. Similar results have been shown after Pb exposure in animal studies (Abdel Moneim et al., 2011b; Pandya et al., 2010; Dogru et al., 2008; Yu et al., 2008; Adegbesan and Adenuga, 2007; Lee et al., 2005). Enhanced lipid peroxidation has been found in Pb treated (50 µg, 1-4 hours) rat brain homogenates (Rehman et al., 1995), rat proximal tubular cells (0.5-1 µM, 12 hours) (Wang et al., 2011b), and in specific brain regions, hippocampus and cerebellum, after Pb exposure (500 ppm, 8 weeks) to rats (Bennet et al., 2007). Overall, there was a correlation between the blood Pb level and measures of lipid peroxidation often measured by MDA levels.

Interestingly, many species of plants, invertebrates, and vertebrates exhibit increased lipid peroxidation with Pb exposure (Sections 7.2.4, 7.3.5, and 7.3.12). 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 that may contribute to the production of ROS after Pb exposure (Ni et al., 2004; Vaziri et al., 2003). Vaziri et al. (2003) found increased protein expression of the NAD(P)H subunit gp^{91}phox in the brain, heart, and renal cortex of Pb treated rats (100 ppm in drinking water, 12 weeks). This upregulation was present in Pb-treated (1-10 ppm) human coronary artery endothelial cells, but not vascular smooth muscle cells (VSMC), which

do not express the protein (Ni et al., 2004). It is possible that NAD(P)H oxidase serves as a potential source of ROS in cells that express this protein.

5.2.4.4 Antioxidant Enzyme Disruption

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

Studies of the effects of Pb on the activities of SOD and CAT give divergent results. These metalloprotein enzymes require various essential trace elements for proper structure and function, making them a target for Pb toxicity. CAT is a heme containing protein that requires 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 (Pandya et al., 2010; Ergurhan-Ilhan et al., 2008; Mohammad et al., 2008; Yu et al., 2008; Patil et al., 2006a; Patil et al., 2006b; Conterato et al., In Press), whereas others have observed increased activity following Pb exposure (Ahamed et al., 2008; 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 ppm, blood Pb level 20.4 and 24.5 µg/dL); however hepatic SOD activity increased at low level Pb administration and decreased after high level exposure (Uzbekov et al., 2007). It is possible that the increased 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. Short-term administration of Pb in vitro (0.1 µM) and biomarkers of Pb exposure in humans (18 µg/dL mean blood Pb level) have been associated with decreased blood and organ GSH and cysteine content, which may be due to increased GSH efflux from tissues (Pandya et al., 2010; Pillai et al., 2010; Ahamed et al., 2009; Ahamed et al., 2008; Flora et al., 2007; Ahamed et al., 2005; Chetty et al., 2005; Nakagawa, 1991, 1989). Long-term Pb exposure 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 (Daggett et al., 1998; Corongiu and Milia, 1982; Hsu, 1981; Conterato et al., In Press). However, other studies have found that long-term Pb exposure resulting in mean blood Pb levels between 6.6 and 22 µg/dL, causes the depletion of GSH (Mohammad et al., 2008; Lee et al., 2005; Ercal et al., 1996). Thus, the time of exposure is important to consider when measuring GSH levels.

Glutathione reductase is able to reduce GSSG back to GSH. Therefore, an increased GSSG/GSH ratio is considered to be indicative of oxidative stress. Epidemiologic studies have found higher blood Pb levels to be associated with increases in the GSSG/GSH ratio (Mohammad et al., 2008; Ercal et al., 1996; Sandhir and Gill, 1995). In one study, this association was observed in a population of children with a mean blood Pb level below 10 µg/dL (Diouf et al., 2006). Studies have found mixed effects on GR activation. GR possesses a disulfide at its active site that is a target for inhibition by Pb. Studies in animals and cells have reported decreased (Bokara et al., 2009; Sandhir and Gill, 1995; Sandhir et al., 1994), increased (Sobekova et al., 2009; Howard, 1974), and no change (Hsu, 1981) in GR activity after Pb exposure. This could be because the effect of Pb on GR varies depending on sex (Sobekova et al., 2009) and organ or organ region (Bokara et al., 2009).

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, evidence indicates that Pb decreases the activity of GPx and GST (Pillai et al., 2010; Yu et al., 2008; Lee et al., 2005; Nakagawa, 1991; Schrauzer, 1987). Similar to other antioxidant enzymes, compensatory upregulation of these enzymes is described after Pb exposure in animals and in Pb-exposed workers (painters with a mean blood Pb level of 5.4 µg/dL) (Bokara et al., 2009; Ergurhan-Ilhan et al., 2008; Conterato et al., 2007; Daggett et al., 1998; Conterato et al., In Press). However, in another study, these enzymes were not able to compensate for the increased Pb-induced ROS, further contributing to the oxidative environment (Farmand et al., 2005).

Recently, γ-glutamyltransferase (GGT) within its normal range has been regarded as an early and sensitive marker of oxidative stress. This may be because cellular GGT metabolizes extracellular GSH to be used in intracellular GSH synthesis. Thus, cellular GGT acts as an antioxidant enzyme by increasing the intracellular GSH pool. However, the reasons for the association between GGT and oxidative stress have not been fully realized (Lee et al., 2004). In one study, occupational Pb exposure (mean blood Pb level of 29.1 µg/dL) was associated with increased serum GGT levels (Khan et al., 2008). Interestingly, higher blood Pb level was similarly associated with higher serum GGT levels in a sample of the U.S. adult population (NHANES III) with lower blood Pb levels (Lee et al., 2006a). In this study of nonoccupationally-exposed individuals, a concentration-dependent relationship was observed at blood Pb levels <7 µg/dL.

5.2.4.5 Nitric Oxide Signaling

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

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

Soluble Guanylate Synthase

Many biological actions of •NO, such as vasorelaxation, are mediated by cyclic guanosine monophosphate (cGMP), which is produced by soluble guanylate cyclase (sGC) from the substrate guanosine triphosphate. Soluble guanylate cyclase is a heterodimer requiring one molecule of heme for enzymatic activity (Boerrigter and Burnett, 2009). In VSMC, sGC serves as the •NO receptor. Marked reduction in plasma concentrations and urinary excretion of cGMP is observed after Pb exposure to rats (5 ppm for 30 days and 100 ppm for 12 months [resulting in a mean blood Pb level of 29.4 µg/dL]) (Marques et al., 2001; Khalil-Manesh et al., 1993b). In addition, Pb exposure downregulated the protein abundance of sGC in vascular tissue (Farmand et al., 2005; Courtois et al., 2003; Marques et al., 2001). This downregulation in sGC was prevented by antioxidant therapy (ascorbic acid) suggesting that oxidative stress also plays a role in Pb-induced downregulation of sGC (no change in blood Pb level was observed after ascorbic acid treatment) (Marques et al., 2001).

5.2.5 Inflammation

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

Traditional immune-mediated inflammation can be seen with bronchial hyperresponsiveness, asthma, and respiratory infections associated with exposure to Pb. But it is important to recognize that any tissue or organ can be affected by immune-mediated inflammatory dysfunction given the distribution of immune cells as both permanent residents and infiltrating cell populations (Mudipalli, 2007; Carmignani et al., 2000). Pb 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 (mean blood Pb level of 18 µg/dL or 100 ppm exposure for 14 weeks) (Rodriguez-Iturbe et al., 2005; Ramesh et al., 2001), which has been coupled with activation of the redox sensitive nuclear transcription factor kappa B (NFκB) and lymphocyte and macrophage infiltration in rats (100 ppm for 14 weeks resulting in mean blood Pb levels ranging 23-27 µg/dL,) (Bravo et al., 2007). These events could be in response to the oxidative environment arising from

Pb exposure, since Pb-induced inflammation and NFκB activation can be ameliorated by antioxidant therapy (Rodriguez-Iturbe et al., 2004).

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 occupationally-exposed humans (mean blood Pb level 48 µg/dL) (Cardenas et al., 1993) and animal and cell models (e.g., 0.01 µM, 48 h) (Chetty et al., 2005; Flohe et al., 2002; Knowles and Donaldson, 1997; Lee and Battles, 1994). Dietary Pb supplementation of animals (500 ppm, 19 days) can increase the percentage of cell membrane AA, the precursor of cyclooxygenase and lipoxygenase metabolism to PGs and leukotrienes (Knowles and Donaldson, 1990). Additionally, Pb (1 µM) may promote the release of AA via activation of phospholipase A_2, as shown in isolated VSMC (Dorman and Freeman, 2002).

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

5.2.5.1 Cytokine Production

There are three modes by which Pb affects immune cytokine production. First, Pb can act on macrophages to elevate the production of pro-inflammatory cytokines such as TNF-α and interleukin (IL)-6 (Cheng et al., 2006; Chen et al., 1999; 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 T-derived lymphocyte helper (Th)1 responses are suppressed and Th2 responses are promoted (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) (Lynes et al., 2006; Heo et al., 1996). In contrast, Th2 cytokines such as IL-4, IL-5, and IL-6 are elevated (Gao et al., 2007; Kim and 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. (2006a) reported that low blood Pb concentrations produced significant changes in cytokine levels in mice. At a low dietary Pb concentration (0.11 ppm, blood Pb level of 1.6 µg/dL), IL-2 and IFN-γ were decreased compared to the controls (0.02 ppm, 0.8 µg/dL), indicating a suppressed Th1 response. As the dietary and blood Pb concentrations increased (resulting in blood Pb levels 12-61 µg/dL), a Th2 phenotype was observed with suppressed IFN-γ and IL-2 and elevated IL-4 production. These findings support the notion that the immune system is remarkably sensitive to Pb-induced functional alterations and that nonlinear effects may occur at low Pb exposures. TGF-β production 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 with respect to Pb-induced changes. Depending upon the protocol it can be slightly elevated in production or unchanged. Recently, Gao et al. (2007) found that Pb-treated dendritic cells (25 µM) promoted a slight but statistically significant increase in IL-2 production among lymphocytes. Proinflammatory cytokines have been measured in other organs and cell types after Pb exposure. Elevation of IL-1β and TNF-α were observed in the hippocampus after Pb exposure (15 ppm, i.p., daily for 2 weeks, blood Pb level of 30.8 µg/dL) and increased IL-6 was found in the forebrain (Struzynska et al., 2007).

Consistent with animal studies, epidemiologic studies also found higher concurrent blood Pb levels in children and occupationally-exposed adults to be associated with a shift toward production of Th2 cytokines relative to Th1 cytokines. The evidence in children was based on comparisons of serum cytokine levels among groups with different blood Pb levels without consideration of potential confounding factors. Among children ages 9 months to 6 years in Missouri, Lutz et al. (1999) found that children with concurrent blood Pb levels 15-19 µg/dL had higher serum levels of IL-4 and IgE (Section 5.6.3) than did children with lower blood Pb levels. These results were consistent with the mode of action for IL-4 to activate B cells to induce B cell class switching to IgE. In another study of children in grades 5 and 6 in Taiwan, investigators did not group children by blood Pb levels but by potential for Pb exposures due to age of home and location of residence (Hsiao et al., 2011). Concurrent blood Pb levels did not differ by residence in old versus new homes or by urban versus rural residence (means: 3.2-3.8 µg/dL) but were higher among children living near an oil refinery, in particular, among children with known respiratory allergies (mean: 8.8 µg/dL). This latter group of children also had the lowest serum levels of IFN-γ and highest levels of IL-4. There was no direct comparison of cytokine levels between blood Pb level groups in the population overall; however, cytokine levels were similar between healthy and allergy groups in the other Pb source groups that had similar blood Pb levels. Thus, the differences in cytokine levels between healthy and allergic children living near the oil refinery may have been influenced by differences in their blood Pb levels.

Evidence of association between blood Pb levels and cytokine levels in nonoccupationally-exposed adults was equivocal. Among adult university students in Incheon, Korea, Kim et al. (2007) found associations of concurrent blood Pb level with serum levels of TNF-α and IL-6 that were larger among male students with blood Pb levels 2.51-10.47 µg/dL. Notably, the relative contributions of lower recent versus higher past Pb exposures to these effects is not known. In models that adjusted for age, sex, BMI, and smoking status, a 1 µg/dL increase in blood Pb level was associated with a 23% increase (95% CI: 4, 55%) in log of TNF-α and a 26% increase in log of IL-6 (95% CI: 0, 55%). The association between levels of blood Pb and plasma TNF-α was greater among men who were GSTM1 null or had the TNF-α GG genotype. For the association between blood Pb level and plasma IL-6, the effect estimate was slightly elevated in TNF-α GG genotype but not elevated in the GSTM1 positive group. The effects of Pb on several physiological systems have been hypothesized to be mediated by the generation of ROS (Daggett et al., 1998). Thus, the null variant of GSTM1, which is associated with reduced elimination of ROS, may increase the risk of Pb-associated immune effects. The results for the TNF-α polymorphism 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. Among adults in Italy, blood Pb levels were not correlated with either Th2 or Th1 cytokine levels in men (Boscolo et al., 1999) or women (Boscolo et al., 2000)

Results from studies of occupationally-exposed adults also suggested that Pb exposure may be associated with decreases in Th1 cytokines and increases in Th2 cytokines; however, analyses were mostly limited to comparisons of levels among different occupational groups with different mean blood Pb levels (Di Lorenzo et al., 2007; Valentino et al., 2007; Yucesoy et al., 1997a). The exception was a 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 information on concentration-response relationships was not provided, higher blood Pb level was associated with higher IL-10 and TNF-α. Levels of IL-2, IL-6, and IL-10 also increased from the lowest to highest blood Pb group. In contrast with most other studies, both exposed worker groups had lower IL-4 levels compared with controls. In a similar analysis, DiLorenzo et al. (2007) separated exposed workers into intermediate (9.1-29.4 µg/dL) and high (29.4-81.1 µg/dL) blood Pb level groups, with unexposed workers comprising the low exposure group (blood Pb levels 1-11 µg/dL). Mean TNF-α levels showed a monotonic increase from the low to high blood Pb group. Levels of granulocyte colony-stimulating factor (G-CSF) did not differ between the intermediate and high blood Pb groups among the Pb recyclers; however, G-CSF levels were higher in the Pb recyclers than in the unexposed controls. Furthermore, among all subjects, blood Pb showed a strong, positive correlation with G-CSF. Yucesoy

et al. (1997a) found lower serum levels of the Th1 cytokines, IL-1β and IFN-γ, in workers (mean blood Pb level of 59.4 µg/dL) compared with controls (mean blood Pb level of 4.8 µg/dL); however levels of the Th2 cytokines, IL-2 and TNF-α levels, were similar between groups. As most occupationally-exposed cohorts represent populations highly exposed to Pb, with mean blood Pb levels >22 µg/dL, effects observed within these cohorts may not be generalizable to the population as a whole. However, animal, general population, and occupational studies suggest that exposure to Pb increases the production of pro-inflammatory cytokines, skews the ratio of Th1 and Th2 cytokines to favor Th2 responses, and suppresses lymphocyte cytokine production.

5.2.6 Endocrine Disruption

5.2.6.1 Hypothalamic-Pituitary-Gonadal Axis

Pb is a potent endocrine disrupting chemical found to be associated with reproductive and developmental effects 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. Long-term Pb exposure in animals has been shown to reduce serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and estradiol (Biswas and Ghosh, 2006; Rubio et al., 2006). Similar changes in serum HPG hormones have been observed after high-level Pb exposure in animals, resulting in blood Pb levels > 20 µg/dL (Dearth et al., 2002; Ronis et al., 1998b; Foster, 1992; Sokol and Berman, 1991). Increases in LH and FSH have been associated with increasing concurrent blood Pb levels in adult women from the NHANES cohort (Krieg, 2007). The change in HPG hormones likely occurs through the inhibition of LH secretion and the reduction in the expression of the steroidogenic acute regulatory protein (StAR) (Huang and Liu, 2004; Srivastava et al., 2004; Huang et al., 2002; Ronis et al., 1996). StAR expression is the rate-limiting step essential in maintaining gonadotropin-stimulated steroidogenesis, which results in the formation of testosterone and estradiol. Prenatal Pb exposure (resulting in 3 µg/dL blood Pb) was found to decrease basal StAR synthesis, but not gonadotropin-stimulated StAR synthesis, suggesting that Pb may not directly affect ovarian responsiveness to gonadotropin stimulation (Srivastava et al., 2004). Instead, Pb may act at the hypothalamic-pituitary level to alter LH secretion, which is necessary to drive StAR production and subsequent sex hormone synthesis. Release of LH and FSH from the pituitary is controlled by gonadotropin-releasing hormone (GnRH). Pb exposure (10 µM, 90 min) in rat brain median eminence cells can block GnRH release (Bratton et

al., 1994). Pb may also interfere with release of pituitary hormones through interference with cation-dependent secondary messenger systems, 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) to rats was able to decrease the number of estrogen, LH, and FSH receptors found in the uterus or ovaries and receptor binding affinity (Wiebe et al., 1988; Wiebe and Barr, 1988). Altered hormone binding ability may be due to the ion binding properties of Pb, resulting in changes in receptor tertiary structure that will disrupt ligand binding. In addition, Pb-induced changes in hormone levels that act as inducing agents for receptor synthesis may affect the number of hormone receptors produced.

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

The liver is often associated with the HPG axis due in part to its production of insulin-like growth factor 1 (IGF-1). Children with increased blood Pb levels (>4 µg/dL) (Huseman et al., 1992) and Pb-exposed animals (blood Pb level of 14 µg/dL) (Pine et al., 2006; Dearth et al., 2002) and gonadal cells (50 ppm Pb exposure) (Kolesarova et al., 2010) show a decrease in plasma IGF-1, which may be the result of decreased translation or secretion of IGF-1 (Dearth et al., 2002). IGF-1 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., 1992; Huseman et al., 1987). This decrease in GH could be a result of decreased release of GH releasing hormone (GHRH) from the hypothalamus or disrupted GHRH binding to its receptor, which has been reported in vitro after Pb treatment (IC_{50} free Pb in solution $5.2x10^{-5}$ µM,

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 (blood Pb levels >7.3 µg/dL) have shown that elevated blood Pb are associated with lower thyroxine (T_4) and free T_4 levels without alteration in triiodothyronine (T_3), suggesting that long-term Pb exposure may depress thyroid function in workers (Dundar et al., 2006; Tuppurainen et al., 1988; Robins et al., 1983). However, animal studies on thyroid hormones have shown mixed results. Pb-exposed cows (blood Pb levels >51 µg/dL) were reported to have an increase in plasma T_3 and T_4 levels (Swarup et al., 2007), whereas mice and chickens manifested decreased serum T_3 concentrations after Pb exposure accompanied by increased lipid peroxidation (Chaurasia et al., 1998; Chaurasia and 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; however, no data were provided to exclude changes in Pb kinetics as the mechanism of protection (Chaurasia and Kar, 1997).

Decreased T_4 and T_3 may be the result of altered pituitary release of thyroid stimulating hormone (TSH). However, several studies have reported higher TSH levels in high-level Pb-exposed workers (blood Pb levels >39 µg/dL) (Lopez et al., 2000; Singh et al., 2000; Gustafson et al., 1989), which would result in increased T_4 levels. Overall, results on the effects of Pb on the HPT axis are inconclusive.

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 regarding whether observed genotoxic effects are due to chromate

or Pb in studies using this form of inorganic Pb, only studies utilizing other forms of inorganic Pb (e.g., Pb nitrate, acetate) are discussed below.

5.2.7.1 DNA Damage

A number of studies in human populations have observed associations between Pb exposure and increased DNA damage, as measured as DNA strand breaks. Most of these associations have been observed in occupationally-exposed populations (Grover et al., 2010; Minozzo et al., 2010; Shaik and Jamil, 2009; Danadevi et al., 2003; Hengstler et al., 2003; Palus et al., 2003; Fracasso et al., 2002; de Restrepo et al., 2000). It is important to note that occupationally-exposed adults have very high blood Pb levels, and in one study (de Restrepo et al., 2000) the association between blood Pb level and DNA damage was observed only in workers with blood Pb levels 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 Palus et al. (2003) (mean blood Pb level: 50.4 µg/dL [range: 28.2-65.5 µg/dL]) and Minozzo et al. (2010) (mean [SD]: 59.43 µg/dL [28.34]) observed no correlation. Lastly, Pb-exposed workers are also potentially exposed to other genotoxic materials, making it difficult to rule out confounding co-exposures. However, Hengstler et al. (2003) examined workers exposed to Pb, cadmium, and cobalt and observed that neither blood (mean: 4.4 [IQR: 2.84-13.6] µg/dL) nor air Pb levels (mean: 3.0 [IQR: 1.6-50.0] µg/m^3) were associated with DNA damage when examined alone, but that blood Pb influenced the occurrence of single strand DNA breaks when included in a multiple regression model along with cadmium in air and blood and cobalt in air indicating lack of confounding. 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 mean (range) blood Pb levels of 19.5 (11.3-49.2) and 28.6 (11.4-47.5) µg/dL, respectively, compared to blood Pb level of 4.6 (0.1-8.7) µg/dL for children living distant to the smelter. DNA damage was increased in children living nearest to the smelter, compared to the children at the intermediate distance, but was not different from children living farthest away from the smelter. Multivariate analysis (which considered children urinary As levels, highest in children farthest from the smelter), revealed no statistically significant associations between DNA damage and blood Pb level. Further, DNA repair ability was also observed to be unrelated to blood Pb levels. Alternatively, Yanez et al. (2003) observed that children living close to a mining complex (mean [range] blood Pb level: 11.6 [3.0 to 19.5] µg/dL) did have higher levels

of DNA damage compared to control children who lived further away from the mining facility (mean [range] blood Pb level: 8.3 [3.0-25.0] 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 concentration (Devi et al., 2000). The three highest concentrations had responses which were similar in magnitude and were actually lower than the responses to lower concentrations tested. In mice exposed to Pb (blood Pb level of 0.68 µg/dL) via inhalation for up to 4 weeks, differential levels of DNA damage were observed in different organ systems, with only the lung and the liver demonstrating statistically greater DNA damage compared to the respective organ controls after acute exposure (Valverde et al., 2002). Statistically elevated levels of DNA damage were observed in the kidneys, lungs, liver, brain, nasal cavity, bone marrow, and leukocytes of mice exposed over a period of 4 weeks, although variability was high in all groups. The magnitude of the DNA damage was characterized as weak and did not increase with increasing durations of exposure. Xu et al. (2008) exposed mice to 10-100 mg/kg Pb-acetate via gavage for four weeks and observed a concentration-dependent increase in DNA single strand breaks in white blood cells that was statistically significant at 50 and 100 mg/kg. The authors characterized the observed DNA damage as severe. Pb nitrate induced DNA damage in primary spermatozoa in Pb-exposed rats (blood Pb levels of 19.5 and 21.9 µg/dL) compared to control rats (Nava-Hernandez et al., 2009). The level of DNA damage was not concentration dependent and was comparable in both exposure groups. Narayana and Al-Bader (2011) observed no increase in DNA damage in the livers of rats exposed to 0.5 or 1% Pb nitrate in drinking water for 60 days. Interestingly, although the results were not statistically significant and highly variable within exposure groups, DNA fragmentation appeared to be lower in the exposed animals.

Studies investigating Pb-induced DNA damage in human cell cultures were contradictory. Pb-acetate did not induce DNA strand breaks in human HeLa cells when exposed to 500 µM Pb-acetate for 20-25 hours or 100 µM for 0.5-4 hours (Hartwig et al., 1990; Snyder and Lachmann, 1989). Pb nitrate, administered to lymphoma cells at 1000-10,000 µM for 6 hours, did not result in any DNA-protein crosslinks (Costa et al., 1996). Pb-acetate was observed by Woźniak and Blasiak (2003) to result in DNA single and double strand breaks in primary human lymphocytes exposed to 1-100 µM for 1 hour, although the pattern of damage was peculiar. DNA damage was greater in cells exposed to 1 or 10 µM, compared to those exposed to 100 µM. DNA-protein crosslinks were only observed in the 100 µM exposure group, suggesting that the decreased strand breaks observed in the high exposure group may be a result of increased crosslinking in this group. Pasha Shaik et al. (2006) also observed DNA damage in human lymphocytes exposed to 2,100-3,300 µM Pb nitrate for 2 hours. Although there was a concentration-

dependent increase in DNA damage from 2,100-3,300 µM, no statistics were reported and no unexposed control group was included making it difficult to interpret these results. Gastaldo et al. (2007) observed that exposure of human endothelial cells to 1-1,000 µM Pb nitrate for 24 hours resulted in a concentration-dependent increase in DNA double strand breaks.

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

Pb-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 repair, but increased two other repair pathways, MRE11-dependent and Rad51-related repair (Gastaldo et al., 2007). Interestingly, in contrast to the above studies, exposure of lung carcinoma cells to 100, 300, or 500 µM Pb-acetate for 24 hours resulted in an increase in nucleotide excision repair efficiency (Li et al., 2008a). Roy and Rossman (1992) observed an increase in UV-induced mutagenicity when CHV79 cells were co-exposed to 400 µM Pb-acetate (a nonmutagenic concentration of Pb-acetate), indicating an inhibition of DNA repair. Treatment of Chinese hamster ovary cells to 0.5-500 µM Pb-acetate resulted in a concentration-dependent accumulation of apurinic/apyrimidinic site incision activity, indicating that DNA repair was diminished (McNeill et al., 2007).

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 occupational exposures to Pb or a number of other heavy metals and organic contaminants. Higher blood Pb level (range 1.6-5.2 µg/dL) was associated with greater HPRT mutation frequency in the total population. Also, women with high blood Pb levels (i.e., greater than the population median, not reported) demonstrated a greater mutation frequency compared to women with lower blood Pb levels.

Pb-induced mutagenicity was investigated in 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 500-2,000 µM for 24 hours. Pb-acetate remained nonmutagenic in the presence of 3-aminotriazole, a catalase inhibitor, indicating that oxidative metabolism did not play a part in potential mutagenicity of Pb. Exposure to Pb-acetate alone did not induce mutagenicity in lung carcinoma cells (100-500 µM for 24 hours) or fibroblasts (300-500 µM for 24 hours) (Li et al., 2008a; Wang et al., 2008c). However, pretreatment with PKC inhibitors before Pb treatment did result in statistically significant increases in mutagenicity in both cell lines.

Results from investigations into Pb-induced mutagenicity using animal cell lines were as equivocal as were the findings from human cell line studies, although the mixed findings may be reflective of specific Pb compounds used. Pb-acetate was observed to be nonmutagenic (HPRT assay) in 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 1,700 µM for 5 days (Roy and Rossman, 1992). Pb-acetate was observed to be nonmutagenic (HPRT assay) in Chinese hamster ovary cells exposed to 5 µM for 6 hours (McNeill et al., 2007). The observation of mutagenicity in the second study is complicated by the concurrent observation of severe cytotoxicity at the same concentration. Pb nitrate was alternatively found to be nonmutagenic in CHV79 cells (gpt assay) exposed to 0.5-2,000 µM for 5 days (Roy and Rossman, 1992), but mutagenic in the same cell line (HPRT assay) exposed to 50-5,000 µM for 5 days (Zelikoff et al., 1988). However, mutagenicity was only observed at 500 µM, and was higher than that observed at higher concentrations. Pb sulfate was also observed to be mutagenic in CHV79 cells (HPRT assay) exposed to 100-1,000 µM for 24 hours, but as

with Pb nitrate, it was not concentration-dependent (Zelikoff et al., 1988). Pb chloride was the only Pb compound tested in animal cell lines that was consistently mutagenic: three studies from the same laboratory observed concentration-dependent mutagenicity in the gpt assay in Chinese hamster ovary cells exposed to 0.1-1 µM Pb chloride for one hour (Ariza and Williams, 1999; Ariza et al., 1998; Ariza and Williams, 1996).

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 levels (means: 10.48 - 86.9 µg/dL) and sister chromatid exchange (SCE) was observed in a number of occupational studies (Wiwanitkit et al., 2008; Duydu et al., 2005; Palus et al., 2003; Duydu et al., 2001; Pinto et al., 2000; Bilban, 1998; Anwar and Kamal, 1988; Huang et al., 1988). However, there are numerous methodological issues that limit firm conclusions from being drawn. Most notably, occupational co-exposures to other genotoxic materials were possible, although some studies excluded workers with exposures to known mutagens (Pinto et al., 2000; Huang et al., 1988). In most studies that attempted to investigate the concentration-response relationship in workers, no association was observed between increasing blood Pb levels and the number of SCE (Palus et al., 2003; Duydu et al., 2001; Pinto et al., 2000). However, Huang et al. (1988) did observe increased SCE in exposed workers in the two highest blood Pb groups (52.1 and 86.9 µg/dL), with a statistically significant association observed in the 86.9 µg/dL group. Pinto et al. (2000) did report an association with duration of exposure (range of years exposed: 1.6-40). Two studies reported no correlation between occupational exposure to Pb and number of SCE (Rajah and Ahuja, 1996; Rajah and Ahuja, 1995). However, these two studies may have suffered from limited statistical power to observe an effect as they included very small numbers of Pb-exposed workers. Mielzynska et al. (2006) found no association between blood Pb level and SCEs in children in Poland. Children had an average blood Pb level of 7.69 µg/dL and 7.87 SCEs/cell.

Pb exposure has been observed to induce SCEs in multiple laboratory animal studies. In mice exposed to up to 100 mg/kg Pb-acetate i.p., Pb induced SCEs with 50 and 100 mg/kg (Fahmy, 1999). Pb nitrate, also administered i.p. and induced the formation of

SCEs in a concentration-dependent manner (10-40 mg/kg) in the bone marrow of exposed mice (Dhir et al., 1993). Nayak et al. (1989b) exposed pregnant mice to 100-200 mg/kg Pb nitrate via i.v. injection and observed an increase in SCEs 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 i.p. injection on alternating days for 11 or 21 days, for a total of 5 or 10 exposures. Induction of SCEs in the bone marrow of exposed rats was increased over controls in a statistically significant duration-dependent manner. It is important to note that all three of these studies utilized an injection route of exposure that may not be relevant to routes of exposure in the general population (e.g., air, drinking water exposure).

Few studies were found that investigated SCE formation in human cell lines due to Pb exposure. Statistically significant, concentration-dependent increases in SCEs were observed in human lymphocytes obtained from a single donor when incubated with 1, 5, 10, or 50 µM Pb nitrate (Ustundag and Duydu, 2007). Melatonin and N-acetylcysteine were reported to ameliorate these effects, indicating Pb may induce SCEs 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., In Press).

Studies investigating SCE in rodent cells were more equivocal than those in human cells. Pb sulfate, acetate, and nitrate were found not to 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 power to detect Pb-induced SCEs. Cai and Arenaz (1998), on the other hand, used 100 cells per treatment and observed that exposure to 0.05-1 µM Pb nitrate for 3-12 hours resulted in a weak, concentration-dependent increase in SCEs in Chinese hamster ovary cells. Lin et al. (1994) also observed a concentration-dependent increase in SCEs in Chinese hamster cells exposed to 3-30 µM Pb nitrate for 2 hours.

Micronucleus Formation

Pb-induced micronucleus formation was observed in numerous occupational studies (Grover et al., 2010; Khan et al., 2010b; Minozzo et al., 2010; Shaik and Jamil, 2009; Minozzo et al., 2004; Palus et al., 2003; Vaglenov et al., 2001; Pinto et al., 2000; Bilban, 1998; Vaglenov et al., 1998). 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 level of 10.48 µg/dL compared with unexposed controls. In studies investigating the correlation between blood Pb levels and micronucleus formation, no association was observed (Minozzo et al., 2010; Minozzo et al., 2004; Palus et al., 2003;

Pinto et al., 2000), although Pinto et al. (2000), Grover et al. (2010), and Minozzo et al. (2010) did report an association between micronuclei formation and duration of exposure. Only one study was found that investigated micronucleus formation in a nonworker population; Mielzynska et al. (2006) reported a statistically significant positive correlation between blood Pb levels and micronuclei frequency in children in Poland. Children, with an average blood Pb level of 7.69 µg/dL, were observed to have 4.44 micronucleated cells per 1,000 cells analyzed. Children with blood Pb levels greater than 10 µg/dL had significantly more micronucleated cells than did children with blood Pb levels less than 10 µg/dL.

Micronucleus formation in response to Pb exposure has been observed in rodent animal studies. Celik et al. (2005) observed that exposure of female rats to 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. (2008b) exposed rats to 100 ppm 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 LD_{50}) via i.p. injection on alternating days for 11 or 21 days, for a total of 5 or 10 exposures. Formation of micronuclei in the bone marrow of exposed rats was increased over that in controls in a significant duration-dependent manner. Two further studies investigated formation of micronuclei in the bone marrow of exposed mice: Roy et al. (1992) exposed mice to 10 or 20 mg/kg Pb nitrate i.p. and observed a concentration-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 concentration-dependent. Mice exposed to 1 g/L Pb-acetate via drinking water, a more environmentally relevant route of exposure, for 90 days had statistically significant increases in micronucleated PCEs (Marques et al., 2006).

A few studies were found that reported increased micronucleus formation in human cell lines treated with Pb. Concentration-dependent micronucleus formation was observed in human lymphocytes when exposed to either 1, 5, 10, or 50 µM Pb nitrate or 3 or 5 ppm Pb chloride (Ustundag and Duydu, 2007; Turkez et al., In Press). Gastaldo et al. (2007) also observed a dose-dependent increase in micronuclei in human endothelial cells exposed to 1-1,000 µM Pb nitrate for 24 hours. Two animal cell culture studies investigating micronuclei formation produced contrasting results. One study observed that micronuclei were not induced in Chinese hamster cells exposed to 3-30 µM Pb nitrate for 2 hours (Lin et al., 1994), whereas the other observed that Pb-acetate induced a concentration-dependent increase in Chinese hamster cells when administered at 0.03-10 µM for 18 hours (Bonacker et al., 2005).

Chromosomal Aberrations

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

The majority of animal studies investigating Pb-induced genotoxicity focused on the capacity of Pb to produce chromosomal damage. Fahmy (1999) exposed mice to 25-400 mg/kg Pb-acetate i.p., either as a single exposure or repeatedly for 3, 5, or 7 days. Chromosomal damage was observed to increase in bone marrow cells (100-400 mg/kg) and spermatocytes (50-400 mg/kg) in a concentration-dependent manner after both exposure regimens. Pb nitrate was also observed to produce concentration-dependent chromosomal damage in mice exposed i.p. to a single exposure of 5, 10, or 20 mg/kg (Dhir et al., 1992b). 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 concentration-dependent response as the response was similar in all concentrations tested. Nayak et al. (1989b) 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 exposure. 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 concentration-dependent response reported and few cells were analyzed (Nayak et al., 1989a). 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 exposure concentrations. Dhir et al. (1992a) also observed a concentration-dependent increase in chromosomal damage in mice exposed via gavage, albeit at much lower concentrations: either 5 or 10 mg/kg. Nehez et al. (2000) observed a Pb-induced increase in aneuploidy and percent of cells with damage after exposure to 10 mg/kg administered by gavage 5 days a week for 4 weeks. In the only study that investigated dietary exposure, El-Ashmawy et al. (2006) exposed mice to 0.5% Pb-acetate in feed, and observed an increase in abnormal cells and frequency of chromosomal damage.

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

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. A few additional studies in humans observed that higher bone Pb levels were associated with lower DNA methylation patterns in adults and cord blood of newborns (Wright et al., 2010; Pilsner et al., 2009). Changes in DNA methylation patterns could potentially lead to dysregulation of gene expression and altered tissue differentiation.

Mitogenesis

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

Many studies have investigated the ability of Pb to induce mitogenesis in animal models, and have consistently shown that Pb nitrate can stimulate DNA synthesis and cell proliferation in the liver of animals exposed to 100 µM/kg via i.v. injection (Nakajima et al., 1995; Coni et al., 1992; Ledda-Columbano et al., 1992; Columbano et al., 1990; Columbano et al., 1987). Shinozuka et al. (1996) observed that Pb-induced hepatocellular proliferation was similar in magnitude to that induced by TNF-α at 100 µM/kg, and Pb was observed to induce TNF-α in glial and nerve cells and NF-κB, TNF-α, and iNOS in liver cells in exposed animals at 12.5 mg/kg and 100 µM/kg, respectively (Cheng et al., 2002; Menegazzi et al., 1997). In the only study that examined Pb exposure via inhalation, exposure to 10,000 µM 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 and Tonner, 1995) and primary oligodendrocyte progenitor cells (1 µM for 24 hours) (Deng and Poretz, 2002) but had no observable effects on growth in glioma cells (0.01-10 µM for 12-72 hours) (Liu et al., 2000). Pb glutamate had no effect on cell growth in human lung cells, but did increase the mitotic index (250-1,000 µM for 24 hours) (Wise et al., 2005).

The increase in the mitotic index was attributed to an arrest of the cell cycle at M-phase, and was not attributed to an actual increase of cell growth and proliferation. Gastaldo et al. (2007) also reported S and G2 cell cycle arrests in human endothelial cells following exposure to 100 µM Pb nitrate for 24 hours. Conflicting results with regard to DNA synthesis were reported, with a concentration-dependent inhibition of DNA synthesis reported in hepatoma cells (1-100 µM for 72 hours) (Heiman and Tonner, 1995), but an induction of synthesis observed in astrocytoma cells (1-50 µM for 24 hours) (Lu et al., 2002).

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

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 GST-P with both Pb compounds. The induction of GST-P by Pb was observed to occur on the transcriptional level and to be dependent on the direct activation of the cis-element GPEI enhancer. Degawa et al. (1993) reported that i.v. exposure to 20, 50, or 100 µmol/kg Pb nitrate selectively inhibited CYP1A2 levels. Pb was shown not to 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 GST 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).

Additional animal studies provide further evidence that exposure to Pb compounds can perturb gene expression. Zawia and Harry (1995) investigated whether the observed Pb-induced disruption of myelin formation in rat pups exposed postnatally was due to altered gene expression. In pups exposed to 0.2% Pb-acetate via lactation from postnatal day (PND)1-20, the expression of proteolipid protein, 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, was similarly elevated in exposed animals, although not significantly so. The expression of both genes returned to control levels 5 days following the termination of exposure. These data suggest that altered gene expression in structural myelin proteins due to Pb exposure may be responsible for observed alterations in abnormal conduction of nerve impulses. Long et al. (2011) investigated the Pb-induced induction of ABCC5, an ATP-binding cassette transporter, in embryonic and adult zebrafish. In the initial in vitro portion of the study, exposure of zebrafish fibroblasts to 20 µM Pb nitrate for 24 hours significantly increased the induction of ABBC5 mRNA 2.68-fold over controls. Similar levels of induction were observed when embryonic zebrafish were exposed to 5 µM 24 to 96 hours; specifically, induction of ABCC5 was seen in the livers of developing embryos. In adult fish, induction of ABCC5 was observed in the brains, intestines, and kidneys of exposed fish, but decreased in their livers. Induction of ABCC5 was observed to attenuate the toxicity of Cd, but not Hg or As, in developing embryos, the attenuation of Pb-induced toxicity was not investigated. However, these findings indicate that increased expression of ABCC5 due to heavy metal exposure may play a part in cellular defense mechanisms.

5.2.7.6 Apoptosis

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

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). While this section draws from older literature as well as newer lines of evidence, the inclusion of new evidence does not qualitatively change the conclusions regarding individual modes of action. However, the new evidence strengthens the conclusions. Evidence for the majority of these modes of action is observed at low blood Pb levels in humans, between 2 and 17 µg/dL, with supporting evidence from in vitro assays. As many of these studies are examining adults with likely higher past than current Pb exposures, uncertainty exists as to the Pb exposure level, duration, and timing leading to these blood Pb levels associated with these modes of action. These observable effect levels are reflective of the data and methods available and do not imply that these modes of action are not acting at lower Pb exposure or blood Pb levels or that these concentrations represent the threshold of the effect. The observable effect levels in humans, reported in Table 5-2, are drawn from the available data; and, do not imply that theses modes of action are not acting at lower exposure levels or that these doses represent the threshold of the effect. Also, the data presented in this table does not inform regarding the exposure frequency and duration required to elicit a particular MOA.

Table 5-2 MOAs, their related health effects, and information on concentrations eliciting the MOAs

Mode of Action [Related Health Effects (ISA section)]	Concentrations or doses (Conditions)[a]	
	Blood Pb	Dose
Altered Ion Status [All Health Effects of Pb]	3.5 µg/dL (mean in cord blood; association with cord blood Ca^{2+}-ATPase pump activity) Huel et al. (2008)	0.00005 µM free Pb^{2+} (In vitro; 30 minutes; calmodulin activation assay) Kern et al. (2000)
Protein Binding [Renal (5.5), Heme Synthesis and RBC Function (5.7)]	17.0 µg/dL (concurrent mean in adult workers with wildtype metallothionein expression; increased BP susceptibility) Chen et al. (2010a)	50 µM Pb glutamate (In vitro; 24 hours; increased nuclear protein in neurological cell) Klann and Shelton (1989)
Oxidative Stress [All Heath Effects of Pb]	5.4 µg/dL (concurrent mean in adult male workers; decreased CAT activity in blood) Conterato et al. (In Press)	0.1 µM Pb-acetate (In vitro; 48 hours; decreased cellular GSH in neuroblastoma cells) Chetty et al. (2005)
Inflammation [Nervous System (5.3), Cardiovascular (5.4), Renal (5.5), Immune (5.6), Respiratory (5.6.4), Hepatic (5.9.1)]	2.5 µg/dL (concurrent minimum in adult males; increased serum TNF-α and blood WBC count) Kim et al. (2007)	0.01 µM Pb-acetate (In vitro; 48 hours; increased cellular PGE_2 in neuroblastoma cells) Chetty et al. (2005)
Endocrine Disruption [Reproductive and Developmental Effects (5.8), Endocrine System 5.9.3), Bone and Teeth (5.9.4)]	1.7 µg/dL (concurrent minimum in women with both ovaries removed; increased serum FSH) Krieg (2007)	10 µM Pb nitrate (In vitro; 30 minutes; displaced GHRH binding to rat pituitary receptors) Lau et al. (1991)
Cell Death/Genotoxicity [Cancer (5.10), Reproductive and Developmental Effects (5.8), Bone and Teeth (5.9.4)]	3.3 µg/dL (concurrent median in adult women; increased rate of HPRT mutation frequency) Van Larebeke et al. (2004)	0.03 µM Pb-acetate (In vitro; 18 hours; increased formation of micronuclei) Bonacker et al. (2005)

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

The alteration of cellular ion status (including disruption of Ca^{2+} homeostasis, altered ion transport mechanisms, and perturbed protein function through displacement of metal cofactors) appears to be the major unifying mode of action underlying all subsequent modes of action (Figure 5-1). Pb will interfere with endogenous Ca^{2+} homeostasis, necessary as a cell signal carrier mediating normal cellular functions. $[Ca^{2+}]_i$ has been shown to increase after Pb exposure in a number of cell types including bone, erythrocytes, brain cells, and white blood cells, due to the increased flux of extracellular Ca^{2+} into the cell. This disruption of ion transport is due in part to the alteration of the activity of transport channels and proteins, such as Na^+-K^+ ATPase and voltage-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 has been shown to be 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 perturbation 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 an important protein in the formation of inclusion bodies and mitigation of the toxic effects of Pb.

A second major mode of action of Pb is 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 decreased production of related cytokines.

Evidence indicates that 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 investigating HPT are mixed and require further investigation.

The association of Pb with increased genotoxicity and cell death has been investigated in humans, animals, and cell models. Occupational Pb exposure in humans has been associated with increased DNA damage, however lower blood Pb and exposure levels have been associated with these effects in experimental animals and cells. Results vary on the effect of Pb on DNA repair activity, however a number of studies reported decreased repair processes following Pb exposure. There is evidence of mutagenesis and clastogenicity in highly-exposed humans, however weak evidence has been shown in animals and cell based systems. Human occupational studies provide limited evidence for micronucleus formation (blood Pb levels >10 µg/dL), supported by Pb-induced effects in both animal and cell studies at higher exposure levels. Animal studies have also provided evidence for Pb-induced chromosomal aberrations. The observed increases in clastogenicity may be the result of increased oxidative damage to DNA due to Pb exposure, as co-exposures with antioxidants ameliorate the observed toxicities. Limited evidence of epigenetic effects is available, including DNA methylation, mitogenesis, and gene expression. Pb may alter gene expression by displacing Zn from multiple transcriptional factors, thus perturbing their normal cellular activities. Consistently positive results have provided evidence of increased apoptosis following Pb exposure.

Similar to Pb, other polyvalent metal ions (e.g., Cd, Cr, Be, Ba, Se, Sr, As, Al, Cu) have demonstrated molecular mimicry and displacement of biological cation (Garza et al., 2006). In this manner, these metal ions share with Pb a common central mode of action of disruption of ion status. Specifically, these metals have been shown to disrupt cellular processes as diverse as Ca^{2+} homeostasis, cell signaling, neurotransmitter release, cation membrane channel function, protein-DNA binding, and cellular membrane structure

(Pentyala et al., 2010; Huang et al., 2004; Atchison, 2003; Jehan and Motlag, 1995; Richardt et al., 1986; Cooper and Manalis, 1984; Habermann et al., 1983). Additionally, presumably through their shared central mode of action, some of these metal ions also display corresponding downstream modes of actions such as oxidative stress, apoptosis, and genotoxicity (Jomova and Valko, 2011; Jomova et al., 2011; Matović et al., 2011; Agarwal et al., 2009; Méndez-Gómez et al., 2008; Rana, 2008; Hengstler et al., 2003).

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

5.3 Nervous System Effects

5.3.1 Introduction

The 2006 Pb AQCD concluded that the "overall weight of the available evidence provides clear substantiation of neurocognitive decrements being associated in young children with blood-Pb concentrations…" (U.S. EPA, 2006b). This conclusion was based on evidence from several prospective and cross-sectional studies conducted in diverse populations and after adjusting for potential confounding by socioeconomic status (SES), parental intelligence, and caregiving environment. 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). Associations between blood Pb levels and decrements in intelligence quotient (IQ), mental development, memory, and other specific indices of cognitive function in children ages 6 months to 17 years were most strongly indicated in children with population mean blood Pb levels (measured at various lifestages) in the range of 5-10 µg/dL; however, several results indicated associations in groups of children (ages 2-10 years) with mean blood Pb levels in the range of 3-5 µg/dL (Bellinger, 2008; Canfield, 2008; Hornung, 2008; Rojo-Tellez, 2008). Based on fewer available studies, the 2006 Pb AQCD described consistent associations of blood Pb levels with behavioral outcomes, including inattention, and antisocial and delinquent behavior assessed in children ages 6 to 13 years (U.S. EPA, 2006b).

Toxicological studies provided coherence with similarly consistent findings for Pb-induced impairments in learning and behavior in rodents and monkeys (U.S. EPA, 2006b). In contrast with studies in children, Pb exposure was not found consistently to affect memory of animals. Effects on learning were largely demonstrated as poorer performance on Morris water maze and discrimination reversal tasks, and effects on

behavior were largely demonstrated as distractibility, impulsivity, and insensitivity to changes in reinforcement. These effects in animals were predominantly found with Pb exposures that resulted in blood Pb levels between 20-50 µg/dL; however, some studies observed learning and behavioral impairments in animals with steady-state blood Pb levels of 10-15 µg/dL (Cory-Slechta, 1994; Altmann et al., 1993; Rice and Karpinski, 1988; Gilbert and Rice, 1987). Toxicological studies also provided biological plausibility by characterizing modes of action for Pb-induced nervous system 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 in children being causally associated with deficits in multiple functional domains such as cognitive function, motor function, memory, mood, and behavior. Additional biological plausibility was provided by associations observed of childhood blood Pb levels with changes indicative of neuronal damage and altered brain physiology assessed in young adults using magnetic resonance imaging techniques (Yuan et al., 2006; Cecil et al., 2005; Meng et al., 2005; Trope et al., 2001).

A common finding across several epidemiologic studies of children was a supralinear concentration-response relationship between blood Pb level and neurocognitive deficits, i.e., a larger decrement in neurocognitive function per unit increase in blood Pb levels in children in the lower range of the population blood Pb level distribution (Kordas et al., 2006; Schnaas et al., 2006; Tellez-Rojo et al., 2006; Lanphear et al., 2005; Rothenberg and Rothenberg, 2005; Bellinger and Needleman, 2003; Canfield et al., 2003a). Most of these epidemiologic results were based on the analysis of concurrent blood Pb levels and a cut-point of 10 µg/dL to define lower and higher blood Pb levels. These findings were corroborated by findings from analyses of the pooled cohort data indicating that a nonlinear relationship fit the data better than a linear relationship did (Lanphear et al., 2005; Rothenberg and Rothenberg, 2005). Consistent with epidemiologic findings, toxicological studies observed nonlinear Pb concentration-response relationships for outcomes such as neuronal activation (Lewis and Pitts, 2004), neurogenesis (Gilbert et al., 2005), and retinal responses (Fox et al., 1991; Fox and Farber, 1988; Fox and Chu, 1988).

Another area of focus included the comparison of various lifestages of Pb exposure in terms of risk of neurodevelopmental deficits. Toxicological studies clearly demonstrated that in utero with or without early postnatal exposure to Pb was an especially sensitive window for Pb-induced neurodevelopmental effects. Nonetheless, not all endpoints in animal toxicology studies had a single defined window of sensitivity but instead were shown to be affected by exposures at multiple periods during the lifespan of the

organism. Epidemiologic studies observed cognitive deficits in children ranging from 2 to 10 years of age in association with prenatal, peak childhood, cumulative childhood, and concurrent blood Pb levels. Although examined in few studies, tooth or bone Pb levels were associated with cognitive and behavioral deficits in children and adolescents (Wasserman et al., 2003; Greene and Ernhart, 1993; Bellinger et al., 1991; Needleman et al., 1979), also pointing to an effect of cumulative childhood Pb exposure. Among studies of children (ages 2-10 years) that examined blood Pb levels measured at multiple lifestages, several found that concurrent blood Pb was associated with an equal or larger decrement in IQ (Chen et al., 2005; Lanphear et al., 2005; Wasserman et al., 1994; Dietrich et al., 1993b; Bellinger et al., 1992). A common limitation of epidemiologic studies of children was the high correlation among blood Pb levels at different ages, making it difficult to ascertain which developmental periods of Pb exposure were associated with the greatest risk of neurodevelopmental decrements (Lanphear et al., 2005). The issue of persistence of the neurodevelopmental effects of Pb exposure also was considered, with some evidence suggesting that the associations of biomarkers of early childhood Pb exposure (e.g., deciduous tooth, blood at age 2 or 6 years) with neurodevelopmental outcomes persisted into adolescence and young adulthood (Ris et al., 2004; Tong et al., 1996; Needleman et al., 1990). Some studies in rats and monkeys also demonstrated that the effects of in utero and early postnatal Pb exposures on neurodevelopmental outcomes persisted into adulthood (Cory-Slechta, 1994; Altmann et al., 1993; Rice and Karpinski, 1988; Gilbert and Rice, 1987).

In epidemiologic studies of adults, a range of nervous system effects (e.g., impaired memory, attention, reaction time, visuomotor tasks and reasoning, alterations in visual or brainstem evoked potentials, postural sway) were mostly clearly indicated in Pb-exposed workers with blood Pb levels in the range of 14 to 40 μg/dL (Iwata et al., 2005; Bleecker et al., 1997; Baker et al., 1979; Cantarow and Trumper, 1944). In the limited literature examining nonoccupationally-exposed adults, the weight of evidence supported associations of bone Pb levels with cognitive function (Weisskopf et al., 2004; Wright et al., 2003b) but not concurrent blood Pb levels (Krieg et al., 2005; Nordberg et al., 2000; Payton et al., 1998; Muldoon et al., 1996). These findings suggested that rather than recent exposures, past or cumulative Pb exposures contributed to cognitive deficits in nonoccupationally-exposed adults. With regards to neurodegenerative diseases, whereas a few toxicological studies demonstrated Pb-induced amyloid plaques commonly associated with Alzheimer's disease pathophysiology (Basha et al., 2005; Zawia and Basha, 2005), epidemiologic studies did not indicate that Pb exposure was associated with Alzheimer's Disease in adults. Pb biomarker (blood or bone) levels were inconsistently associated with amyotrophic lateral sclerosis (ALS) in adults in the general population; however, some case-control studies found that history of occupational Pb exposure was more prevalent among ALS cases than controls (Kamel et al., 2002;

Chancellor et al., 1993). Associations were reported for essential tremor and symptoms of anxiety and depression, but each was examined in only a few studies.

As discussed throughout this section, recent epidemiologic and toxicological studies continued to demonstrate associations of Pb exposure and biomarkers of Pb exposure with nervous system effects. The weight of evidence continued to be derived from associations observed of Pb exposure and blood Pb levels in young animals and children, respectively, with cognitive function decrements, inattention, and impulsivity. Expanding upon the previous body of evidence, several recent studies in children found similar associations with lower population mean (or quantile) blood Pb levels (1-5 µg/dL). Whereas previous evidence was inconsistent, several new studies in children reported associations between concurrent blood Pb levels and attention deficit hyperactivity disorder (ADHD). Recent studies in adults focused primarily on cognitive function decrements but also provided additional evidence for Pb-associated mood disorders, ALS, Parkinson's Disease, and essential tremor. New toxicological studies expanded evidence for the effects of prenatal and postnatal Pb exposure on learning, memory, and attention and provided insight into the contribution of social stress to this paradigm. New or expanded areas of toxicological research related to Pb exposure included mood disorders, neurofibrillary tangle formation, and adult dementia after early life Pb exposures. Historically important areas of toxicological research were further expanded with recent findings for Pb-induced 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 nervous system effects associated with Pb exposure.

5.3.2 Cognitive Function and Learning

5.3.2.1 Epidemiologic Studies of Cognitive Function in Children

Epidemiologic studies have assessed global cognitive function most frequently by full-scale IQ (FSIQ) and its verbal and performance subscale components in children ages 3 to 17 years and by the Bayley Scales of Infant Development in children ages 6 months to 3 years. These indices have strong psychometric properties and are among the most rigorously standardized measures. A large body of evidence also comprises associations of blood Pb levels with specific cognitive abilities, including memory and learning, executive function, language, and visuospatial processing. These specific indices of cognitive function are reflected in global measures of intelligence and also are more comparable to tests of learning and memory in animals. Fewer studies have examined

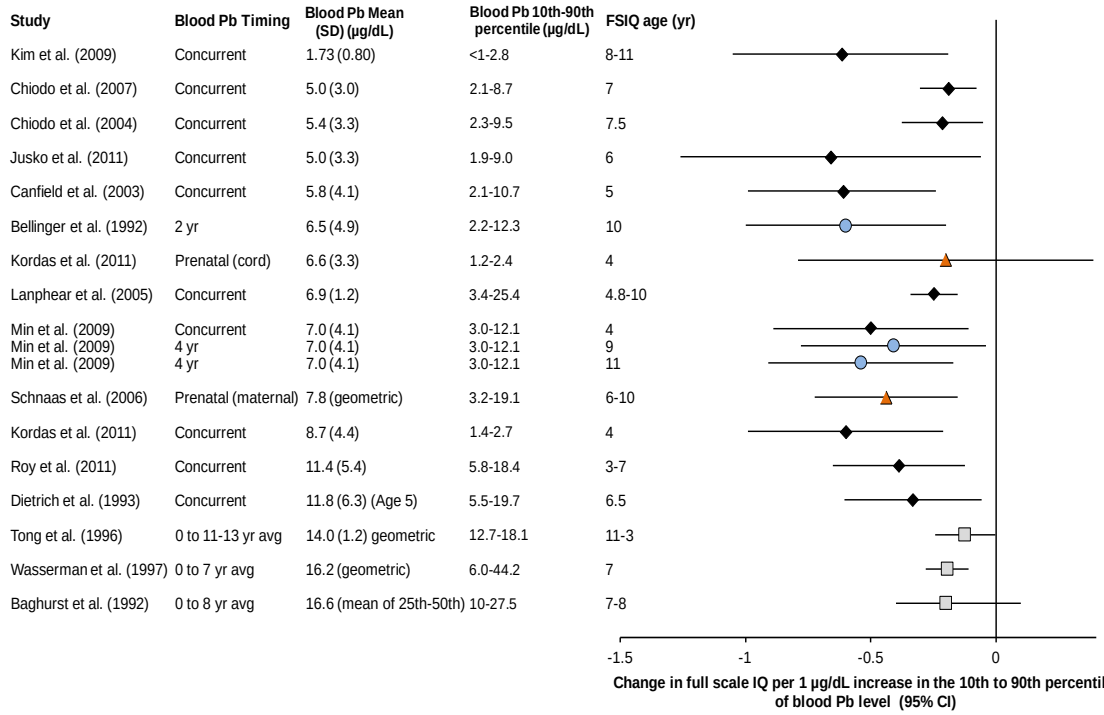
academic performance and achievement; however, these outcomes may provide information on the impact of Pb exposure on life success. The epidemiologic evidence for each of these categories of outcomes was evaluated separately in order of increasing weight of evidence. Emphasis was placed on prospective studies with repeated assessments of blood Pb levels and cognitive function and on studies examining blood Pb levels similar to those measured in contemporary U.S children (i.e., less than 5 µg/dL), and younger children whose blood Pb levels are less influenced by higher past Pb exposures.

Many factors have been shown to influence the intellectual abilities of children, including parental SES, parental education, parental IQ, quality and stability of the caregiving environment, nutritional status, and birth weight (Nation and Gleaves, 2001; Wasserman and Factor-Litvak, 2001). These and other influences on neurodevelopment often are correlated with blood Pb levels. Thus, due to their association with both blood Pb level and outcome, these other risk factors may potentially confound (i.e., bias due to an association with blood Pb level and causal association with the outcome) the associations observed between blood Pb level and indices of cognitive function. In the evaluation of the effect of Pb independent from the effects of the other variables, greater weight was given to studies that accounted for potential confounding in the study design or in statistical analyses. A detailed discussion of the collective weight of evidence for the independent associations between Pb exposure and nervous system effects in relation to the adequacy of control for confounding by other risk factors is located in Section 5.3.11.

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 blood Pb levels and cognitive outcomes, examining the persistence of cognitive deficits to older ages, and comparing risk estimates among blood Pb levels measured 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 the range of blood Pb levels, race/ethnicity, and SES. Individual cohort studies in diverse populations were consistent in demonstrating associations between higher blood Pb and lower FSIQ in populations of school-aged children with mean blood Pb levels in the range of 5 to 10 µg/dL (Schnaas et al., 2006; Bellinger and Needleman, 2003; Canfield et al., 2003a; Wasserman et al., 1997; Dietrich et al., 1993a; Baghurst et al., 1992; Bellinger et al., 1987) (Figure 5-2 and Table 5-3). In analyses restricted to children in the lower range of the blood Pb distribution (e.g., < 10 µg/dL), associations were observed in groups of children with mean blood Pb levels 3-4 µg/dL (Bellinger, 2008; Canfield, 2008; Hornung, 2008). Across cohort studies,

associations were observed with concurrent, prenatal, early childhood (e.g., age 2 years), and childhood average blood Pb levels (e.g., 0-8 year average) (Figure 5-2 and Table 5-3). 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 prospective and cross-sectional studies (Pocock et al., 1994; Schwartz, 1994; Needleman and Gatsonis, 1990).



Note: Studies generally are presented in ascending order of mean blood Pb level. To facilitate comparisons among effect estimates across studies with different distributions of blood Pb levels and model structures (e.g., linear, log-linear), effect estimates are standardized to a 1 µg/dL increase in blood Pb level within the 10th to 90th percentile interval. The percentiles are estimated using various methods and are only approximate values. Effect estimates are assumed to be linear within the 10th to 90th percentile interval of blood Pb level. The various tests used to measure FSIQ are scored on a similar scale (approximately 40-160). Black diamonds, blue circles, orange triangle, and gray squares represent associations with concurrent, earlier childhood, prenatal, and lifetime average blood Pb levels, respectively.

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 represented in Figure 5-2

Study	Population/ Location	Blood Pb Data (µg/dL)	Statistical Analysis	FSIQ Assessment[a]	Effect Estimate (95% CI)[b,c]
Kim et al. (2009a)	279 children in Seoul, Seongnam, Ulsan, and Yeoncheon, Korea, ages 8-11 yr in April-December 2007	Concurrent Mean (SD): 1.73 (0.80) 10th-90th: < 1-2.8	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	-0.62 (-1.05, -0.19)
Chiodo et al. (2007)	506 African-American children in Detroit, MI area followed from birth (1989-1991) to age 7 yr. Large proportions of children with prenatal exposure to cocaine or marijuana	Concurrent Mean (SD): 5.0 (3.0) 10th-90th: 2.1-8.7	Regression model adjusted for prenatal alcohol and drug use, SES, Symptom Checklist (component of HOME score), maternal IQ	WISC-III at age 7 yr	-0.19 (-0.30, -0.08)[b]
Chiodo et al. (2004)	246 African-American children in Detroit, MI area followed from birth (not reported) to age 7.5 yr. Large proportions of children with prenatal exposure to cocaine or marijuana	Concurrent Mean (SD): 5.4 (3.3) 10th-90th: 2.3-9.5	Log linear 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	-0.22 (-0.38, -0.05)[b]
Jusko et al. (2011)	194 children in Rochester, NY followed from age 6 mo (1994-1995) to age 6 yr.	Concurrent Mean (SD): 5.0 (3.3) 10th-90th: 1.9-9.0	Linear regression model adjusted for sex, birth weight, transferrin saturation, maternal race, maternal IQ, maternal education, HOME score, family income, and maternal prenatal smoking	WPPSI-R at age 6 yr	-0.66 (-1.26, -0.06)
Canfield et al. (2003a)	172 children in Rochester, NY born 1994-1995 followed from age 6 mo to age 5 yr.	Concurrent Mean (SD): 5.8 (4.1) 10th-90th: 2.1-10.7	Linear regression model adjusted for sex, maternal race, prenatal smoking, maternal education, child iron status, household income, maternal IQ, HOME score, birth weight	Stanford-Binet at age 5 yr	-0.61 (-0.99, -0.24)
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) 10th-90th: 2.2-12.3	Linear regression model adjusted for HOME score (age 10 and 5 yr), child stress, race, maternal IQ, SES, sex, birth order, marital status	WISC-R at age 10 yr	-0.58 (-0.99, -0.18)
Lanphear et al. (2005)	1,333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts	Concurrent Mean (SD): 6.9 (1.2) 10th-90th: 3.4-25.4	Log linear regression model adjusted for HOME score, birth weight, maternal IQ, maternal education	FSIQ measured at ages 5-10 yr	-0.25 (-0.34, -0.15)
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.	Age 4 yr Mean (range): 7.0 (1.3-23.8) 10th-90th: 3.0-12.1	Linear regression model adjusted for HOME score, caregiver's vocabulary test, sex, parity, maternal marital status, head circumference at birth	WISC-R at age 4 yr (concurrent) 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)
Schnaas et al. (2006)	150 children in Mexico City, Mexico followed from birth (1987-1992) to age 10 yr.	Prenatal (maternal 28-36 weeks) Geometric mean (95% CI): 7.8 (2.5-24.5) 10th-90th: 3.2-19.1	Log linear mixed effects regression model adjusted for sex, SES, maternal IQ, HOME score, birth weight, postnatal blood Pb, random slope for subject	McCarthy GCI at ages 6-10 yr	-0.44 (-0.73, -0.15)

Study	Population/ Location	Blood Pb Data (µg/dL)	Statistical Analysis	FSIQ Assessment[a]	Effect Estimate (95% CI)[b,c]
Kordas et al. (2011)	186 children in Mexico City, Mexico followed from birth (1994-1995) to age 4 yr	Prenatal (cord) Mean (SD): 6.6 (3.3) 10th-90th: 1.2-2.4 Concurrent Mean (SD): 8.7 (4.4) 10th-90th: 1.4-2.7	Linear regression model adjusted for sex, birth weight, gestational age, maternal age, years of schooling, IQ, smoking status, marital status, crowding in home, type of floor in home	McCarthy GCI at age 4 yr Prenatal Concurrent	 -0.20 (-0.79, 0.39) -0.60 (-0.99, -0.21)
Roy et al. (2011)	717 children in Chennai, India ages 3-7 yr	Concurrent Mean (SD): 11.4 (5.4) 10th-90th: 5.8-18.4	Log linear model adjusted for mid-arm circumference, age, sex, family income, parental education, parental IQ, family size	Binet-Kamat at ages 3-7 yr	-0.39 (-0.65, -0.13)
Dietrich et al. (1993a)	253 children in Cincinnati, OH followed from birth (1979-1985) to age 20-23 yr.	Concurrent NR Age 5 yr Mean (SD): 11.8 (6.3) 10th-90th: 5.5-19.7	Linear regression model adjusted for HOME score, maternal IQ, birth weight, birth length, child sex, maternal cigarette consumption during pregnancy	WISC-R at age 6.5 yr	-0.33 (-0.60, -0.06)
Tong et al. (1996)	375 children in Port Pirie, Australia followed from birth (1979-1982) to age 11-13 yr.	Lifetime avg Geometric mean (GSD): 14.0 (1.2) 10th-90th: 12.7-18.1	Regression model adjusted for sex, age, school grade, 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	-0.12 (-0.24, -0.003)
Wasserman et al. (1997)	290 children in Kosovo, Yugoslavia followed from birth (1985-1986) to age 10-12 yr.	Lifetime avg Geometric mean: 16.2 10th-90th: 6.0-44.2	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	-0.20 (-0.28, -0.11)
Baghurst et al. (1992)	494 children in Port Pirie, Australia followed from birth (1979-1982) to age 11-13 yr.	Lifetime avg Mean of 25-50th: 16.6 10th-90th: 10-27.5	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	-0.20 (-0.40, 0.10)

[a]WISC = Wechsler Intelligence Scale for Children, WPPSI = Wechsler Preschool and Primary Scale of Intelligence, GCI = General Cognitive Index
[b]Effect estimates are standardized to a 1 µg/dL increase in blood Pb level within the 10th to 90th percentile interval. Effect estimates are assumed to be linear within the 10th to 90th percentile interval of blood Pb level. The percentiles are estimated using various methods and are only approximate values.
[c]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.

The analysis pooling data from seven prospective studies included 1,333 children ages 5-10 years of age with a median (5th-95th percentile) concurrent blood Pb level of 9.7 µg/dL (2.5-33.2 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 (assessment of physical environment, parental responsivity, learning stimulation, emotional climate, and family interactions), birth weight, and maternal education, higher concurrent, peak, average lifetime, and early childhood blood Pb levels were associated with lower FSIQ measured at age 5-10 years, with the largest decrement in FSIQ estimated for concurrent blood Pb level (-0.25 points [95% CI: -0.34, -0.15] per 1 µg/dL increase in blood Pb level in the 10th to 90th percentile interval [2.2-12.3 µg/dL]

of blood Pb level)[1]. Various models were investigated to characterize the shape of the blood Pb concentration-response relationship. Consistent with findings from several individual cohort studies, Lanphear et al. (2005) found that a log-linear model best fit the data, with a greater decrease in FSIQ estimated for an increase in concurrent blood Pb from 2.4-10 µg/dL (3.9 points [95% CI: 5.3, 2.4]) than an increase from 10 to 20 µg/dL (1.9 points [95% CI: 2.6, 1.2]). Among children with concurrent blood Pb less than 10 µg/dL, the median blood Pb level was 4.2 µg/dL (Hornung, 2008).

A key additional strength of the pooled analysis by Lanphear et al. (2005) was the extensive examination of the potential for confounding by several factors related to SES and the caregiving environment. Variables such as HOME score, birth weight, maternal IQ, and maternal education were statistically significantly associated with FSIQ and were included in the final model with blood Pb level. While a smaller decrement in FSIQ was estimated for concurrent blood Pb level in this adjusted model than in the unadjusted model (-0.43 points [95% CI: -0.53, -0.33] per 1 µg/dL increase in blood Pb level in the 10th to 90th percentile interval of blood Pb level), the adjusted blood Pb effect estimate was nonetheless statistically significant. HOME score was not available in the Rochester study; however, exclusion of data from that cohort resulted in a 3% less negative effect estimate, indicating the lack of a strong influence of HOME score alone on blood Pb level-IQ associations. Other variables such as child sex, tobacco exposure during pregnancy, alcohol use during pregnancy, maternal age at delivery, marital status, and birth order were not statistically significantly associated with FSIQ and did not alter the effect estimate for concurrent blood Pb level. The individual study populations represented a wide range of SES, maternal education, and cultural backgrounds. Sensitivity analyses, in which one study was successively excluded, revealed that no single study was responsible for driving the results. Per 1 µg/dL increase in blood Pb level in the 10th to 90th percentile interval of blood Pb level, effect estimates excluding one study at a time ranged between -0.22 and -0.27, indicating the robustness of the concurrent blood Pb level effect estimate despite between-study variability in the distributions of potential confounding factors.

The small number of studies published since the 2006 Pb AQCD continued to demonstrate associations between higher blood Pb level (primarily concurrent) and lower FSIQ in children between ages 3 and 11 years (Figure 5-2 and Table 5-3). Similar to studies reviewed in the 2006 Pb AQCD, most recent studies demonstrated associations between blood Pb level and lower FSIQ in populations with mean blood Pb level between 5 to 10 µg/dL. New results from the prospective cohorts were limited. Mazumdar et al.

[1]To facilitate comparisons among effect estimates across studies with different distributions of blood Pb levels and model structures (e.g., linear, log-linear), effect estimates are standardized to a 1 µg/dL increase in blood Pb level within the estimated 10th to 90th percentile interval. Effect estimates are assumed to be linear within the 10th to 90th percentile interval of blood Pb level.

(2011) reported on the follow-up of the Boston cohort to age 28-30 years (55 out of the original 249 enrolled at birth). Blood Pb levels measured at age 6 months, 4 years, 10 years, and levels averaged over childhood were associated with decrements in FSIQ in adults. The effect estimates were similar in magnitude for all childhood blood Pb measures, except for 6 month blood Pb level, which was associated with a smaller FSIQ decrement. These findings indicated that the effect of childhood Pb exposures may persist to adulthood.

Jusko et al. (2011; 2008) affirmed the findings in the Rochester cohort previously reported by Canfield et al. (2003a), who examined the cohort at age 5 years (Figure 5-2 and Table 5-3). Jusko et al. (2011) examined the Rochester cohort at age 6 years and, similar to Canfield et al. (2003a), found that a 1 µg/dL increase in concurrent blood Pb level was associated with a 0.66-point decrease (95% CI: -1.26, -0.06) in FSIQ. Model covariates were the same as those analyzed by Canfield et al. (2003a): sex, race, family income, maternal education, race, prenatal smoking, birth weight, transferrin saturation, maternal IQ, and HOME score. At both age 5 and 6 years, higher peak and lifetime average blood Pb levels also were associated with lower FSIQ, and incremental increases in blood Pb levels tended to be associated with larger FSIQ decrements in analyses restricted to children with blood Pb levels less than 10 µg/dL (Jusko et al., 2008; Canfield et al., 2003a). Canfield et al. (2003a) provided additional information on the extent of confounding by SES- and caregiving-related variables. The effect estimate in the covariate-adjusted model was 40% smaller than it was in the model with concurrent blood Pb level alone; however, the association with concurrent blood Pb level remained statistically significant.

Additional evidence recently was provided for children in Mexico City, albeit in a separate cohort of children born later with lower blood Pb levels at corresponding ages. Among children born 1987-1992, Schnaas et al. (2006) previously reported larger Pb-associated decrements in FSIQ for prenatal maternal (28-36 weeks) blood Pb levels than for concurrent blood Pb levels between ages 1 and 10 years. In contrast, Kordas et al. (2011) found that concurrent blood Pb level was associated with a larger decrement in FSIQ at age 4 years than was cord blood Pb level. Children in the latter study were born between 1994 and 1995 and at age 4 years had a mean (SD) blood Pb level of 8.7 (4.4) µg/dL. In Schnaas et al. (2006), the geometric mean (95% CI) blood Pb level at age 4 years was 10.3 (4.2, 20.5) µg/dL. It is not clear whether different temporal patterns of Pb exposure may have contributed to the contrasting associations for prenatal and concurrent blood Pb levels in the two studies.

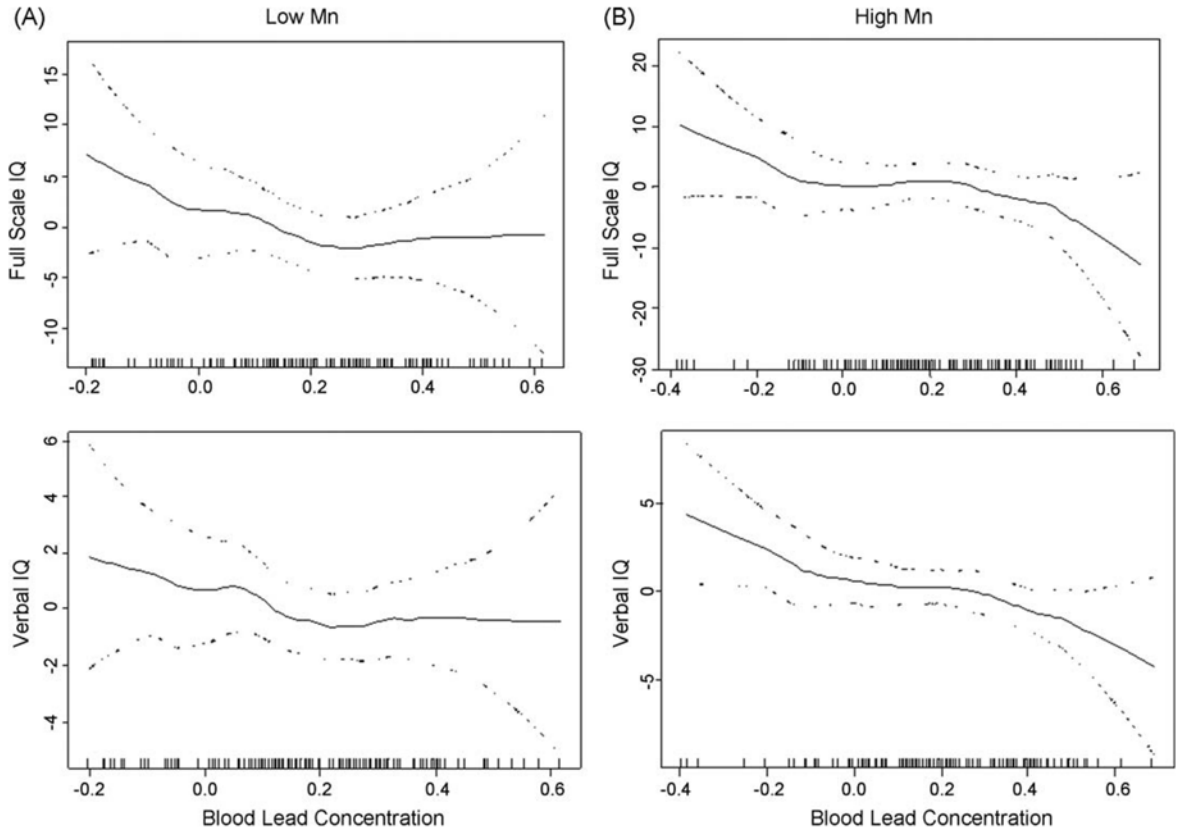
Surkan et al. (2007) examined children from urban Boston, Massachusetts and rural Farmington, Maine participating in a trial designed to assess the effect of amalgam dental

fillings on child neurodevelopment. 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. A strength of the study was that the range of concurrent blood Pb level in this study, 1-10 µg/dL, was lower than that examined in previous studies. Thus, it provided information on associations between blood Pb level and IQ decrements within a lower range of blood Pb levels. However, consistent with several previous studies, Surkan et al. (2007) found lower FSIQ in the group of children with concurrent blood Pb levels of 5 to 10 µg/dL. For example, adjusting for age, race/ethnicity, birth weight, SES, and primary caregiver IQ, children with blood Pb levels 5-10 µg/dL had lower FSIQ scores (-6.04 points [95% CI: -10.7, -1.36]) compared with children who had levels 1-2 µg/dL (referent group). Children with blood Pb levels 2-5 µg/dL did not have FSIQ scores (-0.12 [95% CI: -3.30, 3.06]) that differed from children with blood Pb levels 1-2 µg/dL. Another strength of this study was the analysis of potential confounding by a larger list of variables than that included in the final models. Although HOME score was not examined, several variables such as SES, caregiver education, parenting stress, marital status of caregiver, and maternal utilization of prenatal or annual health care were not significantly ($p > 0.20$) associated with child IQ either independently or in a model with blood Pb level (Surkan et al., 2007).

Other recent studies found blood Pb-associated lower FSIQ in populations of children in Asia with lower blood Pb levels (Kim et al., 2009b; Zailina et al., 2008) than were previously examined. Kim et al. (2009b) examined children ages 8 to 11 years in Korea (born mid- to late-1990s) with a mean (range) concurrent blood Pb level of 1.73 (0.42-4.91) µg/dL. Children were tested using the Korean Educational Development Institute-WISC, which assesses vocabulary, arithmetic, picture arrangement, and block design. In a log-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 higher concurrent blood Pb level was associated with a 0.64-point lower (95% CI: -1.05, -0.19) FSIQ within the 10th-90th percentile interval of blood Pb level (< 1 to 2.8 µg/dL). The potential confounders examined in this study were not found to have a strong influence on the findings as a similar magnitude of effect was estimated in a model that included only blood Pb level (-0.73 points [95% CI: -1.19, -0.27] per 1 µg/dL increase in blood Pb level in the 10th to 90th percentile interval). Although several important SES-related confounders were considered, there was no direct assessment of the home environment and the primary caregiver IQ in this study, which are notable limitations.

Kim et al. (2009b) also examined effect modification of the blood Pb-FSIQ relationship by concurrent blood manganese (Mn) levels. The mean (range) blood Mn level was

14.3 (5.3-29.02) µg/dL. 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 and verbal IQ (VIQ) used concurrent blood Pb level as the predictor variable in the low and high Mn groups. As illustrated in Figure 5-3, the associations of concurrent blood Pb level with FSIQ and VIQ was larger in magnitude and statistically significant for children in the high Mn group compared with children in the low Mn group (-0.65 points [95% CI: -1.26, -0.05] in high Mn group versus -0.50 points [95% CI: -1.23, 0.22] in low Mn group per 1 µg/dL increase in blood Pb level in the 10th to 90th percentile interval). However, higher blood Pb level was associated with lower FSIQ in children in the low Mn group.



Source: Reprinted with permission of Elsevier Science, Kim et al. (2009b).
Note: High and low Mn refer to levels above and below the median of 14 µg/dL, respectively.

Figure 5-3 Effect modification of the association between concurrent blood Pb level and FSIQ by blood Mn level.

Higher concurrent blood Pb level also was associated with a lower IQ score (as assessed by the McCarthy General Cognitive Index) among children ages 6-8 years in Malaysia with a mean blood Pb level of 3.69 µg/dL (Zailina et al., 2008). The concentration-response function was not reported; however, in a multivariate model, blood Pb level and not potential confounding factors such as paternal education, maternal education, household income, number of siblings, and birth order was reported to be significantly associated with FSIQ. As with Kim et al. (2009b), a notable limitation was the lack of consideration of confounding by the caregiving environment.

Pb has long been known to impact dopaminergic neurons, inhibit depolarization-evoked neurotransmitter release, and stimulate spontaneous neurotransmitter release (Section 5.3.8.8). Further, dopaminergic activity is a key process in regulating cognitive function. Thus, variants in dopamine-related genes have the potential to act as effect modifiers of Pb-associated neurodevelopmental effects. Recent studies in children that examined effect modification by variants in dopamine genes produced contrasting results. In a study of children at ages 2 and 4 years, Kordas et al. (2011) did not find differences in association between concurrent blood Pb level and FSIQ by the Taq A1/A1 dopamine receptor 2 (DRD2) genotype, which is associated with reduced dopamine receptor density. Roy et al. (2011) examined children of similar age (3-7 years) in Chennai, India and found that among children with the Taq A1/A1 genotype, a 1 µg/dL higher blood Pb level was associated with a 0.84-point lower (95% CI: -1.66, -0.01) FSIQ within the 10th-90th percentile interval of blood Pb level (5.8-18.3 µg/dL). The same increment in blood Pb level was associated with a 0.36-point lower (95% CI: -0.76, -0.04) FSIQ in children with the Taq A2/A2 higher receptor density genotype. Concurrent blood Pb level was associated with FSIQ in both studies in analyses including all subjects. However, there were many differences between study populations that may have contributed to differences in effect modification by the DRD2 variant. Compared with the group in India, the group in Mexico had a lower mean blood Pb level and lower mean FSIQ score. Additionally, children in the Mexico City group with the Taq A1/A1 genotype had a higher mean FSIQ score, whereas FSIQ scores were similar between Taq genotypes in the children in India.

Other recent studies examined children in the U.S.; however, in these studies, there were high proportions of children with prenatal exposure to alcohol, tobacco, marijuana, or cocaine that may limit the generalizability of findings. Chiodo et al. (2007) examined a population of African American children (age 7 years, born 1989-1991) in the Detroit, MI area with relatively low blood Pb level (mean [SD]: 5.0 [3.0]) but high prevalence of prenatal exposure to cocaine (38%) or marijuana (35%). In a detailed analysis of potential confounding factors, investigators found that cocaine exposure did not meet the criterion for inclusion in the model (i.e., $p > 0.10$ for association with FSIQ). Prenatal marijuana

exposure was associated with FSIQ ($p < 0.10$) and was included in a multivariate model along with caretaker education, SES, HOME score, symptom checklist, number of children in the home, maternal IQ, sex, and prenatal cigarette smoking. In this multivariate model, higher blood Pb level was associated with lower FSIQ (Figure 5-2 and Table 5-3). These findings supported an independent association of blood Pb level despite the high prevalence of prenatal drug exposure in the study population.

Min and colleagues (2009) followed children in Cleveland, OH from age 4 to 11 years who were exposed prenatally to alcohol (77%), cigarette smoking (61%), marijuana (31%), or cocaine (51%). The study population was primarily African-American (86%) and of low SES (98%); 39% of mothers had not finished high school, and 86% were unmarried at the time of enrollment. The mean (range) blood Pb level, measured at age 4 years, was 7.0 µg/dL (1.3-23.8). Several lines of evidence indicated that prenatal drug exposure did not heavily influence the association between blood Pb level and FSIQ. Prenatal alcohol exposure was correlated with blood Pb level but was not statistically significantly associated with IQ ($p > 0.10$) and did not change the blood Pb level effect estimate by more than 10%. Prenatal cocaine exposure was associated with FSIQ at age 9 years; however, blood Pb level at age 4 years remained associated with a decrement in FSIQ at age 9 years after adjusting for cocaine exposure and several SES-related factors (Table 5-3). Based on the examination of interaction terms, associations between blood Pb level and FSIQ were not statistically significantly different between drug-exposed and -unexposed children.

Similar to previous studies with repeated assessments of cognitive function over time, Min et al. (2009) found that the association between blood Pb level at age 4 years and FSIQ persisted to older ages. Higher blood Pb level at age 4 years was associated with similar magnitudes of decrements in FSIQ at ages 4, 9 and 11 years (Figure 5-2 and Table 5-3). Researchers also examined the shape of the concentration-response function using a restricted cubic spline function. Although the cubic spline term did not attain statistical significance ($p = 0.19$), qualitative analysis indicated that the association between blood Pb level and FSIQ decrements persisted and was greater at lower levels blood Pb (< 7 µg/dL). These findings were consistent with those from the pooled analysis (Lanphear et al., 2005) and other individual studies (Tellez-Rojo et al., 2006).

Bayley Scales of Infant Development

The Bayley Scales of Infant Development are the most widely used tests of infant intelligence. The Mental Development Index (MDI) is statistically analogous to IQ, i.e., both scores have a population-standardized mean of 100 and standard deviation of 15. While MDI assesses general cognitive function in infants, it is important to note that

MDI scores are not necessarily correlated with IQ scores measured later in childhood. Associations between blood Pb levels and MDI scores were found in most of the prospective cohort studies in children between age 6 months and 3 years and mean blood Pb levels 5-10 µg/dL (Table 5-4). Recent studies examined children who were born in the mid-1990s to mid-2000s and continued to demonstrate associations between higher cord and concurrent blood Pb level and lower MDI scores (Table 5-4). Studies that found associations with concurrent blood Pb levels also tended to find associations with prenatal cord or maternal blood Pb levels. Recent studies provided new information on effect modification by nutritional status, maternal self-esteem, co-exposure to Mn, and genetic variants. While studies adjusted for multiple SES-related variables including maternal IQ and education, most did not consider confounding by the caregiving environment. Concurrent and cord blood Pb levels were associated with MDI, adjusting for HOME score in diverse cohorts (Solon et al., 2008; Wasserman et al., 1992; Bellinger et al., 1987). In the Cleveland cohort, associations of cord and postnatal blood Pb levels with MDI at ages 6 months to 2 years became null after adjusting for covariates including HOME score (Ernhart et al., 1988; Ernhart et al., 1987). In the analysis of the Cincinnati cohort, HOME score was not significantly associated with blood Pb level or MDI and thus, did not meet the criteria for model inclusion (Dietrich et al., 1987a). Collectively, evidence does not indicate that Pb-associated MDI decrements are driven by confounding by quality of the caregiving environment.

Table 5-4 Associations of blood Pb level with Bayley MDI in children ages 6 months to 3 years[a]

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Cognitive Index	Effect Estimate (95% CI)[b]
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) 10th-90th: 1.2-1.3	Log linear regression model adjusted for maternal education, birth order, prenatal smoking, sex, and within-subject MDI correlation	at age 12 mo at age 24 mo at age 36 mo	-1.9 (-3.8, 0.12) -2.6 (-5.0, -0.21) -2.3 (-4.3, -0.30)
Claus Henn et al.	455 children born 1997-2000 followed prenatally to age 36 mo Mexico City	12 month Mean (SD): 5.1 (2.6) 10th-90th: 2.5-8.4	Linear mixed effects regression adjusted for sex, hemoglobin, gestational age, maternal IQ, maternal education, blood Pb-blood Mn interaction	assessed ages 12 to 36 mo 12 mo blood Mn < 2.0 µg/dL 12 mo blood Mn 2.0-2.8 µg/dL 12 mo Mn > 2.8 µg/dL	 -3.0 (-5.22, -0.78) -0.07 (-0.39, 0.25) -2.2 (0, 4.44)
Tellez-Rojo et al. (2006)	294 children born 1997-1999 followed prenatally to age 24 mo Mexico City Same cohort as above	Concurrent 12 month Mean (SD): 4.7 (2.9) 10th-90th: 1.9-8.2 24 month Mean (SD): 5.3 (4.1) 10th-90th: 2.1-10.7	Log linear regression model adjusted for sex, age, birth weight, maternal IQ, cohort	at age 12 mo at age 24 mo	-0.26 (-0.79, 0.26) -0.89 (-1.32, -0.46)
Dietrich et al. (1993a)	96 to 302 children born 1979-1984 followed prenatally to age 6 mo Cincinnati, OH	Prenatal (cord) Mean (SD): 6.3 (4.5) 10th-90th: 2.3-11.7 Neonatal (10 day) Mean (SD): 4.6 (2.8) 10th-90th: 1.9-8.1 3 month Mean (SD): 5.9 (3.4) 10th-90th: 2.6-10.1	Linear regression model adjusted for birth weight, gestation, maternal age, race, sex, SES	assessed at 6 mo Cord blood Pb Neonatal blood Pb 3-month blood Pb	 -0.66 (-1.4, 0.07) -3.49 (-6.0, 0.96) -0.48 (-1.0, 0.05)
Bellinger et al. (1987)	249 children born 1979-1981 followed prenatally to age 24 mo	Prenatal (cord) Low: < 3 Medium: 6-7 High: ≥ 10	Regression model adjusted for maternal age, race, maternal IQ, maternal education, number of years of smoking, number of alcohol drinks per week in 3rd trimester of pregnancy, SES, HOME, sex, birthweight, gestational age, birth order	assessed ages 6 to 24 mo High vs. low cord blood High vs. medium cord blood	 -4.8 (-7.3, -2.3) -3.8 (-6.3, -1.3)
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) 10th-90th: 2.0-12.4	Linear mixed effects regression model adjusted for sex, maternal age, maternal IQ, maternal education, parity, alcohol consumption, smoking, cohort, maternal self-esteem	assessed ages 12 to 36 mo All subjects High maternal self-esteem Low maternal self-esteem	 -0.18 (-0.45, 0.09) 0.36 (-0.50, 1.2) -0.31 (-0.60, -0.02)
Pilsner et al. (2010)	255 children age 24 mo born 1994-1995 Mexico City, Mexico	Prenatal (cord blood) Mean (SD): 6.7 (3.6) 10th-90th: 3.5-10.5	Linear regression model adjusted for maternal age, maternal IQ, marital status, parity, gestational age, inadequate folate intake, MTHFR genotype	at age 24 mo	-0.73 (-1.2, -0.23)

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Cognitive Index	Effect Estimate (95% CI)[b]
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 (SD): 7.1 (5.1) 10th-90th: 2.5-13.1 3rd trimester Mean (SD): 6.9 (4.2) 10th-90th: 2.8-12.1 Cord blood Mean (SD): 6.2 (3.9) 10th-90th: 2.5-11.0 Concurrent Mean (SD): 4.8 (3.7) 10th-90th: 1.6-9.1	Log linear regression model adjusted for sex, maternal age, current weight, height-for-age Z score, maternal IQ, concurrent blood Pb (in models examining blood Pb at other lifestages)	assessed at age 24 mo Prenatal 1st trimester Prenatal 3rd trimester Cord blood Concurrent	 -0.64 (-1.3, -0.03) -0.38 (-1.0, 0.24) -0.06 (-0.82, 0.70) -0.23 (-0.92, 0.45)
Solon et al. (2008)	502 children ages 6-35 mo, born 1997-2004 Visayas, Philippines	Concurrent Mean (SD): 7.1 (7.7) 10th-90th: 1.6-14.9	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)	assessed ages 6 to 35 mo	-3.32 (-5.02, -1.60)
Vimpani et al. (1985)	592 children followed prenatally to age 24 mo Port Pirie, Australia	20% subjects had 24-month blood Pb levels > 30	Linear regression model adjusted for maternal age, paternal education, maternal education, paternal workplace, maternal workplace, parental relationship, maternal prenatal marital status, child birth rank, mouthing activity, oxygen use at birth, apgar score, neonatal jaundice, size for gestational age, maternal IQ	at age 24 mo Maternal avg prenatal blood Pb Cord blood Pb 6 mo blood Pb 24 mo blood Pb Lifetime avg	 -0.64 0.03 -0.40, $p < 0.05$ -0.06 -0.31, $p < 0.05$
Wasserman et al. (1992)	392 children followed prenatally to age 24 mo Kosovska Mitrovica and Pristina, Yugoslavia	Concurrent Means: 35.5 (K. Mitrovica) 8.4 (Pristina)	Log linear regression model adjusted for sex, birth order, birth weight, ethnic group, HOME, maternal education, maternal age, maternal IQ	at age 24 mo Cord blood Pb 6 mo blood Pb 12 mo blood Pb 18 mo blood Pb 24 mo blood Pb	Per tripling blood Pb -1.7, $p = 0.12$ -1.1, $p = 0.34$ -1.7, $p = 0.17$ -1.8, $p = 0.16$ -2.5, $p = 0.03$

MDI = Mental Development Index, MTHFR = methylenetetrahydrofolate reductase
[a]Studies are presented in order of increasing population mean blood Pb level.
[b]Except where noted, effect estimates are standardized to a 1 µg/dL increase in blood Pb level within the 10th to 90th percentile interval of blood Pb level.

In a population of children in Krakow, Poland with lower cord blood Pb levels (median 1.23 µg/dL, 95% CI: 1.24, 1.34 µg/dL) than those previously examined, increasing cord blood Pb level was associated with similar magnitudes of decrease in MDI at ages 12, 24, and 36 months (Jedrychowski et al., 2009b) (Table 5-4). Consistent with the hypothesis that the developing male central nervous system may be more vulnerable than that of females to environmental insults resulting in later behavioral problems (Moffitt et al., 2001), investigators estimated a larger in MDI per unit increase in cord blood Pb level among the 233 males than among the 223 females (Figure 5-4). Although mean cord blood Pb levels were similar between males (1.35 µg/dL) and females (1.41 µg/dL), the mean 36-month MDI score was lower among males than among females (101 and 105, respectively, $p = 0.0001$).

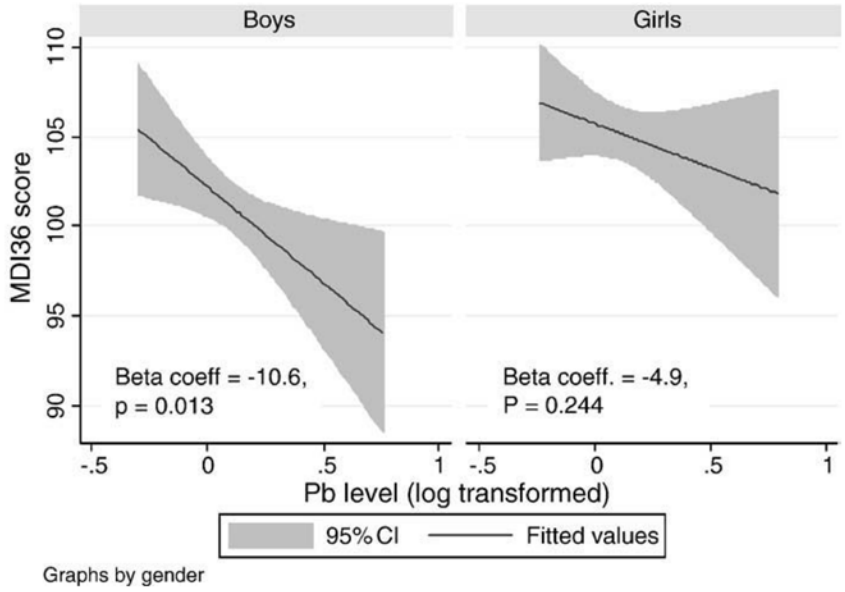


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Figure 5-4 Regression of fitted MDI score at 36 months on log-transformed cord blood Pb level by sex.

Solon et al. (2008) examined children in the Philippines, ages 6 to 35 months. A key strength of this study was the adjustment for HOME score as well as maternal education, maternal smoking, premature birth, region of residence, and years of schooling of child. Not only did investigators find an association between higher concurrent blood Pb level and lower MDI score (Table 5-4), but they also 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 marginal association with lowering MDI scores in the range of 0.80 to 2.44 points. Among children with higher folate levels, blood Pb level was not estimated to have a negative marginal impact. These findings from Solon et al. (2008) indicated that children with folate deficiencies may be at increased risk of Pb-associated decreases in cognitive function. The results were consistent with observations that higher folate level is associated with lower blood Pb level since folate improves Pb excretion by inhibiting the binding of Pb to blood elements.

Multiple studies in different Mexico City mother-child pair cohorts recently reported associations between blood Pb levels (e.g., maternal, cord blood, or child postnatal) and decrements in Bayley MDI in children between age 12 and 36 months (Claus Henn et al.; Pilsner et al., 2010; Surkan et al., 2008; Hu et al., 2006; Tellez-Rojo et al., 2006). Recent studies extended findings from Tellez-Rojo et al. (2006) with follow-up of the same

cohort to age 3 years and observations of effect modification by maternal self-esteem (Surkan et al., 2008) and blood Mn levels (Claus Henn et al.).

Claus Henn et al. examined interactions between blood Mn and Pb levels. Investigators selected mid-range (2.0-2.8 µg/dL) blood Mn levels as the reference group based on previous observations that MDI scores were least affected by increases in blood Mn level in this group. Among subjects with age 12-month blood Mn levels less than 2.0 µg/dL, a 1 µg/dL higher age 12-month blood Pb level was associated with a 3.0-point (95% CI: -5.2, -0.78) lower MDI. Among subjects with blood Mn levels greater than 2.8 µg/dL, a 1 µg/dL higher age 12-month blood Pb level was associated with a 2.2-point (-4.4, 0) lower MDI. Interactions were not found using age 24-month blood Mn and Pb levels. Effects estimates were similar in magnitude before and after adjustment for sex, gestational age, hemoglobin, maternal IQ, maternal education, and visit. Findings in this Mexico City cohort added to those from Kim et al. (2009b) in older children that associations between blood Pb level and cognitive function may be influenced by Mn co-exposure, and results from both studies indicated that blood Pb level was associated with decrements in cognitive function with both lower and higher levels of blood Mn. Biological plausibility for the interactive effects of Pb and Mn exposure is provided by observations that Mn has similar modes of action and cellular targets as does Pb, i.e., altering Ca^{+2} metabolism, inducing oxidative damage to neuronal cells, diminishing dopamine transmission. Claus Henn et al. also indicated that Pb-Mn co-exposure may affect cognitive function in children as young as 12 to 36 months of age.

Surkan et al. (2008) stratified data by the level of maternal self-esteem as reported by mothers. Higher age 24-month blood Pb level was associated with lower MDI score among children with mothers in the lowest three quartiles of self-esteem but not among children with mothers in the highest quartile of self-esteem (Table 5-4). Model covariates included cohort, sex, maternal IQ, maternal age, maternal education, parity, maternal smoking, maternal alcohol consumption, and maternal self-esteem. While HOME score was not examined, maternal self-esteem may incorporate many of the same aspects as HOME score, including mother-child interactions, educational interactions, and dietary practices. These findings indicated that higher maternal psychosocial functioning (e.g., lower stress, anxiety, and depression and higher self-esteem) may contribute to better caregiving, which in turn may improve neuropsychological functioning of the child. The biological plausibility of these findings in children is well-supported by observations in animals unrelated to Pb that environmental enrichment reverses the effects of early stress experiences on reactions such as depressed behavior, HPA activation, and immunosuppressant (Laviola et al., 2008; Laviola et al., 2004; Moreley-Fletcher et al., 2003; Francis et al., 2002). With specific regards to Pb exposure, social isolation or enrichment has been found to exacerbate or protect against, respectively, Pb

exposure-induced learning impairments in animals (Section 5.3.2.2). 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 and Jirtle, 2008).

Hu et al. (2006) compared associations among prenatal blood Pb levels measured at different stages of gestation among 146 mother-child pairs 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 measurement of child blood Pb level at 24 months when the 24-month MDI was ascertained. Among blood Pb levels measured at various lifestages, 1st trimester maternal blood Pb level (whole or plasma Pb) was associated with larger decreases in subsequent 24-month MDI scores compared with maternal 3rd trimester, cord, and child concurrent blood Pb (Table 5-4). These results were adjusted for sex, 24-month blood Pb level, height-for-age Z score, weight, maternal age, and maternal IQ. HOME score was not included in the final model; however, investigators examined the potential for confounding by a larger list of unspecified variables. Prenatal Pb exposure effects also were indicated in a study conducted in Mexico City that examined FSIQ repeatedly during ages 6-10 years (Schnaas et al., 2006). Third trimester (weeks 28-36) maternal blood Pb level, was associated with larger decreases in FSIQ than were maternal blood Pb level at 12-20 weeks, maternal blood Pb level at delivery, or concurrent child blood Pb levels measured between ages 6 and 10 years.

In another recent study in Mexico City, Mexico, investigators found higher cord blood Pb level to be associated with a lower MDI score in children at age 24 months (-0.73 points [95% CI: -1.2, -0.23] in score per 1 µg/dL increase in cord blood Pb level in the 10th-90th percentile interval [3.5-10.5 µg/dL]) (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, 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). Lower folate levels have been associated with higher blood Pb levels. Although Pilsner et al. (2010) found that both cord blood Pb levels and the MTHFR C677T 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.

Specific Indices of Cognitive Function in Children

In addition to indices of global cognitive function, blood Pb levels also have been associated with specific cognitive abilities, including memory, learning, visuospatial processing, and other executive functions such as planning and problem solving in children and adolescents (Kordas et al., 2006; Tellez-Rojo et al., 2006; Chiodo et al., 2004; Ris et al., 2004; Canfield et al., 2003b; Lanphear et al., 2000; Bellinger and Stiles, 1993; Dietrich et al., 1992; Bellinger et al., 1991; Dietrich et al., 1991; Needleman et al., 1979) (Figure 5-5 and Table 5-5). Studies often found associations with multiple indices of cognitive function, and because several tests are interrelated, it is difficult to attribute the effects of Pb exposure to a specific domain of cognitive function. Evidence that blood Pb levels are associated with a spectrum of cognitive indices provides coherence for the associations observed between blood Pb levels and FSIQ, a global measure of cognitive function. Furthermore, these tests of learning and memory in humans are homologous to tests in animals, and compared with that for FSIQ, evidence for these specific indices may improve understanding of the coherence between findings in humans and animals (Rice, 1996). These complex cognitive functions have been described to be mediated by the actions of neurotransmitters dopamine and glutamate in the hippocampus, prefrontal cortex, and ventral striatum of the brain. In toxicological studies, Pb exposure has been shown to disrupt the function of these systems, which provides biological plausibility for associations observed between blood Pb levels and deficits in a range of executive functions in children.

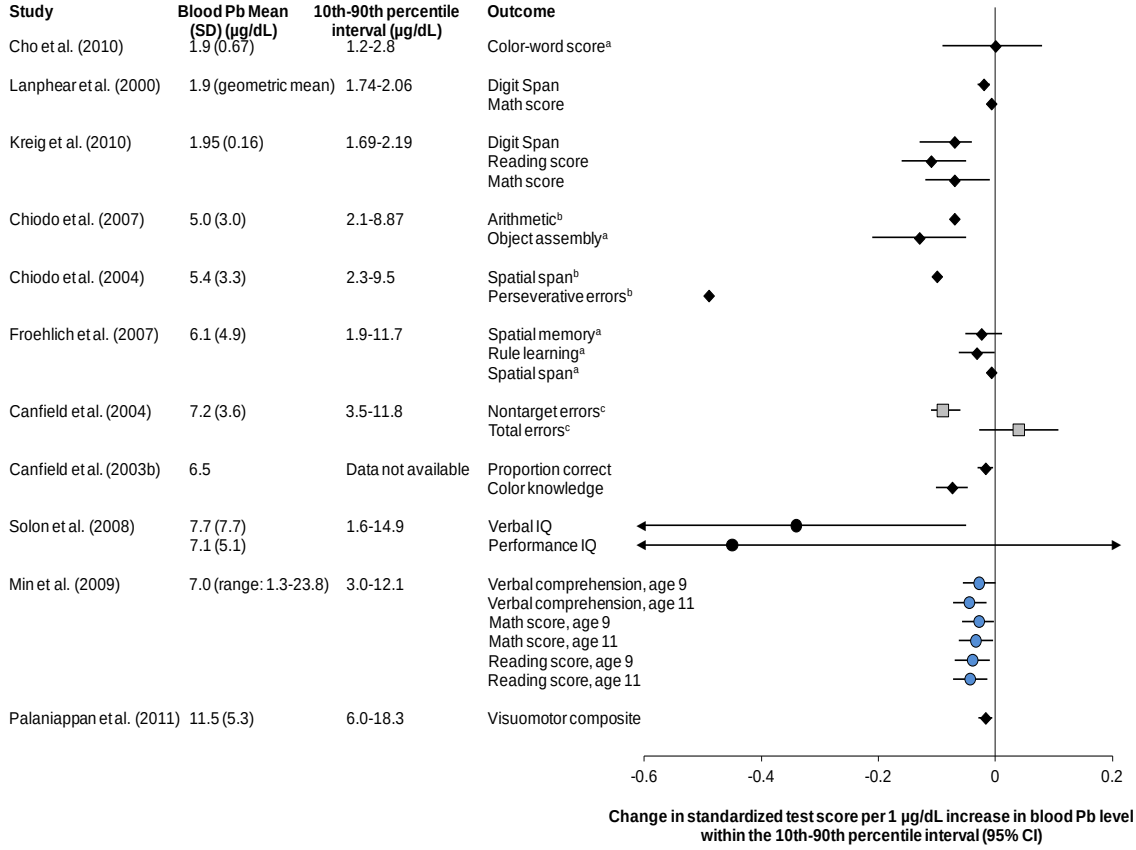
Studies published since the 2006 Pb AQCD added to the evidence for associations between higher blood Pb level (concurrent most frequently examined) and lower performance in specific indices of cognitive function (Figure 5-5 and Table 5-5). The weight of evidence indicated that decrements in specific cognitive indices were associated with concurrent blood Pb level and in populations with mean blood Pb levels between 5-10 µg/dL. Most studies considered potential confounding by multiple SES-related variables, and many found blood Pb-associated decrements in cognitive function in both unadjusted and covariate-adjusted models (Palaniappan et al., 2011; Chiodo et al., 2007; Froehlich et al., 2007). Many studies did not examine potential confounding by HOME score. However, studies that did (Min et al., 2009; Solon et al., 2008; Froehlich et al., 2007; Canfield et al., 2004; Canfield et al., 2003b) and did not adjust for HOME score (Krieg et al., 2010; Jedrychowski et al., 2008; Surkan et al., 2007; Lanphear et al., 2000) both found associations between blood Pb level and decrements in specific cognitive indices.. Several studies found associations after adjusting for different potential confounding factors, including parental IQ or education and HOME score. The set of covariates varied among the specific cognitive endpoints, and HOME score was not associated with every cognitive index. For example, only household income remained

significantly associated with total errors in the spatial working memory task and was included in the final model (Froehlich et al., 2007). Solon et al. (2008) found that in a multivariable model with blood Pb level and several potential confounders, HOME score remained significantly associated with verbal IQ in 5 year-old children, whereas maternal education, maternal smoking, and premature birth did not. Combined, these findings support the independent association of blood Pb level with specific cognitive indices and indicate that the potential confounding variables vary among endpoints and study populations.

Several analyses of the Rochester cohort at ages 4 and 5 years indicated associations of blood Pb level with various indices of cognitive function, including visual and working memory, planning, and problem solving (Froehlich et al., 2007; Canfield et al., 2004; Canfield et al., 2003b). In particular, the associations observed with tests of memory and rule learning and reversal provided strong coherence with observations of Pb-induced impaired performance in animals in homologous tests of the Morris Water Maze, the delayed spatial alternation, and discrimination reversal learning (Section 5.3.2.2). Compared with other prospective cohort studies, findings from the Rochester cohort demonstrated associations between blood Pb level and decrements in executive functions in younger children with a lower mean blood Pb level (6.5 and 6.0 µg/dL at ages 4 and 5 years, respectively). An additional strength of these studies was the detailed analysis of potential confounding by various demographic and SES-related variables (including HOME score) and other environmental exposures. Covariates were selected for specific models based on their association with a particular test ($p \leq 0.20$), and several models adjusted for maternal IQ, maternal education, and HOME score.

Canfield et al. (2003b) reported associations of concurrent blood Pb level with various parameters of the Shape School tasks, which test the ability of children to recall correctly shapes and colors that are attributed to specific cartoon characters. Concurrent blood Pb level was associated with color knowledge, shape knowledge, and number of correct responses at age 4 years (Figure 5-5 and Table 5-5). At age 5 years, higher lifetime average blood Pb level (area under the curve calculation using repeat measurements between age 6 months and 5 years) was associated with poorer performance on multiple tasks related to learning, working and spatial memory, and planning as assessed by the Cambridge Neuropsychological Testing Automated Battery (CANTAB) in both unadjusted and covariate-adjusted analyses (Canfield et al., 2004). These memory tests in children share homology with the Morris water maze test in animals in that both test the ability of subjects to use efficient search strategies to identify target locations, retain spatial information, and access remembered items in working memory. Both measure the time to complete the task and the number of errors made. At age 4 and 5 years, associations of concurrent blood Pb level with several indices of executive function were

robust to additional adjustment for attentiveness and child FSIQ, indicating that higher blood Pb level is associated with specific aspects of associative learning that are not represented by attentiveness and general knowledge base.



[a]Standard error was estimated from p-value.

[b]Sufficient data were not provided to calculate 95% CIs.

[c]Direction of effect estimate was changed to indicate that a negative estimate represents poorer performance and a positive estimate represents improved performance. Black diamonds, gray squares, and blue circles represent associations with concurrent, lifetime average, and earlier childhood blood Pb levels

Note: Regression coefficients were standardized to their standard deviation to facilitate comparisons among tests with different scales. Studies generally are presented in order of increasing mean blood Pb level. Effect estimates are standardized to a 1 µg/dL increase in blood Pb level within the 10th-90th percentile interval. The percentiles are estimated using various methods and are only approximate values. Effect estimates are assumed to be linear within the 10th to 90th percentile interval of blood Pb level.

Figure 5-5 Standardized regression coefficients describing the associations of blood Pb levels with specific indices of cognitive function in children.

Table 5-5 Additional characteristics and quantitative results for studies represented in Figure 5-5

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Cognitive Index	Effect Estimate (95% CI)a
Cho et al. (2010)	667 children ages 8-11 yr in 2008 Five Korean cities	Concurrent Mean (SD): 1.9 (0.67) 10th-90th: 1.2-2.8	Log linear regression model adjusted for age, sex, parental education, maternal IQ, child IQ, birth weight, urinary cotinine	Color-word score[c] using Stroop test at ages 8-11 yr	0 (-0.09, 0.08)[b]
Lanphear et al. (2000)	4,852 children ages 6-16 years U.S. NHANES III (1991-1994)	Concurrent Geometric mean: 1.9 (95% CI: 1.70, 2.10) 10th-90th: 1.74, 2.06	Linear regression model adjusted for sex, race/ethnicity, poverty index ratio, reference adult education, serum ferritin levels, serum cotinine levels	Digit Span (WISC-R) Math Score (WRAT-R) at ages 6-16 yr	-0.02 (-0.03, -0.01) -0.007 (-0.01, -0.001)
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) 10th-90th: 1.69-2.19	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	-0.10 (-0.18, -0.02) -0.08 (-0.13, -0.04) -0.11 (-0.16, -0.05) -0.07 (-0.12, -0.01)
Chiodo et al. (2007)	506 African-American children in Detroit, MI area followed from birth (1989-1991) to age 7 yr. Large proportions of children with prenatal exposure to cocaine or marijuana	Concurrent Mean (SD): 5.0 (3.0) 10th-90th: 2.1-8.7	Regression model adjusted for SES, caretaker education, maternal IQ (all outcomes) Plus: age, HOME, Symptom Checklist (arithmetic and mazes) Plus: sex, prenatal cigarettes/day, prenatal marijuana use (arithmetic)	Arithmetic Mazes Object assembly WISC-III at age 7 yr	-0.07, p > 0.05c -0.11 (-0.18, -0.04) -0.13 (-0.21, -0.05)
Chiodo et al. (2004)	246 African-American children in Detroit, MI area followed from birth (not reported) to age 7.5 yr. Large proportions of children with prenatal exposure to cocaine or marijuana	Concurrent Mean (SD): 5.4 (3.3) 10th-90th: 2.3-9.5	Log linear regression model adjusted for SES, maternal education, sex (all outcomes) Family Environmental Symptom (family functioning), number of children < 18 years, caregiver vocabulary, prenatal alcohol use (Perseverative errors) Prenatal cocaine use (conceptual level responses) HOME score, Symptom Checklist, caregiver age (Spatial span)	Corsi Spatial Span WCST Perseverative errors WCST Conceptual Level Responses at age 7.5 yr	-0.10, p > 0.05[c,d] -0.49, p > 0.05[c,d] -0.77 (-1.5, 0)[b]
Froehlich et al. (2007)	174 children born 1994-1995 followed from birth to age 5 yr Rochester, NY	Concurrent Mean (SD): 6.1 (4.9) 10th-90th: 1.9-11.7	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 Rule learning and reversal Spatial span Problem solving using CANTAB at age 5 yr	-0.02 (-0.05. 0.01)[b] -0.03 (-0.06, -0.001)[b] -0.007 (-0.01, 0)[b] -0.04 (-0.09, 0.01)[b]
Canfield et al. (2004)	174 children 174 children born 1994-1995 followed from birth to age 5 yr Rochester, NY	Lifetime avg Mean (SD): 7.2 (3.6) 10th-90th: 3.5-11.8	Linear regression model adjusted for maternal IQ, duration of breastfeeding, household income, maternal ethnicity, first prenatal visit (spatial span length), NICU admission, sex, spatial span length, spatial working memory problem (total nontarget errors, total errors), age at testing (nontarget errors),	Maximum spatial span length Total nontarget errors Total errors Using CANTAB at age 5 yr	-0.17 (-0.25, 0.59)[d] -0.09 (-0.11, -0.06)[d] 0.04 (-0.07, 0.007)[d]

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Cognitive Index	Effect Estimate (95% CI)a
Canfield et al. (2003b)	170 children born 1994-1995 followed from age 6 mo to age 4 yr Rochester, NY	Concurrent Mean: 6.5 10th-90th: data not available	Linear mixed effects model adjusted for: Sex, gestational age, birth order, maternal IQ, maternal education, prenatal smoking (correct responses) Age, birth order, maternal IQ, maternal education, household income, marital status, HOME score, prenatal smoking (color knowledge) Age, maternal IQ, household income, marital status, HOME score, race (shape knowledge)	Proportion correct responses Color knowledge Shape knowledge using Shape School Task at ages 4 and 4.5 yr	-0.017 (-0.027, 0.007) -0.074 (-0.101, -0.047) -0.057 (-0.094, -0.020)
Solon et al. (2008)	502 children ages 6-35 mo 377 children 3-5 yr, 2003-2004 Visayas, Philippines	Concurrent Mean (SD): 7.1 (7.7) 10th-90th: 1.6-14.9	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)	Verbal IQ Performance IQ using WPPSI-III at ages 3-5 yr	-0.34 (-0.63, -0.05)[b] -0.45 (-2.37, 1.48)[b]
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.	Age 4 yr Mean (range): 7.0 (1.3-23.8) 10th-90th: 3.0-12.1	Linear regression model adjusted for HOME score, caregiver's vocabulary test, sex, parity, maternal marital status, head circumference at birth	Verbal comprehension age 9 yr Verbal comprehension age 11 yr Perceptual reasoning age 9 yr Perceptual reasoning age 11 yr Math at age 9 yr Math at age 11 yr Reading at age 9 yr Reading at age 9 yr Using WISC-R and WJTA	-0.028 (-0.055, 0) -0.044 (-0.072, -0.015) -0.033 (-0.064, -0.003) -0.045 (-0.074, -0.016) -0.028 (-0.057, 0.002) -0.033 (-0.062, -0.004) -0.039 (-0.069, -0.009) -0.043 (-0.073, -0.014)
Palaniappan et al. (2011)	815 children ages 3-7 years in 2003-2006 in Chennai, India	Concurrent Mean (SD): 11.5 (5.3) 10th-90th: 6.0-18.3	Generalized estimating equation adjusted for sex, hemoglobin level, maternal education, parental education, average monthly income, clustering at school and class level	Visuomotor composite Using WRAVMA at ages 3-7 yr	-0.017 (-0.029, -0.005)
Studies not included in figure due to unavailability of blood Pb-cognitive function continuous effect estimates					
Jedrychowski et al. (2008)	452 children followed from birth (2001-2003) to age 6 mo	Prenatal (cord) Mean (95% CI): 1.42 (1.35, 1.48) 10th-90th: 1.36-1.46	Logistic regression model adjusted for maternal education, parity, sex No maternal IQ	Verbal recognition memory score < 52.2 Using FTII at 6 mo	OR (95% CI) 1.47 (1.07, 2.01)
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	WIAT at age 6-10 yr Reading score Math score Visual memory score Perseveration errors Stroop color-word interference	blood Pb level 5-10 µg/dL vs. 1-2 µg/dL[e] -5.20 (-9.45, -0.95) -4.02 (-7.6, -0.43) -6.47 (-11.9, -1.1) -9.19 (-14.6, -3.7)[d] 0.75 (-1.6, 3.1)
Kordas et al. (2006)	294 children with blood Pb levels < 10 µg/dL	Concurrent Range: 2.1-10.0	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	Visual memory span < 10 correct vs. ≥ 10 correct: 0.90 Stimulus discrimination < 20 correct vs. 20 correct	OR (95% CI) 0.90 (0.74, 1.10) 0.85 (0.63, 1.13)

Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Cognitive Index	Effect Estimate (95% CI)a
Bellinger and Stiles (1993)	148 children in the Boston, MA area followed from birth (1979-1981) to age 10 yr	57 month and concurrent blood Pb Exact levels NR, mean reported to be < 8	Linear regression model adjusted for HOME score, family stress, race, marital status	Perseverative errors 57 month blood Pb Concurrent blood Pb Percent conceptual responses 57 month blood Pb Concurrent blood Pb Using WCST at age 10 yr	 -0.05 (-0.09, -0.01)[d] -0.05 (-0.11, 0.01)[d] -0.93 (-2.0, 0.13) NR
Bellinger et al. (1994a)	79 children in the Boston, MA area followed from birth (1979-1981) to age 19-20 yr	Deciduous tooth Pb Q1: 2.9-5.9 µg/g Q2: 6.0-8.7 µg/g Q3: 8.8-19.8 µg/g Q4: 19.9-51.8 µg/g	Regression model adjusted for parental IQ, sex, SES, current drug use, current alcohol use, current illicit drug use, maternal education, maternal age, birth order	Stroop Color-word test WCST Number Correct	Mean (SE) Q1: 103 (8.1), Q2: 116 (7.6), Q3: 127 (8.4), Q4: 125 (7.7) Q1: 70.5 (2.9), Q2: 72.7 (2.8), Q3: 74.5 (3.1), Q4: 71.4 (2.9)

WISC = Wechsler Intelligence Scale for Children, WRAT = Wide Range Achievement Test, WCST = Wisconsin Card Sorting Test, CANTAB = Cambridge Neuropsychological Testing Automated Battery, WPPSI = Wechsler Preschool and Primary Scale of Intelligence, WRAVMA = Wide Range of Visual Motor Ability, FTII = Fagan Test of Infant Intelligence, WIAT = Wechsler Individual Achievement Test
[a]Effect estimates are transformed to a z-score and standardized to a 1 µg/dL increase in blood Pb level in the 10th-90th percentile interval.
[b]95% CI was constructed using a standard error that was estimated from a p-value.
[c]Sufficient data were not provided to calculate 95% CI.
[d]The direction of the effect estimate was changed such that a negative estimate represents poorer performance and a positive estimate represents better performance.
[e]Effect estimates compare test performance of children in higher blood Pb groups to children in lowest blood Pb group.

A recent analysis of the Rochester cohort age 5 years extended previous findings with observations that higher concurrent blood Pb level also was associated with lower performance on many of the same tests executive function as assessed by CANTAB (Froehlich et al., 2007) (Figure 5-5 and Table 5-5). In addition to spatial memory, Froehlich et al. (2007) found associations with rule learning and reversal tasks. Pb exposure of animals consistently has been found to induce impairments in homologous tests of cognitive flexibility, including delayed spatial alternation tests (Section 5.3.2.2). Both of these tests assess the ability of children and animals to complete a task according to a change in rules or reinforcement. Impaired performance in both children and animals is indicated by increased response errors, decreased percent of correct responses, and perseverance (i.e., animals repeatedly pressing the same lever without moving between the two locations or children repeatedly selecting the wrong target despite a change in rules or reinforcement. Some effect estimates describing the blood Pb-cognitive function association were attenuated after adjustment for covariates (e.g., spatial span and planning tasks), whereas others were magnified (e.g., total trials in rule learning, total errors in spatial working memory) (Froehlich et al., 2007). Further, despite attenuation, several associations did not change in statistical significance (Figure 5-5 and Table 5-5).

Froehlich et al. (2007) also added to previous findings in the Rochester cohort by indicating that associations were modified by sex and dopamine receptor (DRD4) genetic variants. An increase in blood Pb level was associated with larger decrements in rule

learning and reversal and spatial memory in children with the DRD4 exon III 7-repeat microsatellite (assessed using a blood Pb-DRD4-7 interaction term, p = 0.042). The biological plausibility for these findings is provided by observations that the DRD4-7 variant is associated with reduced dopamine-induced signaling in downstream pathways (e.g., cyclic AMP), that Pb exposure is found to impact dopaminergic activity (Section 5.3.8.8), and that dopamine is a key neurotransmitter that regulates cognitive processes. Associations of concurrent blood Pb level with rule learning and reversal also were greater in boys. Additional support for these observed interactions was provided by observations with children with the DRD4-7 variant and boys had poorer performance on rule learning and reversal tasks.

Several other studies found associations between higher blood Pb level and poorer performance on rule learning and reversal tasks in children and young adults (Surkan et al., 2007; Kordas et al., 2006; Chiodo et al., 2004; Bellinger et al., 1994a; Bellinger and Stiles, 1993). Across studies, the age range of subjects was 6 to 20 years, and most studies examined concurrent blood Pb levels. In the Boston cohort, age 4.5 year blood Pb levels were associated with poorer cognitive flexibility at age 10 years (Bellinger and Stiles, 1993), and deciduous tooth Pb (measured at ages 6-7 years) was associated with poorer cognitive flexibility at ages 19-20 years (Bellinger et al., 1994a).

Blood Pb-associated decrements in executive function in children with mean blood Pb levels < 5 were not as clearly demonstrated. Among children in five Korean studies, ages 8-11 years, blood Pb level was not associated with performance a color-word association test. In the study of children (ages 6-10 years) participating in NECAT, Surkan et al. (2007) provided information on the blood Pb-cognitive function concentration-response relationship. Children with concurrent blood Pb levels 3-4 µg/dL had lower scores compared with children with blood Pb levels 1-2 µg/dL; however, only a few associations were statistically significant (e.g., digit span, memory, motor speed). In analyses adjusted for caregiver IQ, age, SES, race, and birth weight, children with concurrent blood Pb levels of 5 to 10 µg/dL had lower performance in a number of executive functioning domains such as working memory, visuospatial skills, cognitive flexibility, and ability to formulate, test, and adapt hypotheses (Figure 5-5 and Table 5-5). Compared with children with blood Pb levels of 1-2 µg/dL, children with blood Pb levels 5-10 µg/dL had lower scores on the reading (5.2 points [95% CI: 0.95, 9.45]) and math (4.0 points [95% CI: 0.43, 7.6]) composites of the Wechsler Individual Achievement Test. These decrements were robust to adjustment for child FSIQ. Thus, these findings combined with those from the Rochester cohort (Canfield et al., 2004; Canfield et al., 2003b) provide evidence that higher blood Pb level is associated with decrements in specific cognitive functions that may not be represented by FSIQ, a test of global function.

In other studies of populations with mean concurrent or cord blood Pb level < 3 µg/dL, the contributions of likely higher past Pb exposures cannot be excluded. Also, these studies did not have as extensive consideration for potential confounding. In infants in Krakow, Poland with a mean cord blood Pb level of 1.42 µg/dL, higher cord blood Pb level was associated with a lower score on a visual recognition memory test (Jedrychowski et al., 2008) (Table 5-5). The cord blood Pb levels may have been influenced by past Pb exposures of the mother mobilized from bone stores to the blood. While investigators adjusted for maternal education, parity, and sex, they did not adjust for other SES-related variables such as maternal IQ or HOME score.

The influence of past Pb exposures also is uncertain in the NHANES analyses, which included adolescent subjects born in the 1970s. Krieg et al. (2010) examined an older subset of children (ages 12-16 years) from NHANES III (1988-1994) who were previously examined in Lanphear et al. (2000). Among all ages and older adolescents, higher concurrent blood Pb level was associated with lower scores on block design, digit span, reading score, and arithmetic tests (Figure 5-5 and Table 5-5) (Krieg et al., 2010; Lanphear et al., 2000). In analyses of quartiles of blood Pb level Lanphear et al. (2000) additionally found a monotonic decrease in math and reading scores across increasing quartiles in covariate-adjusted models. Per unit increase in blood Pb level, the decrease in arithmetic and reading scores were larger among children with blood Pb levels less than 2.5 µg/dL than among all subjects, with statistically significant associations found for children with blood Pb levels less than 5 µg/dL. While the NHANES results were adjusted for sex, race/ethnicity, test language, and multiple SES-related variables such as family income and caregiver education, information on HOME score was unavailable.

Krieg et al. (2010) 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 some indices but a greater Pb-associated decrement in other indices. 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 decreases in digit span score (95% CI) per 1 µg/dL increase in concurrent blood Pb level were -1.5 points (-2.2, -0.71) for the CC genotype and -0.26 points (-0.99, 0.46) for the TT genotype. Conversely, the TT genotype was associated with the greatest Pb-associated decrease in arithmetic score. The decreases (95% CIs) in math score per

1 µg/dL increase in concurrent blood Pb level were -8.4 points (-11.5, -5.2) for the TT genotype and -2.7 points (-10.1, 4.6) for the CC genotype. Effect modification by VDR rs731236 was more consistent across cognitive tests, with larger blood Pb-associated decrements in cognitive performance found in children with the CC genotype. It is important to recognize that not all of these VDR variants have been linked to functional changes.

Other recent studies conducted in diverse populations of children also found associations between higher blood Pb level and decrements in learning, memory, and visuospatial skills; however, they had limited or no consideration of potential confounding by SES-related variables, which limits the implications of their findings (Nelson and Espy, 2009; Counter et al., 2008; Min et al., 2007; Vega-Dienstmaier et al., 2006).

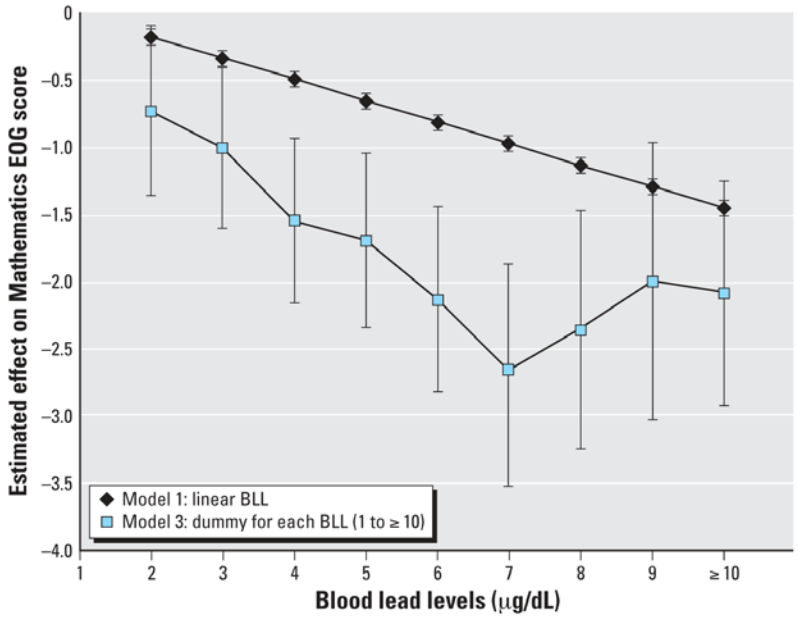
Academic Performance in Children

As described in the preceding sections, a preponderance of evidence demonstrates Pb-associated deficits in FSIQ and specific indices of cognitive function, including math, reading, and vocabulary skills. Deficits in global cognitive function and the aforementioned skills can lead to poorer academic achievement and school performance, which may be more objective measures of 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 the actual knowledge of an individual 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 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 academic performance at older ages (ages 8-18 years), suggesting the effect of early exposure to Pb may be persistent (Leviton et al., 1993; Bellinger et al., 1992; Needleman et al., 1990). Several studies found associations with concurrent blood Pb levels (Kordas et al., 2006; Wang et al., 2002a; Al-Saleh et al., 2001; Lanphear et al., 2000). Among recent studies, academic performance was examined less frequently; however, findings were consistent with the extant body of evidence.

The longitudinal study by Bellinger et al. (1992) was particularly noteworthy for examining associations of blood Pb level at several ages from the prenatal period to age 10 years with Kaufman Test of Educational Achievement (KTEA) scores at age 10 years in the Boston cohort. While blood Pb levels at several ages were associated with lower academic achievement scores when in a model alone, only blood Pb level at age 2 years

showed a statistically significant association with lower predicted academic achievement after adjusting for HOME score, child stress, maternal age, race, maternal IQ, SES, sex, birth order, marital status, and number of residence changes. These covariates were selected because they were associated with blood Pb level and KTEA score, changed the blood Pb effect estimate by more than 10%, or because they were recognized to be important predictors of cognitive development (e.g., maternal IQ, birth order, sex). Age 2 year blood Pb level was associated with a similar magnitude of decrease in the KTEA battery composite score in univariate and multivariate models. Additionally, the association was robust to adjustment for FSIQ, indicating that blood Pb levels may be associated with poorer performance on academic tasks not reflected in FSIQ.

Miranda et al. (2007b) and (2009) linked blood Pb surveillance data collected between ages 0 and 5 years with end-of-grade (EOG) testing data for 8,600 fourth grade children in seven of the largest counties in North Carolina and 57,678 children in the entire state, respectively. A key strength of these analyses was the availability of individual-level data on blood Pb level and achievement score on a large number of children representative of the North Carolina fourth grade population, which provided large numbers of children with lower blood Pb levels (2-5 µg/dL). Thus, in this study, there was greater power to discern differences in achievement scores among children in the lower range of blood Pb levels. However, due to the records-based research design, investigators had a smaller set of available potential confounding variables than those typically considered in analyses of neurodevelopmental outcomes. In addition to adjusting for sex, race, school-type, school district, and age of blood Pb measurement, investigators adjusted for participation in a free or reduced lunch program as a measure of SES, and parental education as a measure of parental IQ. Miranda et al. (2007b) additionally adjusted for daily use of a computer as a measure of a stimulating home environment. Higher early childhood blood Pb levels were associated with lower reading and math EOG scores (Figure 5-6 and 5-7), and in both analyses, children with a blood Pb level of 2 µg/dL had lower EOG scores ($p \leq 0.05$) compared with children with a blood Pb level of 1 µg/dL. Further, across deciles of blood Pb level, the decrease in EOG score generally was monotonic (Figure 5-6). In the statewide dataset, compared with children with an earlier (measured at some point between birth and age 5 years) blood Pb level of 1 µg/dL, children with an earlier blood Pb level of 2 µg/dL had a 0.30-point lower (95% CI: -0.58, -0.01) reading EOG score (Miranda et al., 2009).

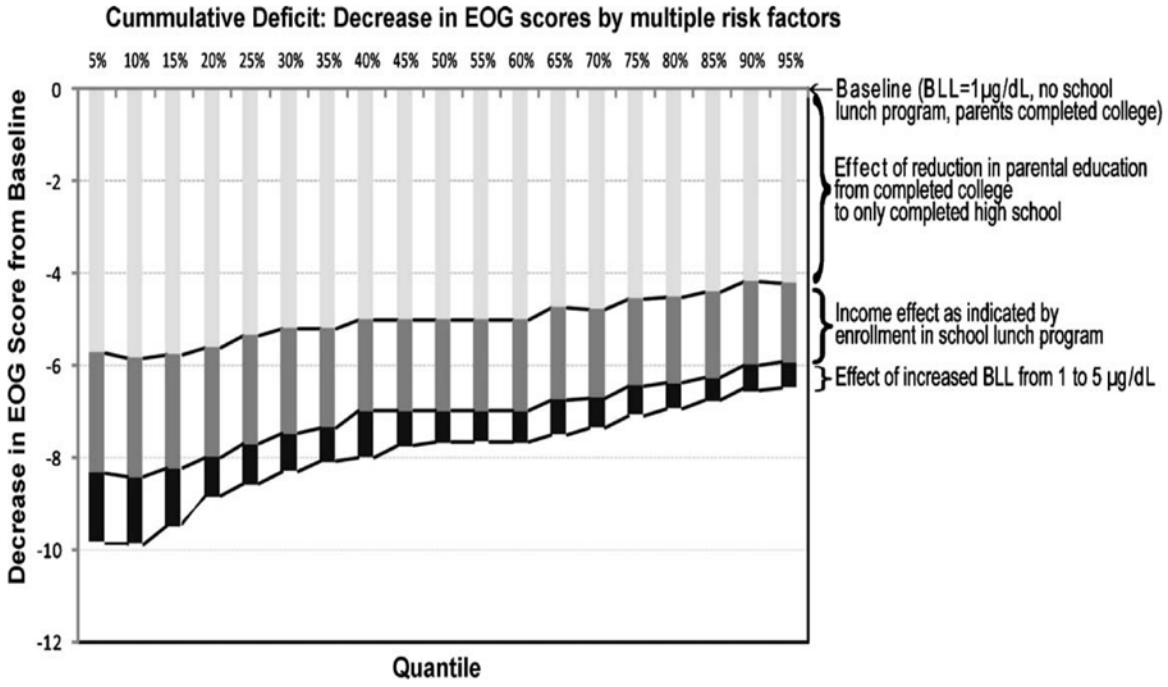


Source: Reprinted with permission of Elsevier Science, Miranda et al. (2007b).

Note: These results illustrate the decrease from a baseline score of 262.6 for a hypothetical referent white female, screened at 2 years of age, living in Wake County, NC, with parents with a high school education, not enrolled in the school lunch program, and who does not use a computer every day (i.e., model covariates = zero).

Figure 5-6 Associations between childhood blood Pb levels and fourth grade End-of-Grade (EOG) math scores.

While the linear regression analyses indicated fractional-point decreases in EOG scores per 1 µg/dL increases in blood Pb level, Miranda et al. (2009) additionally used quantile regression to discern differential effects in various segments of the EOG distribution (i.e., what is the 10th percentile of EOG scores conditioned on early childhood blood Pb levels). Compared with linear regression, quantile regression is more robust in response to outliers and predicts outcomes at the top and bottom tails of the outcome distribution rather than at the mean. With increasing blood Pb level, the lower tail of the EOG distribution was stretched out more so than were the middle or upper portions of the distribution. For example, an increase in blood Pb level from 1 to 5 µg/dL was associated with a greater decrease in EOG score among children in the 5th percentile of EOG than in children in the 95th percentile of EOG score (Figure 5-7). These findings indicated that children residing at the lowest performance regions of the EOG score distribution may be more affected by Pb exposure. Similarly, using quantile regression, Miranda et al. (2009) showed that while cumulative social risk (lower parental education, being enrolled in a school lunch program) had a greater negative association with academic achievement in these children, blood Pb level was independently associated with EOG score decrements that were as large as 1 to 2 points.



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

Note: Baseline score calculated for a hypothetical referent individual with a blood Pb level of 1 µg/dL, parents completed college, and not enrolled in the school lunch program (i.e., model covariates = zero).

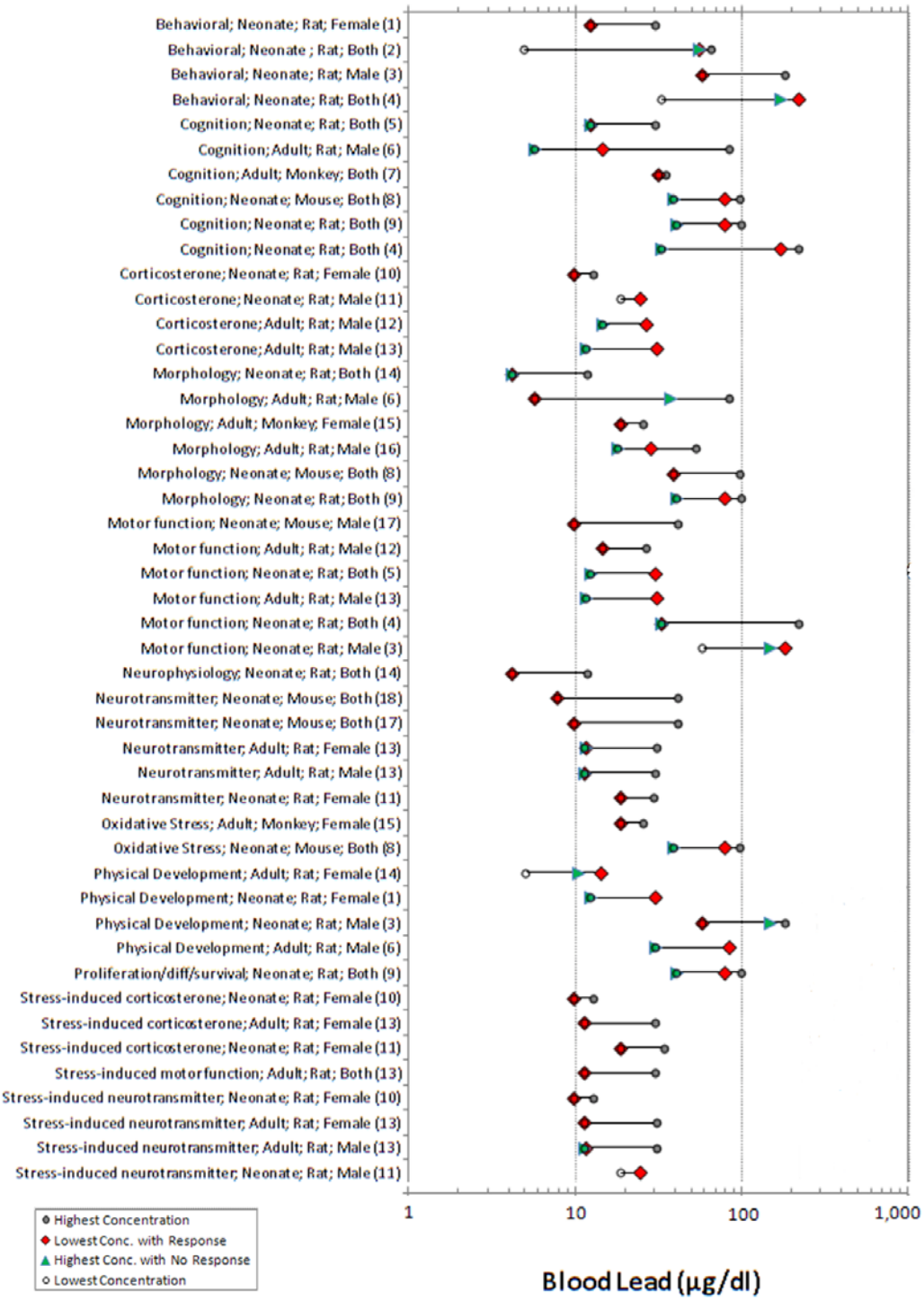
Figure 5-7 Greater reduction in EOG achievement test scores with increasing blood Pb level in lower percentiles of the test score distribution.

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 [SAT] at age 7 years) among participants of the Avon Longitudinal Study of Parents and Children conducted in the U.K. However, in this study, decrements in SAT score were most clearly indicated in children with blood Pb levels of 5 to 10 µg/dL. Compared with children with blood Pb levels 0-2 µg/dL, children with blood Pb levels 5-10 µg/dL scored 0.51 points lower (95% CI: -0.82, -0.32) on the reading test and 0.49 points lower (95% CI: -0.78, -0.31) on the math test, adjusting for maternal education, home ownership, maternal smoking, home facilities score, parental SES, family adversity index, and parenting attitudes. Children with blood Pb levels 2-5 µg/dL did not consistently have lower scores compared with children with blood Pb levels 0-2 µg/dL. While these aforementioned recent studies found associations for early childhood blood Pb levels, unlike the longitudinal assessment by Bellinger et al. (1992), they did not have blood Pb measurements available at other lifestages. Therefore, recent studies could not provide information on associations with blood Pb levels at other lifestages.

5.3.2.2 Toxicological Studies of Cognition, Memory and Learning

As described in Section 5.3.2.1, epidemiologic studies in children have consistently demonstrated associations between blood Pb levels and decrements in a range of executive functions related to memory, reasoning, problem solving, and verbal skills. Studies in animals also demonstrate Pb-induced decrements in various indices of executive function, and several tests conducted in animals share homology with tests conducted in humans.

Data on neurocognition and learning as well as other nervous system endpoints with concentration-response data are shown in Figure 5-8 and accompanying Table 5-6. These data demonstrate that numerous studies in animal models have demonstrated that Pb exposure produces a spectrum of changes in the central nervous system, including deficits in CNS development and plasticity and altered homeostasis of mediators of cognitive function that manifest with deficits in memory, learning, and cognition. It is thought that early life Pb exposure can permanently alter CNS development and other pathways that contribute to these aforementioned memory and learning deficits, but the animal toxicology data show that multiple sensitive developmental windows for Pb exposure exist (Rice and Gilbert, 1990). As illustrated in Figure 5-8 and Table 5-6, Pb-induced impairments in learning primarily previously were observed in animals with blood Pb levels > 30 µg/dL. Several new studies added to the evidence for impaired learning and memory in animals with lower blood Pb levels, 8-17 µg/dL (Cory-Slechta et al., 2010; Li et al., 2009c; Niu et al., 2009; Virgolini et al., 2008a; Stangle et al., 2007). In these studies of animals with lower blood Pb levels, Pb exposures began early, during the gestational or lactation period.



Dosimetric representation reported by blood Pb level. (ID corresponds to Table 5-6.)

Figure 5-8 Nervous system summary array of toxicological outcomes after Pb exposure.

Table 5-6 Summary of findings from neurotoxicological exposure-response array presented in Figure 5-8

Study ID in Figure 5-8	Reference	Blood Pb Level (µg/dL)	Outcome
1	Beaudin et al. (2007)	13 & 31	Behavior, neonate: Lactational Pb exposure, offspring deficient in Reward Omission testing.
			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	Grant et al. (Grant et al., 1980)	57	Behavior, neonate: chronic Pb exposure (drinking water) to dams and pups, Changed behavior, males.
3	Kishi et al. (1983)	59 & 186	Behavior, neonate: Pb exposure (oral gavage of pups) during lactational period, Changed emotional behavior, males and females.
			Motor function, neonate: Pb exposure (oral gavage of pups) during lactational period, motor function (rotarod performance) impaired, both sexes.
			Physical development; Pb exposure during lactation (oral gavage): Delayed development of righting reflex in male rats.
4	Overmann (1977)	33, 174 & 226	Behavior-Pb exposure (oral gavage of pups) during lactation: aversive conditioning affected by Pb exposure, male and females.
			Cognition-Pb exposure (oral gavage of pups) during lactation: Response inhibition impaired, both sexes.
			Motor function- Pb exposure (oral gavage of pups) during lactation: Increased motor activity and impaired motor coordination (rotarod), male and females.
5	Stangle et al. (2007)	13 & 31	Cognition; Developmental Pb exposure (PND1-PND30): Impaired learning with visual discrimination task, heightened response to errors, both sexes.
			Motor function; Developmental Pb exposure (PND1-PND30): Alcove latency and response latency significantly affected by Pb exposure, both sexes.
6	Gong & Evans (1997)	38 & 85	Cognition-Adult male 21 day Pb exposure: Hyperactivity with Habituation to new cage environment.
			Morphology; 21 day Pb exposure to adult males: Marker of neuronal injury-elevated hippocampal glial fibrillary acidic protein (GFAP).
			Physical development; Adult male rats (21 day Pb exposure): Neurotoxicity measured with brain glial fibrillary acidic protein (GFAP).
7	Rice (1990)	32 & 36	Cognition-Chronic Pb exposure from birth: Spatial discrimination reversal task impairment, both sexes.
8	Li et al. (2009c)	80 & 100	Cognition-Gestational & lactational Pb exposure: Morris water maze performance impaired.
			Morphology; Gestational & lactational Pb exposure: Increased levels of inflammatory cytokines & exocytosis related proteins in brains of pups at weaning, both sexes.
			Oxidative stress-gestational and lactational Pb exposure: Elevated hippocampal TNF levels in offspring, males and females.
9	Li et al. (2010b)	80 & 102	Cognition- Gestational & lactational Pb exposure: Morris water maze performance impaired.
			Morphology: Increased levels of Alzheimer disease-associated proteins in mice with gestational and lactational Pb exposure, both sexes.
			Proliferation/diff/survival, gestational & lactational Pb exposure: Increased hippocampal expression of P-tau and amyloid beta in male and female pups.
10	Cory-Slechta et al. (2010)	10 & 13	Corticosterone: Lifetime Pb +/- stress: Correlation between 9-month old's corticosterone level and frontal cortex dopamine levels in behaviorally tested female offspring.
			Stress: Corticosterone-Lifetime Pb plus stress: Affects FI performance, dopamine and serotonin levels in female offspring.
			Stress: Corticosterone-neurotransmitter-Lifetime Pb exposure in female rats plus stress: Dopamine homeostasis affected.
11	Virgolini, Rossi-George, Weston et al. (2008b)	25	Corticosterone: Maternal Pb plus stress: Elevated corticosterone in male offspring with prenatal stress + offspring stress was further enhanced with Pb exposure.
			Stress: Corticosterone-Maternal Pb plus stress: Affects FI performance.
			Neurotransmitter; Gestational and lactational Pb exposure: Induced DA and 5HT changes in rat offspring.

Study ID in Figure 5-8	Reference	Blood Pb Level (µg/dL)	Outcome
			Stress induced neurotransmitter effects, Maternal Pb plus stress: 5-HT and 5-HIAA (5-HT metabolite), and dopamine turnover were significantly affected in males.
12	Virgolini et al. (2005)	15 & 27	Corticosterone: Chronic Pb exposure from weaning: Pb exposure alone decreased basal plasma corticosterone levels at 5 months of age, males.
			Motor function: Chronic Pb exposure from weaning: Locomotor activity significantly decreased FI response rates & increased post-reinforcement pause period in a concentration-dependent manner, males.
			Stress: Corticosterone-Chronic Pb plus stress: Affects neurotransmitters & FI performance
13	Virgolini, Rossi-George, Lisek et al. (2008a)	31	Corticosterone: Maternal Pb exposure (gestation and lactation) +/- stress: Differential basal corticosterone levels between behavioral and non-behavioral tested rats, females.
		11 &/or 31	Stress: Corticosterone-Maternal Pb plus stress: Affects FI performance, dopamine, serotonin, and NE levels.
			Motor function: Maternal Pb +/- stress: Increased locomotor activity (run rate) with Pb and stress exposure.
		31	Neurotransmitter; Gestational and lactational Pb exposure: Induced NE aberrations in adult rat offspring (both sexes).
			Stress induced motor function: Maternal Pb +/- stress: Increased locomotor activity (run rate) with Pb and stress exposure.
			Stress induced neurotransmitter; Gestational and lactational Pb exposure + stress: Induced HVA (monoamine neurotransmitter metabolite) and NE aberrations in female adult rat offspring.
14	Hu et al. (2008b)	4 & 12	Morphology; Gestational Pb exposure: Neurite outgrowth marker PSA-NCAM decreased in rat pups, both sexes.
			Neurophysiology; gestation Pb exposure: decreased hippocampal sialyltransferase activity, both sexes.
			Physical development; t-Gestational Pb exposure: Early brain synapse development impaired (hippocampal PSA-NCAM).
15	Wu et al. (2008a)	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).
			Oxidative stress: Elevated oxidative DNA damage in aged brains of female monkeys (exposed to Pb as infants).
16	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.
17	Leasure et al. (2008)	10 & 42	Motor function; Mouse maternal (dam) Pb exposure: Induced decreased rotarod performance in offspring (1 year-old male offspring).
			Neurotransmitter; Mouse maternal (dam) Pb exposure: Affects 1 year old male offspring dopamine homeostasis, both sexes.
18	Fortune & Lurie (2009)	8 & 43	Neurotransmitter; Mouse maternal (dam) Pb exposure: Affects offspring superior olivary complex (auditory) neurotransmitters, both sexes.

Memory and Learning - Morris Water Maze

Blood Pb levels have been associated with decrements in both verbal (e.g., digit span) and spatial memory in children (e.g., spatial and visual memory span) (Figure 5-5 and Table 5-5). In the 2006 Pb AQCD, the results from different animal studies testing the effect of Pb on memory were mixed with impaired memory observed in animals with blood Pb levels 10-35 µg/dL but improved memory observed in other studies. Low-dose Pb was not found to affect short-term memory, i.e., recall of a learned task. Mixed results for memory also may be due to the fact that memory tests also test inattention. Memory tests may give incorrect results when opportunities exist for impaired attention to contribute to test results (U.S. EPA, 2006b). In animals, spatial memory is tested most

commonly using the Morris water maze. The 2006 Pb AQCD reported deficits in the Morris water maze performance with Pb exposure. New research continued to show Pb-induced impaired Morris water maze performance. The Morris water maze tests spatial memory and learning by having a mouse swim and locate or remember the location of a platform submerged in opaque water. The Morris water maze is homologous to the spatial memory components of the CANTAB and WISC-R, which test the ability of subjects to recall correctly a sequence of locations (Froehlich et al., 2007; Canfield et al., 2004; Chiodo et al., 2004).

Li et al. (2009c) examined Pb exposures from gestation through lactation. During this period, dams received Pb-acetate dissolved in drinking water (0.1%, 0.5%, and 1% with corresponding blood Pb levels of 4, 8 and 10 µg/dL at postnatal day [PND]21). Beginning at weaning, Pb-exposed pups were subjected to Morris water maze performance testing. Pb-exposed pups had statistically 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., 2009c). The pups in Li et al. (2009c) were not separated by sex. Cao et al. (2009) found that chronic administration of the supplement melatonin exacerbated Pb-induced impairments in spatial memory and long-term potentiation. Adult male Wistar rats that had been exposed to Pb-acetate (0.2%) from birth received melatonin (3 mg/kg) from weaning via gastric gavage for 60 days. At this point (PND81-90), animals performed in the Morris water maze, and LTP of the hippocampal dentate gyrus was measured.

Another study found that various dietary supplements or methioninecholine concomitant with Pb exposure in weanling males shortened the escape latency of Pb-exposed pups to resemble more closely the escape latency of control pups (Fan et al., 2010; Fan et al., 2009a). Zinc and methionine were effective dietary supplements in Fan et al. (2009a); glycine, taurine, vitamin C, vitamin B1, tyrosine had no effect on the Pb-associated Morris water maze results. These data on the effect of early life Pb exposure on learning and memory in the Morris water maze affirm earlier findings including those by Kuhlmann et al. (1997) who 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.

Another component of memory is working memory, which is the ability to temporarily keep information in mind while using the information to perform a related or unrelated task. The Morris water maze measures working memory in addition to learning. The 2006 Pb AQCD reported that working memory as assessed by the Morris water maze was

significantly affected in chronic developmentally Pb-exposed (Pb-acetate in feed 10 days prior to mating through PND 21) female offspring at PND 21 (Jett et al., 1997).

Working memory also can be measured by testing delayed spatial alternation (DSA), which specifically tests spatial reversal learning. With DSA, an animal receives rewards based on alternating responses between two separate levers. In children, tests of spatial reversal learning includes the rule learning and reversal components of the CANTAB, Stroop Test, and WCST, and decrements in DSA have been associated with higher blood Pb levels in children (Figure 5-5 and Table 5-5). Studies detailed in earlier Pb AQCDs showed that Pb-exposed animals had deficits under DSA testing (Alber and Strupp, 1996; Rice and Gilbert, 1990; Rice and Karpinski, 1988; Levin et al., 1987; Levin and Bowman, 1986). Studies in nonhuman primates showed Pb-induced behavioral impairment on DSA tasks (Rice and Gilbert, 1990) across many exposure periods, including early-life and chronic Pb exposures. Specifically, there were multiple lifestages during which Pb exposure induced deficits in this task. As in children, deficits in DSA included increased response errors, decreased percent of correct responses, and perseverance at a task (i.e., repeatedly pressing the same lever without moving between the two locations). These observations have been consistently made in nonhuman primates with continuous Pb exposure or juvenile to adult exposure but less consistently made in rats with juvenile only or juvenile to adult exposure. In fact, some rodent studies showed increased accuracy in the delayed alternation trials (Cory-Slechta et al., 1991). Working memory is a subcategory of executive function or goal-oriented problem solving. Pb-induced deficits in working memory may be one of many factors that contribute to associations between blood Pb levels and inattention observed in humans (Min et al., 2007; Surkan et al., 2007; Chiodo et al., 2004; Schweitzer et al., 2000; Stiles and Bellinger, 1993).

Learning - Y Maze

A recent study using the three-branch radial Y-maze showed Pb-induced effects on learning in rat offspring exposed during lactation and into adulthood (Niu et al., 2009). The Y-maze has a light at the end of each branch. The branch with the illuminated light is a safe area whereas the other two branches are electrified and cause a mild electric shock when entered. The Y-maze test evaluates learning based on three criteria: 1) learning days or the number of days required to learn the maze (90% correctly); 2) the error number (EN) or the number of Y-maze runs required to learn the maze; and 3) the total reaction time (TRT) or the total amount of time spent in the maze per test day. Wistar Albino rat pups were exposed to Pb-acetate during lactation until the termination of the experiment at 12 weeks of age (dam drinking water 300 mg/L, blood Pb level: 17 µg/dL at 6 weeks of age. Rats were evaluated on the three Y-maze endpoints at 6, 8, 10 and

12 weeks of age.) Pb induced statistically significant impairments in learning, in particular, at 8, 10 and 12 weeks of age. EN and TRT were significantly affected only at 8 and 10 weeks, respectively. Hippocampal glutamate levels were also significantly attenuated in Pb-exposed animals, and this is discussed in more detail in Section 5.3.8.4.

Learning - Response Inhibition and Schedule-Controlled Behavior Testing

Response inhibition is another measure of executive function and is measured with multiple tests of premature responses, decreased pause time between two scheduled events, and increased perseverance. These tests include Differential Reinforcement of Low Rates of Responding (DRL), Fixed Interval (FI) testing (Table 5-7), FI with Extinction (FI-Ext) or Fixed Ratio (FR)-FI, and Signal Detection with Distraction. Multiple studies from the 2006 Pb AQCD as well as earlier literature showed that early life Pb exposure contributed 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 that in 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. These findings in animals are consistent with observations in children that blood Pb levels are associated with poorer impulse control (Figure 5-14 and Table 5-9). In children, impulsivity is commonly assessed by having parents or teachers rate problem behaviors such as trouble waiting, interrupting others, and responding at inappropriate times. However, studies also have specifically tested response inhibition assessed using the continuous performance test, which similar to animal tests, measures reactions to a stop signal (Section 5.3.3.1).

The 2006 Pb AQCD discussed learning or cognition as measured with schedule-controlled behaviors including FI and FR operant conditioning and found that FI response rate was affected differentially with low-level (as low as 11 µg/dL) and high-level Pb (peak levels of 115 µg/dL) exposures increasing and decreasing FI response rate, respectively. This nonlinear response was since further explored in recent work, much of which also examined the interaction between stress and Pb exposure.

Table 5-7 Summary of effects of maternal and lifetime Pb exposure on FI performance

	Maternal Pb[b]		Lifetime Pb[c]	
Pb (ppm)	Overall rate[a]	PRP[a]	Overall rate[a]	PRP[a]
0 ppm:				
0-PS	No Significant Effect[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. PRP = post-reinforcement pause.

[b]Data from Virgolini et al. (2005). *Denotes significant effect versus 0ppm control ($p < 0.05$).

[c]Data from current study [Rossi-George et al. (2011)]

*Denotes significant effect vs. 0ppm control ($p < 0.05$).

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

Learning Ability with Stress

The combined paradigm of Pb exposure and stress experienced by 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. These studies indicated greater impairments in learning with Pb when combined with stress. These findings provide support for the association between higher blood Pb level and lower MDI score observed in children with mothers with low self-esteem but not children with mothers with high self-esteem (Surkan et al., 2008) (Table 5-4). In children, maternal self-esteem has been linked with the ability to cope with stress and improved maternal-child interactions. As indicated in Figure 5-8 and Table 5-6, Pb exposure has been shown to increase corticosterone levels and exacerbate Pb-induced dopamine release and learning ability. Cory-Slechta and colleagues have conducted multiple investigations in this area. Most recently, they showed enhanced learning deficits in female rat 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 from their dams during the aforementioned period and then via drinking water Pb (50 ppm) through the remainder of their lifetime. The resultant blood Pb levels of dams and pups ranged from 5 to 13 µg/dL.

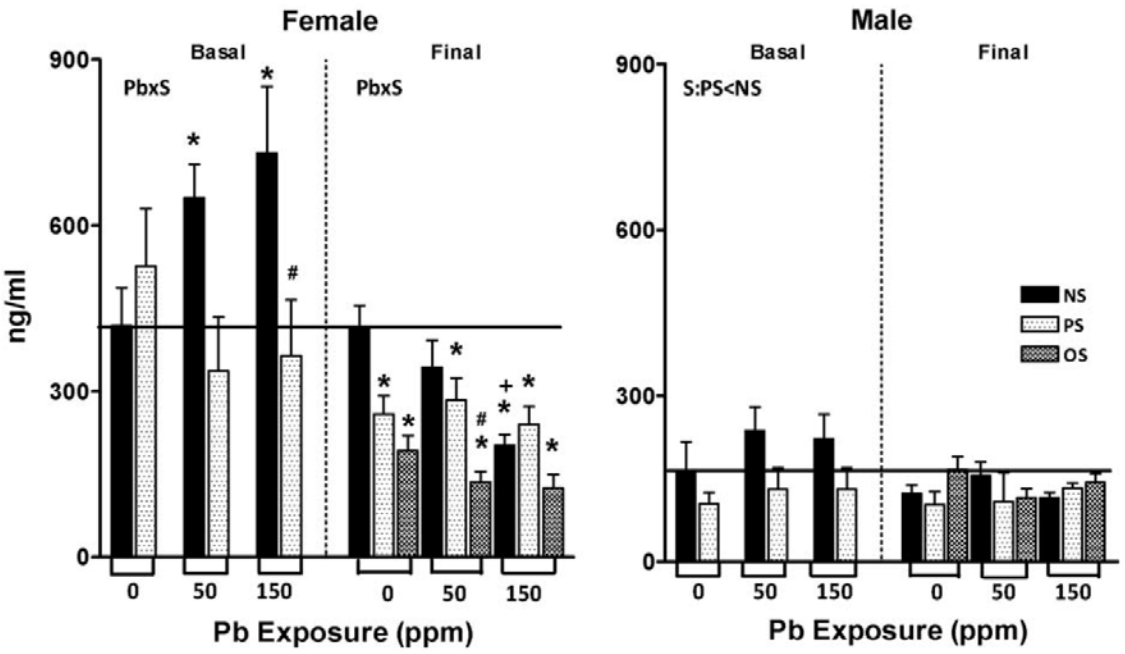
In a separate investigation, Pb plus stress-related outcomes were examined in female offspring of dams who were exposed to Pb from 2 months prior to mating through lactation, i.e., developmental Pb exposure (2 exposure groups: 50 or 150 ppb Pb-acetate in drinking water) (Virgolini et al., 2008a). Dams were subject to restraint stress at GD16-17. Compared with controls, marked increases in response rates on FI performance were found in the Pb plus stress female offspring, 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, the results indicated a potentiation of effects with Pb and stress co-exposures.

Similarly, lifetime Pb exposure (50 or 150 ppm, resulting in blood Pb levels of 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 (Rossi-George et al., 2011) (Table 5-7). Again, the results indicated a potentiation of effects. 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 started responding too early due to a decreased pause or PRP interval. Aberrant FI performance in infants and children has been used as a marker for impulsivity. Separately, overall FI response rate, a hyperactive behavior, was significantly increased with Pb exposure alone and with maternal or offspring stress at the 50 ppm exposure dose. At 150 ppm Pb, 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 striatal 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 affirms Pb-related learning deficits and provides possible mechanistic explanations.

Pb exposure over various developmental windows has been shown to affect corticosterone levels in rodents. These findings indicate that associations of Pb and stress with learning deficits (FI testing in females) may be related to aberrations in corticosterone and dopamine. 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 observed 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). Pb-induced decreases in corticosterone were enhanced with maternal stress. Postnatal exposure of male rodents to Pb (PND 21-5 months of age) produced animals with 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 changes in the HPA axis (corticosterone levels) in both sexes that were dynamic as the animal aged.

Mechanistic understanding of the cognitive deficits observed 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., 2011; Virgolini et al., 2008a). This study used the same model of developmental Pb exposure as is detailed in the preceding paragraph. Outcomes were examined in both male and female offspring. At 2 months of age without stress, basal corticosterone in females was significantly increased with 150 ppm Pb (resulting in blood Pb level of 32 µg/dL at PND21) and 50 ppm Pb (resulting in blood Pb level of 19 µg/dL) (Figure 5-9). Pb plus stress attenuated the Pb-induced elevations in corticosterone to baseline levels (Figure 5-9). At age 10 months, Pb and stress accelerated the age-dependent decrease in corticosterone levels in females. In males, basal corticosterone levels were not affected significantly by Pb and/or stress at 2 or 10 months of age (Figure 5-9). These authors also explored the function of the glucocorticoid negative feedback loop using the dexamethasone suppression test and found that Pb and/or maternal stress significantly impacted this negative feedback by increasing nuclear glucocorticoid receptor levels. This negative feedback loop was impacted more at the lower dose (50 ppm versus 150 ppm Pb-acetate). As summarized in Table 5-7, the results indicate that lifetime Pb exposure when combined with stress can exacerbate impairments in learning. The interaction between Pb and stress may be mediated via effects on corticosterone and dopamine.



Source: Rossi-George et al. (2011)

Note: *denotes significantly different from NS control; # denotes significantly different from corresponding Pb-NS value; + differs from 50-NS. (0, 50, 150 ppm) and/or stress (PS [dam stress] or OS [PS followed by offspring stress]).

Figure 5-9 Mean basal corticosterone levels of female and male offspring exposed to lifetime Pb.

Another study examined the effects of Pb on the HPA axis but examined the interaction with an outside stress administered using control vehicle injections (Rossi-George et al., 2009). The corticosterone response to this vehicle injection stress was prolonged in a nonlinear concentration-dependent manner in both sexes with the most profound effects observed at the lower 50 ppm Pb 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 DEX in males induced prolonged corticosterone suppression or failure to return to baseline as was observed in control animals. In summary, dam Pb exposure induced HPA negative feedback hypofunction in both sexes of offspring with an inverse U concentration-response function.

These studies of lifetime exposure (2011; Rossi-George et al., 2009) reported different basal stress hormone levels with Pb exposure. Males with lifetime Pb exposure had no statistically significant corticosterone response to Pb exposure; whereas males with dam Pb exposure had statistically significant decreases in corticosterone at 5 months of age in

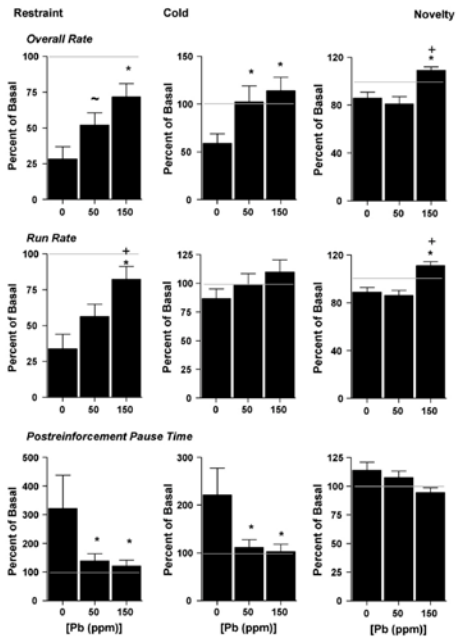
the 50 ppm exposure group only (not in 150 ppm Pb exposure group). On the other hand, females had concentration-dependent corticosterone responses to Pb exposure in both exposure models (lifetime Pb exposure and dam Pb exposure). Maternal stress alone also led to HPA axis negative feedback hypofunction. 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 suppression in males. These data together show that HPA axis alterations could provide a link between the contribution of Pb and stress to health effects.

Schedule-controlled 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 that 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. (2008b) 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 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 (Virgolini et al., 2008b). 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 showed a U shaped concentration-response curve with 50Pb animals having significantly less corticosterone than did control or 150Pb animals. 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). These data demonstrate that behaviorally trained animals have altered HPA axis and response to Pb exposure versus animals that are housed under conditions without daily handling by caregivers.

Virgolini et al. (2008b) also expanded evidence for Pb exposure-stress interactions through the examination of the effects additional intermittent stress as an adult. The authors proposed that associations of Pb and stress with learning deficits (FI testing in females) may be related to aberrations in corticosterone and dopamine. Dam exposure to Pb (50 or 150 ppm Pb-acetate) followed by intermittent stress (cold, novelty or restraint) in offspring as adults induced statistically significant changes in FI response rate. Females were more sensitive to the adult intermittent stressors at the higher dose of Pb

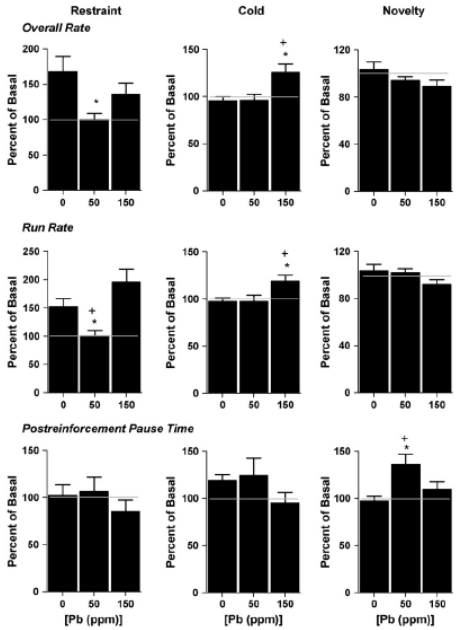
(150 ppm) with statistically significant increases in FI response rate and decreased PRP, i.e., increased impulsivity (Figure 5-10). Males were more sensitive (decreased FI response rate due to decreased run rate) to the restraint stress at the lower Pb dose (50 ppm). At the higher dose of Pb, males were more sensitive to the cold stress (increased FI response rate and increased run rate) (Figure 5-11). Corticosterone levels were examined in this study and showed concentration-dependent correlations with FI outcomes in females but were independent of dose in males.

Another study examined female rats with lifetime Pb exposure combined with prenatal stress and found enhanced learning deficits (drinking water 50 ppm Pb-acetate, resulting in offspring blood Pb levels: 7-13 µg/dL) (Cory-Slechta et al., 2010). Learning was evaluated with multiple schedule of repeated learning (RL) and performance testing. RL was impaired, but performance was not affected with Pb exposure. The Pb-impaired RL was further enhanced with prenatal stress. There were statistically significant associations between Pb/stress and corticosterone 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 statistically significant decreases in hippocampal nerve growth factor (NGF) versus controls. Thus, this study demonstrated that lifetime Pb exposure with or without prenatal stress induced learning deficits in female mice.



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

Figure 5-10 Changes in FI performance (FI overall performance, run rate, PRP) in female offspring with maternal Pb exposure plus various stressors (restraint, cold, novelty) in adulthood.



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

Figure 5-11 Changes in FI performance (FI overall performance, run rate, PRP) in male offspring with maternal Pb exposure plus various stressors (restraint, cold, novelty) in adulthood.

Cognitive Flexibility

Cognitive flexibility is a component of executive function that measures the ability to reallocate mental resources when situations change (Monsell, 2003). This flexibility is assessed as the ability to alter behavioral responses according to the context of the situation and incorporates the function of attention, working memory, and visual processing. Discrimination reversal learning and concurrent random interval (RI-RI) scheduling are used to measure cognitive flexibility. The 2006 Pb AQCD reported discrimination reversal learning deficits in monkeys with blood Pb levels 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 and Strupp, 1997). Interestingly, recent work has shown that N-Methyl-D-aspartic acid or N-Methyl-D-aspartate (NMDA) receptors and D2-like receptors, two well-characterized 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 Pb AQCD reported monkeys with Pb-induced cognitive flexibility impairment under RI-RI (Newland et al., 1994). Coherence for these observations of Pb-induced deficits in cognitive flexibility is provided by consistent evidence in animals and children for associations of Pb with

individual tests of inattention, memory, and visual processing. Studies in children also demonstrate associations between blood Pb levels and decrements in specific tests of cognitive flexibility that are homologous to tests in animals such as discrimination reversal learning, the WCST, and Stoop test (Figure 5-5 and Table 5-5). While tests in animals measure the ability to complete a task according to changes in reinforcement, tests in humans assess the ability of subjects to complete a task according to changes in rules.

5.3.2.3 Toxicological Studies on the Effects of Chelation

Earlier work in the animal toxicological 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 and Evans, 1997) and forced-swim immobility (Stewart et al., 1996). A more recent study examined the effect of succimer treatment on various behavioral and cognitive 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 were 1.5; 12.6; 31; 2.8; and 8.5 µg/dL, respectively. Brain Pb levels at the same time for the same groups were 41 (control), 1,040 (m-Pb), 3,690 (h-Pb); and 196 (m-Pb+succimer) and 1,370 (h-Pb+succimer) ng/g dry weight. 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 but did not improve the impaired inhibitory control (Stangle et al., 2007). These are important findings because they provide evidence that certain neurobehavioral or cognitive impairments associated with Pb exposure appear to be reversible with chelation therapy.

In another study, a 3-week course of Pb-acetate (PND 1-17, dam drinking water) plus or minus succimer/chelator (PND 31-52) treatment was given 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 treatment had normalized reactivity to reward omission and errors (Beaudin et al., 2007). Pb-induced behavioral abnormalities were attenuated with chelation therapy.

The interaction between Pb and methionine choline also has been examined (Fan et al., 2009a). Pb-exposed rats were supplemented with methionine choline to provide

information on its effect on Pb disposition in various tissues (blood, bone, brain) and its contributions 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 and Blusztajn, 1994). In this model, methionine choline attenuated Pb-induced memory and learning deficits (Section 5.3.2.2). Exposure of weanling male rats to Pb-acetate in drinking water (300 mg/L) through PND60 produced a blood Pb level of 60 µg/dL, bone Pb level of 165 µg/g, and brain Pb level of 0.63 µg/g. Methionine choline supplementation significantly attenuated blood and bone Pb levels but produced a nonsignificant attenuation of brain Pb (0.51 µg/g) in rats 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 were neuroprotective for Pb-related effects in the ALS mouse neurodegenerative model or Tg(SOD1-G93A) model (Petri et al., 2007).

In summary, succimer or chelation treatment appears to be able to restore Pb-dependent impairments of learning and arousal and be neuroprotective in a concentration-dependent fashion. In these studies, succimer use was more efficacious at lower doses of Pb exposure. Chelation did not restore Pb-impaired inhibitory control. Chelation with the antioxidant supplement affected the disposition of Pb in various tissues, significantly attenuating blood and bone Pb levels and nonsignificantly attenuating brain Pb.

5.3.2.4 Integrated Summary of Cognitive Function in Children

Results from recent epidemiologic studies and animals studies expand the strong existing evidence base demonstrating that Pb exposure is associated with impaired cognitive function in children. A large epidemiologic evidence base demonstrates associations of higher blood Pb level with lower FSIQ in school-aged children, lower MDI scores in children ages 6 months to 3 years, and lower scores on tests of specific cognitive functions in children ages 6 months to age 16 years (Figure 5-2 and Figure 5-5; Table 5-3, Table 5-4, and Table 5-5). Blood Pb level has been associated with a range of cognitive indices, including memory, verbal and math skills, and cognitive flexibility. There was no clear indication that blood Pb level was more strongly associated with performance in a particular domain of cognitive function. Studies in animals clearly demonstrated impaired performance on Morris Water Maze, the delayed spatial alternation, and discrimination reversal learning with Pb exposure. These animal observations provided strong coherence with associations in children with homologous tests of spatial memory and rule learning and reversal (Figure 5-5 and Table 5-5). Several studies found that blood or dentin Pb levels measured at an early age (ages 2-8 years) were associated with poorer academic performance at older ages (ages 8-18 years),

suggesting the effect of early exposure to Pb may be persistent and have important implications for success later in life.

The weight of epidemiologic evidence for Pb-associated cognitive function decrements was provided by several prospective studies conducted in diverse populations that found associations with blood Pb levels measured during various lifestages (concurrently, prenatally (cord or maternal), earlier in childhood, and averaged over multiple years) and had extensive evaluation of potential confounding variables. The association with FSIQ 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). Several new studies added evidence for associations of FSIQ with concurrent blood Pb level in populations with lower mean blood Pb levels. Previously, the weight of evidence supported these associations in populations with mean blood Pb levels in the range of 5-10 µg/dL. However, several new studies shifted the weight of evidence to lower blood Pb levels (primarily concurrent), with populations means in the range of 2-7 µg/dL (2011; Kim et al., 2009b; Min et al., 2009; Zailina et al., 2008; Chiodo et al., 2007). Studies of specific cognitive indices and academic performance did not consistently find decrements in performance in children with blood Pb levels < 5 µg/dL (Cho et al., 2010; Miranda et al., 2010; Chandramouli et al., 2009; Surkan et al., 2007). Several new toxicological studies added to the evidence for impaired learning and memory in animals with lower blood Pb levels, 8-17 µg/dL (Cory-Slechta et al., 2010; Li et al., 2009c; Niu et al., 2009; Virgolini et al., 2008a; Stangle et al., 2007). A large body of new evidence from Cory-Slechta and colleagues demonstrated that lifetime Pb exposure when combined with stress in animals exacerbated impairments in learning (2011; Cory-Slechta et al., 2010; Rossi-George et al., 2009; Virgolini et al., 2008a). Findings also indicated that these interactions potentially were mediated via effects on corticosterone and dopamine.

With regards to important lifestages of Pb exposure, the weight of toxicological evidence demonstrates impaired learning and memory in animals exposed to Pb gestationally with or without early postnatal exposure. In particular, impairments in learning and memory observed with lower blood Pb levels (8-17 µg/dL), were found with Pb exposures that began during the gestational or lactation period. The prospective epidemiologic studies found decrements in cognitive function of children in association with concurrent, prenatal (cord and maternal), early childhood, and cumulative average blood Pb levels. However, collectively, based on the frequency of examination, the weight of evidence demonstrates associations between cognitive function decrements and concurrent blood Pb levels in children ages 4-10 years. Among studies that examined MDI in children ages 6 months to 3 years, several found stronger associations of MDI with prenatal (maternal

or cord) blood Pb than with postnatal child blood Pb (Hu et al., 2006; Bellinger et al., 1987; Dietrich et al., 1987a; Vimpani et al., 1985). Child concurrent or lifetime average blood Pb levels also were associated with MDI scores (Claus Henn et al.; Solon et al., 2008; Surkan et al., 2007; Tellez-Rojo et al., 2006; Wasserman et al., 1992; Vimpani et al., 1985). Thus, both postnatal child and maternal Pb exposures may contribute to lower cognitive function in young children. It is important to note that in studies that examined maternal pregnancy or cord blood Pb levels, there is greater uncertainty regarding the relative contributions of maternal past and recent Pb exposures that contributed to associations. The influence of past or cumulative maternal Pb exposures was supported by observations that maternal patella Pb levels were associated with MDI among children at age 2 years in Mexico City (Gomaa et al., 2002). Nonetheless, results for prenatal blood Pb levels and concurrent child blood Pb levels measured during specific postnatal ages indicate that relatively short-duration Pb exposures (< 1 year) influence the cognitive development of children between birth and age 3 years. Short-duration Pb exposures may be especially important during early childhood because processes such as neurogenesis and synaptic pruning are highly active during the first few years of life (Rice and Barone, 2000; Landrigan et al., 1999).

Epidemiologic studies found independent associations of blood Pb level after adjusting for multiple potential confounding factors, including parental IQ, SES, household income, and HOME score. In most studies that provided unadjusted and adjusted effect estimates, blood Pb level was found to be associated with a smaller but statistically significant decrement in FSIQ after adjusting for potential confounding factors (Palaniappan et al., 2011; Kim et al., 2009b; Chiodo et al., 2007; Froehlich et al., 2007; Lanphear et al., 2005; Canfield et al., 2003a). Analyses of associations of covariates with blood Pb level and cognitive function indicated that the potential confounding variables may vary across populations and endpoints. HOME score was not associated with every cognitive index. Studies that adjusted for multiple SES-related factors but did not examine HOME score produced similar magnitudes of associations as did studies that adjusted for HOME score (Table 5-3, Table 5-4, toTable 5-5). Thus, confounding by HOME score may be minimized when other correlated variables are accounted for in analyses. Further, it is uncertain the extent to which HOME score alone may confound blood Pb-IQ associations as studies have not reported the magnitude of change in the blood Pb effect estimate with just the addition of HOME score in the model. Thus, while the caregiving environment can be an important confounder, the overall weight of evidence indicates that it does not mitigate the strong findings linking higher blood Pb levels with lower FSIQ in children. Prenatal drug exposure also was not found to have a large influence on the relationship between blood Pb level and FSIQ (Min et al., 2009; Chiodo et al., 2007). That Pb exposure induces impairments in tests of learning and memory in animals further that are directly homologous to tests conducted in children

further demonstrates that confounding by SES and HOME does not fully account for the associations between blood Pb levels and cognitive function observed in children.

The evidence from epidemiologic and toxicological studies linking Pb exposure with decrements in multiple cognitive functional domains is strengthened by the well-characterized toxicological evidence for Pb exposure interfering with development of the brain and activity of neurochemical processes that mediate cognitive function (Section 5.3.8). Pb has been shown to increase the permeability of the blood-brain barrier and deposit in the target central nervous system. Pb has been shown to impair neurogenesis, synaptic architecture, and neurite outgrowth. The high activity of these processes during fetal and infant development provides biological plausibility for young children being particularly at increased risk for Pb-associated impairments in cognitive function. Cognitive function is mediated by the cortical and subcortical structures of the brain that integrate function in the hippocampus, prefrontal cortex, and nucleus accumbens using dopamine and glutamate as primary neurotransmitters. Experimental studies have shown that Pb induces changes in dopamine release in these regions. Numerous studies also have shown Pb-induced changes in hippocampal function, including changes in glutamate release, receptor binding, and long-term potentiation. Thus, several lines of toxicological evidence establish a neuroanatomical and neurochemical basis for the effect of Pb on cognitive function

5.3.2.5 Epidemiologic Studies of Cognitive Function in Adults

Adults without Occupational Lead Exposures

As described in the preceding section, Pb exposure of animals that begins in gestation and lasts through the early postnatal period or for a lifetime has been shown to induce learning impairments in adult animals. Less well characterized are learning impairments in adult animals due to adult-only Pb exposures. In contrast, epidemiologic studies have examined cognitive performance in adults primarily in association with concurrently measured blood and bone Pb levels. For nonoccupationally-exposed adults, the 2006 Pb AQCD cited some evidence for associations of cognitive performance with bone Pb levels but not blood Pb levels (U.S. EPA, 2006b). Studies published since the 2006 Pb AQCD continued to indicate that bone Pb levels were associated more consistently with cognitive function in nonoccupationally-exposed adults (Table 5-8). Despite the large number of available publications, it is important to recognize that several studies are variants on analyses in the same population (e.g., NHANES or the Normative Aging Study [NAS]) and should not be considered as all independent assessments of the Pb-cognitive function relationship. Another point to consider is that although cross-sectional

and longitudinal studies have been conducted, most examine the associations between one measurement of cognitive function and one concurrent measurement of blood Pb level. Such a design has the inherent weakness that temporality cannot be determined, which limits the causal inference regarding the effects of Pb exposure. When bone Pb level is used as the exposure measure, this issue is mitigated somewhat because bone Pb is a biomarker that reflects many cumulative years of exposure. Additionally, with single measurements of both blood and bone Pb level, it is difficult to characterize the specific timing, duration, and level of Pb exposure that contributed to the associations observed with cognitive function. This uncertainty may apply particularly for assessments of blood Pb levels, which are influenced both by current exposures and cumulative Pb stores in bone that are mobilized during bone remodeling compared with occupationally-exposed adults (Sections 4.3 and 4.7.3).

Several analyses of the large prospective Baltimore Memory Study and NAS have contributed to greater understanding of the relationship between biomarkers of Pb and cognitive effects in adults. In both cohorts, subjects were evaluated periodically with in-person clinical assessments and self-administered questionnaires. Both studies were noted for comparisons of associations between bone and blood Pb levels within the same cohort and for their repeated assessments of Pb biomarker levels and cognitive function over time. In particular, the repeated assessments permitted the examination of associations of Pb biomarkers with changes in cognitive function over time, which allowed investigators to establish temporality between Pb biomarker levels and subsequent changes in cognitive function. The Baltimore Memory Study (BMS) included men and women, 50-70 years of age, residing in Baltimore, MD. A total of 1,140 out of 2,351 (48.5%) subjects participated from neighborhoods that represented a diversity of race and SES. Of particular note, this study was unique in that it included a large proportion of African-Americans (n=395). In comparison, the NAS involved only men (original n = 2,280) residing in the Greater Boston area. Subjects primarily were white and at enrollment were aged 21 to 80 years and had no current or past chronic medical conditions. Studies differed with respect to the potential confounding variables analyzed. The most notable difference was the inclusion of age, smoking and alcohol intake as covariates in the NAS analyses. Despite differences in study population characteristics and potential confounding variables considered, findings were similar between cohorts.

In the BMS, longitudinal analyses were conducted with repeat cognitive testing of study subjects at approximately 14-month intervals. Most subjects completed follow-up; 91% of the original cohort returned for a second round of testing and 83% for a third round each (Bandeen-Roche et al., 2009). An interquartile range higher tibia Pb level (12.7 μg/g) was associated with a 0.019 units per year decrease in eye-hand coordination z-score, adjusting for age, sex, interviewer, race and SES. Tibia Pb was associated with a

larger decrease among African Americans than among whites (Table 5-8). Tibia Pb levels were weakly associated with other time-related decreases in other indices of cognitive function (e.g., language, processing speed, executive function).

Cross-sectional analyses of the BMS included comparisons of the associations for blood and bone Pb level. The 991 adults in Shih et al. (2006) had a mean (SD) blood and tibia Pb levels of 3.46 (2.23) μg/dL and 18.7 (11.2) μg/g, respectively, Whereas higher concurrent blood and bone Pb level both were associated with poorer performance in the domains of language, processing speed, eye-hand coordination, executive function, verbal memory and learning, visual memory, and visuoconstruction, associations with tibia Pb level tended to be larger in magnitude (per SD increase) and statistically significant (Table 5-8). Tibia 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 (potential risk factor for Alzheimer's Disease). The magnitudes of associations were attenuated with additional adjustment for education, race, and SES. In these fully-adjusted models, higher tibia Pb levels were associated with poorer performance in domains except language and processing speed, with a borderline statistically significant association observed for visuoconstruction. In linear models, visuoconstruction scores were 0.0044 SDs (95% CI: -0.0091, 0.0003) lower per 1 μg/g bone higher tibia Pb level. Analysis of a quadratic term for tibia Pb indicated no evidence of nonlinearity.

Other cross-sectional analyses indicated effect modification by race and neighborhood psychosocial hazards. In contrast with the longitudinal results, race-stratified analyses of persistent effects in cross-sectional analyses indicated that tibia Pb levels were associated with greater decreases in performance on tests of eye-hand coordination, executive functioning, and verbal memory and learning among whites than among African Americans (Bandeen-Roche et al., 2009) (Table 5-8).

Table 5-8 Associations of blood and bone Pb levels with cognitive function in adults[a]

Study	Population/ Methods	Cognitive Test	Subgroup/ Model	Blood Pb Effect Estimate (95% CI)[b]	Bone Pb Effect Estimate (95% CI)[b]
Bandeen-Roche et al. (2009)	1,140 adults, ages 50-70 yr Baltimore Memory Study (BMS) cohort Baltimore, MD Marginal linear regression models adjusted for age, household wealth, education, race/ethnicity	Longitudinal associations Eye/hand coordination African-Americans White Cross-sectional associations Verbal memory/learning African-Americans White Executive Function African-Americans White		NOT EXAMINED	Tibia Mean (SD): 18.8 (11.6) µg/g Per 12.7 µg/g (IQR) increase -0.032 (-0.052, -0.012) per year -0.009 (-0.024, 0.006) per year 0.006 (-0.09, 0.10) -0.076 (-0.15, 0.001) 0.009 (-0.05, 0.07) -0.07 (-0.12, -0.01)
Shih et al. (2006)	985 adults, mean age: 59 yr BMS, Baltimore, MD Linear regression adjusted for: Model A: age, sex, technician, presence of APOE-ε4 allele Model B: Model I, years of education, race/ethnicity, wealth	Raven's Colored Progressive Matrices Language Eye-hand coordination Executive functioning Visuoconstruction	 Model A Model B Model A Model B Model A Model B Model A Model B	Concurrent blood Pb Mean (SD): 3.5 (2.2) µg/dL -0.013 (-0.064, 0.037) -0.004 (-0.044, 0.036) -0.024 (-0.0704, 0.022) -0.017 (-0.057, 0.024) -0.031 (-0.075, 0.012) **-0.022 (-0.059, 0.015)** **-0.042 (-0.101, 0.017)** -0.031 (-0.084, 0.021)	Tibia Mean (SD): 18.7 (11.2) µg/g **-0.0896 (-0.146, -0.034)** 0.007 (-0.034, 0.047) **-0.0896 (-0.134, -0.045)** **-0.034 (-0.067, 0)** **-0.0896 (-0.123, -0.056)** -0.016 (-0.056, 0.025) -0.024 (-0.190, 0.141) **-0.049 (-0.101, 0.002)** **TIBIA Pb STRONGER**
Glass et al. (2009)	1,001 adults, mean age 59 yr BMS, Baltimore, MD Multilevel hierarchical regression model adjusted for age, sex, race/ethnicity, education, testing technician, time of day	Raven's Colored Progressive Matrices Language Eye-hand coordination Executive functioning Visuoconstruction	 Middle NPH High NPH Middle NPH High NPH Middle NPH High NPH Middle NPH High NPH	NOT EXAMINED	Tibia Pb Mean (SD): 18.8(11.1) µg/g 0.011 (-0.089, 0.111)[c] -0.09 (-0.189, -0.011)[c] -0.04 (-0.133, 0.044)[c] -0.067 (-0.167, 0.033)[c] -0.022 (-0.111, 0.067)[c] -0.111 (-0.1998, -0.022)[c] -0.003 (-0.155, 0.149)[c] -0.007 (-0.189, 0.175)[c]
Weisskopf et al. (2007a)	1,089 males, mean age 68.7 yr; Normative Aging Study (NAS), Boston, MA 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	CERAD, Neurobehavioral Evaluation System, WIAS-R, MMSE, VMI Visuospatial, pattern comparison (+ = poorer performance) Executive function verbal fluency Short-term memory, word list		NOT EXAMINED	Mean (IQR): Tibia: 20 (15) µg/g Patella: 25 (20) µg/g Estimates per IQR increase: Tibia: 0.79 (0.40, 1.2) over time Patella: 0.73 (0.40, 1.2) over time Tibia: -0.40 (-1.6, 0.80) over time Patella: -0.86 (-2.00, 0.30) over time Tibia: -0.28 (-1.2, 0.60) over time Patella: -0.81 (-1.7, 0.05) over time

Study	Population/ Methods	Cognitive Test	Subgroup/ Model	Blood Pb Effect Estimate (95% CI)[b]	Bone Pb Effect Estimate (95% CI)[b]
Wang et al. (2007a)	358 males, median ages: 67.2 yr (HFE wild-type) 67.7 yr (HFE variant) Normative Aging Study, Boston, MA 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	NOT EXAMINED	Median: 19 µg/g (Tibia), 23 µg/g (Patella) -0.20 (-1.0, 0.70) per year -1.40 (-3.3, 0.40) per year -6.3 (-10.4, -2.1) per year
Weuve et al. (2006)	720 males, ages ≥ 45 yr NAS, Boston, MA 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 wildtype ALAD2 carrier ALAD wildtype ALAD2 carrier	Concurrent blood Pb Mean (IQR): 5.2 (3) µg/dL -0.013 (-0.053, 0.027) -0.087 (-0.180, 0.007)	Median (IQR): Tibia: 19 (15) µg/g; Patella: 27 (21) µg/g Tibia **-0.03 (-0.14, 0.07)** **-0.11 (-0.30, 0.06)** Patella **-0.03 (-0.11, 0.04)** **-0.12 (-0.30, 0.06)**
Rajan et al. (2008)	720 males, ages ≥ 45 yr Normative Aging Study, Boston, MA Linear regression adjusted for blood Pb main effect, ALAD genotype, age at cognitive test, education, alcohol consumption, cumulative smoking, English as first language	CERAD, Neurobehavioral Evaluation System, WIAS-R		Concurrent blood Pb Mean (SD): 5.3 (2.9) µg/dL (ALAD wildtype); 4.8 (2.7) µg/dL (ALAD2 carriers)	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)
		Visuospatial, constructional praxis		-0.048 (-0.216, 0.120)[d]	**Tibia: -0.216 (-0.419, -0.013)**[d] Patella: 0.018 (-0.182, 0.218)[d]
		Executive function verbal fluency		-0.028 (-0.2044, 0.148)[d]	**Tibia: -0.089 (-0.292, 0.1143)**[d] Patella: -0.018 (-0.218 0.1082)[d]
		Verbal memory, word recall		0.003 (-0.168, 0.174)[d]	Tibia: 0.064 (-0.127, 0.254)[d] Patella: 0.127 (-0.728, 0.989)[d]
		Perceptual speed, mean latency		**-0.168 (-0.392, 0.056)**[d]	Tibia: -0.152 (-0.495, 0.191)[d] Patella: -0.146 (-0.40, 0.109)[d] **TIBIA Pb STRONGER**
Weuve et al. (2009)	587 females, ages 47-74 yr Nurses' Health Study, Boston, MA 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	Telephone Interview for Cognitive Status and East Boston Memory Test		Concurrent blood Pb Mean (SD): 2.9 (1.9) µg/dL	Mean (SD) Tibia Pb: 10.5 (9.7) µg/g Patella Pb: 12.6 (11.6) µg/g
		Composite cognitive score		-0.015 (-0.068, 0.038)	**Tibia: -0.039 (-0.087, 0.0097)** Patella: -0.012 (-0.058, 0.035)
		Composite except letter fluency		0.015 (-0.070, 0.101)	**Tibia: -0.049 (-0.097, 0)** Patella: -0.035 (-0.081, 0.012) **TIBIA Pb STRONGER**

Study	Population/ Methods	Cognitive Test	Subgroup/ Model	Blood Pb Effect Estimate (95% CI)[b]	Bone Pb Effect Estimate (95% CI)[b]
Krieg and Butler (2009)	2,823 adults, ages 20-59 yr, U.S. NHANES III (1991-1994)	Neurobehavioral Evaluation System 2		Concurrent Blood mean (SD): 2.88 (6.91) µg/dL	NOT EXAMINED
	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)	Ages 20-39 yr Ages 40-59 yr	-0.097 (-0.422, 0.228)[e] -0.290 (-0.601, 0.0207)[e]	
		Serial digit learning score	Ages 20-39 yr Ages 40-59 yr	-0.117 (-0.463, 0.228)[e] 0.401 (-0.193, 0.995)[e]	
Krieg et al. (2009)	2,090 adults, ages 20-59 yr 1976 adults, ages ≥ 60 yr U.S. NHANES III (1991-1994)	Neurobehavioral Evaluation System 2		Concurrent Blood Pb Mean (SD): 20-59 yr: 2.85 (7.31) µg/dL; ≥ 60 yr: 4.02 (3.56) µg/dL	NOT EXAMINED
	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)	Ages 20-59 yr ALAD GG ALAD CC/CG	-0.132 (-0.358, 0.095)[e] -0.526 (-1.118, 0.066)[e]	
		Serial digit learning score	Ages 20-59 yr ALAD GG ALAD CC/CG	-0.022 (-0.526, 0.482)[e] 0.025 (-0.406, 0.456)[e]	
		Word recall	Ages ≥ 60 yr ALAD GG ALAD CC/CG	-0.075 (-0.285, 0.135)[e] 0.025 (-0.406, 0.456)[e]	
		Story recall	Ages ≥ 60 yr ALAD GG ALAD CC/CG	0.085 (-0.0997, 0.271)[e] -0.466 (-1.072, 0.139)[e]	

Study	Population/ Methods	Cognitive Test	Subgroup/ Model	Blood Pb Effect Estimate (95% CI)[b]	Bone Pb Effect Estimate (95% CI)[b]
Krieg et al. (2010)	2,093 adults, ages 20-59 yr 1,799 adults, ages ≥ 60 yr U.S. NHANES III (1991-1994)	Neurobehavioral Evaluation System 2		Concurrent blood Pb Mean (SD): 20-59 yr: 2.85 (7.32) µg/dL; ≥ 60 yr: 4.02 (3.39) µg/dL	NOT EXAMINED
	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)	Ages 20-59 yr	-3.916 (-8.638, 0.805)[e]	
			VDR haplotype CC	0.139 (-0.278, 0.556)[e]	
			VDR haplotype CT	-0.505 (-1.025, 0.015)[e]	
			VDR haplotype TC	-0.695 (-0.783, 0.871)[e]	
		Serial digit learning score (+ = poorer performance)	VDR haplotype TT	-2.533 (-4.868, -0.198)[e]	
			Ages 20-59 yr	-0.322 (-0.922, 0.278)[e]	
			VDR haplotype CC	0.447 (0.542, 0.351)[e]	
			VDR haplotype CT	0.044 (-0.783, 0.871)[e]	
			VDR haplotype TC		
			VDR haplotype TT		
		Word recall	Ages 20-59 yr	-0.766 (-1.817, 0.285)	
			VDR haplotype CC	-0.085 (-0.40, 0.21)	
			VDR haplotype CT	-0.034 (-0.471, 0.403)	
			VDR haplotype TC	-0.095 (-0.895, 0.705)	
			VDR haplotype TT		
		Story recall	Ages ≥ 60 yr	0.146 (-1.674, 1.966)	
			VDR haplotype CC	0.003 (-.193, 0.20)	
			VDR haplotype CT	0.034 (-0.322, 0.3899)	
			VDR haplotype TC	-0.166 (-0.434, 0.102)	
			VDR haplotype TT		
			Ages ≥ 60 yr		
			VDR haplotype CC		
			VDR haplotype CT		
			VDR haplotype TC		
			VDR haplotype TT		
Gao et al. (2008)	188 adults, mean age 69.2 yr Sichuan and Shandong Provinces, China ANCOVA adjusted for age, sex, education, BMI, APOE ε4	CERAD, CSID, IU story recall, Animal fluency test, IU token test Composite cognitive score		Concurrent plasma Pb Mean (SD): 0.39 (0.63) µg/dL -0.006 (-0.016, 0.004)	NOT EXAMINED

Study	Population/ Methods	Cognitive Test	Subgroup/ Model	Blood Pb Effect Estimate (95% CI)[b]	Bone Pb Effect Estimate (95% CI)[b]
Van Wijngaarden et al. (2009)	47 adults, mean age 61.5 yr Rochester, NY Linear regression adjusted for age, gender, educational level, history of hypertension	CANTAB and Montreal Cognitive Assessment Delayed matching, % correct Total trials		NOT EXAMINED	Mean (SD): Tibia: 2.0 (5.2) µg/g; Calcaneus: 6.1 (8.5) µg/g Calcaneus Lowest tertile: 87.56[f] Medium tertile: 86.67 Highest Tertile: 80.67, p = 0.027 Tibia Lowest tertile: 85.42[f] Medium tertile: 87.08 Highest tertile: 82.44, p = 0.25 Calcaneus Lowest tertile: 2.54[f] Medium tertile: 2.61 Highest tertile: 2.72, p = 0.21 Tibia Lowest tertile: 2.62[f] Medium tertile: 2.59 Highest tertile: 2.66

[a]Studies are presented by cohort then generally in the order of discussion in the text.

[b]Effect estimates have been standardized to the standard deviation of the cognitive test scores and standardized to an SD or IQR increase in blood or bone Pb level.

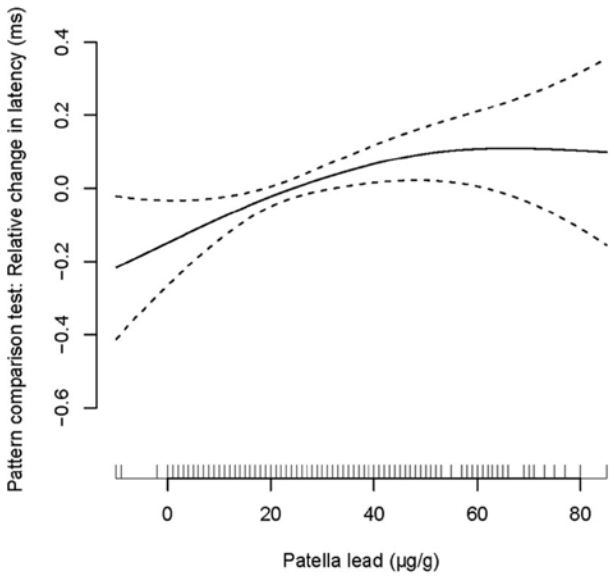
[c]Effect estimates indicate interactions between Pb and category of neighborhood psychosocial hazard (NPH), with the lowest tertile of NPH serving as the reference group.

[d]Effect estimates indicate interactions between Pb and ALAD genotype.

[e]The directions of effect estimates were changed to indicate a negative slope as a decrease in cognitive performance.

[f]Results refer to mean cognitive function scores among tertiles of bone Pb.

Similar to the BMS, in the NAS cohort, higher tibia Pb levels were associated with decreases in cognitive performance over time (Weisskopf et al., 2007a). Weisskopf et al. (2007a) expanded evidence by finding associations with patella Pb levels. Two measurements of cognitive function, collected approximately 3.5 years apart were available for 60-70% of participants. Longitudinal analyses were conducted with repeated measures plus a bone Pb-time interaction term in order to estimate the association between bone Pb level and decline in cognitive test score over time. Although bone Pb levels were associated with increased response latency on a pattern comparison test, they were associated with fewer errors on the same test. The authors proposed that this may be related to slowing reaction time to improve accuracy. When the nine men with the highest bone Pb levels were removed, the association with fewer errors was no longer statistically significant. However, the authors did not indicate whether the point estimate changed. In the analysis with patella Pb, Weisskopf et al. (2007a) found a nonlinear association, with latency times becoming worse over time (i.e., larger values or slower response time) up to approximately 60 µg/g patella Pb, but the change over time leveling off at higher levels (Figure 5-12). Below 60 µg/g, a 20 µg/g difference in patella Pb level was associated with an increase in latency of approximately 0.15 ms.



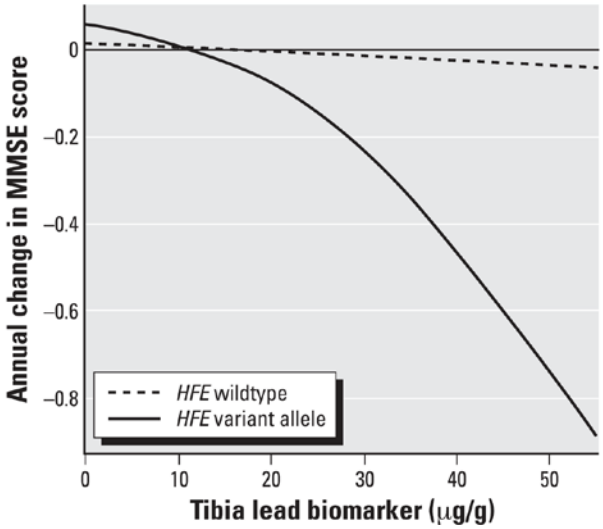
Source: Reprinted with permission of Williams & Wilkins, Weisskopf et al. (2007a).

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. (reference = 0 at mean of patella Pb concentration).

Figure 5-12 Nonlinear association between patella Pb level and the relative change over time in response latency on the pattern comparison test.

Longitudinal analysis of the NAS cohort also indicated effect modification by hemochromatosis (HFE) gene variants (Wang et al., 2007a). 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 higher tibia 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 either the H63D and C282Y variant. The association was found to be nonlinear, with larger Pb-associated declines observed at higher tibia Pb levels (Figure 5-13). This difference was comparable to the difference in MMSE score between men who were 4 years apart in age in their study sample. Tibia Pb level was associated with a smaller decline in MMSE score in men with only HFE wildtype alleles (Figure 5-13). HFE variants, H63D and C282Y, are associated with hemochromatosis, a disease characterized by higher iron body burden. Higher iron body burden has been linked with lower Pb absorption, thus the apparent interaction could be related to altered Pb toxicokinetics rather than some other direct biological interaction. For example, the relation between tibia Pb level and Pb dose at the biologically relevant site(s) could be shifted to the left among for HFE variant allele carriers such that for the same tibia Pb level, Pb the dose at the relevant biological site(s)

may be higher leading to a greater annual decline in MMSE score as indicated in Figure 5-13 (solid curve). This implies that similar decreases in MMSE score would still be found in wildtype HFE carriers only at higher tibia Pb levels.



Source: Wang et al. (2007a).

Note: The lines indicate curvilinear trends estimated from the penalized spline method. Among HFE wild-types, 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). 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 5-13 Exploration of nonlinear association of tibia Pb level with annual rate of cognitive decline, by hemochromatosis (HFE) gene variant.

The 2006 Pb AQCD described associations of blood and tibia Pb levels with poorer cognitive performance among 141 NAS men (Payton et al., 1998). Several new and larger cross-sectional NAS analyses corroborated previous findings for bone Pb but generally indicated weak associations with concurrent blood Pb levels and only in groups with specific genetic variants. In contrast with the longitudinal analyses, Weisskopf et al. (2007a) found that repeat measures of bone Pb levels were not consistently associated with cognitive function in cross-sectional analyses. In a study of Mini-Mental State Examination (MMSE) tests scores (test of general cognitive function) among 720 men 45 years of age and older, higher concurrent blood Pb levels were associated with lower MMSE scores among ALAD2 carriers (Weuve et al., 2006). A 3 µg/dL higher concurrent 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 did not find a consistent

direction of modification of the association between blood or bone Pb levels and other tests of cognitive function by ALAD genotype (Rajan et al., 2008). Nonetheless, Rajan et al. (2008) found tibia Pb levels to be associated more strongly and consistently with poorer cognitive performance compared with concurrent blood or patella Pb levels (Table 5-8).

Effect modification by stress was examined in both the NAS and BMS cohorts. In the NAS cohort, higher bone Pb levels were associated with poorer cognitive function among individuals with higher individual-level perceived stress (Peters et al., 2007). Higher tibia Pb levels also were associated with larger decrements in cognitive performance among BMS subjects living in neighborhoods with a greater number psychosocial hazards (e.g., number of violent crimes, emergency calls, off-site liquor licenses) (Glass et al., 2009) (Table 5-8). These observation of effect modification by environmental stress in adult humans is supported by several observations of Pb-stress interactions in impaired learning and memory and adult animals with Pb exposures from gestation through post-weaning and lifetime Pb exposures (Section 5.3.2.2).

Weuve et al. (2009) studied the association of blood and bone Pb levels with cognitive function in a subset of 587 healthy women in the Boston, MA area participating in the Nurses' Health Study. Blood and bone Pb levels were measured between the ages of 47 and 74 years, and the mean (SD) blood Pb level in this group was 2.9 (1.9) µg/dL measured in samples collected an average of 5 years before cognitive testing. As in the aforementioned studies of adults, tibia and patella Pb levels were more consistently associated with cognitive performance than was blood Pb levels (Table 5-8). Contrary to expectation, higher patella and tibia Pb levels were associated with higher scores on the "f" naming test (naming words that begin with f). In separate models, the "f" naming test was omitted from a composite index of all cognitive tests, and a one SD (10 µg/g bone) higher tibia Pb level was associated with 0.051-point lower (95% CI: -0.010, -0.003) standardized composite score. A similar magnitude of decrease was estimated for an increase in age of 3 years in these women. The magnitude of association was slightly smaller for an SD unit increase in patella Pb level (-0.033 [95% CI: -0.080, 0.014).

Several studies analyzed data from the U.S.-representative NHANES III (1991-1994) population of men and women and investigated effect modification by age and genetic variants. Only blood Pb levels were available and were measured in samples collected concurrently with cognitive testing. Krieg and Butler (2009) did not find blood Pb level consistently to be associated with poorer performance on cognitive testing among 2,090 adults 20-59 years of age or among 1,796 adults 60 years of age and older. Because different types and numbers of tests were conducted in the two age groups, it is difficult to compare findings between age groups. In the subset of the population with genetic

analysis, blood Pb-cognitive function associations were not found to be modified by ALAD genetic variants in the same direction (Krieg et al., 2009). Among adults ages 20-59 years with the CC and CG ALAD genotype groups combined (i.e., ALAD2 carriers), reaction time improved (i.e., faster reaction time) by 38 ms per 10-fold increase in concurrent blood Pb level. In contrast, among ALAD2 carriers, higher blood Pb levels were associated with poorer performance on a symbol-digit substitution task. The potential direction of effect modification by the ALAD2 genotype is not immediately clear as the greater affinity of the ALAD2 enzyme subunit for Pb may increase susceptibility to Pb-associated health effects by increasing blood Pb levels or it may diminish Pb-associated health effects by decreasing Pb bioavailability by maintaining it in a sequestered state in the bloodstream. Krieg et al. (2010) did find differences in the association between concurrent blood Pb level and scores on a symbol-digit substitution test by the VDR variants, rs731236 and VDR rs2239185, and by the VDR haplotype. Similar to observations in adolescent NHANES participants (Section 5.3.2.1), results were not uniform 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.

Other studies with smaller numbers of subjects generally produced results consistent with those from the larger studies above. A cross-sectional study of 188 rural Chinese men and women found a weak association between higher plasma Pb levels and a lower composite cognitive score based on a battery of in-person administered tests (Gao et al., 2008). 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 cross-sectional study of 47 men and women in Rochester, NY (55-67 years of age), subjects in the higher two tertiles of calcaneal bone (trabecular bone with higher turnover rate than tibia) Pb level performed worse on delayed matching-to-sample and paired associated learning tasks (Van Wijngaarden et al., 2009) (Table 5-8). In analyses of tibia Pb levels, subjects in the highest tertile of tibia Pb level did not consistently perform worse on cognitive tests (Table 5-8). The exact calcaneal and tibia Pb levels in tertiles were not reported.

Adults with Occupational Lead Exposures

The 2006 Pb AQCD concluded that in adults, blood Pb levels were associated with cognitive function most consistently among those with occupational Pb exposures. These findings were supported by results from a few recent studies of occupationally-exposed

adults. 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 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 found to be associated with poorer performance on a battery of neurocognitive tests (Schwartz et al., 2005; Schwartz et al., 2001). Dorsey et al. (2006) found higher patella Pb levels to be associated with poorer manual dexterity, poorer sensory function, and greater depression symptoms. These associations for patella Pb levels were not as strong as the previously reported associations were with either blood or tibia Pb levels in this occupational cohort.

A follow-up study of the original 1982 Lead Occupational Study was conducted 2001-2004 with 83 of the original 288 Pb-exposed workers and 51 of the original 181 controls (Khalil et al., 2009b). 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 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 worse than did the controls in total cognitive score and in the spatial and general intelligence domains ($p < 0.05$). They also performed worse in all other domains (e.g., motor, executive, and memory) although the differences were not as large. A similar pattern was observed in analyses using tibia Pb levels measured at the follow-up visit to represent exposure. Weaker associations were observed with concurrent blood Pb levels (median among the exposed: 12 μg/dL). Among the former Pb workers, higher 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 poorer performance in other domains as well. As in nonoccupationally-exposed adults, the stronger findings for tibia Pb levels in former Pb-exposed workers indicate stronger effects of higher past Pb exposures than lower current exposures on cognitive function.

Additional studies aimed to characterize factors that either mediate or modify the association between Pb biomarkers and cognitive function. A study of 61 current Pb smelter workers with a mean age of 40 years and blood Pb level of 29.1 μg/dL found that both a working lifetime time-weighted integrated blood Pb level (an index of cumulative exposure) ($p = 0.09$) and tibia Pb level ($p = 0.08$) were associated with longer times to complete the grooved pegboard test (Bleecker et al., 2007a). Among 112 Pb smelter workers, working lifetime time-weighted integrated blood Pb level was associated with poorer performance on attention and digit symbol tasks among those with low cognitive

reserve (assessed by performance on the Wide Range Achievement Test-R for reading) (Bleecker et al., 2007b).

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. ApoE-ε4 carriers are at two-fold increased risk of developing Alzheimer's disease, although the majority of such individuals still do not develop the disease. Thus, it is biologically plausible that ApoE-ε4 carriers may be biologically susceptible to cognitive dysfunction. A study of occupationally-exposed adults found that among individuals with at least one ApoE-ε4 allele, blood Pb level was associated with poorer performance on digit symbol, pegboard assembly, and complex reaction time tests (Stewart et al., 2002).

Studies also indicated that Pb-exposed workers may be at increased risk of motor dysfunction. Among Pb smelter workers, working lifetime time-weighted integrated blood Pb level was associated with poorer motor performance ($p < 0.05$). Iwata et al. (2005) examined the cross-sectional 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, higher blood Pb level was associated with greater sagittal sway with eyes open ($p < 0.05$) and eyes closed ($p < 0.01$) and transversal sway with eyes closed ($p < 0.05$). The authors calculated a benchmark dose level (Budtz-Jorgensen et al., 2001; NRC, 2000) of 14.3 μg/dL from a linear concentration-response model of their data. A supralinear concentration-response function was found to fit the data slightly better than was a linear function.

Summary of Cognitive Function in Adults

In summary, among nonoccupationally-exposed adults, there is weak evidence that cognitive function is associated with concurrently measured blood Pb levels. The strongest evidence was provided by NHANES analyses, in which concurrent blood Pb levels were associated with lower cognitive function in particular age and genetic variant subgroups (Krieg et al., 2010; Krieg and Butler, 2009; Krieg et al., 2009). These analyses did not have bone Pb measures for comparison. It is important to note that because bone Pb is a major contributor to blood Pb levels, blood Pb level also can reflect to a large extent longer term exposures, including higher past exposures, especially among adults without occupational exposures. Thus, in the NHANES analyses of adults, it is difficult to characterize the relative contributions of recent and past Pb exposures to the

associations observed between concurrent blood Pb level and cognitive function. Consistent with the conclusion of the 2006 Pb AQCD, several recent studies found associations between bone Pb levels and cognitive function in adults (Table 5-8). Much of the evidence was provided by analyses of two cohorts: BMS and NAS. Recent longitudinal analyses demonstrated that higher bone Pb levels measured at baseline were associated with subsequent declines in cognitive function over 2- to 4-year periods (Bandeen-Roche et al., 2009; Weisskopf et al., 2007a). These findings suggested that long-term Pb exposure may contribute to ongoing declines in cognitive function in adults. Recent studies that analyzed both blood and bone Pb levels generally found stronger associations for bone Pb levels, in particular tibia Pb levels, across the various cognitive tests that were performed (Table 5-8). The discrepant findings for blood and bone Pb levels indicate that cumulative Pb exposure that likely included higher past exposures, may be a better predictor of cognitive function in adults than is blood Pb level. Patella and tibia Pb levels were examined in the NAS and Nurses' Health Study; however, evidence did not consistently indicate that tibia Pb levels were more strongly associated with decreases in cognitive performance (Weuve et al., 2009; Weisskopf et al., 2007a). Additional support for the effects of cumulative or past Pb exposure is provided by analyses of the Boston prospective cohort as adults. Deciduous tooth Pb was associated with decrements in specific cognitive indices at ages 19-20 years (Bellinger et al., 1994a), and blood Pb levels measured at age 6 months, 4 years, 10 years, and levels averaged over childhood were associated with decrements in FSIQ at ages 28-30 years (Mazumdar et al., 2011).

Although based on limited examination, there is some indication that certain variants in HFE, ALAD, or VDR genes modify the association between Pb and cognitive function in nonoccupationally-exposed adults; however, results were uniform across the various cognitive tests performed. Aside from identifying populations potentially at increased risk and elucidating underlying modes of action, such effect modification also serves to strengthen the basic inference about associations between Pb biomarkers and cognitive function. Specifically, when effect modification is identified, potential confounding factors would have to vary by levels of the modifying factor, which is usually unlikely, particularly when considering genotype. It also is important to bear in mind that it is not always clear whether the observed effect modification reflects a change in the toxicokinetics of Pb and therefore a change in dose at the biological site of action or a direct biological interaction that increases the toxicity of Pb at a particular target organ or tissue.

In contrast with nonoccupationally-exposed adults, in adults with current occupational Pb exposures, cognitive function was associated with both blood and bone Pb levels. These findings indicate that among adults with occupational Pb exposures, both current and

cumulative exposures affect cognitive function. In the study of former Pb workers, blood Pb levels (median: 12 μg/dL) and findings were more similar to those from nonoccupationally-exposed adults. Among former Pb workers, tibia Pb levels were associated more strongly with cognitive performance than were blood Pb levels (Khalil et al., 2009b). Thus, in the absence of higher current Pb exposures, cumulative Pb exposures may have a greater effect on cognitive function in adults.

5.3.3 Behavioral Effects

5.3.3.1 Epidemiologic Studies of Behavioral Effects in Children

Noncognitive effects of Pb are more complex to study relative to IQ tests. There are fewer objective tests of behavior, and existing tests do not have as strong psychometric properties and are less rigorously standardized compared with IQ tests. In several studies, behaviors are assessed frequently using teacher and/or parent ratings and thus are subject to greater measurement error. 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) (White et al., 2009). Several epidemiologic studies reviewed in the 2006 Pb AQCD reported associations between blood Pb levels and a wide range of behavioral effects, with the weight of evidence supporting associations with inattention and hyperactivity and smaller bodies of evidence indicating associations with misconduct and delinquent behaviors and withdrawn and depressive behaviors (Bellinger and Rappaport, 2002; Needleman et al., 2002; Dietrich et al., 2001; Burns et al., 1999; Wasserman et al., 1998; Needleman et al., 1996; Bellinger et al., 1994a). Coherence was provided by similar findings of inattention, impulsivity, and changes in social behavior in Pb-exposed animals (Section 5.3.3.2). Epidemiologic studies found that blood Pb levels were associated with decrements in cognitive function and behavioral problems within the same population of children, which demonstrates the strong relationship between the two neurodevelopmental domains. The strong relationship between cognitive function and behavior in children also is demonstrated by the fact that the schedule controlled behavior tests in animals measure both memory and inattention. Thus, behavioral problems associated with Pb exposure may contribute to problems with learning, which may progress to antisocial and delinquent behavior later in life.

Most previous epidemiologic 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 (e.g., 7-22 years of age). Most studies examined

associations with blood Pb levels assessed at a single time point; however, even the prospective studies with serial measurements of blood Pb levels, found a range of behavioral problems to be associated with both prenatal and early childhood blood Pb levels (Dietrich et al., 2001) and lifetime average blood Pb levels (Burns et al., 1999). Thus, evidence did not conclusively identify a single lifestage of Pb exposure that was associated with the greatest risk of behavioral problems and misconduct. Recent studies strengthened the evidence for associations of blood Pb levels with inattention and aggression (Figure 5-14 and Table 5-9). Based on a smaller number of studies, new evidence demonstrates associations of blood Pb levels with ADHD diagnosis and diagnostic indices as well as with conduct disorders (Table 5-10 and Table 5-11). As with cognitive function, the epidemiologic evidence for each category of outcomes was evaluated separately in order of increasing weight of evidence. Emphasis was placed on prospective studies with repeated assessments of blood Pb levels and behavior, studies assessing effects relevant to blood Pb levels in contemporary U.S. children (i.e., less than 5 µg/dL), and studies of younger children whose blood Pb levels are less influenced by higher past Pb exposures.

Similar to cognitive function, associations between blood Pb levels and behavioral outcomes may be potentially confounded by factors such as parental SES, parental education, parental IQ, quality and stability of the caregiving environment, and nutritional status. Accordingly, in assessing whether blood Pb-behavior associations were independent of the effects of the other variables, greater weight was given to studies that accounted for potential confounding in the study design or in statistical analyses.

Inattention and Hyperactivity in Children

Consistent with previous evidence, recent studies provided strong evidence that blood Pb level were associated with various endpoints related to inattention, hyperactivity, and impulsivity in children after adjusting for potential confounding by multiple SES-related variables and co-exposures (Figure 5-14 and Table 5-9). Evidence was equally consistent for inattention assessed using teacher and parent ratings and objective tests that measure sustained attention such as the continuous performance test (CPT). The associations observed with CPT, in particular, provide strong coherence with findings in animals for Pb-induced impairments in homologous tests of response inhibition in Schedule Controlled Behavior Tests (Section 5.3.3.2). Both tests measure reactions to stop signals, i.e., premature responses, reaction time. Most of the recent evidence is derived studies of non-U.S. children. Whereas previous studies primarily examined associations with blood Pb levels measured earlier in childhood, recent studies indicated associations with concurrent blood Pb levels. Further, many earlier studies of inattention and impulsivity included children with higher blood Pb levels than those observed in contemporary

children. Recent studies provided new evidence of blood Pb-associated inattention and impulsivity in populations of children with mean blood Pb levels ranging from 2 to 5 µg/dL (Cho et al., 2010; Plusquellec et al., 2010; Chiodo et al., 2007; Plusquellec et al., 2007).

Previous results from prospective studies indicated that early childhood Pb exposures were associated with attention deficits in adolescence and young adulthood (Ris et al., 2004; Bellinger et al., 1994a). Bellinger et al. (1994a) found that compared with young adults (ages 19-20 years) who had deciduous tooth Pb levels 2.9-5.9 ppm, adults with deciduous tooth Pb levels > 19.9 ppm committed more errors on the CPT and performed worse on Stroop and WCST, which measure the ability to shift focus and execute a different task. In the CLS cohort, Ris et al. (2004) found increases in attention as measured by CPT in association with prenatal maternal, 3-60 month average, and 78 month blood Pb levels in adolescents ages 15-17 years, adjusting for maternal IQ, SES, HOME, and adolescent marijuana use. Prenatal blood Pb level was associated with slightly larger increases in attention, and larger associations were estimates for males (Figure 5-14 and Table 5-9).

Studies in children in Korea with relatively low blood Pb levels (means < 3 µg/dL) both demonstrated associations with measures of inattention (Cho et al., 2010; Min et al., 2007). In a population of children ages 8-11 years, Cho et al. (2010) found relationships of concurrent blood Pb levels with ADHD symptoms (i.e., inattentiveness, hyperactivity, and total score) rated by teachers and parents, with the association with teacher ADHD rating attaining statistical significance. In addition to the low blood Pb levels in this study (mean: 1.9 µg/dL [range: 0.53-6.16]), a strength of this study was the comparison of effect estimates with and without adjustment for potential confounders such as age, sex, paternal education, maternal IQ, child IQ, city of residence, birth weight, and urinary cotinine. In multivariate models, effect estimates decreased by 2 to 14%; however, associations remained statistically significant. Mean ADHD ratings by teacher and parents were similar (both 9.1); however, parental ratings had greater variability (SD: 11.5 for parents and 8.6 for teachers), which may have contributed to differences in association. Although higher blood Pb levels were associated with more errors (responding to a nontarget) on the CPT test, they were not consistently associated other indicators of inattention on the CPT or Stroop test (Figure 5-14 and Table 5-9). Further, effect estimates lost statistical significance when urinary cotinine was included in models.

Cho et al. (2010) did not examine potential confounding by HOME score but they did examine parental history of neuropsychiatric disease (e.g., ADHD, learning disability, depression, obsessive-compulsive disorder). Mean blood Pb levels were similar in children with and without parental history of neuropsychiatric disease (1.80 and

1.93 µg/dL, respectively, p = 0.32). Thus, the relationship between blood Pb level and ADHD-related symptoms in the child was not likely confounded by parental neuropsychiatric disease. Results from this study also indicated that the relationship between blood Pb level and ADHD-related symptoms was independent of the relationship between blood Pb level and IQ. Min et al. (2007) examined a population of children (ages 7-16 years) in Korea with a mean concurrent blood Pb level of 2.9 µg/dL and adjusted for maternal score on attention tests. Investigators found that a 1 µg/dL increase in concurrent blood Pb level was associated with a 16.8 ms increase (95% CI: -1.08, 0.06) in reaction time, a test of attention. Although this study did not consider confounding by SES and caregiving environment, the findings was consistent with those from Cho et al. (2010).

Similar results were obtained in a study of children, ages 8-12 years, in Romania, except that concurrent blood Pb level was associated with similar magnitudes of increase in inattention, hyperactivity, and impulsivity as assessed by both parents and teachers (Nicolescu et al., 2010). Results for parent and teacher ratings were strengthened by observations that blood Pb levels also were associated with increased false-alarm rates in responses to stop signals (Figure 5-14 and Table 5-9). Blood Pb levels in this study also were relatively low (median: 3.7 [95% CI: 1.7, 11.1]), and removing five children with blood Pb levels at or above 10 µg/dL had minimal impact on observed associations. Exposures to aluminum and mercury, other neurotoxic metals, also were examined, and blood Pb level was associated with the largest, statistically significant increases in inattention, impulsivity, and hyperactivity.

The aforementioned studies did not consider confounding by the caregiving environment, i.e., HOME score. Studies that did adjust for HOME score, also found associations of blood Pb level with inattention and hyperactivity (Chandramouli et al., 2009; Chiodo et al., 2007). In their longitudinal study of children in the U.K., Chandramouli et al. (2009) found association of higher blood Pb level at age 30 months with hyperreactivity at ages 7 and 8 years; however, this association was observed primarily in children with blood Pb levels greater than 10 µg/dL (Table 5-9) and the strongest for ratings given by teachers. Children with elevated blood Pb levels did not consistently have elevated odds of inattention as assessed using stop signal tasks. Similar to Cho et al. (2010), this association was not influenced by the inclusion of child IQ in the model. Therefore, these findings add support for increasing blood Pb levels having effects on behavior independent of effects on cognitive function. Chiodo et al. (2007; 2004) found that concurrent blood Pb level was associated with inattention in 7 year-old children in Detroit, MI, as assessed using teacher ratings and CPT (Figure 5-14 and Table 5-9). Potential confounders were selected based on the association of each with a particular endpoint, thus model covariates varied among endpoints. HOME score was associated

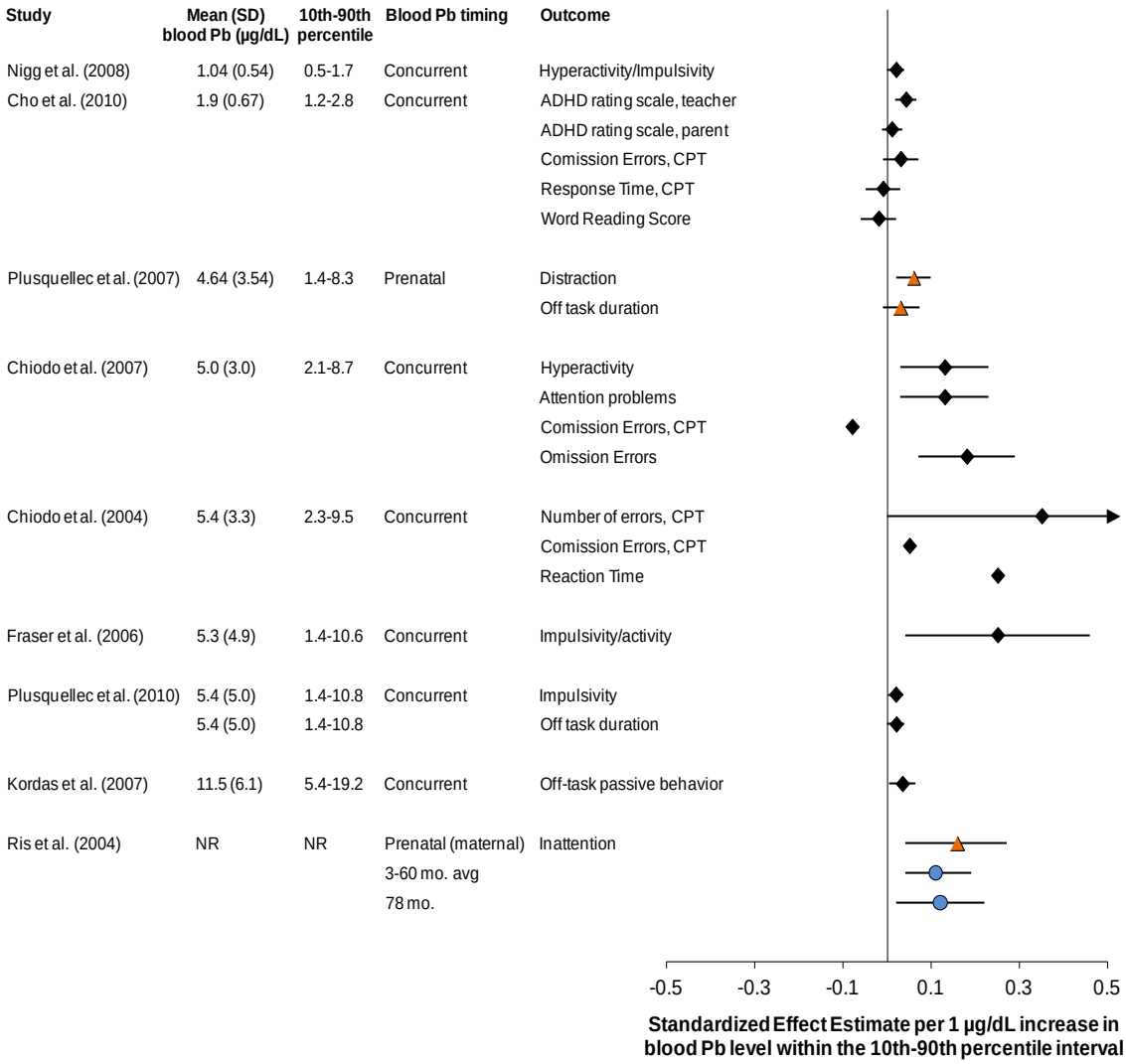
with inattention teacher ratings (Chiodo et al., 2007) but not with all CPT indices of inattention (2007; Chiodo et al., 2004). Blood Pb level was associated with inattention alone and adjusted for covariates. These findings indicated that HOME score did not account for all of the association between blood Pb level and behavioral outcomes. As was discussed in Section 5.3.2.1, this cohort comprised large proportions of children with prenatal exposure to alcohol and drugs. While this may limit the generalizability of findings, it is important to note that alcohol and drug use were not associated with every inattention index or in each study. Blood Pb level remained associated with inattention in a model that included alcohol and marijuana use along with other demographic and SES-related variables.

A series of studies conducted in Inuit children living in northern Quebec, Canada reported associations between blood Pb levels and measures of inattention after extensive consideration of potential confounding variables (Plusquellec et al., 2010; Plusquellec et al., 2007; Fraser et al., 2006). Plusquellec et al. (2007) found higher cord blood Pb level to be associated with higher ratings by investigators for distractibility in completing tasks, frenetic movement, and duration of off task behaviors at age 11 months (Figure 5-14 and Table 5-9). In the same cohort of children, concurrent blood Pb level but not cord blood Pb level was associated with impulsivity, irritability, and duration of off task behavior at ages 4-6 years (Plusquellec et al., 2010). Fraser et al. (2006) additionally indicated that at ages 4-6 years, the relationship between concurrent blood Pb level and motor function (i.e., transversal sway, reaction time) may be mediated by the effects of blood Pb level on an inattention/impulsivity index. These studies conducted in Inuit children evaluated confounding by examining associations of demographic, SES, and co-exposures with outcomes independently and with blood Pb level in models. HOME score was not associated with inattention or impulsivity. These findings further indicate that confounding by HOME score cannot fully account for the collective body of evidence for associations between blood Pb level and measures of inattention and impulsivity.

Consistent with findings in a group of children in Korea (Cho et al., 2010) and children in the U.K. (Chandramouli et al., 2009), Nigg et al. (2008) found that associations of blood Pb level with a hyperactivity/impulsivity index in a group of U.S. children, ages 8-17 years, were independent of associations with IQ. Whereas other studies assessed direct and indirect effects of blood Pb level on behavioral outcomes by including IQ in the model as a covariate, Nigg et al. (2008) specifically used regression-based path analysis, a more rigorous method to characterize the impact of one variable on the association of another in the model after controlling for other previous variables. After adjusting for sex and income, investigators found that concurrent blood Pb level had a direct association with hyperactivity/impulsivity that was not completely mediated by the blood Pb-IQ association. Instead, the association between blood Pb level and IQ was

found to be mediated via the association with hyperactivity/impulsivity. Based on associations between hyperactivity/impulsivity slower stop signal reaction time and response variability, the authors concluded that the association between blood Pb level and hyperactivity/impulsivity was mediated via poorer response on the stop task. It is important to note that other potential confounders including parental IQ and HOME score were not examined

Several other recent studies found associations between concurrent blood Pb levels and inattention; however, their results should be interpreted with caution due to their limited consideration of confounding by SES-related variables (Li et al., 2008d), their examination of children living near Pb sources who likely have limited applicability to contemporary children in the U.S. general population (Bao et al., 2009; Kordas et al., 2007), or both (Liu et al., 2011a).



Note: Test scores were standardized to their standard deviation to facilitate comparisons among tests with different scales. Studies generally are presented in order of increasing mean blood Pb level. Effect estimates are standardized to a 1 µg/dL increase in blood Pb level within the 10th-90th percentile interval. Black diamonds, orange triangles, and blue circles represent associations with concurrent, prenatal (maternal), and earlier childhood blood Pb levels, respectively.

Figure 5-14 Associations of blood Pb levels with behavioral indices of inattention, hyperactivity, and impulsivity.

Table 5-9 Additional characteristics and quantitative results for studies presented in Figure 5-14

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[a]
Nigg et al. (2008)	150 children ages 8-17 yr. Year of birth and location NR	Concurrent Mean (SD) 8-11 yr: 1.04 (0.53) 12-17 yr: 1.03 (0.54)	Regression-based path analysis adjusted for sex and income. **No parental IQ or education**	Hyperactivity/impulsivity using Teacher ADHD Rating Scale and Kiddie Schedule for Affective Disorders and Schizophrenia at ages 8-17 yr.	0.21 (0, 0.42)[b]
Cho et al. (2010)	667 children ages 8-11 yr in 2008 Five Korean cities	Concurrent Mean (SD): 1.9 (0.67) 10th-90th: 1.2-2.8	Log linear regression model adjusted for age, sex, parental education, maternal IQ, child IQ, city, birth weight, urinary cotinine	ADHD rating scale, teacher ADHD rating scale, parent Korean ADHD Rating Scale IV commission errors, CPT Response time, CPT Word reading score, Stroop assessed at ages 8-11 yr.	0.042 (0.017, 0.067) 0.010 (-0.013, 0.033) 0.03 (-0.01, 0.07) -0.01 (-0.05, 0.03) -0.02 (-0.06, 0.02)[c]
Plusquellec et al. (2007)	148-164 children ages 11 mo born 1995-2002 Inuit communities Quebec, Canada	Prenatal (cord) Mean (SD): 4.6 (3.5) 10th-90th: 1.4-8.3	Log linear regression model adjusted for delivery complication, home organization, low birth weight (distractibility) and sex, maternal anxiety, prenatal alcohol exposure (off task duration) **Several more examined**	Distraction Off task duration using Bayley Behavioral Rating Scale at age 11 mo	0.06 (0.021, 0.098) 0.03 (-0.01, 0.073)
Chiodo et al. (2007)	506 African-American children in Detroit, MI area followed from birth (1989-1991) to age 7 yr. Large proportions of children with prenatal exposure to cocaine or marijuana	Concurrent Mean (SD): 5.0 (3.0) 10th-90th: 2.1-8.7	Linear regression model adjusted for child age, sex **Several more examined**	Hyperactivity Attention problems using PROBS-14 and Conners' Teacher Rating Scale-39 at age 7 yr Comission Errors (%) Omission Errors (%)	0.13 (0.03, 0.23)[b] 0.13 (0.03, 0.23)[b] -0.08[d] 0.18 (0.07, 0.29)[c]
Chiodo et al. (2004)	246 African-American children in Detroit, MI area followed from birth (not reported) to age 7.5 yr. Large proportions of children with prenatal exposure to cocaine or marijuana	Concurrent Mean (SD): 5.4 (3.3) 10th-90th: 2.3-9.5	Log linear regression adjusted for SES, maternal vocabulary score (Number of errors and errors of commission); Number of children > 18 yr (Number of errors); Child age (errors of commission); Education, sex, prenatal cocaine exposure (reaction time) **Several more examined**	Number of errors Comission Errors Reaction time Using CPT at age 7.5 yr	0.35 (0, 0.69)[b,c] 0.05 (p > 0.05)[d] 0.25 (p > 0.05)[d]
Fraser et al. (2006)	90 children ages 5-6 yr born 1993-1996 Inuit communities Quebec, Canada	Concurrent Mean (SD): 5.3 (4.9) 10th-90th: 1.4-10.6	Linear regression model adjusted for binge drinking in pregnancy **Several more examined**	Impulsivity/activity using modified Infant Behavioral Rating Scale at ages 5-6 yr	0.25 (0.04, 0.46)
Plusquellec et al. (2010)	95-98 children ages 5-6 yr born 1993-1996 Inuit communities Quebec, Canada	Concurrent Mean (SD): 5.4 (5.0) 10th-90th: 1.4-10.8	Log linear regression model adjusted for birth weight, sex, parity, caregiver education (impulsivity) and birth weight, SES, child blood hemoglobin (off task duration) **Several more examined**	Impulsivity Off task duration using modified Infant Behavioral Rating Scale at ages 5-6 yr.	0.019 (0.001, 0.036) 0.02 (0, 0.039)

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[a]
Kordas et al. (2007)	168 children ages 6-8 yr. born 1993-1995 Torreon, Mexico Residence near metal foundry	Concurrent Mean (SD): 11.5 (6.1) 10th-90th: 5.4-19.2	Linear regression model adjusted for age, sex, SES, home ownership, crowding in home, maternal education, family structure, forgetting homework	Off task passive behavior using instrument developed by investigator at ages 6-8 yr	0.034 (0.005, 0.063)
Ris et al. (2004)	195 children, ages 15-17 years, born 1979-1985	NR	Linear regression adjusted for sex, maternal IQ, HOME, SES, adolescent marijuana use **Several more examined**	Inattention using CPT at ages 15-17 yr Prenatal (maternal) 3-60 mo avg 78 mo	 0.16 (0.04, 0.27) 0.11 (0.04, 0.19) 0.12 (0.02, 0.22)
Studies not included in figure because of categorical analysis of Pb biomarker or outcome:					
Chandramouli et al. (2009)	488 children ages 7-8 yr born 1991-1992 Avon, UK	Age 30 mo Mean (SD): NR	Linear regression model adjusted for maternal education, home ownership, maternal smoking, HOME score, maternal SES, family adversity index, parenting attitudes at 6 mo.	Hyperactivity, teacher using Strengths and Difficulties Questionnaire at ages 7-8 yr Selective inattention using Test of Everyday Attention for Children at ages 7-8 yr	OR: 0.84 (0.47, 1.52)[e] blood Pb 2-5 vs. 0-2 µg/dL 1.25 (0.67, 2.33)[e] blood Pb 5-10 vs. 0-2 µg/dL 2.82 (1.08, 7.35)[e] blood Pb > 10 vs. 0-2 µg/dL OR: 0.97 (0.62, 1.52)[e] blood Pb 2-5 vs. 0-2 µg/dL 1.01 (0.64, 1.61)[e] blood Pb 5-10 vs. 0-2 µg/dL 0.88 (0.42,1.85)[e] blood Pb > 10 vs. 0-2 µg/dL
Nicolescu et al. (2010)	83 children ages 8-12 yr born 1995-1999 Bucharest and Pantelimon, Romania	Concurrent Median (IQR): 3.7 (2.6) 10th-90th: 2.0-8.5	Log linear regression model adjusted for city, sex, age, computer experience, handedness, eye problems, number of siblings, parental education, prenatal smoking, parental psychological/psychiatric problem	Inattention Parent rating Inattention Teacher rating Premature Response using German version of Test battery for attention performance at ages 8-12 yr.	1.3% (-4.0, 6.9)[f] 4.3% (-0.02, 10.4) 8.3% (-0.02, 19.0)
Bellinger et al. (1994a)	79 young adults, ages 19-20 yr, born 1970, Boston, MA area	Deciduous tooth (age 5-8 yr) Q1: 2.9-5.9 ppm Q2: 6.0-8.7 ppm Q3: 8.8-19.8 ppm Q4: 19.9-51.8 ppm	Regression model adjusted for parental IQ, sex, SES, current drug use, current alcohol use, current illicit drug use, maternal education, maternal age, birth order **Several more examined**	Correct Responses on CPT	Q1: 98.0 (1.0)[g] Q2: 97.6 (1.1) Q3: 96.9 (1.1) Q4: 94.6 (1.1)

[a]Effect estimates are standardized to a 1 µg/dL increase in blood Pb level within the 10th to 90th percentile interval and standardized to the standard deviation of the test score to facilitate comparisons among tests that are scored on different scales.
[b]Standard error was estimated from the reported p-value.

[c]Direction of effect estimate was changed to indicate an improvement in performance.

[d]Sufficent data were not provided to calculate 95% CIs.

[e]Odds in higher quantile of blood Pb level compared to that in lowest quantile of blood Pb level.

[f]Results represent the change in false alarm rate.

[g]Results represent the mean (SD) score in each quartile.

Attention Deficit Hyperactivity Disorder in Children

Studies of ADHD were included in the 2006 Pb AQCD; however, previous studies did not conclusively demonstrate that blood Pb level was associated with ADHD itself. Importantly, previously studies examining ADHD were fewer in number and limited by smaller sample sizes (Gittleman and Eskenazi, 1983; David et al., 1972). Recent studies consistently reported associations of blood Pb levels with ADHD in children. Coherence and biological plausibility for these associations are provided by the consistent body of epidemiologic and toxicological evidence indicating Pb-associated increases in inattention, hyperactivity, and impulsivity.

Several recent studies reported associations between concurrent blood Pb level and ADHD diagnosis or diagnostic indices in children between the ages of 8 and 17 years (Table 5-10). While none of the studies examined the potential for confounding by HOME score, they did evaluate confounding by several other demographic and SES-related variables, as well as parental history of psychopathology, including ADHD (Cho et al., 2010; Nicolescu et al., 2010). Recent studies also provided evidence of association between blood Pb level and ADHD in populations born in the mid- to late-1990s with relatively low concurrent blood Pb levels (means: 1.9 and 3.7 µg/dL) (Cho et al., 2010; Nicolescu et al., 2010). However, the temporal trends in Pb exposure in these non-U.S. populations may have differed from those in U.S. children of the same age.

Table 5-10 Associations between blood Pb level and ADHD diagnosis or diagnostic indices in children

Study	Population/Location	Blood Pb Levels (µg/dL)[a]	Statistical Analysis	Outcome	Effect Estimate (95% CI)[b]
Nigg et al. (2008)	150 children ages 8-17 yr. Year of birth and location NR	Concurrent Mean (SD) 8-11 yr: 1.04 (0.53) 12-17 yr: 1.03 (0.54) 10th-90th: 0.5-1.7	Three-group analysis of covariance with sex and income. **No parental IQ or education**	ADHD Dx by two licensed clinicians	F(2,145) = 6.08, p = 0.04
Braun et al. (2006)	4,704 children ages 4-15 yr born 1984-1998 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 vs. <0.8 µg/dL[c] 2.1 (0.7, 6.8), blood Pb 1.1-1.3 vs. <0.8 µg/dL[c] 2.7 (0.9, 8.4), blood Pb 1.4-2.0 vs. <0.8 µg/dL[c] 4.1 (1.2, 14.0), blood Pb > 2.0 vs. <0.8 µg/dL[c]
Froehlich et al. (2007)	2,588 children, ages 8-15 yr born 1986-1996 U.S. NHANES 2001-2004	Concurrent 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[c]
Cho et al. (2010)	667 children ages 8-11 yr born 1997-2000 Five Korean cities	Concurrent Mean (SD): 1.9 (0.67) 10th-90th: 1.2-2.8	Log linear regression model adjusted for age, sex, parental education, maternal IQ, child IQ, city, birth weight, urinary cotinine	Total ADHD rating, teacher Total ADHD rating, parent using Korean ADHD Rating Scale IV at ages 8-11 yr.	0.042 (0.017, 0.067) 0.010 (-0.013, 0.033)
Nicolescu et al. (2010)	83 children ages 8-12 yr born 1995-1999 Bucharest and Pantelimon, Romania	Concurrent Median (IQR): 3.7 (2.6) 10th-90th: 1.8-7.1	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, parent ADHD score, teacher using German version of Conner's scales at ages 8-12 yr.	OR: 1.04 (1.00, 1.10) OR: 1.06 (1.00, 1.12)
Roy et al. (2009a)	756 children ages 3-7 yr tested 2005-2006 Chennai, India	Concurrent Mean (SD): 11.4 (5.3) 10th-90th: 5.8-18.3	Log linear regression model adjusted for age, sex, hemoglobin, average monthly income, parental education, number of other children, clustering in school and classroom	ADHD index z-score using Conners' ADHD/DSM-IV Scales at ages 3-7 yr.	0.002 (0, 0.033)
Chen et al. (2007)	780 children in TLC trial followed ages 2-7 yr Baltimore, MD; Cincinnati, OH; Newark, NJ; Philadelphia, PA Children underwent chelation therapy	Concurrent Mean (SD): 12.0 (5.2) 10th-90th: 6.5-18.7	Regression-based path analysis adjusted for city, race, sex, language, parental education, parental employment, single parent, age at blood Pb measurement, caregiver IQ	ADHD index Using Conners' Parent Rating Scale-Revised at age 7 yr.	0.54 (-1.22, 2.30) Direct 0.90 (0.35, 1.45) Indirect

[a]Studies are presented in order of increasing quantile or population mean blood Pb level.
[b]Except where noted, effect estimates represent regression coefficients. All effect estimates are standardized to a 1 µg/dL increase in blood Pb level within the 10th-90th percentile interval.
[c]Odds ratio in higher quantile of blood Pb level, with children in the lowest quantile of blood Pb level serving as the reference group.

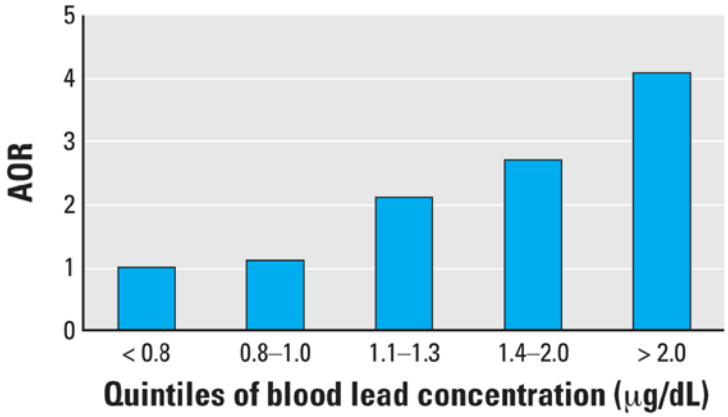
In addition to finding associations of concurrent blood Pb level with inattention and hyperactivity, Cho et al. (2010) found a statistically significant relationship with a total ADHD index as rated by teachers in population of children ages 8-11 years in Korea. In a

multivariate model that included age, sex, paternal education, maternal IQ, child IQ, city of residence, birth weight, and urinary cotinine, the effect estimate decreased by 12%; however, the association remained statistically significant (Table 5-10). Neuropsychiatric disease (e.g., ADHD, learning disability, depression, obsessive-compulsive disorder) in parents was not associated with child blood Pb level, and thus, likely did not confound the relationship between blood Pb level and ADHD index in the child. Results from this study also indicated that the relationship between blood Pb level and ADHD index was independent of the relationship between blood Pb level and IQ.

Similar to Cho et al. (2010), Nicolescu et al. (2010) found higher concurrent blood Pb levels to be associated with greater inattention, impulsivity, and total ADHD score among children in Romania, ages 8-12 years. As with individual symptoms, only concurrent blood Pb level, and not aluminum or mercury, was associated with a higher total ADHD rating (4% increase [95% CI: 0, 10] per 1 µg/dL increase in concurrent blood Pb level within the 10th-90th percentile interval [1.8-7.1 µg/dL]). The association did not change substantially in an analysis that excluded the 5 children with blood Pb levels at or above 10 µg/dL. Adjustment for potential confounding by city, sex, age, computer experience, handedness, eye problems, number of siblings, parental education, prenatal smoking exposure, prenatal alcohol exposure, and parental history of psychological or psychiatric problems resulted in a decrease in statistical significance for blood Pb level associations, although investigators did not report whether the magnitude of association changed. It is important to note that model covariates were selected a priori but not all were associated with both blood Pb level and behavioral endpoint. Only sex, number of siblings, and maternal education were reported to be significantly associated with both blood Pb level and total ADHD rating. Parental history of psychological or psychiatric problems was significantly correlated with parental but not teacher rating of ADHD total score; however, ORs were fairly similar for ADHD score rated by teachers and parents. Despite the potential for over-adjustment in this study, higher blood Pb level was associated with a higher ADHD total score rating.

A recent analysis of data from NHANES 1999-2002 found a relationship between higher blood Pb level and greater odds of ADHD (parent-report of a diagnosis of ADHD or use of stimulant medication) among children ages 4-15 years (Braun et al., 2006). Strengths of this study included the large sample representative of U.S. children (n = 4704) and the low concurrent blood Pb levels at which associations were observed. These authors reported a monotonic increase in ORs from the lowest to highest quintile of blood Pb level, adjusting for age, race, prenatal smoking exposure, smoker in the home, preschool/child care attendance, health insurance coverage, and ferritin levels (Figure 5-15). With children in the lowest quintile serving as the reference group (<0.8 µg/dL), children in the fifth quintile of concurrent blood Pb level (>2.0 µg/dL, maximum not

reported) had the highest prevalence of ADHD (OR: 4.1 [95% CI: 1.2, 14]). A similar OR was estimated when children with blood Pb levels > 5.0 µg/dL were excluded from the highest group. Children in the other three higher quintiles of blood Pb level (range: 0.8-2.0 µg/dL) also had a higher prevalence of ADHD relative to the reference group (Table 5-10).



Source: Braun et al. (2006)

Note: Adjusted for child age, gender, race/ethnicity, preschool attendance, serum ferritin, prenatal tobacco smoke exposure, smoker in the household, and insurance status (p for trend = 0.012). Data from ages 4-15 years from NHANES 1999-2002 by quintile of concurrent blood Pb level.

Figure 5-15 Adjusted odds ratios for Attention Deficit Hyperactivity Disorder (ADHD) among U.S. children.

In the NHANES 2001-2004 dataset restricted to children ages 8-15 years, Froehlich and colleagues (2009) found an interaction between prenatal tobacco smoke exposure (maternal report) and concurrent blood Pb levels. Investigators found ADHD to be independently associated with 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. These results were adjusted for current household smoke exposure, sex, age, race/ethnicity, income, preschool attendance, maternal age, and birth weight. As in the younger NHANES dataset, a similar OR was estimated when children with blood Pb levels > 5.0 µg/dL were excluded from the highest tertile. The strongest association was observed in children with both high blood Pb level and prenatal tobacco smoke exposure. 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 highest prevalence of ADHD (OR: 8.1 [95% CI: 3.5, 18.7]). Although ADHD was associated with low concurrent blood Pb levels (1.3-5 µg/dL), it is

important to acknowledge that the adolescents in both NHANES analyses were born in the late 1980s and may have had higher Pb exposures earlier in childhood that contributed to the observed associations.

Consistent with findings in the NHANES population, Nigg et al. (2008) found an association between concurrent blood Pb level and ADHD diagnosis among children 8-17 years. Concurrent blood Pb levels in this population also were low, ranging between 0.40 and 3.47 µg/dL. However, the examination of adolescents adds uncertainty regarding the relative contributions of higher past Pb exposures and current exposures to the observed associations. A key strength of this study over other studies was the verification of parental reports of ADHD by licensed clinicians using the same diagnostic criteria. Although the results were adjusted for household income, other potential confounders including parental IQ and HOME score were not examined.

Roy and colleagues (2009a) examined associations between concurrent blood Pb level and a range of behavioral problems in 756 children, ages 3-7 years, in Chennai, India. In this population, the mean blood Pb level was higher than that in most other studies (11.4 [SD: 5.3] µg/dL); however, this study demonstrated associations of concurrent blood Pb level with inattention, hyperactivity, and a total ADHD index (assessed by teachers using Conners' ADHD/Diagnostic and Statistical Manual for Mental Disorders) in younger children compared with other studies. In generalized estimating equations, higher blood Pb level was associated with a higher ADHD index score adjusting for age, sex, hemoglobin, average monthly income, parental education, number of other children, and clustering at school and classroom levels (Table 5-10). In analyses that did not adjust for potential confounding variables, mean ADHD scores were similar in the first three quartiles of blood Pb level and elevated in children with blood Pb levels > 18.71 µg/dL, suggesting that in this population, associations may have been driven by children with the highest blood Pb levels.

Whereas several studies indicated the direct effects of increasing blood Pb level on inattention and hyperactivity indices, Chen et al. (2007) found stronger indirect effects of blood Pb level on an ADHD index among children at age 5 years (Table 5-10). Important limitations of this study include the high blood Pb levels of children at ages 12 to 33 months (20-44 µg/dL) that made them eligible for a randomized controlled trial of chelation. Because this study only enrolled children with Pb poisoning, it is difficult to extend findings to children with lower blood Pb levels. Blood Pb levels of study subjects remained high at age 5 years (mean: 12.0 µg/dL) and out the range of the current U.S. general population of children.

In recent commentaries to studies reporting associations between blood Pb level and ADHD in children, Brondum (2011, 2007) asserted the need for studies to consider

confounding by parental history of ADHD. Given the highly heritable nature of ADHD, parental history of ADHD is a strong risk factor for ADHD in children (Faraone and Doyle, 2001). Therefore, the risk associated with parental history of ADHD may mask the smaller magnitudes of risk associated with environmental exposures, including Pb. However, little information is available on an association between ADHD in the parent and blood Pb level in the child, thus, it is uncertain whether parental ADHD confounds and fully accounts for the associations observed between blood Pb level and ADHD in children. It should be noted that for parental ADHD to be a confounder, parental Pb levels would have to be highly associated with ADHD in the parent and with blood Pb level in the child. Among children in Korea, Cho et al. (2010) found that parental history of psychopathology (ADHD or several other conditions) was not associated with child blood Pb level. Among children in Romania, parental history of psychopathology was associated with a higher ADHD rating in the child as assessed by parents but not teachers (Nicolescu et al., 2010). Higher blood Pb level was associated with higher ADHD ratings in children in models with blood Pb level alone and in models that adjusted for parental psychopathology plus other covariates. Further, associations of blood Pb level with ADHD rating assessed by parents and teachers were similar in magnitude (Table 5-10). While available data are limited, they do not provide strong evidence that parental psychopathology fully accounts for the associations observed between blood Pb level and ADHD in children.

Social Misconduct and Delinquent Behavior

The 2006 Pb AQCD described several studies in which higher levels of blood and bone Pb were associated with higher frequency or risk of misconduct in children or delinquent behavior in adolescents (U.S. EPA, 2006b). Previous studies primarily indicated associations with measures of cumulative Pb exposure, including early childhood average blood Pb level, lifetime average blood Pb level, or bone Pb level. Recent studies added to the collective body of evidence by demonstrating associations for concurrent blood Pb level and for a variety of conduct problems including aggression (Chiodo et al., 2007), conduct disorder (Braun et al., 2008), oppositional defiant disorder (Nigg et al., 2008) and more serious behaviors such as criminal arrests (Wright et al., 2008) (Table 5-11). Associations were found with early childhood and concurrent blood Pb levels and were demonstrated in populations with lower blood Pb levels (means: 1-8 µg/dL) than those examined in the 2006 Pb AQCD. Nonetheless, the weight of cumulative evidence supports associations in populations with mean blood Pb levels between 5 and 11 µg/dL. However, because some studies examined outcomes in adolescents and young adults born in the 1970s and 1908s, there is greater uncertainty regarding the level, timing, frequency, and duration of Pb exposure that contributed to the observed associations.

Several other studies examined children and adolescents born in the 1990s, who likely did not have as high historical Pb exposures earlier in childhood (Chandramouli et al., 2009; Braun et al., 2008; Chiodo et al., 2007).

Table 5-11 Associations between blood Pb level and misconduct and delinquent behavior in children and young adults[a]

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[b]
Blood Pb level as a continuous variable					
Chiodo et al. (2007)	506 African-American children in Detroit, MI area followed from birth (1989-1991) to age 7 yr. Large proportions of children with prenatal exposure to cocaine or marijuana	Concurrent Mean (SD): 5.0 (3.0) 10th-90th: 2.1-8.7	Linear regression model adjusted for HOME, child sex, current marijuana use (both outcomes) Caretaker education, alcohol use during pregnancy, (delinquent behavior Age, caretaker psychopathology, maternal IQ (inappropriate behavior) **Several more examined**	Delinquent behavior Inappropriate behavior using Achenbach Teacher Report Form at age 7 yr	0.09 (0, 0.18)[c] 0.09 (0, 0.18)[c]
Dietrich et al. (2001)	195 children followed from birth (1979-1985) to age 15-17 yr Cincinnati, OH	0-6 yr avg: NR	Linear regression model adjusted for HOME score, parental IQ, current SES	Delinquent behavior using the Self-Report of Delinquent Behavior at ages 15-17 yr.	1.21 (1.08, 1.37)
Wright et al. (2008)	250 adults followed from birth (1979-1985) to age 19-24 yr Cincinnati, OH	Age 6 yr Mean (SD): 8.3 (4.8) 10th-90th: 3.9-14.5	Negative binomial regression models adjusted for maternal IQ, sex, SES, maternal education **Several more examined**	Criminal arrests Violent arrests assessed from county records at ages 19-24 yr	RRs: 1.05 (1.00, 1.09) prenatal 1.05 (1.01, 1.09) 6 yr 1.01 (0.97, 1.05) 0-6 yr avg 1.06 (0.97, 1.15) prenatal 1.08 (1.03, 1.14) 6 yr 1.05 (1.01, 1.10) 0-6 yr avg
Chen et al. (2007)	780 children participating in TLC trial followed between ages 2-7 yr Baltimore, MD; Cincinnati, OH; Newark, NJ; Philadelphia, PA Children underwent chelation therapy	Concurrent Mean (SD): 12.0 (5.2) 10th-90th: 6.5-18.7	Regression-based path analysis adjusted for city, race, sex, language, parental education, parental employment, single parent, age at blood Pb measurement, caregiver IQ	Externalizing behavior, parent Externalizing behavior, teacher using Behavior Assessment System for Children at age 7 yr.	1.024 (0.996, 1.053) Direct 1.008 (1.002, 1.014) Indirect 1.036 (1.003, 1.069) Direct 1.004 (0.998, 1.010) Indirect

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[b]
Blood Pb level as a categorical variable					
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 Using Diagnostic Interview Schedule for Children-Caregiver Module at age 8-15 yr	OR: 7.24 (1.06, 49.47), blood Pb level 0.8-1.0 µg/dL vs. blood Pb <0.8 µg/dL[d] 12.37 (2.37, 64.56), blood Pb level 1.1-1.4 µg/dL vs. blood Pb <0.8 µg/dL[d] 8.64 (1.87, 40.04), blood Pb level 1.5-10 µg/dL vs. blood Pb <0.8 µg/dL[d]
Chandramouli et al. (2009)	488 children ages 7-8 yr born 1991-1992 Avon, UK	Age 30 mo Mean (SD): NR	Linear regression model adjusted for maternal education, home ownership, maternal smoking, HOME score, maternal SES, family adversity index, parenting attitudes at 6 mo.	Antisocial activities using Antisocial Behavior Interview at ages 7-8 yr	OR: 0.93 (0.47, 1.83)[d] blood Pb 2-5 vs. 0-2 µg/dL 1.44 (0.73, 2.84)[d] blood Pb 5-10 vs. 0-2 µg/dL 2.90 (1.05, 8.03)[d] blood Pb > 10 vs. 0-2 µg/dL

[a]Results with continuous blood Pb are presented first followed by results with categorical blood Pb. Within each group, studies generally are presented in order of increasing mean blood Pb level.
[b]Effect estimates are standardized to a 1 µg/dL increase in blood Pb level.
[c]95% CI was estimated from a reported p-value of 0.05.
[d]Odds in higher quantile of blood Pb level compared to that in lowest quantile of blood Pb level.

The consistency of association of Pb biomarker levels with social misconduct and delinquent behavior was corroborated in a recent meta-analysis (Marcus et al., 2010) that included 19 studies (those reviewed in the 2006 Pb AQCD plus several recent studies) with a total of 8,561 children and adolescents (mean ages ranging from 3.5 years to 18.4 years). Effect estimates were converted to Pearson correlation coefficients, and the combined effect estimate was r = 0.19 (95% CI: 0.14, 0.23). The key finding of this study was the robustness of associations to considerations of heterogeneity in study design, definition and assessment method of conduct problems, potential confounding variables examined, and blood Pb levels. The major source of heterogeneity in effect estimates was the biomarker of Pb examined. A larger magnitude of effect was estimated for hair Pb levels compared with bone or blood Pb levels; however, similar effect sizes were estimated for blood and bone 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 the meta-analysis, effect sizes did not differ significantly between longitudinal and cross-sectional studies, among studies that examined different conduct problems (i.e., opposition defiance, delinquency, externalizing problems), or among studies that assessed conduct disorders using self-report, teachers report, or criminal records. Controlling for covariates such as SES, birth weight, parental IQ, and home environment did not attenuate the relationship between blood Pb level and conduct problems. In

addition to strengthening the evidence for the independent associations of Pb biomarker levels with conduct disorders, the results indicate that the lack of adjustment for any particular covariate, including HOME score, does not warrant limiting inferences from a particular study.

Studies of children ages 7-8 years found associations between blood Pb level and misconduct adjusting for HOME score and several other potential confounding variables. They examined different lifestages of blood Pb level and did not clearly indicate associations between blood Pb level and misconduct with blood Pb levels less than 5 µg/dL. In their longitudinal study of children in the U.K. who were born in the 1990s, Chandramouli et al. (2009) found associations of age 30 month blood Pb level not only with hyperreactivity but also with antisocial behavior at age 7-8 years. Results were adjusted for maternal education, home ownership, maternal smoking, HOME score, maternal SES, family adversity index, and parenting attitudes at 6 months. In analyses of blood Pb level as a categorical variable, greater antisocial activity was most clearly indicated in children with blood Pb levels greater than 10 µg/dL (Table 5-11). Chiodo et al. (2007) examined children, ages 7.5 years in Detroit, Michigan with a mean (SD) concurrent blood Pb level of 5.0 (3.0) µg/dL. Higher blood Pb was associated with higher teacher ratings of delinquent behaviors and inappropriate behaviors, adjusting for HOME score and other variables. The association with inappropriate behavior additionally adjusted for caregiver psychopathology. As with cognitive function and inattention outcomes, prenatal alcohol and drug exposure were not found to influence associations for blood Pb level. Blood Pb level was associated with behavioral problems in unadjusted and adjusted analyses, corroborating the independent associations for blood Pb level.

In the U.S. NHANES 2001-2004 dataset of children ages 8-15 years (born in the 1990s), Braun et al. (2008) analyzed blood Pb as a categorical variable and found higher prevalence of conduct disorder with concurrent blood Pb levels in the range of 0.8 to 1.0 µg/dL, the lowest level among all studies examined. Compared with children with blood Pb levels less than 0.8 µg/dL, the OR (95% CI) in children with blood Pb levels 0.8-1.0 µg/dL was 7.24 (1.06, 49.47). Odds ratios also were elevated in children with blood Pb levels higher than 1.0 µg/dL (Table 5-11). The wide 95% CIs likely were due to the small numbers of cases of conduct disorder, as assessed using DSM-IV criteria. For example, there were 22 cases of conduct disorder among children with blood Pb levels 0.8-1.0 µg/dL. Poisson regression models showed that children with blood Pb levels 0.8-1.0 µg/dL had 1.55 (95% CI: 1.09, 2.22) times as many conduct disorder symptoms as did children with blood Pb levels < 0.8 µg/dL. Investigators had data available on a limited set of potential confounders, and poverty income ratio was used to control for potential confounding by SES.

While other recent studies supported associations between biomarkers of Pb exposure and misconduct and delinquent behavior, they examined Pb levels in blood or tooth samples collected in the 1980s when Pb exposures were much higher (Fergusson et al., 2008; Wright et al., 2008). Although this evidence may be less informative as to the relationship between current blood Pb levels and misconduct and delinquent behavior in adolescents and young adults, it is important to note the consistency of findings among populations with widely varying blood Pb levels and historical Pb exposures.

In the CLS cohort, prenatal and postnatal blood Pb levels previously were reported to be associated with self- and parent-reported delinquent and social acts at ages 16-17 years (Dietrich et al., 2001). Wright et al. (2008) extended these findings to include associations of prenatal and postnatal blood Pb levels with criminal arrests (ascertained from county records) at ages 19-24 years. Mean blood Pb levels were 8.3 µg/dL (range 1-26) for the prenatal period (maternal blood), 13.4 µg/dL (range 4-37) for the average between birth and age 6 years, and 8.3 µg/dL (range 2-33) at age 6 years. 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. Blood Pb levels measured during these lifestages also were associated with increased risk of violent criminal arrests (Table 5-11). Although interactions terms for 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]). Results from the two CLS studies suggest that in addition to the prenatal blood Pb levels, 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. Therefore, these studies do not provide information on the potential effects of more recent Pb exposures or differences in association between earlier and more recent blood Pb levels.

A strength of Wright et al. (2008) was the detailed examination of potential confounding by a large number of variables. All of the examined covariates were weakly correlated with blood Pb levels ($r = 0.24$-0.35), thereby minimizing the potential for confounding. Nonetheless, variables such as maternal IQ, maternal education, and SES were included in the model because they were associated with arrests in the full multivariate model or changed the blood Pb level by more than 10%. HOME score was similar between subjects with and without criminal arrest records and did meet the criteria for inclusion in the final model.

In the New Zealand cohort of children born in 1977, dentin Pb levels measured between ages 6 and 8 years were associated with self-reported and documented violent or property convictions at ages 14-21 years (Fergusson et al., 2008). Similar to those from the meta-analysis, these findings pointed to an effect of cumulative childhood Pb exposure. Analyses were adjusted for SES, parental criminal record, parental education, and parental alcoholism, and although effects estimates decreased in adjusted models, associations remained statistically significant.

Several studies of misconduct and delinquent behavior aimed to characterize whether associations with biomarkers of Pb exposure were independent of effects on IQ and educational attainment. Most studies found that associations of Pb biomarkers with misconduct and delinquent behavior remained statistically significant in a model that additionally adjusted for child IQ or educational attainment, indicating that Pb exposure may have a direct effect on misconduct independent of its effect on IQ (Chandramouli et al., 2009; Fergusson et al., 2008; Burns et al., 1999). It is important to note that because a decrement in IQ may on the causal pathway to behavioral problems, including both IQ and behavioral problems in the same model may result in an underestimate of the effect on behavior. Chen et al. (2007) used path analysis to characterize the direct effects and indirect effects (mediated through child IQ) of blood Pb level on externalizing problems (i.e., outbursts of behavior); however, results were inconclusive. A direct effect was estimated for externalizing problems assessed by teachers and an indirect effect was estimated for problems assessed by parents. These findings may have limited applicability 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, 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.

5.3.3.2 Toxicological Studies of Behavior

Neurobehavioral Changes

The effects of Pb on neurobehavioral changes in animals are well characterized with various targeted sites including the prefrontal cerebral cortex, cerebellum, and hippocampus; affected functions include cognition, execution of motor skills, and memory/behavior. As discussed in earlier Pb AQCDs, young animals are especially susceptible to the effects of Pb due to the ongoing development of the nervous system 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 capability of Pb to mimic calcium. Very early toxicological research on neurobehavioral endpoints failed to capture the disposition of Pb and its resulting body burden or blood Pb level and was thus difficult to use in risk assessment.

The 1986 Pb AQCD reported findings of Pb-induced aberrant operant conditioning tasks in rodents and non-human primates (some with resulting blood Pb levels: 11 to 15 µg/dL) as well as hyperactive or inappropriate Pb-mediated responses (U.S. EPA, 1986b). It was indicated that these effects were possibly of hippocampal origin and showed a curvilinear response, decreasing at higher Pb doses possibly due to impairment of motor function (Ma et al., 1999; Crofton et al., 1980). Pb exposure in laboratory animals resulted in distractibility, reduced to adapt to changes in behavior, impaired ability to inhibit inappropriate responses, and perseveration (U.S. EPA, 2006b). Pb has been shown to impair learning in Fixed Interval tasks (FI) as indicated by premature responses in the absence of a fixed schedule of reinforcement or reward (Section 5.3.2.2). These findings in animals are consistent with observations in children that blood Pb levels are associated with poorer impulse control as rated by parents or teachers or assessed using the continuous performance test (Figure 5-14 and Table 5-9). Interresponse rates and overall run rate are the two subcomponents of FI response rate. The 2006 Pb AQCD reported consistent findings for Pb exposure (resulting in blood Pb levels: 58 to 94 µg/dL) affecting FI response rates, by means of decreased interresponse times. Some studies indicated decreased interresponse times in animals with blood Pb levels of 11 µg/dL (U.S. EPA, 2006b). Discrimination reversal has been shown to be especially sensitive to Pb exposure. Spatial and non-spatial discrimination reversal or reversal of a previously learned habit was significantly affected after developmental Pb exposure and was exacerbated with distracting stimuli. Repeat-acquisition testing revealed that these deficits were likely not due to sensory or motor impairment at this dose. Together, the data from the 2006 Pb AQCD showed that social behavior and learning in rodents and nonhuman primates is significantly affected by Pb exposure that results in blood Pb levels 15-40 µg/dL.

In the new literature, gestationally and early postnatally (gestation to PND10, G+P) Pb-exposed male mice (low and high dose Pb: 10 and 42 µg/dL blood Pb level at PND10, respectively) were significantly less active than were control mice, and low dose mice were significantly less active than were high dose mice, demonstrating a nonlinear concentration-response relationship (Leasure et al., 2008). A similar nonlinear concentration-response relationship was observed for changes in corticosterone in male mice exposed post-weaning to Pb (Virgolini et al., 2005). Activity level of G+P Pb-

exposed female mice versus controls was unaffected (Leasure et al., 2008). Amphetamine-induced motor activity was monitored in male and female G+P Pb-exposed mice at 1 year of age. Amphetamine-induced activity of male low and high dose G+P Pb-exposed offspring was significantly elevated over that in controls; G+P Pb-exposed females had no change in sensitivity to amphetamine-induced motor activity (Leasure et al., 2008).

Herring gull chicks injected with a single i.p. bolus dose of Pb (100 mg/kg Pb-acetate, a dose created to be similar to that which wild herring gulls are exposed in the wild) on PND2 were found to have neurobehavioral deficits and 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 mouth of the parent bird, decreased time spent in the shade (behavioral thermoregulation), decreased learning in food location, decreased recognition of familiar individuals (caretaker or sibling), and slower development of motor skills (treadmill test) versus control birds (Burger and Gochfeld, 2005). The impaired thermoregulation with Pb exposure agrees with earlier work in Pb-exposed rat pups that also showed impaired thermoregulatory behavior, i.e., impaired ultrasonic vocalization (Davis, 1982). These studies in herring gull chicks demonstrate that a single dose of Pb early in life can induce neurobehavioral deficits that affect survival skills.

Rhesus monkeys exposed to Pb in daily milk formula from PND8 to 1 or 2 years of age (Pb-acetate/50% glucose in 4 cc of commercial milk formula producing blood Pb of 35-40 µg/dL) were assessed for tactile defensiveness using the Sensory Processing Scale for Monkeys, an adaptation of laboratory observational measures of sensory processing for children (Baranek and Berkson, 1994). Tactile defensiveness in children is defined as "feelings of discomfort and a desire to escape the situation when certain types of tactile stimuli are experienced" (Ayres, 1964) and is associated with emotional dysregulation, inattention, and difficult social relations. Other reports have shown that tactile defensiveness in monkey offspring is affected by prenatal stress alone without Pb exposure (Schneider et al., 2008), a factor that has been shown to affect other Pb-related outcomes.

Attention

Epidemiologic studies consistently have reported associations between higher blood Pb level and deficits in attention (inattention, distractibility, impulsivity, or ADHD) in children (Section 5.3.3.1). Animal toxicological studies in the 2006 Pb AQCD detailed attention deficits in animals undergoing various tests including FR/waiting-for-reward

testing and tests that employed signal detection with distraction, a test recording omissions after exposure to an external distraction.

With FR testing, rats are trained on a FR/waiting-for-reward behavioral baseline, learning to produce food delivery by pressing a lever 50 times. Rodents can earn additional food by withholding lever presses by waiting; free food is given at increasing time intervals after completion of the FR. Brockel and Cory-Slechta (1998) exposed male Long-Evans rats to 0, 50, or 150 ppm Pb-acetate in water from weaning, which produced respective blood Pb levels of <5, 11, and 29 µg/dL after 3 months of exposure. After 40 days of exposure, rats were trained on a FR schedule, and the higher dose animals had significantly more frequent response rates and resets of the waiting period than did the low dose group and controls. In the waiting behavior component, wait time was significantly lower in both treated groups compared to controls. The higher dose group animals also had an increased number of reinforcers and a higher response to reinforcement ratio than did low dose and controls. Mechanistic understanding of the aforementioned FR deficits was studied using the same FR schedule with similar postweaning dosing of 0, 50, and 150 ppm Pb that yielded respective blood Pb levels of <5, 10, and 26 µg/dL after 3 and 7 months of exposure. Administration of dopamine receptor antagonists attenuated Pb-induced effects on FR schedule testing, suggested a role for D2 receptors in Pb-induced behavioral impairments.

Another study used a similar postweaning Pb exposure as that in earlier studies (Cory-Slechta et al., 1998) to yield blood Pb levels of <5, 16, and 28 µg/dL and evaluate sustained attention using testing of signal detection during a distraction (Brockel and Cory-Slechta, 1999). Rats earned food rewards by discriminating correctly between a target and distracter light. A 13 second time-out was given for incorrect responses. Pb exposure produced no deficits in attention with this testing. Further work since the 2006 Pb AQCD affirmed these findings. Testing for signal detection with distraction showed no effect after postnatal Pb exposure in female rats (Stangle et al., 2007). The two dose groups (20 ppm or 300 ppm Pb-acetate in drinking water with lactational, and drinking water exposure, PND1-PND30) yielded blood Pb levels of 13 µg/dL and 31 µg/dL (Stangle et al., 2007). Another recent study reported that impaired auditory threshold task related behavioral testing was likely due to inattention in Pb-exposed animals. The inability of some of the monkeys to engage or focus on the task at hand yielded fewer available measurements in Pb-exposed animals versus control animals (Laughlin et al., 2009). These rhesus monkeys were exposed to Pb-acetate gestationally (dam drinking water, 3 months prior to mating) through age 5.5 months (weaning) and had resulting bone Pb levels at 11 years of 7 and 13 µg/dL for prenatal and postnatal groups, respectively, and blood Pb levels during Pb exposure of 35 and 46 µg/dL, respectively. Animals were tested at age 13 years when blood Pb levels had returned to baseline levels.

Thus, the inattention literature demonstrates that impulsivity, waiting-for-reward behavior, and sustained attention may all be affected by Pb with the first two possibly affected to a greater extent than is sustained attention.

5.3.3.3 Epidemiologic Studies of Mood in Children

A majority of investigation of the effects of Pb on behavior in children has focused on externalizing behaviors such as inattention, hyperactivity, aggression, and delinquency. However, studies also have linked biomarkers of Pb exposure with internalizing behaviors related to emotional dysfunction, including withdrawn behavior, depression, fearfulness, and anxiety. These behaviors were assessed frequently in school-aged children using the Child Behavior Checklist, and as with inattention, hyperactivity, and misconduct, often were rated by parents and/or teachers.

Several earlier results were provided by the prospective cohort studies in Boston, Cincinnati, Port Pirie, Australia, and Yugoslavia. Collectively, these studies found higher ratings for internalizing behaviors in association with concurrent blood Pb level as well as biomarkers of cumulative Pb exposure such as multiyear average blood, tooth, and bone Pb levels (Burns et al., 1999; Wasserman et al., 1998; Bellinger et al., 1994b; Dietrich et al., 1987b). While several studies demonstrated associations with biomarkers of long-term, cumulative exposures, results from the Cincinnati cohort provided support for the effects of shorter-duration exposures. Among CLS infants, blood Pb level at age 3 months and average blood Pb level at age 6 months was associated with less positive mood in white infants at age 6 months (Dietrich et al., 1987b). In the Boston cohort, subjects were examined at age 8 years and 19-20 years. Pb levels measured in deciduous teeth but not cord blood were associated with a higher rating of internalizing behaviors at age 8 years but not in adulthood, indicating the lack of persistence of effects of early exposure (Bellinger et al., 1994a; Bellinger et al., 1994b). Wasserman et al. (1998) also examined blood Pb levels measured at various lifestages in the Yugoslavia cohort and found that concurrent blood Pb was associated more strongly with anxious-depressed and withdrawn behaviors at age 3 years than were blood Pb levels measured prenatally or between ages 6 months and 2 years. Thus, while biomarkers of Pb exposure were consistently associated with poorer mood and emotional state in children, there was no clear indication of differences in association among biomarkers measured at various lifestages. Associations between Pb biomarkers with mood also were consistently reported in recent studies, with most studies examining concurrent blood Pb level (Liu et al., 2011a; Bao et al., 2009; Roy et al., 2009b; Chiodo et al., 2004). However, several had limited generalizability to the general population of U.S. children due to the examination

of children residing near Pb sources or high prevalence of prenatal alcohol or drug exposure (Liu et al., 2011a; Bao et al., 2009; Chiodo et al., 2004).

Associations of Pb biomarkers with mood and emotional state in children were observed after adjusting for a wide range of potential confounding variables including parental education and HOME score. Several studies that each adjusted for a different set of covariates found similar effect estimates in univariate and multivariate models (Burns et al., 1999; Wasserman et al., 1998; Bellinger et al., 1994b), supporting the robustness of associations with Pb biomarkers. Associations between blood Pb level and internalizing behaviors also were observed in studies that adjusted for maternal psychopathology (Burns et al., 1999; Wasserman et al., 1998). Thus, the evidence indicates that associations observed between Pb biomarkers and internalizing behaviors in children are not driven by confounding by any particular measured variable.

In the collective body of evidence, associations between blood Pb level and internalizing behaviors were observed in populations of school-aged children with mean blood Pb levels between 8 and 28 µg/dL. Mean blood Pb levels were not related to the age of the study population. In the limited investigation of populations with mean blood Pb levels of approximately 5 µg/dL, results were inconclusive. Chiodo et al. (2004) found an association with internalizing behaviors in children (age 7 years) in Detroit, in whom the prevalence of prenatal alcohol and drug use was high. Neither exposure was found to influence associations with blood Pb level. Another study that examined Inuit children (age 5 years) in Quebec, Canada, did not find an association between concurrent blood Pb level and internalizing behaviors (Plusquellec et al., 2010).

A common observation across studies was finding that Pb biomarkers were associated with multiple indices of neurodevelopmental function, i.e., FSIQ, executive function, externalizing, and internalizing behaviors, within the same population. Whereas some studies found stronger associations for externalizing behaviors than for internalizing behaviors (Plusquellec et al., 2010; Bellinger et al., 1994a; Sciarillo et al., 1992), most did not find a clear difference in the strength of association (Roy et al., 2009b; Chiodo et al., 2004; Wasserman et al., 1998; Bellinger et al., 1994b). In the Port Pirie population, Burns et al. (1999) found that lifetime average blood Pb levels were associated with externalizing behaviors more strongly in boys and with internalizing behaviors more strongly in girls, indicating potential sex-based differences. These findings demonstrating Pb effects on a wide spectrum of cognitive and behavioral indices are not surprising given that Pb exposure is linked to changes in the HPA axis and dopaminergic and GABAergic systems, which are involved in mediating cognitive function, behavior, and mood. Dietrich et al. (1987b) provided support for Pb exposure affecting a wide range of neurodevelopmental outcomes by characterizing the direct and indirect effects of Pb

using structural equations. The associations of prenatal Pb exposure (maternal and infant day 10 blood) with poorer mood in infants aged 6 months were found to be mediated through lower birth weight and/or shorter gestation. These results suggested that Pb may exert its effects by impairing nervous system development. It is well known that the fetal period is an active period for neuronal differentiation, dendritic branching, and synaptogenesis. Thus, it is not surprising that prenatal Pb exposure effects on nervous system development could result in a wide range of neurodevelopmental effects assessed later in childhood.

5.3.3.4 Epidemiologic Studies of Mood and Psychiatric Effects in Adults

Examination of the potential effects of Pb exposure on mood and psychiatric outcomes in adults has received far less attention than that in children and cognitive function in adults. Nonetheless, evaluation of mood states is an integral part of the neurocognitive test battery of the World Health Organization (WHO), and it has been suggested that indices of the Profile Of Mood States may be particularly sensitive to toxicant exposures (Johnson et al., 1987). As with other nervous system endpoints in adults, 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 related to mood disorders and anxiety among Pb-exposed workers than unexposed controls (mean blood Pb levels ranging from 15-38 µg/dL) (Schwartz et al., 2005; Maizlish et al., 1995; Parkinson et al., 1986; Baker et al., 1985; Baker et al., 1984; Lilis et al., 1977).

While comprising a smaller body of evidence, studies of adults without occupational exposures demonstrated associations of blood and bone Pb level with mood. Analyses of men ages 48-70 years in the NAS indicated associations of both concurrent blood (mean: 6.3 µg/dL [SD: 4.16]) and tibia (mean: 21.9 µg/g [SD: 13.5]) Pb levels with greater self-reported symptoms of depression and anxiety (Rhodes et al., 2003). As bone Pb is a major contributor to blood Pb levels in adults without current occupational Pb exposure, associations with both biomarkers may indicate effects of cumulative Pb exposure. In a subsequent analysis of the same dataset, Rajan et al. (2007) found effect modification of the associations for patella and tibia Pb levels by ALAD genotype, although results were not in a consistent direction for a particular genotype. For a majority of the mood symptoms considered, tibia bone Pb levels were associated with larger ORs among men with the ALAD 1-1 genotype. In contrast, ORs for associations between patella Pb levels and several mood symptoms such as depression and positive symptom distress index were larger among ALAD 1-2/2-2 carriers. In the NAS, inconsistent effect modification

by ALAD genotype also was observed for associations between tibia Pb levels and cognitive performance (Rajan et al., 2008) (Section 5.3.2.5).

A cross-sectional study of 1,987 adults age 20-39 years participating in NHANES 1999-2004 was the largest study of mood and included both men and women (Bouchard et al., 2009). However, only concurrent blood Pb levels were available for analysis. Investigators assessed major depressive disorder (MDD), panic disorder and generalized anxiety disorder (GAD) using the WHO Composite International Diagnostic Interview, which follows criteria defined in DSM. Adults with concurrent blood Pb levels higher than 0.7 µg/dL had higher prevalence of all three symptoms. Adults in the highest quintile of blood Pb level (≥ 2.11 µg/dL) had the highest OR for MDD (OR: 2.32 [95% CI: 1.13, 4.75]) and panic disorder (OR: 4.94 [95% CI: 1.32, 18.48]) compared with adults with blood Pb levels < 0.7 µg/dL. For all endpoints, ORs were larger in analyses excluding current smokers. While this study demonstrated associations with relatively low concurrent blood Pb levels, it is important to note the uncertainty of the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations.

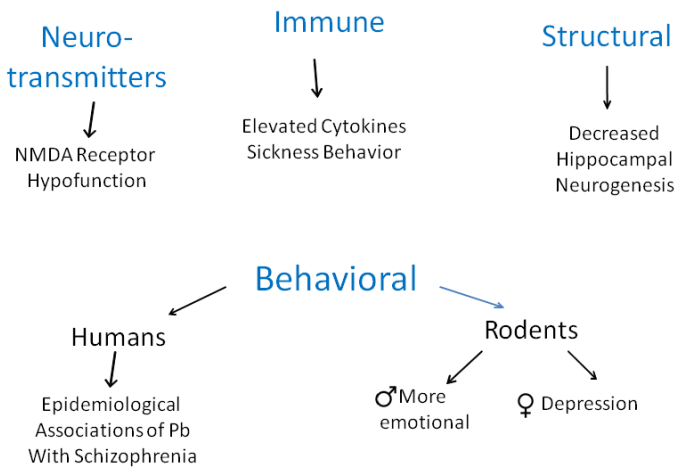
In analyses of cohorts in California and New England, Opler et al. (2008; 2004) reported associations between higher prenatal levels of plasma δ-ALA and subsequent diagnosis of schizophrenia spectrum disorder (ascertained using DSM-IV criteria) in adolescence and adulthood. In the absence of blood Pb levels, investigators measured δ-ALA levels in stored serum samples to serve as a surrogate for Pb exposure citing previous observations of a high correlation (0.90) between δ-ALA levels ≥ 9.05 ng/mL and blood Pb levels ≥ 15 µg/dL. In the California cohort, a δ-ALA level ≥ 9.05 ng/mL was associated with schizophrenia spectrum disorder with an OR (95% CI) of 2.43 (0.99, 5.96), adjusting for maternal age at delivery. In analyses combining the California and New England cohorts, a δ-ALA level ≥ 9.05 ng/mL was associated with schizophrenia spectrum disorder with an OR (95% CI) of 1.92 (1.05, 3.52), adjusting for maternal age and education. A covariate-adjusted OR was not presented for the New England cohort alone, and it appeared that the association in the combined cohorts was driven by that observed in the California cohort. Studies in other populations with direct measurements of blood Pb levels are warranted to characterize the potential effects of Pb exposure on schizophrenia. These limited available data indicate associations with higher blood Pb levels (≥ 15 µg/dL) in individuals born in the 1950s and 1960s who likely had higher early Pb exposures. Thus, these findings may have limited relevance to current lower levels of Pb exposure.

5.3.3.5 Toxicological Studies of Mood, Emotional, and Psychotic Changes

As in epidemiologic studies, neurotoxicological studies often focus more on motor, sensory, behavioral or cognitive outcomes and less on psychological pathologies. As described in Section 5.3.3.3 above, epidemiologic studies indicating associations of Pb biomarkers with mood disorders in children and adults have grown in recent years. Likewise, studies increasingly have found that developmental Pb exposure (gestation and lactation) affects emotional state and mood disorder-like behavior in adult animal offspring. Offspring of Wistar rats treated with 10 mg of Pb-acetate daily by gavage during pregnancy (G) or pregnancy plus lactation (G+L) were tested in the open field test and the forced swimming test also known as the Porsolt test (de Souza Lisboa et al., 2005). Blood Pb levels in the pups at PND70 were 5-7 µg/dL. The open field test monitors activity levels and movements of animals in three dimension and is a measure of hyperactivity, emotion (grooming or freezing), and exploratory behavior (rearing). In the forced swim test, animals are placed in a tank and monitored for helplessness, a response common in animal models of depression. (G+L) Pb-treated male rats had increased emotionality with the open field test as indicated by decreased rearing and increased immobility. (G+L) Pb-exposed female offspring had a significantly increased depressive phenotype in the forced swim test as indicated by increased immobility (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 with males showing increased emotionality and females showing elevated depressive-like symptoms (Figure 5-16). In another study, anxiety was assessed in offspring after G+L Pb exposure. Dams received Pb-enriched drinking water (2.84 mg/mL Pb-acetate trihydrate) and produced pups with blood Pb levels of 698 ng/g at PND25. Using the elevated plus maze test, Pb-exposed animals displayed no signs of anxiety (Molina et al., 2011). However, it is important to note the high concentrations of Pb exposure used in this study. Another study found that female pups exposed to Pb-acetate (0.2% to pups PND1-30) had increased anxiety at PND30 as measured by a decreased percentage of entries and time spent in the open arms of the plus-maze (Fox et al., 2010).

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; Pace and Miller, 2009). Some researchers use sickness behavior and its associated malaise as a model for depression. Sickness behavior is characterized by overall malaise, decreased food intake, immobility and changes in core body temperature. Dyatlov and Lawrence (2002) observed in mice that sickness behavior, which is due to an interaction of the immune system and the CNS, was potentiated by Pb exposure (resulting in blood Pb level:

17 µg/dL) and was correlated with depletions in specific thymic T-cell populations (Figure 5-16). Pb exposure also potentiated the infection-dependent elevation in IL-1β, a cytokine that has been shown to inhibit hippocampal glutamate release in young but not aged animals. Sickness behavior was induced with *Listeria monocytogenes* infection; Pb exposure occurred from birth through lactation and was continued for a brief period after weaning until the experiment was terminated.



Note: The blue headings provide four independent possible contributors to development of psychiatric disorders in Pb-exposed individuals.

Figure 5-16 Animal toxicology evidence of possible Pb-induced contributors to the development of mood and psychotic 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), and prenatal Pb exposure, assessed using ALAD activity as a biomarker, was linked to schizophrenia in a group of adolescents and young adults in California (Opler et al., 2008; Opler et al., 2004). Because of these observations, the animal toxicological field 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 (Figure 5-16 to Figure 5-18). Pb may bind a divalent cation site in the NMDAR and allosterically inhibit glycine binding (Hashemzadeh-Gargari and Guilarte, 1999); human studies of patients with schizophrenia

have shown aberrations at this site (Coyle and Tsai, 2004). These findings are consistent with the glutamatergic hypothesis of schizophrenia which purports 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 (Maeda et al., 2007) and in animal models with developmental Pb exposure (Verina et al., 2007; Jaako-Movits et al., 2005) (Figure 5-16 to Figure 5-18). 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 to treat 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. Another study gives insight into pathways involved in Pb-induced changes in neurogenesis in the developing brain. Exposure of zebrafish embryos to Pb (50–700 µM Pb-acetate in embryo medium from 0 to 6 days post hatch) caused significant apoptosis (increased TUNEL positive brain cells) and impaired neurogenesis as shown by decreased levels of gfap and huC in the brain; however two other genes involved in neurogenesis, crestin and neurogenin1, were unaffected in brains of Pb exposed embryos (Dou and Zhang, 2011). Thus, toxicological studies indicate that Pb exposure may result in mood disorders via behavioral, neurochemical, and ultrastructural changes.

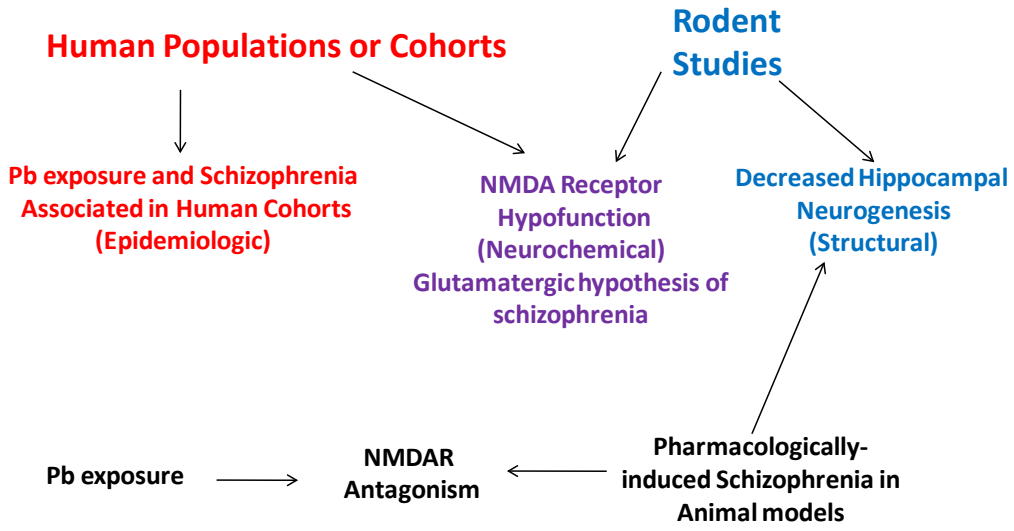
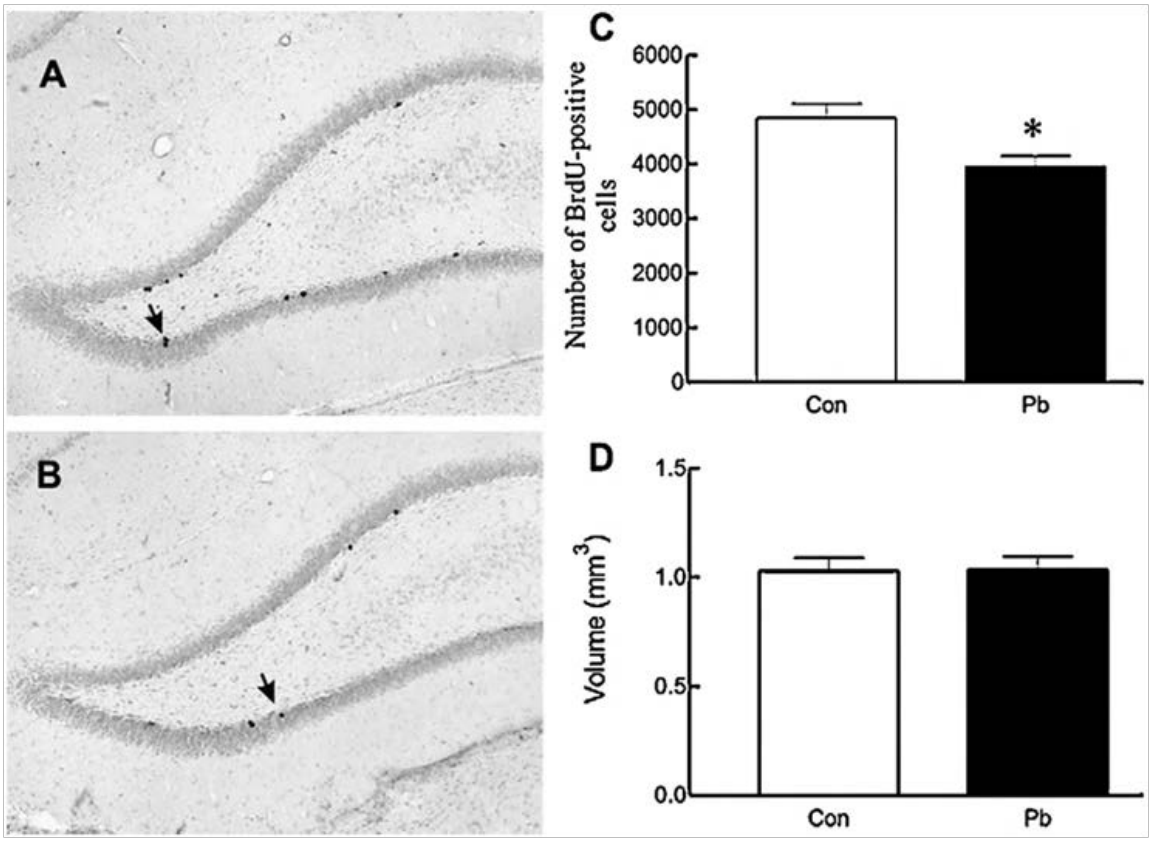


Figure 5-17 Schematic representation of the contribution of Pb exposure to the development of a phenotype consistent with schizophrenia.



Source: Reprinted with permission of Elsevier Science, Fox et al. (2010)

Note: (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 vs. control.

Figure 5-18 Neurogenesis (production of new cells) in the rat dentate gyrus after postnatal Pb exposure.

5.3.3.6 Integrated Summary of Behavior and Mood

Epidemiologic studies in children demonstrate associations of higher blood Pb levels with a range of behavioral problems, with the weight of evidence demonstrating associations with inattention and hyperactivity as rated by parents or teachers and as assessed using objective neuropsychological tests. Previous studies found associations with early childhood blood or tooth Pb level (i.e., ages 2-6 years), and recent studies expanded evidence to include associations with concurrent blood Pb level. Recent epidemiologic studies consistently found associations with inattention and hyperactivity in children ages 1 to 12 years with mean concurrent blood Pb levels of 2 to 5 µg/dL (Cho et al., 2010; Nicolescu et al., 2010; Plusquellec et al., 2010; Chiodo et al., 2007; Plusquellec et al., 2007), similar to those associated with cognitive function decrements. The epidemiologic findings are strengthened by observations in animals of Pb-induced

inattention and impulsivity in homologous tests of response inhibition (e.g., DSA, FI, DR). Tests in children such as CPT, Stroop, WCST share homology with tests in animals such as DSA, DR, and FR tasks in that they all test the ability to inhibit inappropriate responses, organize behavior in response to varying stimuli, and learn from the consequences of previous actions. Inattention and impulsivity in animals are most clearly indicated with gestational and early postnatal Pb exposures that result in blood Pb levels of 10 to 40 µg/dL. These findings in children and animals for Pb-associated dysfunction in response inhibition and response variability may be explained by observations that Pb affects dopaminergic neurons of the frontal striatum of the brain by altering dopamine release and receptor density . The circuitry in this regions is thought to mediate response inhibition. Whereas previous evidence was inconsistent, several recent epidemiologic studies indicate associations between higher concurrent blood Pb level and higher prevalence or incidence of ADHD diagnosis and its contributing diagnostic indices in children ages 8–17 years (Cho et al., 2010; Nicolescu et al., 2010; Roy et al., 2009a; Nigg et al., 2008; Braun et al., 2006). The biological plausibility for associations with ADHD is strongly supported by the large epidemiologic and toxicological evidence base demonstrating Pb-associated increases in inattention and impulsivity, both of which are primary symptoms of ADHD. A smaller but equally consistent body of evidence indicated associations of concurrent and early childhood blood Pb levels with social misconduct in children and delinquent behaviors in adolescents and young adults (Chandramouli et al., 2009; Braun et al., 2008; Wright et al., 2008; Chiodo et al., 2007). Associations of blood Pb levels with ADHD, misconduct, and delinquency were observed in populations of children with a wide range of blood Pb levels, 1 to 11 µg/dL, all similar in the strength of evidence.

While mood and emotional state have been examined less frequently compared with inattention and misconduct, several studies found associations of biomarkers of cumulative Pb exposure (i.e., tooth or childhood average blood Pb) and concurrent blood Pb levels with parental or teacher reports of withdrawn behavior or depression in children with mean blood Pb levels 8-28 µg/dL (Section 5.3.3.3). These findings in children are supported by a small body of toxicological studies in which prenatal plus lactational Pb exposure resulted in depression-like behavior in rodents.

Rather than examining externalizing behaviors and criminal behavior, a small body of studies of behavior in nonoccupationally-exposed adults examined and found associations of blood (Bouchard et al., 2009) and tibia (Rajan et al., 2008) Pb levels with depression and anxiety symptoms. All of these studies used single assessments of Pb biomarker levels and outcomes and analyzed associations in a cross-sectional manner. Thus, there is uncertainty regarding the critical level, timing, frequency, and duration of Pb exposure associated with mood and psychiatric symptoms. The associations with bone

Pb levels indicate an effect of cumulative Pb exposure. Concurrent blood Pb levels in adults reflect both cumulative and recent exposure, and it is uncertain what are the relative contributions of past versus recent Pb exposures to the observed associations. Associations of Pb biomarkers with withdrawn behavior and anxiety in children and adults may by explained by evidence for Pb-induced changes in the HPA axis, dopaminergic and GABAergic CNS processes. These processed have been shown to mediate anxiety and depression. While it may seem that Pb exposure affects different behaviors in children and adults, it is important to acknowledge differences in the evidence base. Studies of behavior in children and young adults have focused on inattention and misconduct; studies of older adults did not examine externalizing behaviors or misconduct. Differential effects in children and adults also may be expected given the predominance of different neurophysiological processes operating at different ages, for example, neurogenesis and brain development in children and neurodegeneration in adults.

5.3.4 Sensory Organ Function

5.3.4.1 Epidemiologic Studies of Sensory Organ Function in Children

Although not as widely examined as cognitive and behavioral outcomes, several studies found associations of higher blood Pb level with higher hearing thresholds and poorer auditory processing in children (U.S. EPA, 2006b). Such evidence is limited largely to studies described in the 2006 Pb AQCD. The prospective CLS with repeat measurements of blood Pb prenatally to age 5 years provided information on potentially important lifestages of exposure. In this cohort with higher blood Pb levels than those in contemporary U.S. children (lifetime average mean: 17.4 [SD: 8.8] µg/dL), poorer auditory processing was associated with higher prenatal (maternal), neonatal (10-day), early childhood, and lifetime average blood Pb levels, with the strongest associations observed for neonatal blood Pb level. A 1 µg/dL higher neonatal blood Pb level was associated with a 0.20-point ($p \leq 0.01$) and 0.26-point ($p \leq 0.10$) lower score on the total and left ear Filtered Word test (indicative of incorrectly identified filtered or muffled words), after adjusting for hearing screen, social class, HOME score, birth weight, gestational age, obstetrical complications, alcohol consumption, and prenatal and postnatal blood Pb levels (Dietrich et al., 1992). Overall, the findings pointed to an effect of early Pb exposure during infancy.

Large U.S. studies, including NHANES II (Schwartz and Otto, 1987) and the Hispanic Health and Nutrition Examination Survey (HHANES) also found associations between higher blood Pb level and lower auditory function; however, their cross-sectional design precluded comparisons among various lifestages of exposure (Schwartz and 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). Higher concurrent blood Pb level also was associated with higher hearing thresholds 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 concurrent blood Pb levels below 10 µg/dL.

Mechanistic support for these observations in children was provided by Otto and colleagues (Otto and Fox, 1993; Otto et al., 1985b), who found associations of blood Pb level with lower brainstem auditory evoked potentials in children. Rothenberg et al. (1994b) 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 lower interpeak intervals in auditory evoked potentials at age 5-7 years. Prenatal maternal blood Pb level showed a biphasic relationship, with a lower evoked potentials at blood Pb levels of 1-8 µg/dL and higher evoked potentials at blood Pb levels of 8-30 µg/dL. Recent studies also aimed to identify the locus in the auditory system where Pb may exert its effects on auditory function. Investigation was limited to a population of children with high blood Pb levels (means 33 and 37 µg/dL) living in Pb glazing communities in Ecuador (Buchanan et al., 2011; Counter et al., 2011). In these studies, concurrent blood Pb level was not correlated with the acoustic stapedius reflex (Counter et al., 2011) or distortion product otoacoustic emissions (Buchanan et al., 2011), indicating lack of effect on the auditory brainstem or inner ear, respectively. Other loci were not examined.

5.3.4.2 Epidemiologic Studies of Sensory Organ Function in Adults

Studies of auditory function reviewed in the 2006 Pb AQCD provided consistent evidence of association between blood Pb levels and changes in auditory evoked brainstem potentials in occupationally-exposed adults, but less consistent findings for hearing thresholds (U.S. EPA, 2006b). A few new studies of Pb workers found Pb-associated increases in hearing thresholds. A recent study provided new evidence in nonoccupationally-exposed adults for associations of tibia Pb levels with hearing loss.

Men in the NAS study were free of hearing loss at baseline and had hearing tested repeatedly (median 5 observations per subject) over a median of 23 years (Park et al., 2010). Higher tibia Pb level, measured up to 20 years after initial hearing testing was associated with a faster rate of increase in hearing threshold for frequencies of 1, 2, and 8 kHz and a pure tone average. Blood Pb was not examined in this study. 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 (measured within 5 years of hearing test) was associated with a higher hearing thresholds for frequencies greater than 1 kHz. A 21 μg/g (IQR) increase in patella bone Pb level was associated with pure tone average hearing loss with an OR of 1.48 (95% CI: 1.14, 1.91) in adjusted analyses. Similar, but slightly weaker associations were found for tibia bone Pb levels.

With new investigation, the weight of evidence indicates that blood Pb levels are associated increased hearing thresholds in adults with occupational Pb exposures. In a study of 183 Pb workers with relatively low blood Pb levels, 1 to 18 μg/dL, higher blood Pb level was correlated with higher hearing threshold at 4 kHz (Forst et al., 1997). Other studies examined workers with much higher blood Pb levels. In a study of 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, sex, and duration of employment. Another cross-sectional study examined 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 (Hwang et al., 2009). The participants had a mean (SD) concurrent blood Pb level of 54.3 (34.6) μg/dL. Average noise levels also were measured in work areas and dichotomized at 80dB. In analyses adjusted for age and work area noise (dichotomized at 80 dB), workers with blood Pb level ≥ 7 μg/dL had a statistically significant higher prevalence (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.

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 based on measurements in samples collected at the time of the study. In models that adjusted for age, smoking, alcohol consumption, years of noise exposure, as well as Mn, As, and Se

levels in blood, higher blood Pb levels were associated with a statistically significant higher average hearing threshold (0.5-6 kHz).

In summary, together, the findings from NAS (Park et al., 2010) and studies of Pb-exposed workers indicate that biomarkers of Pb exposure are associated with lower auditory function in adults. Evidence for association with concurrent blood Pb levels is provided studies of adults with current occupational Pb exposures. Because only bone Pb levels measured after auditory testing were examined in the NAS study, further investigation is required to characterize the timing, level, frequency, and duration of Pb exposure contributing to lower auditory function in adults without occupational exposures.

5.3.4.3 Toxicological Studies of Sensory Organ Function

The 1986 and 2006 Pb AQCDs detailed the effects of Pb in animals on vision, including the retina and CNS visual processing areas, as well as the auditory system and described possible or known mechanisms of action where available. Pb exposure effects on sensory dysfunction and impaired sensory processing have been postulated to contribute to Pb-associated effects on neurocognition and attention. In support of this hypothesis, Dietrich et al. (1992) found that higher prenatal (maternal) and postnatal blood Pb levels were associated with both lower performance on the screening test for auditory processing disorders and lower cognitive function in 5 year-old CLS children (Section 5.3.4.1). New research in this area expands upon the extant evidence by exploring sensory function in animals with lower Pb exposures and blood Pb levels.

Auditory Effects

The 2006 Pb AQCD discussed impaired auditory function in nonhuman primates exposed to Pb from gestation through age 8-9 years (resulting in blood Pb levels 33-56 µg/dL during Pb exposure period). Brainstem auditory evoked responses (BAER), 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 levels had returned to baseline. In Pb-exposed animals (birth to age 13 years), half of the pure tone detection thresholds were outside of the control range at certain frequencies (Rice, 1997). In concordance with the data from developmentally Pb-exposed laboratory animals, elevated auditory thresholds, as measured with brainstem auditory evoked response (BAER), have also been associated with blood Pb levels in children (Rothenberg et al., 2000; Rothenberg et al., 1994a). In addition to indicating hearing loss, BAER can indicate impaired synaptic maturation and incomplete neuron axon myelination leading to

impaired neuronal conduction (Schwartz and Otto, 1991; Gozdzik-Zolnierkiewicz and Moszyński, 1969. Thus, the findings from Rice (1997) indicated that early life Pb exposure impaired auditory function. The cochlear nerve in both developing and mature humans appeared to be especially sensitive to the Pb insult. At low to moderate Pb exposures, elevated thresholds and increased latencies were observed in brainstem auditory evoked potentials.

In a recent study, Laughlin et al. (2009) studied rhesus monkeys exposed to Pb-acetate gestationally through age 5.5 months (dam drinking water, 3 months prior to mating until weaning, resulting in bone Pb levels at 11 years of 7 and 13 µg/dL for prenatal and postnatal groups, respectively and blood Pb levels during Pb exposure of 35 and 46 µg/dL, respectively) and conducted auditory threshold testing and threshold task testing at 13 years of age after blood Pb levels had returned to baseline. At birth, animals were cross fostered, creating a control group, a prenatal Pb group, and a postnatal Pb group; however, Pb exposed animals were analyzed as a single group. Pb exposure induced small elevations in auditory thresholds in animals. Auditory threshold task-related behavioral testing was also impaired in Pb-exposed animals. This study has multiple limitations which likely contributed to its not finding statistically significant aberrations. These limitation included limited power with the examination of 5 animals per group, the inability of some of the monkeys to engage or focus on the task at hand and thus had fewer available measurements, differences between the sexes in inattention, and mixing of the postnatal Pb and prenatal Pb animals into one group (Pb-exposed animals).

In summary, studies in nonhuman primates have shown lower BAER due to chronic Pb exposure (gestation through adulthood) that result in blood Pb levels in the range of 33-56 µg/dL. In concordance with these findings, previous epidemiologic studies described lower BAER in children with higher prenatal maternal blood Pb levels and concurrent blood Pb levels. Collectively, these findings provide support for the associations observed between higher blood Pb levels and increased hearing thresholds (concurrent blood Pb) and lower auditory processing in children (neonatal) (Section 5.3.4.1). The animal evidence for long-term Pb exposure effects provide coherence with findings in adults with occupational Pb exposures for associations between blood Pb levels and increasing hearing thresholds and in nonoccupationally-exposed adults, for associations with biomarkers of cumulative Pb exposure (i.e., tibia and patella Pb level) (Section 5.3.4.2).

Effects on Vision

In toxicological studies, Pb has been shown to affect multiple aspects of the visual system from the retina, to the sensory processing areas of the brain, to neurons involved in vision. The selective action of Pb on retinal rod cells and bipolar cells is well documented in earlier Pb AQCDs (Fox et al., 1997; Fox and Sillman, 1979) and research in this area continues to date. Pb exposure during perinatal development and adulthood has also been shown to affect the visual cortex (Costa and Fox, 1983) and subcortical neurons (Cline et al., 1996). Extensive work in nonhuman primates with various Pb exposure paradigms (development or lifetime Pb) showed sensory impairment, i.e., dysfunction of spatial and temporal visual function (Rice, 1998). Environmentally-relevant doses of Pb (10^{-3} µM) administered to tadpoles inhibited the growth of developing neurons in the subcortical retinotectal pathway, the main efferent from the retina (Cline et al., 1996). Functional tests like electroretinograms (ERGs) show Pb-related aberrations in children, rodents and nonhuman primates.

The animal toxicological data show that developmental windows and the dose of Pb contribute to the complex and variable effects of Pb with the retina. Table 5-12 summarizes Pb-related effects in retinal ERG studies. Female rats exposed postnatally to moderate and high levels of Pb (0.02 or 0.2% Pb-acetate exposure in dam drinking water from birth through weaning, resulting in weaning blood Pb levels of 19 and 59 µg/dL, respectively) had subnormal scotopic ERGs (decreased A- and B-wave amplitudes) with decreased sensitivity and temporal resolution when assessed at 90 days of age (Fox et al., 1991) (Table 5-12). Similar results were obtained in multiple studies conducted in in vitro models (Otto and Fox, 1993; Fox and Farber, 1988; Fox and Chu, 1988). Monkeys exposed to moderately high levels of Pb continuously from the prenatal period to age 7 years (350 or 600 ppm Pb-acetate, resulting in blood Pb levels of 40 and 50 µg/dL, respectively) had persistently increased maximal retinal ERG amplitude (B-wave only, supernormality) and increased mean ERG latency when assessed 2 years after termination of Pb exposure when blood Pb levels were <10 µg/dL (Lilienthal et al., 1988) (Table 5-12).

Table 5-12 Summary of Pb-related retinal ERG studies

Study	Species	Sex[a]	Pb Exposure Protocol/Dose	Maximal Blood Pb Level (µg/dL)	ERG Abnormality	Progenitor cell proliferation	Retinal Cellular Apoptosis	Retinal Dopamine Levels	Retinal Cell Layer Thickness
Fox et al. (2008)	Long-Evans Rat	F	Prenatal-PND10[b] DW						
			Low 27 ppm	12	Supernormal	Yes	Not affected	Dose-dependent ↓	↑
			Moderate, 55 ppm	24	Supernormal	Yes	Not affected	Dose-dependent ↓	↑
			High, 109 ppm	46	Subnormal	No	Yes	Dose-dependent ↓	↓
Lilienthal et al., (1988)	Rhesus Monkey	M & F	Pre- and Post-natal (lifetime), DW						
			350 ppm	~50	Supernormal		—	—	—
			600 ppm	~115	Supernormal	—	—	—	—
Fox et al. (1997)	Long-Evans Rat	F	PND1-PND21						
			0.02% DW	19	Subnormal	—	Yes		↓
			0.2% DW	59	Subnormal		Yes		↓
Rothenberg et al. (2002a)	Human children	M & F	Prenatal 1st trimester	≥ 10.5	Supernormal	—	—	—	—
Guguchkova et al. (1972)	Human	M	Occupational		Subnormal	—	—	—	—
Otto and Fox (1993)	Human	M	Occupational		Subnormal	—	—	—	—

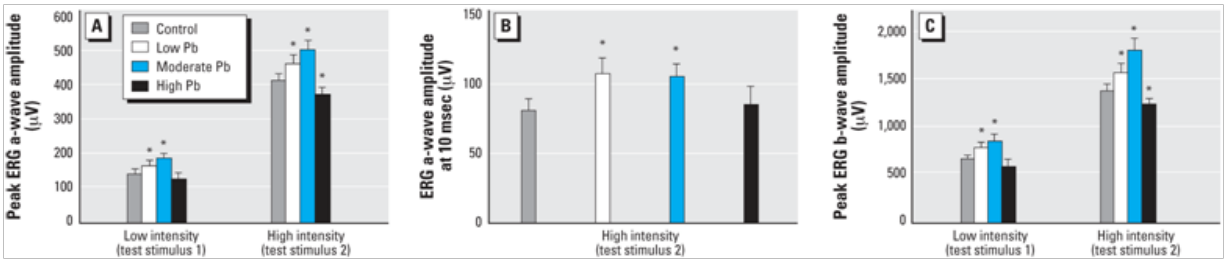
[a] F: Females; M: Males

[b] PND: postnatal day

"—" Denotes not measured.

A recent study exposed female Long-Evans 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 (G+P exposure) (Fox et al., 2008) (Table 5-12). Blood Pb levels in G+P Pb-exposed pups in the three groups were 10-12 µg/dL (lower), 21-24 µg/dL (moderate), and 40-46 µg/dL (higher). This developmental window in the retina of the rat is equivalent to gestational human retinal development. Results of this rodent study demonstrated persistent supernormal scotopic rod photoreceptor-mediated ERGs (lower and moderate Pb exposure) similar to the associations observed between ERG and prenatal maternal blood Pb levels ≥ 10.5 µg/dL in male and female children (Rothenberg et al., 2002a). Supernormal scotopic ERGs may be recorded without other overt opthalogical changes and are rarely seen in the clinical setting (Terziivanov et al., 1983). Lower and moderate levels of G+P Pb exposure increased neurogenesis of rod photoreceptors and rod bipolar cells without affecting Müller glial cells and statistically significantly increased the number of rods in central and peripheral retina. Higher-level G+P Pb exposure (109 ppm, blood Pb level of 46 µg/dL) or moderate to higher level postnatal exposure (PND1-21, blood Pb levels 19 and 59 µg/dL) statistically significantly decreased the number of rods in central and peripheral retina, induced scotopic ERG subnormality in adult rats (Table 5-12), and statistically significantly decreased the retinal

Zn concentration. Pb exposure induced concentration-dependent decreases in adult rat retinal dopamine synthesis and utilization/release (Fox et al., 2008). Thus, the dose of Pb and the exposure window produce complex interactions in the retina that differentially affect retinal histology and functional tests, i.e., ERG as summarized in Table 5-12.



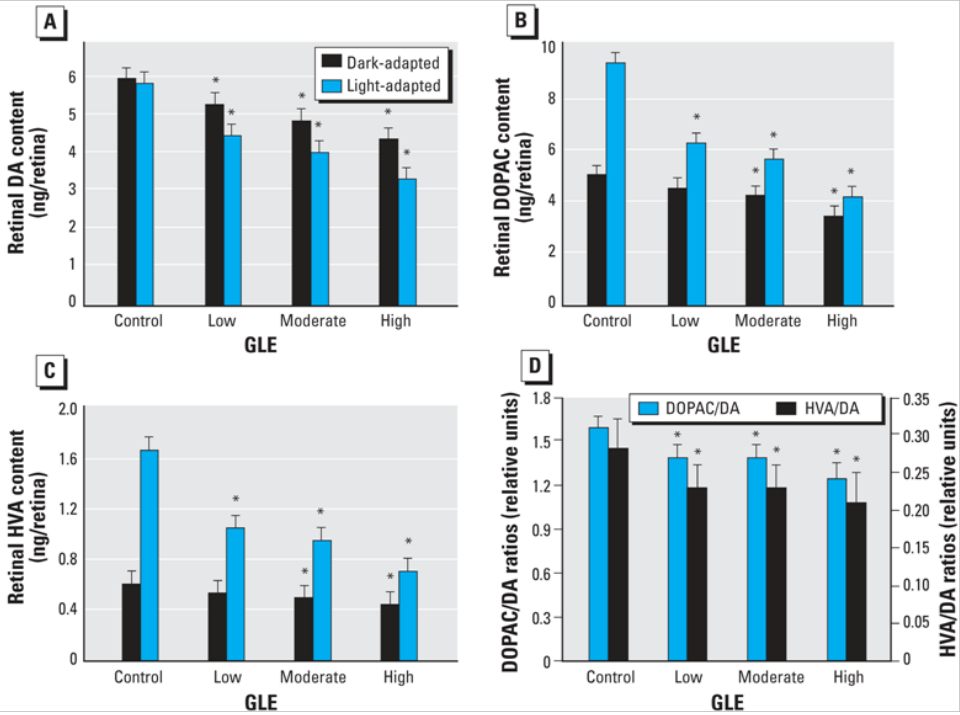
Note: *p <0.05
Source: Fox et al. (2008)

Figure 5-19 Retinal a-wave and b-wave ERG amplitude in adult rodents after prenatal and early postnatal Pb exposure.

A recent study of adult zebrafish that were exposed as embryos (2 to 24 hours post-fertilization) to water containing 0.03 μM $PbCl_2$ showed fish with impaired response to visual stimulation (visual response to a rotating bar) under low light conditions. These zebrafish also failed to respond normally to mechanosensory stimulation (0.01 and 0.03 μM $PbCl_2$), showing a significantly impaired startle response. These data show that low-level developmental Pb exposure causes sensorimotor deficits in fish (Rice et al., 2011). Another study evaluating visual spatial acuity in rhesus monkeys (Laughlin et al., 2008) exposed to Pb-acetate postnatally (PND8-26 weeks of age via commercial milk formula, achieving a target blood Pb of 35-40 μg/dL) found no effects of Pb exposure on spatial acuity as assessed with modified the Teller preferential looking paradigm.

Mechanistic understanding of the effect of Pb on the visual system includes its capability to displace divalent cations, act as an inhibitor of physiological enzymes, regulate cell proliferation and apoptosis, impair and perturb normal neuroanatomy formation, i.e., cytoarchitecture in the brain, and affect neurotransmitters. The effects of Pb on the retina have been shown to by mediated by its capability to act as a cGMP phosphodiesterase (PDE) inhibitor (Srivastava et al., 1995; Fox and Farber, 1988). The drug sildenafil citrate, another cGMP PDE inhibitor, can also cause visual problems including alterations in scotopic ERGs (Laties and Zrenner, 2002). With postnatal exposure of animals or in vitro Pb exposure of isolated rods, Pb has been shown to induce elevated cGMP which contributes to elevated rod calcium concentration (Fox and Katz, 1992) and subsequently to apoptotic cell death in a concentration-dependent fashion. In

separate work in mice, low and moderate doses of Pb (27 or 55 ppm Pb-acetate in dam drinking water from gestation to PND10, resulting in blood Pb levels of 12 and 25 μg/dL, respectively), induced greater rod and rod bipolar cell neurogenesis (proliferation) and greater thickness and cell number of the outer and inner neuroblastic layers of the retina (Giddabasappa et al., 2011; Fox et al., 2008). Rodents with moderate dose G+P Pb exposure (resulting in blood Pb level of 25 μg/dL) had 27-fold greater and prolonged retinal progenitor cell proliferation (Giddabasappa et al., 2011); at higher doses of Pb (109 ppm Pb-acetate, resulting in blood Pb levels of 56 μg/dL) there was no rod neurogenesis. Nitric oxide has been shown to regulate retinal progenitor cell proliferation in chick embryos (Magalhaes et al., 2006). Thus, these authors postulated that impaired NO production may contribute to aberrant retinal cell proliferation (Giddabasappa et al., 2011). Pb exposure has been shown to impair NO synthase activity in other organs (Section 5.2.4.5). Pb has been shown to affect a plethora of neurotransmitters in the brain and it has recently been shown to affect neurotransmitters in the retina. In the aforementioned model of G+P rodent Pb exposure through PND10, Pb decreased dopamine (DA) synthesis and use in a concentration-dependent manner (Fox et al., 2008) (Figure 5-20). These new data provide further insight into retinal changes by showing increased proliferation of Pb-exposed retinal progenitor cells without changes in apoptosis in G+P exposed rats (Fox et al., 2008).



Note: *p <0.05
Source: Fox et al. (2008)

Figure 5-20 Retinal dopamine metabolism in adult control and gestationally lead-exposed (GLE) rats.

As discussed in the 2006 Pb AQCD, Pb-induced decreased Na/K+ ATPase activity have been reported in vitro and in vivo. Also, structural changes from chronic Pb exposure (birth to age 6) included cytoarchitectural changes in visual projection areas of the brain of monkeys; maximum blood Pb level in the low and high dose group reached 20 µg/dL and 220 µg/dL, respectively (Reuhl et al., 1989). Within the visual system, Pb has been shown to affect multiple pathways including signaling, enzyme inhibition, neurotransmitter levels, neuroanatomy formation, and cell proliferation and apoptosis.

In summary, developmental windows of exposure as well as exposure dose (Table 5-12) are important factors that contribute to Pb-induced changes in retinal function. The retina is a sensitive target for Pb-induced altered histology and retinal function. Prenatal, postnatal, lifetime or occupational exposures provide windows with different effects on the retina, effects that are modified by dose of Pb.

5.3.5 Motor Function

Some studies in children have assessed fine motor function, i.e., response speed, dexterity, eye-hand coordination, as part of a battery of cognitive function and behavioral testing, and most have found associations with blood Pb level. Fewer studies have examined gross motor function, i.e., postural balance, action tremor, agility but also have found associations with blood Pb level.

Multiple studies were conducted in the CLS cohort at age 6 years and in adolescence at ages 12 and 15-17 years. Assessments in younger children indicated associations of concurrent and lifetime average blood Pb levels but not prenatal blood Pb levels with poorer visuomotor control and upper limb dexterity (Dietrich et al., 1993b) and poorer postural balance (Bhattacharya et al., 1995). Associations with both fine and gross motor function were adjusted for HOME score and race. Additional covariates included maternal IQ, SES, and sex for fine motor functions (Dietrich et al., 1993b) and height, BMI, birth weight, bilateral ear infection, and foot area for postural balance (Bhattacharya et al., 1995). Blood Pb levels were associated with fine and gross motor function in unadjusted and adjusted analyses, indicating that bias from the measured confounders was not driving the observed associations. Subsequent analyses in the CLS demonstrated associations of earlier childhood average blood Pb levels (0-5 year average or 78 month average) with poorer fine (Ris et al., 2004) and gross motor function (Bhattacharya et al., 2006) assessed in adolescence. While these findings suggest the persistence of Pb effects or early, long-term exposure effects, it is important to note that blood Pb levels measured later in childhood or concurrently with motor function were not examined.

Studies in other populations found associations of blood Pb level (primarily concurrent) with poorer fine motor function in children ranging in age from 3 to 16 years (Palaniappan et al., 2011; Min et al., 2007; Wasserman et al., 2000). Among children in New England participating in NECAT (described in Section 5.3.2.1) Surkan et al. (2007) found higher blood Pb levels to be associated with lower FSIQ but better fine motor function as indicated by faster finger tapping speed. Gross motor function also was assessed in other populations of children. In the Yugoslavian cohort, lifetime average blood Pb level was not associated with gross motor function (Wasserman et al., 2000). Concurrent blood Pb level was associated with greater sway oscillation, alternating arm movements, and action tremor in a group of Inuit preschool children in Quebec, Canada (Despres et al., 2005). Investigators considered potential confounding by several variables including HOME score, maternal education and nutrient levels. In animal studies, Pb exposure has shown mixed effects on endurance, balance and coordination as measured by rotarod performance. G+P Pb-exposed male mice had significantly shorter

mean latencies to fall from the rotarod compared with controls; females were unaffected. Further, low dose G+P Pb-exposed male mice had significantly poorer rotarod performance (i.e., fell off more quickly) than did high dose G+P male mice (resulting blood Pb levels at PND10: 10 µg/dL and 42 µg/dL, respectively), indicative of a nonlinear concentration-dependent relationship (Leasure et al., 2008). Other rotarod experiments at higher doses of Pb exposure and at various speeds of rotarod rotation yielded mixed results (Kishi et al., 1983; Grant et al., 1980; Overmann, 1977).

The collective body of evidence demonstrates that within the same population of children, blood Pb levels are associated with decrements in cognitive function, attention, and fine and gross motor function. Studies found associations with concurrent and childhood average blood Pb levels mostly in populations with mean levels ranging from 11 to 28 µg/dL. Despres et al. (2005) found impaired gross motor function in a children with a mean blood Pb level of 5 µg/dL. Min et al. (2007) found impaired fine motor function in children with a mean concurrent blood Pb level of 2.9 µg/dL; however, the results were not adjusted by SES-related variables. motor skills are the result of the coordination of complex cognitive and physical processes in the cortex, cerebellum, vestibular systems, and visual system, and biological plausibility for the associations of blood Pb levels with fine motor skills observed in children is provided by observations that Pb exposure affects development and function of these systems.

5.3.6 Seizures in Animals

One neurological sign of high dose Pb exposure has been the development of epileptic form activity or seizures in animals (Krishnamoorthy et al., 1993). However, earlier studies in the animal toxicological literature exploring the effects of Pb on seizure activity and threshold showed mixed results. Pb-acetate (250, 500, or 1,000 ppm for 30 days in drinking water to PND60 male Wistar rats, resulting in blood Pb levels of ~20, 35, and 42 µg/dL, respectively) significantly decreased the elapsed time required to develop the first myoclonic jerk and tonic-clonic seizure (Arrieta et al., 2005); also, the dose of pentylenetetrazol (PTZ) required to induce seizures was significantly decreased across all Pb dose groups. Some studies showed no effect of Pb on kindled animals (Schwark et al., 1985; Alfano and Petit, 1981). Other studies showed differential susceptibility to convulsant-inducing agents in developmentally Pb-exposed rats (Chen and Chan, 2002). Sprague Dawley rats were exposed to Pb-acetate (0.2% w/v in drinking water from PND1-25, followed by 25 days with no Pb exposure). Seizures were induced at PND25 or PND50. At PND25, Pb exposure significantly decreased (PTZ)-, picrotoxin (PIC)-, and strychnine (STRY)-induced convulsion thresholds, but increased N-methyl-D-aspartate (NMDA) and 4-aminopyridine (4-AP)-induced convulsion thresholds. At

PND50, the effects of PTZ, NMDA, and 4-AP remained similar to those at PND25, but PIC and STRY-dependent convulsion thresholds were reversed and significantly increased (Chen and Chan, 2002). Chen and Chan (2002) hypothesized that this differential effect may be due to selective effects on inhibitory and excitatory neurotransmission as an effect of age and blood Pb level.

Recent investigation expands on the work by Arrieta et al. (2005) by showing similar effects in another rodent species, BALB/c mice. Adult male BALB/c mice were exposed to Pb for 30 days via drinking water (resulting in blood Pb levels in control and 50, 100, 200 and 400 ppm Pb groups of 0.02, 6, 11, 15 and 18 µg/dL, respectively) (Mesdaghinia et al., 2010). Exposure to 50 ppm Pb did not affect PTZ-induced seizure threshold, but all other doses significantly reduced the thresholds of face and forelimb clonus, myoclonic twitch, running and bouncing clonus, and tonic hindlimb extension. These studies show that Pb exposure may modulate seizure activity in animals.

5.3.7 Neurodegenerative Diseases

5.3.7.1 Epidemiologic Studies of Neurodegenerative Diseases in 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. Among NAS men, higher bone Pb levels were associated with lower MMSE scores (Weisskopf et al., 2004; Wright et al., 2003b), which indirectly pointed to a potential association with dementia, given that the MMSE is widely used as a screening tool for dementia. Overall, studies had sufficient limitations (e.g., indirect assessment of dementia, comparison of Alzheimer's Disease patients and healthy controls, lack of Pb biomarker data), and findings were inconclusive (U.S. EPA, 2006b). New studies on dementia are not available to assess further the associations with Pb biomarkers. Similarly, new studies examining Alzheimer's disease are not available, and as in 2006, the evidence is still inconclusive regarding the association with Pb biomarkers. 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. Case-control

studies that measured blood Pb levels produced contrasting results. A study of 16 ALS cases (mean blood Pb level: 12.7 µg/dL) and 39 controls (mean blood Pb level: 10.8 µg/dL) found a small difference in the mean concurrent blood Pb level (Vinceti et al., 1997). Another case-control study that examined concurrent blood and bone Pb levels in a New England-area population found higher odds of ALS among subjects with concurrent blood Pb levels ≥ 3 µg/dL (e.g., OR: 14.3 [95% CI: 3.0, 69.3] for blood Pb levels 3-4 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 higher ALS prevalence. For example, the ORs (95% CIs) for patella Pb levels 10-20 µg/g and tibia Pb levels 8-14 µg/g were 2.1 (0.6, 7.4) and 1.6 (0.5, 5.6), respectively. Also in this population, an estimate of cumulative Pb exposure based on occupational history was found to be associated with ALS (Kamel et al., 2002). The stronger findings for blood Pb level were surprising given that bone Pb level is a better biomarker of cumulative Pb exposure. One explanation for these findings is 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, a few additional studies have been conducted with the same New England-area case-control study population. Kamel et al. (2005) reported that the association between blood Pb level and ALS was not modified by the ALAD genotype (Kamel et al., 2005). Another report examined survival of 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, although 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. On the other hand, the decreased mobility due to the disease itself would tend to increase bone resorption and lower bone Pb levels over time. This process might mitigate this effect for bone Pb but would tend to increase blood Pb levels among cases. However, this was not observed in the one study that had bone and blood Pb biomarkers (Kamel et al., 2002). Because the strongest findings for survival were found for tibia Pb, it is unlikely that the findings were biased due to increased survival of cases.

Another case-control study examined concurrent blood Pb levels and ALS among 184 cases (33 were either progressive muscular atrophy or primary lateral sclerosis, mean

blood Pb level: 2.41 µg/dL) and 194 controls (mean blood Pb level: 1.76 µg/dL) (Fang et al., 2010). The cases were recruited from the National Registry of U.S. Veterans with ALS, and controls were recruited from among U.S. Veterans without ALS and frequency matched by age, gender, race, and past use of the Veterans Administration system for health care. A doubling of blood Pb levels was associated with ALS 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 suggest that reverse causation is not likely driving the association between blood Pb level and ALS. Whether other types of reverse causality are contributing, 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, studies have found associations of blood and bone Pb levels with ALS but in relatively few different cohorts. The case-control design of most studies and issues of reverse causality and bias due to survival time make it difficult to draw firm conclusions. Studies in additional cohorts using designs other than case-control comparisons are needed to address the limitations of the available studies and characterize better the potential effects of Pb on ALS.

Parkinson's Disease

A few previous studies, some ecological (Rybicki et al., 1993; Aquilonius and Hartvig, 1986) and some case-control relying on questionnaire data or occupational history (Gulson et al., 1999; Gorell et al., 1997; Tanner et al., 1989) indicated associations between exposure to heavy metals, particularly Pb, and risk of PD. Available evidence was limited and far from conclusive. A recent large case-control study (330 cases, 308 controls) recently reported on associations between biomarkers of Pb and PD in a population with virtually no occupational exposures to Pb (Weisskopf et al., 2010). Subjects in the highest quartile of tibia Pb level (>16.0 µg/g) had higher odds of PD compared to those in the lowest quartile (≤ 5 µg/g) (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 toward the null as these groups are likely to share many exposures.

Manganese exposure has been associated with Parkinsonian symptoms and could potentially confound associations between Pb and PD. Weisskopf et al. (2010) did not adjust for Mn exposure in analyses. However, unlike a setting of occupational exposure to Pb, general environment exposure to Pb is much less likely to be correlated with environmental Mn exposure. Thus, the likelihood of any associations with Pb being confounded by co-occurring Mn exposure is less likely.

Coon et al. (2006) conducted a smaller case-control study of 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 ≥ 15 μg/g) and calcaneus (OR: 1.50 [95% CI: 0.75, 3.00] for levels ≥ 25.29 μg/g) bone Pb levels had higher odds of PD compared to those in the lowest quartiles (0-5.91 μg/g for tibia and 0-11.70 μg/g for calcaneus). The highest OR for PD was estimated for subjects in the highest quartile of whole-body lifetime exposure to Pb (≥ 80.81 μg/g) estimated using PBPK modeling), compared to the lowest quartile of exposure (0-40.04 μg/g) (OR: 2.27 [95% CI:1.13, 4.55] for levels ≥ 80.81 μg/g). These analyses did not adjust for Mn either; however, in this study it was not clear what the extent of occupational exposure to Pb was among the participants. Thus, it is uncertain whether the observed associations were specifically related to Pb exposure or could have resulted from co-occurring Mn exposure.

In summary, a small number of recent studies expand on previous evidence by finding associations of bone Pb levels, biomarkers of cumulative Pb exposure, with PD in adults. Nonetheless, additional investigation is warranted to establish the temporality between Pb exposure and development of PD and to assess potential confounding by Mn exposure.

Essential Tremor

In a relatively small body of literature, concurrent blood Pb levels have been consistently associated with essential tremor, although studies have had relatively small sample sizes and have produced imprecise effect estimates. The 2006 Pb AQCD described case-control studies that found associations between concurrent blood Pb levels and essential tremor in New York City metropolitan area populations (Louis et al., 2005; Louis et al., 2003). In Louis et al. (2005), the magnitude of association was larger among carriers of an ALAD2 allele than among adults with only ALAD1 alleles.

Since 2006, 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). After adjusting for age, sex, education, cigarette smoking, cigarette pack-years, and alcohol use, a 1 μg/dL higher blood Pb level

(measured at the time of study recruitment) was associated with essential tremor with an OR (95% CI) of 4.19 (2.59, 6.78). This OR was much larger than that obtained in the New York area study (OR: 1.19 [95% CI: 1.03, 1.37]) (Louis et al., 2003). The magnitude of association in Dogu et al. (2007) is even more striking because so many of the controls were spouses who are expected to share many environmental exposures as cases. Most of the essential tremor cases were retired at the time of the study, but past occupational history was not reported. Occupational Pb exposures were less likely in the New York area population.

In this small body of studies, associations were observed between blood Pb level and essential tremor in adults. However, due to the case-control design of studies, temporality between exposure and development of essential tremor cannot be established. Further, the level, timing, frequency, and duration of Pb exposure associated with PD is uncertain as all studies examined blood Pb level at the time of study recruitment. Occupational histories were not reported in these studies. Thus, it is not clear what past exposure to Mn may have contributed to the associations observed with blood Pb levels.

5.3.7.2 Toxicological Studies of Neurodegenerative Disease

Although epidemiologic studies have provided weak evidence for associations of blood Pb level and ALS in adults, toxicological studies have found that Pb exposure induces neurophysiologic changes consistent with ALS. For example, chronic Pb exposure (Pb-acetate in drinking water at 200 ppm from weaning onward, resulting 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 did not significantly increase the onset of the ALS disease but increased survival time in SOD1 Tg mice (Barbeito et al., 2010). This finding does provide biological plausibility for the association observed between blood Pb level and longer survival time in patients diagnosed with ALS (Kamel et al., 2008). In mice, astrocyte of vascular endothelial growth factor (VEGF) also was examined to understand its possible contribution to Pb effects on increasing survival time in ALS models. Baseline levels VEGF were elevated in astrocytes from the ventral spinal cord of untreated SOD1 Tg mice versus untreated nontransgenic animals. VEGF was not induced in the astrocytes of Pb-treated nontransgenic mice. Pb-exposed SOD1 Tg mice had significant elevations of astrocyte VEGF versus vehicle-treated SOD1 Tg animals (Barbeito et al., 2010). Nontransgenic animals exposed to Pb showed no elevation in VEGF expression above that in nontransgenic 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

(Yamanaka et al., 2008; Boillee et al., 2006). Thus, the aforementioned findings for Pb-induced effects on astrocytes provide a mechanistic explanation for Pb effects on ALS progression in animals.

Others 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 was 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 was 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 were 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 (Zhang et al., 2010b; Qian and Flood, 2008). 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 can induce cell death or apoptosis in various models including rat brain (Tavakoli-Nezhad et al., 2001), retinal rod cells (He et al., 2003; He et al., 2000), cerebellar neurons (Oberto et al., 1996), and PC12 cells (Sharifi and Mousavi, 2008). These observations indicate that Pb-induced cell apoptosis may mediate its neurodegenerative effects. A recent study reported that chronic (40 days) Pb exposure induced hippocampal apoptosis in young (exposure starting at 2-4 weeks of age) and adult (exposure starting at 12-14 weeks of age) male rats exposed to 500 ppm Pb by drinking water (resulting in blood Pb levels of 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 level at the end of lactation or PND20 was 80 µg/dL. The data showed that 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 showed temporal and regional changes in activation of death protein message levels in male offspring.

Pb exposure has also been shown to induce apoptosis during spinal cord development in chicks exposed to 150 or 450 µg in ovo Pb-acetate exposure at embryonic day 3 or 5 and visualized six days later; TUNEL positive cells were at significantly higher levels in Pb-exposed animals and were visualized in all layers of the developing spinal cords (Müller et al., In Press). Also, levels of glial fibrillary acidic protein (GFAP), a factor important in neuronal migration and cellular differentiation during nervous system development, was significantly attenuated in spinal cords of Pb-exposed chicks. Another recent study examined Pb treatment animals (i.p. injections of 350 mg/kg, twice daily, blood Pb levels at PND14 of 0.15 µg/dL in controls and 8.10 µg/dL in Pb-treated animals) during PND7-14, a known period of sensitivity to NMDA antagonist-dependent neuronal apoptosis (Ikonomidou et al., 1999). Liu et al. (2010b) examined apoptotic effects in 30 day-old male rats that were exposed to Pb-acetate 1x/d for 6 weeks via intragastric infusion. Four dose groups: control, low (2), medium (20), and high (200 mg/kg BW) had blood Pb levels of 1.0 to 7.5 µg/dL; 4.5 to 11 µg/dL; 9 to 42 µg/dL; and 48 to 73 µg/dL, respectively. Liu et al. (2010b) reported Pb-induced apoptosis in the brain with hippocampal XIAP (statistically significant at high dose only) and Smac (statistically nonsignificant trend) downregulation and associated histopathology showing hippocampal neuronal apoptosis (TUNEL positive staining, significant at all doses) at the termination of the 6 week treatment. In another study, Pb exposure (500 ppm Pb-acetate in drinking water for 8 weeks) of adult male rats induced regional-specific changes in brain apoptotic proteins poly(ADP-ribose) polymerase, Bcl-2, caspase-3) with a greater effect observed in the hippocampus and cerebellum and a lesser effect observed in the brainstem and the frontal cortex (Kiran Kumar et al., 2009)

Collectively, studies have shown that Pb exposure induces neuronal apoptosis in animals during various developmental windows: early postnatal and adulthood. The new data continue to show that Pb exposure induced apoptosis in brains of animals.

Lead-Induced Neuronal Plaque Formation

Epidemiologic studies have not provided compelling evidence that Pb exposure is associated with Alzheimer's Disease in adults (Section 5.3.7.1); however, Pb exposure in early life has been shown to promote Alzheimer’s Disease-like pathologies in the brains of aged adult animals. Alzheimer’s disease is characterized by amyloid-beta peptide (Ab) accumulation, hyper-phosphorylation of the tau protein, neuronal death and synaptic loss. In the last decade, the developmental origins of adult health and disease (DoHAD)

paradigm and the similar Barker hypothesis have indicated that early life exposures can result in aberrant adult outcomes. 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 (2008a) 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 toxicants rather than relying on adult exposure alone to predict probable health risks from prenatal, neonatal or juvenile exposure (Dietert and Piepenbrink, 2006). Mechanistically, some of these pathologies have been associated with changes in the epigenome.

The fetal basis of amyloidogenesis has been examined extensively by the Zawia laboratory in both rodents and nonhuman primates. Mechanistically, amyloid plaques originate from the cleavage of the amyloid precursor protein (APP) to Ab, which comprises the plaque. In rodents exposed to Pb as neonates or as adults, neonatal Pb exposure induced amyloidogenesis in the aged animal brains; adult exposure to Pb did not contribute to plaque formation. Basha et al. (2005) exposed male rodents neonatally via lactation to Pb (PND1-PND20 exposure, dam drinking water Pb-acetate 200 ppm, resulting in pup PND20 blood Pb level of 46 µg/dL and cortex 0.41 µg/g wet weight of tissue) and examined cortical APP gene expression over the lifetime. A bimodal response was observed, with a significant increase in APP expression above that in control animals first manifesting neonatally and second manifesting in old age (82 weeks of age) (Basha et al., 2005). A concomitant bimodal response was observed in specificity protein 1 (Sp1), a transcription factor known to be related to APP expression. Ab, the amyloid plaque constituent, was also significantly elevated in these aged animals developmentally exposed to Pb. A subset of rodents exposed to Pb only as aged adults (18-20 weeks of age) was unresponsive in APP or Sp1 expression or Ab production after Pb exposure, indicating the developmental lifestage and not adult lifestage as the susceptible period for Pb-induced amyloidogenesis. The Zawia lab (Wu et al., 2008a) produced similar findings for amyloid plaques in the brains of monkeys that were exposed to Pb as infants (PND1-PND400), i.e., significantly higher gene expression of APP, and Sp1 and significantly higher protein expression of APP and Ab in aged female monkey cortex tissue (23 year-old *Macaca fascicularis*) from a cohort of animals established in the 1980s by Rice (1992, 1990). After weaning but with continued Pb exposure, the monkeys had blood Pb levels of 19-26 µg/dL. However, in old age when amyloid plaques had manifested, blood Pb levels and brain cortex Pb levels had returned to control or baseline levels. Together, the rodent and nonhuman primate toxicological studies concur and show that developmental Pb exposure induced elevations in neuronal plaque proteins in aged animals.

Mechanistic understanding of Ab production and elimination after Pb exposure was explored in human SH-SY5Y neuroblastoma cells exposed to Pb concentrations of 0, 5, 10, 20, and 50 µM for 48 hours. These studies showed that Pb affected two separate pathways to contribute to elevated Ab. Pb exposure induced both the overexpression of APP and repression of neprilysin, a rate-limiting enzyme involved in Ab metabolism or removal (Huang et al., 2011a). Further mechanistic understanding of how Ab peptide formation is affected by Pb exposure was examined by Behl et al. (2009). The choroid plexus is capable of removing beta-amyloid peptides from the brain extracellular matrix; however, Pb was shown to impair this function, possibly via the metalloendopeptidase, insulin-degrading enzyme (IDE), which metabolizes Ab (Behl et al., 2009). In another study, the effect of Pb on transcription factors essential in the regulation of the developing brain was explored. Pb exposure has been shown to perturb DNA binding of transcription factors including SP1 at essential sites like zinc finger proteins. In Long-Evans hooded rat pups exposed to Pb during lactation, these Pb-induced developmental perturbations of SP1 DNA binding were found to be ameliorated by exogenous zinc supplementation (Basha et al., 2003).

An additional study with developmental Pb exposure (gestational plus lactational, dam drinking water solutions of 0.1%, 0.5% or 1%, blood Pb level 40, 80 and 1,00 µg/dL) showed that the hippocampus contained neurofibrillary 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., 2010b).

In summary, the multiple recent studies showed that developmental Pb exposure induced significant increases in neuronal plaque associated proteins, which is the pathology found in humans with Alzheimer's disease. Adult exposure to Pb did not generate this neurofibrillary pathology, further demonstrating that early life Pb is a sensitive window for Pb-induced pathology including Ab-peptide accumulation, activation of Ab-supporting transcription factors, as well as tau hyperphosphorylation. Epidemiologic evidence for Pb-associated Alzhemier's disease is weak, and although several study design limitations were noted (Section 5.3.7.1), the animal evidence indicates that epidemiologic studies assessing concurrent bone or brain Pb levels or occupational Pb exposure may not have examined the etiologically relevant exposure period. Notably, animals were not behaviorally assessed for dementia.

5.3.8 Modes of Action for Lead Nervous System Effects

5.3.8.1 Effects on Brain Physiology and Activity

A small body of available epidemiologic evidence with small sample sizes demonstrated associations of Pb biomarkers with electrophysiologic changes in the brains of young adults as assessed by magnetic resonance imaging (MRI) or spectroscopy (MRS) (Yuan et al., 2006; Cecil et al., 2005; Meng et al., 2005; Trope et al., 2001). By characterizing underlying mechanisms by which Pb exposure may disrupt brain function, these studies have provided biologically plausible evidence for the effects of Pb exposure on cognitive and psychological and behavioral function observed in adults.

Findings such as lower levels of N-acetylaspartate (NAA), creatine (Cr), and choline are linked to decreased neuronal density and alteration in myelin. Notably, Trope et al. (2001) 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 was 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 childhood average 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 subjects that indicated associations of blood Pb level with other indices of language skills in childhood.

Since the 2006 Pb AQCD, studies examining MRI data were largely 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 of adults. These recent studies expanded on previous studies by including larger sample sizes, aiming to characterize important lifestages of Pb exposures, and evaluating potential links between changes in brain activity and functional neurodevelopmental deficits. Whereas previous CLS analyses focused on activity in specific regions of the brain, Cecil et al. (2011) examined brain metabolites in 159 subjects and found that childhood average blood Pb levels were associated with lower levels of NAA and Cr in the basal ganglia and lower levels of choline in white matter. These results were adjusted for age and FSIQ. A recent analysis of 31 men in the NAS cohort similarly reported an

association between biomarkers of cumulative, long-term Pb exposure and changes in brain metabolites in older adults. Weisskopf et al. (2007a) found higher tibia and patella Pb levels to be associated with a higher myoinositol/Cr ratio, which may be indicative of glial activation and is a signal reportedly found in the early stages of HIV-related dementia and Alzheimer's disease.

Other studies in CLS young adults found that childhood average blood Pb levels were associated with altered brain architecture. Brubaker et al. (2009) reported associations of childhood average blood Pb levels with diffusion parameters that were indicative of less organization of fibers throughout white matter. Results were adjusted for potential confounding by variables such as maternal IQ, prenatal alcohol and tobacco exposure, and adult marijuana use. In regions of the corona radiata, higher blood Pb levels were associated with less myelination axonal integrity. In regions of the corpus callosum, higher blood Pb levels were associated with greater myelination and axonal integrity. The differential impact on different neural elements 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 (Cecil et al., 2008). The most affected regions included frontal gray matter, specifically the anterior cingulate cortex and the ventrolateral prefrontal cortex (i.e., areas traditionally related to executive functions, mood regulation, and decision-making). Further, 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 suggested that MRI changes associated with blood Pb levels may be indicative of decrements in cognitive function. The functional relevance of these structural changes in the brain also is supported by observations from other studies that link changes in brain architecture and activity with changes in cognitive function (e.g., visuoconstruction, visual memory, eye-hand coordination) (Schwartz et al., 2007) and behavior (impulsivity, aggression, violence) (Yang et al., 2005; Raine et al., 2000).

In a subsequent comparison of blood Pb levels measured at various lifestages, Brubaker et al. (2010) found that blood Pb levels at older ages were associated with greater losses in gray matter volume than were childhood average or maximum blood Pb levels. Both Cecil et al. (2008) and Brubaker et al. (2010) found that Pb-associated reductions in gray matter were more pronounced in CLS males than females.

Studies of Pb-workers also found associations of blood and bone Pb levels with changes in brain structure and physiology, adding support for the effects of chronic Pb exposure. Pb-associated changes included white matter lesions, smaller brain volumes, less total gray matter, and lower levels of brain metabolites such as NAA and Cr (Hsieh et al.,

2009b; Jiang et al., 2008; Bleecker et al., 2007a; Stewart et al., 2006). In a few of these occupational groups, Pb-associated brain changes were linked to poorer performance in cognitive function tests (Caffo et al., 2008; Bleecker et al., 2007a).

In summary, results in a few different populations indicated associations of blood or bone Pb levels with changes in brain structure and physiology as assessed by MRI or MRS. A majority of evidence was derived from the CLS study. Several studies linked these changes to functional changes in cognitive performance or motor skills. While the collective findings pointed to the effects of long-term or past Pb exposures, it is important to recognize that other lifestages of exposure, including recent adult, were not widely examined.

5.3.8.2 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 adduct 8-hydroxy-2'-deoxyguanosine (8-oxo-dG). The contribution of reactive oxygen or nitrogen species to these Pb induced changes was assayed by examining the ratio of 8-oxo-dG to 2-deoxyguanosine (2-dG). 2-dG is product of oxidative cleavage that can generate 8-oxo-dG from a parent compound forming a DNA adduct during conditions of nitrosative or oxidative stress. The 8-oxo-dG to 2-dG ratio data from rodent male offspring were similar to 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, nonPb-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, similar findings were reported in a monkey study. The ratio of 8-oxo-dG to 2-dG in the brains of aged monkeys (23 years) after being exposed to Pb as infants, was significantly elevated above that in controls (Wu et al., 2008a). Several lines of evidence indicate that oxidative stress is involved in neurodegenerative pathologies including Alzheimer's disease; hydrogen peroxide-induced oxidative stress has been shown to induce intracellular accumulation of amyloid beta-protein (Abeta) in human neuroblastoma cells (Misonou et al., 2000). Similar to the amyloid findings in Pb-exposed animals the oxidative stress markers showed no significant changes above baseline when animals were exposed to Pb only as aged adults (Wu et al., 2008a; Bolin et al., 2006). 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 and an absence of effect with adult only 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 (Verma et al., 2005; Yun and Hoyer, 2000; Regunathan and Sundaresan, 1984; Sterling et al., 1982). Mitochondria produce ATP or energy through oxidative phosphorylation. Aberrant mitochondrial function can decrease the energy pool and contribute to ROS formation via electron transport chain disruption. ATP depletion can also affect synaptic and extracellular neurotransmission. The mitochondrial Na/K ATPase is important in maintaining the inner mitochondrial membrane potential (delta omega sub m) and the functioning of the mitochondria.

To characterize the effect of Pb exposure on these mitochondrial parameters, brains from the offspring of Pb-exposed mice were collected at PND8 (Baranowska-Bosiacka et al., 2011b). Cerebellar granular cells were harvested from PND 8 control and Pb-exposed animals (0.1% Pb-acetate in dam drinking water, resulting in blood Pb levels of 4 µg/dL and cerebella Pb levels of 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 controls. Neuronal Na/K ATPase activity was significantly lower in cortical granule cells from Pb-exposed mice versus cells from controls. Mitochondrial mass was unaffected with Pb treatment, but mitochondrial membrane potential was significantly decreased with Pb exposure. Pb-exposed crayfish that were placed under hypoxic conditions adapted to the situation by decreasing their metabolism (Morris et al., 2005) and showed mitochondrial changes consistent with those observed in granule cells (Baranowska-Bosiacka et al., 2011b). These studies showed 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.

The effects of co-administration of flaxseed oil (FSO) with Pb-acetate on oxidative stress and neurotoxicity were examined in adult male Wistar albino rats (Abdel Moneim et al.). Animals were administered Pb-acetate i.p. for 5 days (20mg/kg, resulting in blood Pb level ~31 µg/dL the day after the last Pb injection) or FSO (oral gavage 1000mg/kg body weight for 5 days, 1 hour prior to Pb dosing, resulting in blood Pb level ~12 µg/dL). Administration of the polyunsaturated fatty acid FSO significantly attenuated the blood Pb level of Pb-exposed animals and control animals, indicating that FSO may alter Pb toxicokinetics in animals. As would be expected with a lower blood Pb level, FSO+Pb exposed animals had significant attenuations in Pb-induced histological neuronal damage

and brain tissue DNA fragmentation. Pb exposure effects on indicators of oxidative stress included decreased antioxidant GSH levels, elevated lipid peroxidation, and decreased antioxidant enzyme activity in brain tissue. These Pb-induced effects were attenuated in FSO+Pb exposed animals. These results indicated that FSO likely attenuates Pb-related neurotoxicological damage and oxidative stress via its action on altering Pb toxicokinetics.

Green tea extract (GTE) was shown to be protective against Pb-induced oxidative stress in the brains of adult male rats (1.5% GTE +/- 0.4% Pb-acetate for 6 weeks) (Hamed et al., 2010). GTE+Pb-exposed animals had significantly less brain Pb than did Pb-exposed animals (1.2 versus 1.9 ppm). Levels of pro-oxidants or antioxidants including lipid peroxides (LPO), nitric oxides (NO), total antioxidant capacity (TAC), glutathione (GSH), glutathione-S-transferase (GST), and superoxide dismutase (SOD) were measured in brain tissue homogenate 24 hours after the termination of Pb exposure. Comparing the Pb-exposed animals to controls, brain LPO was significantly elevated and brain GSH, GST and NO were significantly decreased. GTE and Pb co-exposure significantly attenuated the Pb-related changes. This study reported a positive correlation between whole blood Pb levels and brain tissue LPO levels and a negative correlation between whole blood Pb and NO levels. The antioxidant quercetin (Que) rescued chronic Pb exposure-related (0.2% Pb-acetate in drinking water from birth to PND67, 30 mg/kg BW Que for one week from PND60-67) impaired synaptic plasticity in adult male and female Wistar rat dentate gyrus (DG) (Hu et al., 2008a). Pb-related impaired long-term potentiation, paired-pulse reactions, and input/output functions were significantly attenuated with Que treatment at PND67. Que treated animals had significantly less hippocampal Pb than did the Pb-exposed animals and this decreased Pb brain burden likely contributed to the attenuated response observed with Que exposure.

The vulnerability of the highly energetic brain tissue to stressors and cell death can be exacerbated with an energy imbalance. Baranowska-Bosiacka et al. (2011b) showed that Pb exposure induced energy imbalances in immature rat brains exposed to Pb. Wistar rat pups (PND15) of both sexes were injected daily for 2 weeks with Pb-acetate (15mg/kg BW, i.p., resulting in blood Pb levels of 3 and 30 µg/dL, control and Pb-exposed, respectively) and thereafter sacrificed for analysis of regional brain purines and purinergic receptors. ATP and ADP were significantly decreased in various brain regions with Pb exposure, with the cerebellum and hippocampus more strongly affected than the forebrain cortex. Also, enhanced expression of the proinflammatory P2XR receptor was observed in the glial fraction, indicating the astrocyte pool may be involved in the pathological changes found in Pb-exposed immature rat brains.

5.3.8.3 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 in Pb-exposed animals 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 neuronal nitric oxide synthase (NOS) with perinatal Pb exposure. Namely, with repeated swim tests, control animals more quickly found a submerged platform (i.e., escaped), than did Pb-exposed animals. After either 4 or 8 weeks of Pb exposure to weanling male rats (resulting in blood Pb level of 6.2 µg/dL), hippocampal NOS and NO were significantly decreased. Dietary supplementation with taurine or glycine concomitant with 8 weeks of Pb exposure induced significant increases in hippocampal NOS, whereas Pb plus dietary supplementation with vitamin C, methionine, tyrosine, or vitamin B1 decreased hippocampal NOS. Supplementation also changed Pb-related effects on hippocampal NO. Pb-induced NO increased with taurine and decreased with vitamin C, tyrosine or glycine. Dietary supplementation with tyrosine, methionine, or ascorbic acid after 4 weeks of Pb exposure in weanling males (4-week blood Pb level of 47.6 µg/dL and 8-week blood Pb level of 8.1 µg/dL), induced significant increases in NO. Zinc supplementation in this model had no effect on the NO system. The investigators concluded various combinations of nutrients significantly attenuate Pb-related 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-related decrements in NO/NOS concentrations (Fan et al., 2009a).

5.3.8.4 Synaptic Changes

Previous toxicological studies point to an effect of developmental Pb exposure on synapse development, which mechanistically may contribute to multiple Pb-related aberrant effects, including changes in long-term potentiation (LTP) and facilitation. Earlier work has shown that developmental Pb exposure results in altered density of dendritic hippocampal spines (Király and Jones, 1982; Petit and LeBoutillier, 1979), aberrant synapse elimination (Lohmann and 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 compared with unexposed controls, Pb-exposed larvae had significant increases in intracellular calcium and significant delays in calcium decays back to baseline levels at the pre-synaptic neuronal bouton (as stimulated with multiple action potentials, also called AP trains). Pb-exposed larvae had

reduced activity of the plasma membrane calcium ATPase, which is responsible for extravasations of calcium from the synaptic terminal (He et al., 2009). Intracellular calcium in Pb exposed-larvae was no different from that in controls under resting conditions or in neurons with stimulation by a single action potential. Pb media concentrations in these experiments were 100 or 250 µM with the 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 capability 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 (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 *Drosophil*a. Thus, the neuromuscular junction is a potential site of Pb interaction.

A study by Li et al. (2009c) focused on inflammatory endpoints and synaptic changes after gestational plus lactational dam drinking water Pb exposure (solutions of 0.1%, 0.5% or 1%, resulting in offspring blood Pb levels of 40, 80 and 100 µg/dL, respectively at PND 21). Hippocampal TNF-α was significantly elevated with Pb exposure and proteins that comprise the SNARE complex were all changed with Pb exposure. The SNARE complex of synaptic proteins includes SNAP-25, VAMP-2 and Syntaxin 1a and is essential in exocytotic neurotransmitter release at the synapse (Li et al., 2009c). Thus, Li et al. (2009c) found significant differences in hippocampal synaptic protein composition and increased pro-inflammatory cytokine levels in the brains of Pb-exposed offspring.

Neurotransmission is an energy-dependent process as indicated by the presence of calcium-dependent ATP releases at the synaptic cleft. At the synapse, ATP is metabolized by ectonucleotidases. In heme synthesis, Pb is known to substitute for the cation zinc in another nucleotidase, pyrimidine 5′-nucleotidase, and thus, the nucleotidase is used as a biomarker of Pb exposure. Acute exposure (96 hours) of male and female zebrafish to Pb-acetate (2 µg/dL) in their water induced significant decreases in ATP hydrolysis in brain tissue. This dose is deemed to be environmentally relevant. With chronic exposure (30 days), Pb-acetate promoted the inhibition of ATP, ADP and AMP hydrolysis; these findings were consistent with findings in rodents (Baranowska-Bosiacka et al., 2011b). The authors hypothesized that at 30 days, this Pb-induced change in nucleotide hydrolysis was likely due to post-translational modification because expression of enzymes responsible for the hydrolysis, NTPDase1 and 5′-nucleotidase, were unchanged (Senger et al., 2006). Thus, Pb has been 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 Pb-acetate (2 µg/dL 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 was shown also to affect synaptic homeostasis of AChE in the brains of zebrafish (Richetti et al., 2010).

Pb has been shown to act as an antagonist of the NMDA receptor (NMDAR). The NMDAR is essential for proper presynaptic neuronal activity and function. Primary cultures of mouse hippocampal cells were exposed to Pb (10 or 100 µM solutions in media) during the period of synaptogenesis (Neal et al., 2010a). 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 was found in both GABAergic and glutamatergic neurons. Pb also induced an increase in number of presynaptic contact sites. But, these sites may be nonfunctional as they lack the protein receptor complexes necessary for proper vesicular exocytosis. Another factor involved in growth and signaling of presynaptic neurons is BDNF, which is synthesized and released by postsynaptic neurons. BDNF is regulated by the NMDAR and acts in a retrograde fashion, participating in presynaptic maturation. In hippocampal cells, both pro-BDNF and BDNF release were significantly attenuated with Pb exposure (Neal et al., 2010a). Further, exogenous BDNF administration rescued the aforementioned Pb-related presynaptic effects. Thus, this cell culture model showed that Pb-related presynaptic aberrations are controlled by NMDAR-dependent BDNF effects on synaptic transmission.

Animals exposed to Pb postnatally (Wistar Albino rats, drinking water 300 mg/L Pb-acetate, resulting in blood Pb levels of 17 µg/dL at 6 weeks of age), from birth through lactation and through 12 weeks of age showed decreased learning ability and decreased hippocampal glutamate at 6, 8, 10 and 12 weeks of age (Niu et al., 2009) as well as significant decrements in the hippocampal glutamate synthesis-related enzymes aspartate aminotransferase and alanine aminotransferase.

5.3.8.5 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 BBB, formed by tight junctions at endothelial capillaries forming the zonulae occludens (occludins, claudins, and cytoplasmic proteins), separates the brain from the blood and its oncotic and osmotic forces, allowing for selective transport of materials across this barrier.

Pb exposure during various developmental windows has been shown to increase the permeability of the BBB (Dyatlov et al., 1998; Struzynska et al., 1997b; Moorhouse et al., 1988; Sundstrom et al., 1985). Possibly due the underdevelopment of the BBB early in life, prenatal and perinatal Pb exposure has been found to result in higher brain Pb accumulation than have similar exposures later in life (Moorhouse et al., 1988). Studies reviewed in earlier Pb AQCDs have shown that the chemical form of Pb and its capability to interact with proteins and other blood components affects its capability to penetrate the BBB (U.S. EPA, 2006b). Pb also has been shown to compromise the function of the BCB and decrease the CSF level of transthyretin, a thyroid binding protein made in the choroid plexus. The choroid plexus and cerebral endothelial cells that form the BBB and BCS tight junctions have been shown to accumulate Pb more than other cell types and regions of the CNS do. Hypothyroid status can contribute to impaired learning and IQ deficits (Lazarus, 2005).

Recent research with weanling rats exposed to Pb-acetate via drinking water showed leaky cerebral vasculature, an indication of a compromised BBB, as detected histologically with lanthanum nitrate staining of the brain parenchyma. Cerebral vasculature leakiness was ameliorated or 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 (Wang et al., 2007b). These data demonstrate that Pb induced a leaky BBB in weanling rats with associated decreases in the junctional protein occludin; dietary supplementation with iron ameliorated these Pb-induced impairments of the BBB in male rats. This loss of integrity at the junctional protein level was affirmed with additional experiments using the rat brain vascular endothelial cell line RBE4, in which 10 µM Pb-acetate exposure for 2, 4, 8, 16 and 24 hours resulted in decreases in junctional proteins occludin and claudin 5 as well as scaffold proteins ZO1 and ZO2 (Balbuena et al., 2011). Because expression of these junctional and scaffold proteins did not show decrements, it was determined that these protein decrements were due to post-translational modifications.

A study examined the effects of Pb on transendothelial electrical resistance (TEER), a marker of BBBB integrity, in an in vitro co-culture system employing endothelial cells (RBE4 or bovine brain microvascular endothelial cells) and astrocytes (primary Sprague-Dawley neonatal pup astrocytes, in utero day 21) as the barrier between Pb containing

exposure media and neurons. After 14 hours of exposure to Pb, TEER was significantly impaired in a concentration-dependent manner, with the lowest significant effects found with 1 µM Pb-acetate exposure. Thus, Pb exposure was found to contribute to leakiness of the BBB by decreasing the resistance across the junction.

Adult male rats exposed to Pb-acetate in drinking water for 4 or 12 weeks (50 or 500 ppm, resulting in blood Pb levels of 12 and 55 µg/dL, respectively) were assessed by diffusion weighted imaging for changes in apparent diffusion coefficent (ADC), a measure of tissue water diffusivity that changes under pathological conditions like cerebral edema. After 4 weeks of exposure to 500 ppm Pb, the water ADC was significantly increased in the hippocampus, mesencephalic reticular formation, and cerebellum but unaffected in other brain areas. After 12 weeks of 500 ppm Pb exposure, ADC was significantly increased in the corpus callosum and caudate putamen. Exposure to 50 ppm Pb for 12 weeks increased the ADC values in the cerebellum and mesencephalic reticular formation. The brain areas with elevated ADC also showed increased BBB permeability as measured with evans blue albumin complex. These data show that adult animals with chronic Pb exposure are susceptible to regional edema and regional increased permeability of the BBB, even with low blood Pb levels (12 µg/dL) (López-Larrubia and Cauli, 2011).

5.3.8.6 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 potentially contribute to nervous system barrier function disruption, tissue development dysregulation, immune dysfunction, and affect learning and memory (Prozialeck et al., 2002).

5.3.8.7 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, structural support to neurons, and myelination. Glial cells provide immune surveillance in the brain or contribute to inflammation-mediated pathologies. In the central nervous system, Pb treatment of Wistar rats 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. Glial cells have been shown 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 doses of 0.25 ± 1.0 µM Pb-acetate via Pb-related reduction in glutamine synthetase activity in the astroglia; astroglia take up glutamate after its release and convert it to glutamine. Pb has been shown to induce hypomyelination and demyelination (Coria et al., 1984) mediated through the oligodendrocytes with younger animals being more susceptible to the effects of Pb (Tiffany-Castiglioni et al., 1989). Pb accumulation in young glial cells may contribute to a lifelong exposure of neurons to Pb as Pb is released from the sink over time. Thus, Pb accumulation in glial cells can contribute to continual damage of surrounding neurons (Holtzman et al., 1987).

Glial transmitters

To determine the contribution of the gliotransmitter serine to Pb-mediated changes in long-term potentiation (LTP), Sun et al. (2007) exposed pups to Pb-acetate in utero, lactationally, through PND28 via drinking water and collected hippocampal sections. CA1 section LTPs were examined using in vitro patch clamp monitoring. Chronic Pb exposure impaired the magnitude of hippocampal LTPs, but the magnitude of LTDs was restored with supplementation with D-serine (Sun et al., 2007), which is known to be regulated by the NMDAR (Bear and Malenka, 1994). The use of 7-chlorokynurenic acid, an antagonist of the glycine binding site of the NMDAR, which also is the binding site of D-serine, effectively abolished the rescue to LTP by D-serine. NMDAR-independent LTP hippocampal neurotransmission, which was examined in slices of Pb-exposed mossy-CA3 synapses, was not rescued by exogenous D-serine supplementation. These data indicate that glial transmission is affected with Pb exposure and that the NMDAR may also be involved in this aberrant glial transmission.

5.3.8.8 Neurotransmitters

Pb has been shown to compete with calcium for common binding sites and second messenger activation. When Pb activates a calcium-dependent system in the nervous system, it can contribute to aberrant neurotransmitter regulation and release because this system intimately relies on calcium signaling for its homeostasis. Pb also has been shown to interfere with other physiological divalent cations. Pb-related alterations in neurotransmission are discussed in further detail below.

Monoamine Neurotransmitters and Stress

Combined exposures of maternal stress and Pb exposure can synergistically enhance behavioral and neurotoxic outcomes in offspring of exposed animals and can sometimes potentiate an effect that would otherwise be sub-threshold. Virgolini et al. (2008a) found that CNS effects of developmental Pb exposure (50 or 150 ppm via drinking water, 2 months prior to mating through lactation, resulting in blood Pb levels of 11 µg/dL and 35 µg/dL, respectively) were enhanced with combined maternal and offspring stress. Offspring neurotransmitter concentrations were significantly affected with Pb exposure, but the most interesting findings were those of potentiated effects, i.e., effects that were not observed with Pb exposure alone or stress alone. These potentiated effects were only observed 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 Pb exposure alone. The concentration of 5-Hydroxyindoleacetic acid (5HIAA), the main metabolite of 5HT, was significantly increased with Pb exposure alone in the striatum of male offspring with 150 ppb Pb exposure alone; with the remaining Pb-stress exposure combinations, 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 norepinephrine (NE). Earlier work has shown that perinatal Pb exposure of rats induced increased tyrosine hydroxylase, increased DA and increased cerebral cortex catecholamine neurotransmission (Devi et al., 2005; Leret et al., 2002; Bielarczyk et al., 1996). Earlier publications detailing important time windows of exposure, duration of exposure, and dose of Pb indicated varying effects on monoamine transmitters. In more recent work, these neurotransmitters, among others, have been implicated in Pb effects on auditory function in the brainstem in various integration centers there including the lateral superior olive (LSO), and the superior olivary complex (SOC). Among various functions, the SOC is vital for sound detection in noisy settings. Low-level Pb exposure has been associated with altered processing of auditory temporal signals in animal studies (Lurie et al., 2006; Finkelstein et al., 1998). Blood Pb levels for control, very low Pb (VLPb) and low Pb (LPb) exposure groups were 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-hydroxylase (DbH), a marker for NE. Statistically significant alterations in VMAT2 and DbH were found with both VLPb and LPb exposure; however, decrements in 5HT were statistically significant only 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 involved in auditory integration are affected by developmental Pb exposure via effects on the monoamine neurotransmitter system (Fortune and Lurie, 2009). The Pb-induced effects on the auditory portion of the brainstem at the level of the monoamine system provide possible mechanistic understanding of the animal data showing Pb-induced impaired auditory processing.

Dopamine

The 2006 Pb AQCD detailed evidence for low-dose Pb-related 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 DA metabolism, as indicated by changes in DA and DOPAC, a DA metabolite. Given these findings, a recent study measured DA and DOPAC in various brain regions of year-old male rodents to examine if GLE affected DA metabolism. Low- and high-dose GLE in male rodents induced significant elevations in DOPAC concentration and the DOPAC to DA ratio in the forebrain. In the forebrain, DA was significantly decreased in low-dose GLE males and significantly elevated in high-dose GLE males compared to controls. In the striatum, DOPAC was significantly elevated with both low- and high-dose GLE 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 that in controls. These new data expand upon the monoamine literature base in which indicates low concentration perinatal Pb exposure of rats was found to induce increased sensitivity of the dopamine receptors (D2 and D3) (Gedeon et al., 2001; Cory-Slechta et al., 1992), produce higher DA levels (Devi et al., 2005; Leret et al., 2002), and enhance catecholamine neurotransmission in the cerebral cortex, cerebellum, and hippocampus (Devi et al., 2005).

The interaction of DA and the nitric oxide system in the striatum was studied after prenatal Pb exposure (Nowak et al., 2008). Blood Pb levels were not reported in this study, but similarly treated Wistar rat pups had blood Pb levels at parturition in range of 50-100 µg/dL (Grant et al., 1980). 7-nitroinidazole (7-NI), a selective inhibitor of nNOS, enhanced amphetamine-evoked DA release in the rat striatum (Nowak et al., 2008).

Prenatal Pb exposure attenuated the facilitatory effect of 7-NI on DA release in the striatum. This interaction 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-related dopaminergic changes.

Dopamine and Vision

In various experimental animal models, the loss of retinal DA or zinc is associated with abnormal rod-mediated scotopic ERGs. These effects may explain observations of Pb-associated retinal effects (Rothenberg et al., 2002a; Lilienthal et al., 1994; Lilienthal et al., 1988; Alexander and Fishman, 1984). In the human and animal toxicological literature, prenatal maternal blood Pb level (humans), gestational + early postnatal Pb exposure (rodents, low and moderate dose Pb only), or gestational continuing to lifetime (non-human primate) Pb exposure has been associated with increased amplitude (supernormality) of ERGs (Rothenberg et al., 2002a; Lilienthal et al., 1994; Lilienthal et al., 1988). In the animal toxicological literature, subnormality of the ERGs has been observed with postnatal Pb exposure (Fox et al., 2008; Otto and Fox, 1993; Fox et al., 1991; Fox and Farber, 1988) and high-dose developmental (prenatal + early postnatal, rodent) Pb exposure (Fox et al., 2008). Producing results consistent with observations in humans (Rothenberg et al., 2002a), Fox et al. (2008) showed that low- (LPb) and moderate-level (MPb) gestational+early postnatal Pb exposure in the rat, a period equivalent to the human gestational retinal development period, produced supernormal retinal ERGs. In children, supernormal ERGs were associated with prenatal Pb maternal blood Pb levels >10.5 µg/dL (Rothenberg et al., 2002a). The animal data provide mechanistic information that may begin to explain these supernormal ERGs that are seen in children and rodents, i.e., significant increases in retinal neurogenesis and significant decreases in retinal DA use and dopamine turnover (DOPAC:DA ratio). High-dose developmental (rodent, gestational+early postnatal to PND10) Pb exposure (HPb) produced significant subnormal retinal ERGs. Subnormal ERGs also were found in occupationally-exposed adults (Otto and Fox, 1993; Guguchkova, 1972). Female rats were exposed to Pb-acetate in drinking water from 2 weeks prior to mating throughout gestation and lactation until PND 10, a period of developmental exposure that is equivalent to gestational exposure in humans. Peak blood Pb levels in the offspring at PND1-10 were 12, 24, and 46 µg/dL in the LPb, MPb, and HPb groups, 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 summary, the retina is affected by low-dose Pb exposure and gestational Pb exposure, as indicated by concentration-dependent decreases in DA use and turnover. Inverted U-

shaped Pb concentration-response relationship 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 has been shown to contribute to aberrations in LTP or long term depression (LTD) in the hippocampus via reduced NMDA current, among other mechanisms (Liu et al., 2004). The 2006 Pb AQCD indicated that Pb induced decreases in stimulated glutamate release that affected LTP. Further, the relationships between Pb exposure and decreased magnitude and increased threshold of the LTP in the hippocampus were found to be biphasic or nonlinear. NMDAR subtypes have been shown to be significantly decreased with developmental Pb exposure (Guilarte and McGlothan, 1998). Recent work examining dietary supplement use, found that Pb-related decreases in the gene expression and protein level of NMDAR subunit NR1 in weanling male rats were rescued with methioninecholine co-exposure (Fan et al., 2010). Fan et al. (2010) found that Pb-related suppression of the NMDAR subunits NR2A and NR2B was not rescued with methioninecholine treatment. Other recent mechanistic studies found that pretreatment of primary fetal brain neuronal rat cultures with glutamic acid, a NMDAR agonist, reversed Pb-induced reductions in NMDAR subunits (Xu and Rajanna, 2006) whereas pretreatment with the NMDA antagonist MK-801 exacerbated Pb-induced NMDAR deficits (Xu and Rajanna, 2006).

Studies continue to show that Pb exposure affects neurogenesis or proliferation of new cells in the hippocampus. Earlier work by Schneider et al. (2005) showed that postnatal Pb exposure (30-35 days starting at PND25, 1,500 ppm Pb-acetate in chow, resulting in blood Pb level of 20 µg/dL) of male Lewis rats induced significant decrements in BrdU incorporation (proliferation) at PND50-55. Recent publications affirm this original finding with different sex of animals, dosing and exposure time windows. Postnatal Pb exposure to Wistar rat pups (0.2% Pb-acetate from PND1-30, resulting in blood Pb levels of 34 and 6.5 µg/dL at PND21 and PND80, respectively) induced a statistically significant decrement in the number of new cells (BrdU positive cells) in the dentate gyrus at PND80 (Fox et al., 2010) (Figure 5-18). In another study, developmental/lifetime Pb exposure (1,500 ppm Pb-acetate in chow from 10 days before mating to termination of experiment at PND50, resulting in blood Pb levels of 0.8 µg/dL in controls and 26 µg/dL in Pb-exposed animals) to female Long-Evans rats induced significant decrements in hippocampal granule cell neurogenesis or proliferation of new cells in adult rats (Verina et al., 2007), outcomes that affect LTP, spatial learning, neuronal outgrowth, and possibly mood disorders such as 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 exposure (dam Pb-acetate chow exposure 10 days prior to mating through pregnancy 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. Pb-exposed animals also showed a significant decrease in the length-density of immature or newly-formed neuron in the outer portion of the DG. 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 reduced learning impairment, increased expression of hippocampal NMDA receptor subunit 1, and increased induction of brain derived neurotrophic factor mRNA (Guilarte et al., 2003).

Glutamate Receptor

Glutamate receptors including the ionotropic NMDAR and the metabotropic glutamate receptors (mGluR) are known targets of Pb toxicity with recent findings showing a role for mGluR5 in learning and memory. In vitro (GD18 fetal rat cultures, 100 µM, 1 µM, 0.01 µM $PbCl_2$ in culture media) and in vivo studies (gestational and lactational Pb-acetate exposure; control, 0.05, 0.2, or 0.5% in dam drinking water, with respective weanling blood Pb levels of 3, 18, 57, 186 µg/dL) showed that Pb exposure induced mGluR5 mRNA and protein decrements in a concentration-dependent manner (Xu et al., 2009c).. The Pb-related attenuation of mGlu5 expression may contribute to the effect of Pb on LTP and LTD.

5.3.8.9 Neurite Outgrowth

The 2006 Pb AQCD reported that Pb decreased neurite outgrowth at 20µg/dL and noted that Pb interfered with neurite outgrowth via protein kinase mediated pathways (MAPK/ERK); earlier work had documented decreased primary DA neuron outgrowth with 0.001 µM Pb exposure (Lidsky and Schneider, 2004). Recent studies have shown that exposure of dams to low-dose Pb (resulting in blood Pb level of 4 µg/dL) 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 (Hu et al., 2008b). PSA-NCAM is transiently expressed in newly formed neurons during the period of neurite outgrowth from embryrogenesis until the early postnatal period and is down-regulated in the adults

except in areas known to exhibit synaptic plasticity (Seki and Arai, 1993). NCAM is important for memory formation, plasticity and synapse formation, and early-life Pb exposure was found to affects its expression in laboratory rats.

5.3.8.10 Epigenetics

Many investigators are beginning to show that environmental chemical exposures are associated with epigenetic changes. Air pollution exposure is being linked increasingly with epigenetic changes (Baccarelli and Bollati, 2009; Pavanello et al., 2009; Tarantini et al., 2009; Bollati et al., 2007). Epigenetic changes involve changes in DNA expression without changes in the DNA sequence, and these changes may be heritable. Epigenetic changes are mediated by histone modification, DNA methylation, miRNA changes, or pathways that affect these processes. Differential epigenetic modification has the potential to contribute to disease by silencing or activating genes in an aberrant manner. Monozygotic twins are often used to study epigenetic changes, and a recent study identified differential methylation of a specific locus in twins discordant for schizophrenia (Dempster et al., 2011); Pb was not examined in this study.

DNA methyltransferases catalyze the transfer of a methyl group to DNA and are important in epigenetics (i.e., silencing of genes like tumor suppressors) and imprinting. DNA methyltransferase activity was significantly decreased in cortical neurons from Pb-exposed monkeys (aged animals, blood Pb) and mouse brains (fetal cells exposed to Pb in culture, 0.1 µM Pb) (Wu et al., 2008b). Changes in DNA methyltransferases (Dnmt1, Dnmt3a) were noted in control primate brains as they aged and these changes were further exacerbated by Pb exposure (Bihaqi et al., 2011). Another enzyme involved in DNA methylation, methyl CpG binding protein 2 MECP2, showed a similar trend as the Dnmts. Profiles of the histone modifying gene H34mc2 increases with age in control animals. This age-related increase is significantly attenuated in Pb-exposed animals. The cerebral cortex tissue used in this experiment was obtained from female primates who had received 1.5 mg/kg • day Pb-acetate via diet from birth until 400 days of age (resulting in blood Pb levels 19-26 µg/dL at age 400 days) (Rice, 1990). Methyltransferases catalyze biological methylation reactions and are dependent on the cofactor S-adenosyl methionine (SAM) for this transfer to acceptor molecules. SAM exposure after gestational and lactational Pb exposure (dam Pb-acetate exposure to 1,500 ppm Pb-acetate followed by 20-22 days of daily 20 mg/kg BW SAM exposure) improved hippocampal LTP and Morris water maze performance at PND 44-54. Thus, the impaired cognition and synaptic plasticity induced by developmental Pb exposure were attenuated with SAM treatment (Cao et al., 2008).

5.3.8.11 Cholesterol and Lipid Homeostasis

Various pathological conditions are associated with elevated plasma free fatty acids or elevated cholesterol. Adult male rats exposed to Pb-acetate (200, 300, or 400 ppm) in their drinking water for 12 weeks had increased cholesterogenesis and phospholipidosis in brain tissue (Ademuyiwa et al., 2009). Pb-induced changes in brain cholesterol showed an inverse U concentration-response relationship, with the largest increase in brain cholesterol observed with 200 ppm Pb followed by 300 ppm Pb. Animals exposed to 400 ppm Pb did not have significant changes in brain cholesterol. 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 statistically nonsignificant 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 concentration-dependent manner; plasma triglycerides and cholesterol were unaffected by Pb exposure. The molar ratio of brain cholesterol to phospholipids, an indicator of membrane fluidity (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 levels at 0, 200, 300, and 400 ppm were 7, 41, 61, and 39 µg/dL, respectively. In summary, based on limited examination, 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 these Pb-related changes in phospholipidosis and cholesterogenesis.

5.3.9 Lifestage of Lead Exposure and Neurodevelopmental Deficits

Environmental exposures during critical lifestages can affect key physiological systems that orchestrate plasticity (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 (Rice and Barone, 2000; Landrigan et al., 1999). Synaptic pruning, which is active throughout early childhood (ages 1-4 years), may underlie the elevated risk of young children to environmental exposures. MRI studies have provided understanding of brain development in normally developing children and

adults, ages 3-30 years (Giedd et al., 2009; Lenroot and Giedd, 2006). Total cerebral volume was found to peak at age 10.5 and 14.5 years in females and males, respectively. The volume of the cerebellum was found to peak 2 years after the cerebral volume peaks. Lateral ventricular volume showed the most inter-individual variation and was found to increase throughout childhood and adolescence. White matter volume generally increased throughout childhood and adolescence. Gray matter volume and associated structures show inverted U-shaped developmental trajectories with peak volumes found in late childhood or early adolescence. Females generally had gray matter volume peaks 1 to 3 years earlier than did males. Thus, the anatomical development of the brain during childhood and adolescence is found to be a dynamic process with variation by age, brain region, and sex. Observations that brain development is active throughout childhood and in adolescence indicates that Pb exposure may affect neurodevelopment throughout this period.

The elevated risk of Pb-associated neurodevelopmental deficits in children is well supported by findings in animals that prenatal and postweaning Pb exposure alters brain development via changes in synaptic architecture (Section 5.3.8.4) and neuronal outgrowth (Section 5.3.8.9) and leads to impairments in memory and learning (Section 5.3.2.2) and emotional and depressive changes postnatally (Section 5.3.3.5). Unlike other organ systems, the unidirectional nature of CNS development limits the capability of the developing brain to compensate for cell loss, and environmentally-induced cell death can result in a permanent reduction in cell numbers (Bayer, 1989). Hence, when normal development is altered, the early effects may persist into adult life even in the absence of current exposure, magnifying the public health impact. Supporting evidence is provided by a few available new 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 5.3.7.2).

With repeated assessments of children prenatally to later childhood and early adulthood, the prospective cohort studies have aimed to distinguish among neurodevelopmental effects associated with blood Pb levels measured at different periods of development. In the collective body of evidence, cognitive function decrements in children have been associated with prenatal, early childhood, childhood average, and concurrent blood Pb levels, without clear indication that the risk of neurodevelopmental decrements is greatest for blood Pb levels measured at a particular lifestage. In these studies, the identification of developmental periods when children are at increased risk of Pb-associated neurodevelopmental decrements has been complicated by the high degree of correlation in the blood Pb levels of children over time and the confounding of age and peak blood Pb levels (Lanphear et al., 2005; Dietrich et al., 1993b; 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 (maternal and umbilical cord) with neurodevelopmental deficits throughout childhood and into early adulthood (U.S. EPA, 2006b). Pb exposure during the prenatal lifestage may be associated with increased risk of neurodevelopmental effects not only because of the nervous system developmental processes that are active 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 that may be related to past Pb exposures (Gulson et al., 2003; Hu and Hernandez-Avila, 2002). Pb can cross the placenta to affect the developing fetal nervous system (Rabinowitz, 1988). Maternal and cord blood Pb levels generally have been shown to be highly correlated, indicating that blood Pb levels in a newborn infant reflects that of the mother (Schell et al., 2003).

Prenatal blood Pb levels (maternal and cord) were consistently associated with cognitive function decrements and behavioral problems assessed between infancy and age 3 years (Table 5-4 and Table 5-13). Among studies that had blood Pb measurements at both lifestages, some found stronger associations for prenatal blood Pb levels (Hu et al., 2006; Bellinger et al., 1984), and other found stronger associations for concurrent blood Pb levels (Wasserman et al., 1998; Wasserman et al., 1992). Studies that found associations with concurrent blood Pb levels also tended to find associations with prenatal cord or maternal blood Pb levels. Thus, both postnatal child and maternal Pb exposures may contribute to lower cognitive function in young children. Several studies found that prenatal or neonatal blood Pb levels were associated with neurodevelopmental decrements assessed neonatally (within 30 days) or early in infancy (within 3 months), which indicated that relatively short durations of Pb exposure were associated with neurodevelopmental decrements (Shen et al., 1998; Rothenberg et al., 1989; Dietrich et al., 1987a; Ernhart et al., 1986). In the studies of neurodevelopmental effects in infancy, prenatal blood Pb level may be serving as a surrogate of postnatal blood Pb levels as both are expected to be highly correlated.

Table 5-13 Associations of cognitive function and behavioral outcomes with blood Pb levels measured at different lifestages[a]

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[b]
Assessments in children up to age 3 years					
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)
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[c]
Hu et al. (2006)	146 children born 1997-1999 followed prenatally to age 24 mo Mexico City, Mexico	Prenatal (maternal 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)**
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. Mitrovica: 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	**Concurrent: -4.1 (-6.2, -2.0)** Prenatal: -3.2 (-7.2, 0.86)
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)**
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. Mitrovica: 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	**Concurrent: -4.1 (-6.2, -2.0)** **Prenatal: -3.2 (-5.1, -1.2)**

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[b]
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	**Neonatal: -0.38, p ≤ 0.01**[d] **Prenatal: -0.25, p ≤ 0.01**[d] **Concurrent: -0.19, p ≤ 0.01**[d] **Lifetime avg: -0.16, p ≤ 0.01**[d]
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. (1993b)	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 obstetric 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	**Concurrent: -0.18 (-0.26, -0.10)** Neonatal: -0.15 (-0.33, 0.03) **Lifetime avg: -0.11 (-0.19, -0.03)** Prenatal: -0.04 (-0.20, 0.12)
Dietrich et al. (1993b)	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	**Concurrent: -0.33 (-0.60, -0.06)** Lifetime avg: -0.13 (-0.35, 0.09) Neonatal: -0.03 (-0.42, 0.36) Prenatal: 0.15 (-0.26, 0.56)
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	**Early childhood: -2.0 (-3.8, -0.21)** Lifetime avg: -1.6 (-3.7, 0.52) Prenatal: 0.26 (-0.67, 1.5)
Lanphear et al. (2005)	1,333 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	**Concurrent: -0.23 (-0.32, -0.14)** **Peak: -0.20 (-0.29, -0.11)** **Lifetime avg: -0.15 (-0.22, -0.09)** **Early childhood: -0.14 (-0.23, -0.06)**
Pocock et al. (1994)	Meta-analysis of 5 prospective (over 1,100 children and 14 cross-sectional studies (3,499 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	**Early childhood: -2.7 (-4.1, -1.2)** Postnatal mean: -1.3 (2.9, 0.37) Around birth: 0.26 (-1.5, 2.0)
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): 7.8 (2.5, 24.6) 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: -3.9 (-6.5, -1.4)** Early childhood avg: 0.10 (-3.9, 4.1) Later childhood avg: 0.17 (-1.4, 1.8)

Study	Population/Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[b]
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	**Early childhood: -0.58 (-0.99, -0.17)** Prenatal: -0.48 (-5.7, 4.7), blood Pb >10 µg/dL vs. <3 µg/dL[c] Concurrent: -0.46 (-1.5, 0.56)
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	**Concurrent: 1.45 (0.04, 2.86)** **Prenatal: 1.16 (0.02, 2.3)**
Leviton et al. (1993)	1,923 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. (1994a)	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	**Early childhood: 1.8 (0.49, 3.1)** Prenatal: -0.31 (-1.7, 1.07)
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 at 15-17 yr	**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 at 15-17 yr	**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 = Wechsler Intelligence Scale for Children, NR = Not reported

[a]Studies are organized by age of neurodevelopmental assessment. Effect estimates are presented in order of increasing magnitude, with statistically significant results in bold.

[b]Effect estimates are standardized to a 1 µg/dL increase in blood Pb level in analyses of blood Pb as a continuous variable.

[c]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.

[d]Sufficient data were not provided in order to calculate 95% CI.

Prenatal and neonatal (10 days after birth) blood Pb levels also were associated with cognitive function and behavioral outcomes in children examined at school-age (ages 4-17 years) (Table 5-13). Concurrent blood Pb levels generally were estimated to have similar or larger magnitudes of effect (Wasserman et al., 1998; Wasserman et al., 1994;

Dietrich et al., 1993a; Dietrich et al., 1993b; Bellinger et al., 1992; Dietrich et al., 1992). Studies conducted in the Cincinnati cohort examined diverse neurodevelopmental effects and found that prenatal and neonatal blood Pb levels were associated with impairments in behavior and auditory processing (Ris et al., 2004; Dietrich et al., 2001; Dietrich et al., 1992) but not cognitive function or motor function in children at age 6 years or in adolescence (Bhattacharya et al., 2006; Bhattacharya et al., 1995; Dietrich et al., 1993a; Dietrich et al., 1993b). These findings suggest that the effects of prenatal Pb exposure may vary among different populations and specific endpoints.

Early childhood blood Pb levels were associated with diverse neurodevelopmental effects assessed later in childhood and into early adulthood in both recent (Chandramouli et al., 2009; Min et al., 2009; Miranda et al., 2009) and previous studies that did not compare various lifestages of Pb exposure (Yuan et al., 2006; Cecil et al., 2005; Tong et al., 2000). In a meta-analysis of results from five cohort studies (Pocock et al., 1994), a larger decrease in FSIQ was estimated for an increase in peak (around age 2 years) blood Pb level than for blood Pb level measured around birth or after age 2 years (Table 5-13). 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, associations with early childhood or peak blood Pb levels may reflect the greater reliability of neurodevelopmental assessments at later ages when the processes modalities of children 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. Further, due to the correlation of blood Pb levels over time, it is difficult to assess whether early childhood or peak blood Pb levels were serving as surrogates of concurrent or cumulative blood Pb levels.

As presented in Table 5-13, several studies of school-aged children estimated larger blood Pb-associated decreases in neurodevelopmental function for concurrent or lifetime average (range: 5 to 13 years) blood Pb levels than for 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 (5- to 10-year average), and age 2 year blood Pb levels all were negatively associated with IQ measured between ages 5 and 10 years, with the larger magnitude of decrease associated with increases in concurrent and peak blood Pb levels (Lanphear et al., 2005). Childhood average blood Pb levels (Lanphear et al., 2005; Dietrich et al., 1993a; Dietrich et al., 1993b) and deciduous tooth Pb levels (Bellinger et al., 1994a) have been associated with neurodevelopmental effects. While the ages of children varied among these studies as did the years over which blood Pb levels were averaged, the results nonetheless indicate cumulative Pb exposure over multiple years may contribute to neurodevelopmental effects in children. Associations with concurrent blood Pb level also were demonstrated consistently in

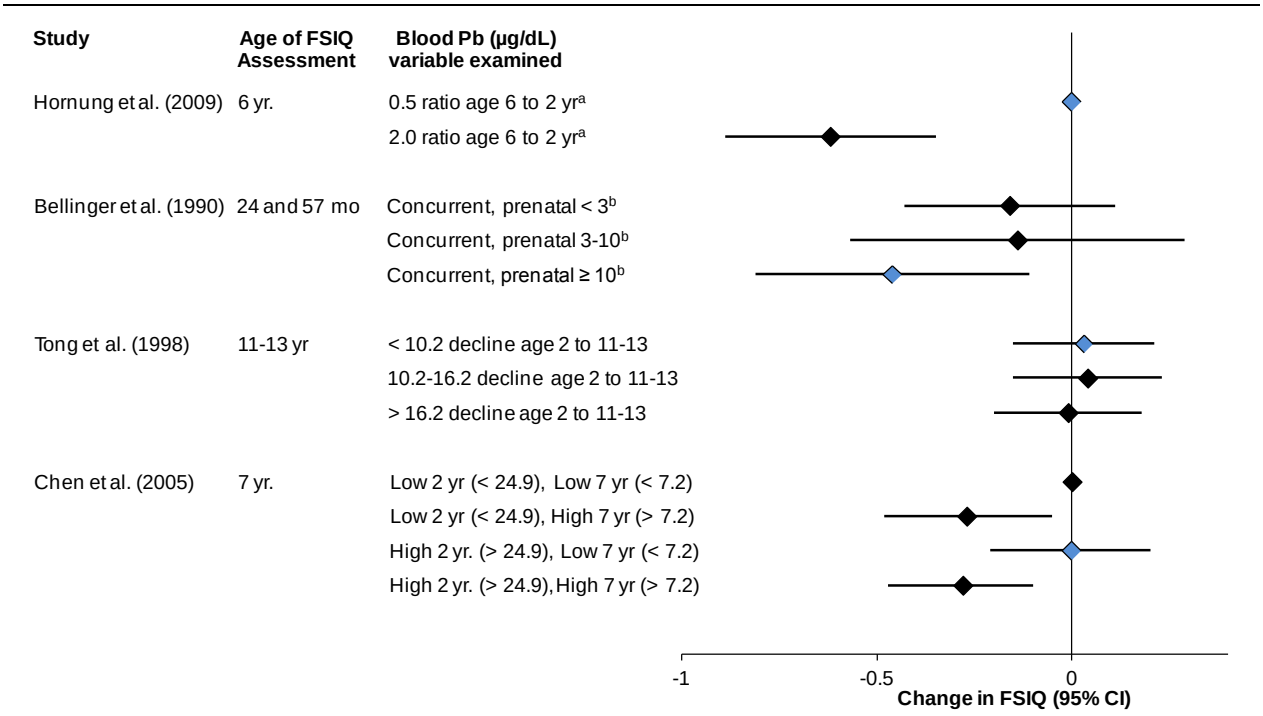
studies without comparisons to blood Pb levels during other lifestages (Figures and Tables in Sections 5.3.2.1 and 5.3.3.1).

Some studies have aimed to improve the characterization of important lifestages of Pb exposure by examining children in whom blood Pb levels are weakly correlated over time (i.e., children whose blood Pb level ranking changed over time) (Hornung et al., 2009; Schnaas et al., 2006; Chen et al., 2005; Tong et al., 1998; Bellinger et al., 1990). Collectively, the results did not conclusively demonstrate stronger findings for concurrent blood Pb level. However, with the exception of Schnaas et al. (2006), the results did not preclude an association with concurrent blood Pb level. Schnaas et al. (2006) followed children in Mexico City prenatally through age 10 years and found prenatal blood Pb levels (maternal blood at 28-36 weeks of pregnancy) to be weakly correlated with repeated measures of blood Pb between ages 1 and 10 years (Pearson $r \leq 0.25$). In a mixed effects model that included prenatal and multiple postnatal blood Pb measures, only prenatal blood Pb level was associated with a decrement in FSIQ (Table 5-13). Analysis of variance inflation factors indicated a lack of collinearity among the serial blood Pb measures.

Tong et al. (1998) found that higher early-life blood Pb level was associated with a larger deficit in IQ (Figure 5-21 and Table 5-14). 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 FSIQ 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 smaller decline (<10 µg/dL). These findings indicated an influence of higher blood Pb levels early in life despite declines in blood Pb with age and a persistence of Pb effects. The results do not preclude an independent association with concurrent blood Pb level.

In several different cohorts of U.S. children, larger decrements in cognitive function were estimated for concurrent blood Pb levels with consideration of blood Pb levels measured earlier in childhood (Hornung et al., 2009; Chen et al., 2005; Bellinger et al., 1990) (Figure 5-21 and Table 5-14). In the Boston cohort, Bellinger et al. (1990) found that at age 57 months, FSIQ, as assessed by McCarthy GCI, was similar between children with higher (≥ 10 µg/dL) and lower (<3 µg/dL) prenatal cord blood Pb levels. Additionally, higher concurrent blood Pb level (age 57 months) was associated with the largest decline in GCI score over time (score at age 57 months-score at age 24 months) among children

with high prenatal blood Pb levels (≥ 10 µg/dL), which pointed to a larger decrease in FSIQ in children with both high early and concurrent blood Pb levels (Figure 5-21 and Table 5-14). The findings indicated that by age 5 years, children with higher prenatal blood Pb levels appeared to recover the Pb-associated decrements in cognitive function unless concurrent blood Pb levels remained high. The investigators also demonstrated that 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. Collectively, these results suggest that cognitive development is not fixed early in childhood and can be affected negatively or positively by postnatal influences.



[a]Values represent the ratio of blood Pb level at age 6 years to that at age 2 years.

[b]Results represent the decrease in FSIQ per 1 µg/dL increase in concurrent blood Pb level in children in different groups of prenatal cord blood Pb levels. FSIQ scores were standardized to their standard deviation. Effect estimates in blue represent associations for higher prenatal or early childhood blood Pb levels relative to concurrent blood Pb levels.

Note: Effect estimates represent associations between concurrent blood Pb level and cognitive function (standardized to standard deviation) in children. Studies are presented in order of increasing prenatal level.

Figure 5-21 Associations of cognitive function in children with different degrees of changes in blood Pb levels over time.

Table 5-14 Additional characteristics and quantitative results for studies presented in Figure 5-21

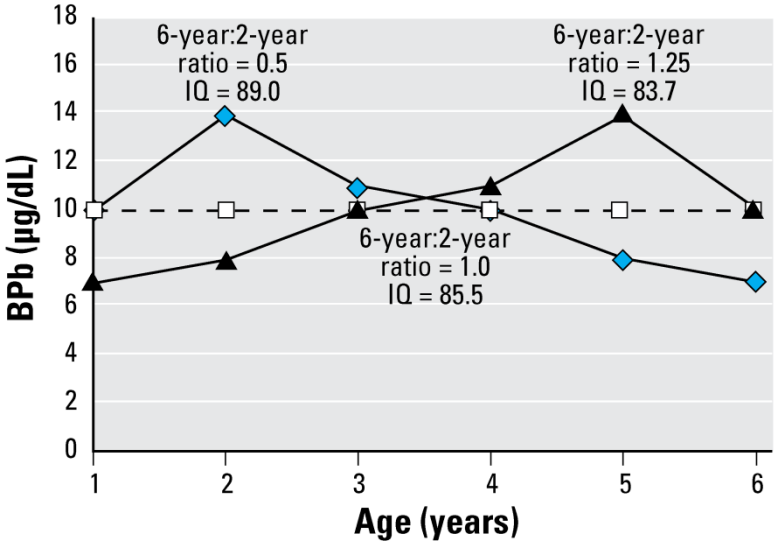
Study	Population/ Location	Blood Pb Levels (µg/dL)	Statistical Analysis	Outcome	Effect Estimate (95% CI)[a]
Hornung et al. (2009)	462 children followed from birth (1979-1984) to age 6 yr Rochester, NY and Cincinnati, OH	Geometric mean (5th-95th): Peak: 13.6 (4.6-34.4) Early childhood: 8.9 (3.0-23.8) 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)
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[b] 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)
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)[c] 10.2-16.2 µg/dL decline: 0.04 (-0.15, 0.23)[c] >16.2 µg/dL decline: -0.01 (-0.20, 0.18)[c]
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 Children underwent chelation therapy	Mean (SD): Age 2 yr: 26.2 (5.1) Age 5 yr: 12.0 (5.2) Age 7 yr: 8.0 (4.0) Low age 2 yr: <24.9 Low age 7 yr: < 6.2	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, Low age 7: 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)

[a]Effect estimates represent the cognitive function score or change in score over time standardized to its standard deviation.
[b]Effects 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.
[c]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.
[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.

Pooling the Cincinnati and Rochester cohorts (n = 397), Hornung et al. (2009) 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) (Figure 5-21, Figure 5-22, and Table 5-14). 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.

As part of the multicenter TLC, Chen et al. (2005) also found higher concurrent blood Pb level (≥ median 7.2 µg/dL) to be associated with lower IQ at age 7 years, regardless of whether blood Pb level at age 2 years was low or high (less than or greater than the median of 24.9 µg/dL, respectively). Blood Pb levels at ages 2 and 7 years were weakly correlated (r = 0.27). It is important to note that children participating in TLC had undergone chelation therapy due to high blood Pb levels (20-44 µg/dL) at age 12 to 33 months, and the findings may have limited application to the general population of children currently living in the U.S. In fact, in all of the studies that examined weakly correlated serial blood Pb measurements, blood Pb levels were higher than those currently measured in U.S. children. Additionally, in several study populations, children experienced large decreases in blood Pb levels over time. It is unclear whether these findings would apply to children 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. (2009)
Note: All three patterns have an identical mean blood Pb level of 10 µg/dL.

Figure 5-22 Estimated IQ in combined Cincinnati and Rochester cohorts, for three 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 (white squares).

To conclude, in the collective body of epidemiologic evidence in children, it is difficult to ascertain which lifestage of Pb exposure is associated with the greatest risk of Pb-associated neurodevelopmental effects. Associations have been observed with prenatal, early-childhood, lifetime average, and concurrent blood Pb levels as well as with childhood tooth Pb levels. Comparisons among different lifestages of exposure is complicated further by the fact that blood Pb levels in children, although highly affected by recent exposure, are also influenced by Pb stored in bone due to rapid growth-related bone turnover in children relative to adults. Thus, concurrent blood Pb level in children also may reflect body burden (Section 4.3.4.6). 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 school-age and into adolescence. With additional results from studies described in Sections 5.3.2.1 and 5.3.3.1, the weight of epidemiologic evidence supports associations of concurrent blood Pb level with neurodevelopmental effects in children. These findings are consistent with the understanding that the nervous system continues to develop throughout childhood.

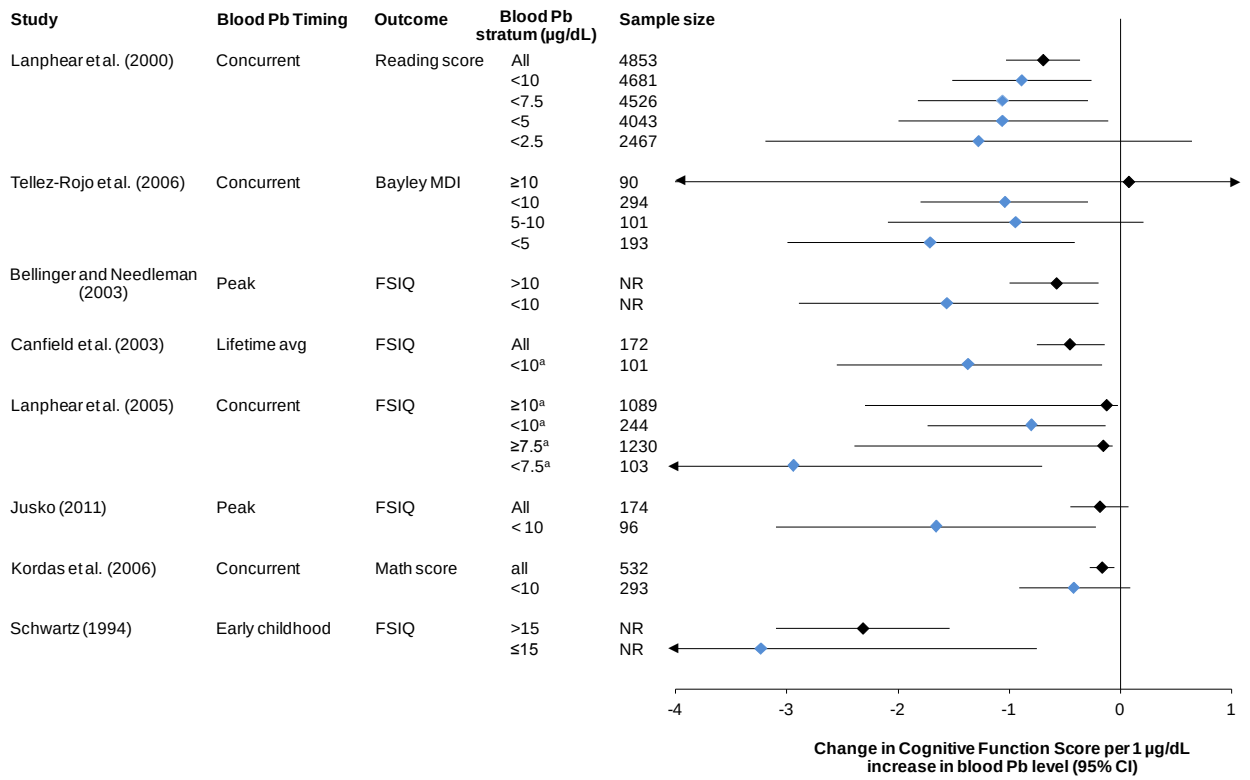
5.3.10 Examination of the Lead Concentration-Response Relationship

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 effects 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 Pb AQCD (U.S. EPA, 2006b). Furthermore, in the 2006 Pb 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 5-23 and Table 5-15). As lower concentrations of Pb exposure are being used experimentally, the toxicological literature also has reported nonlinear concentration-response relationships for various endpoints, including those coherent with cognitive function decrements observed in children. Also consistent with the epidemiologic literature, some toxicological studies have shown larger magnitudes of effect (absolute effects) in lower Pb exposure groups (relative to control groups) than in the higher exposure groups.

In epidemiologic studies, a supralinear concentration-response relationship was observed for concurrent, early childhood, and lifetime average blood Pb levels. Most

epidemiologic studies used a blood Pb level of 10 µg/dL to define lower and higher blood Pb levels. Several of these observations were made in groups of children aged 2 to 10 years, and the mean blood Pb levels in the lower strata of blood Pb levels were in the range of 3-5 µg/dL (Bellinger, 2008; Canfield, 2008; Hornung, 2008; Rojo-Tellez, 2008). Except for the pooled analysis, lower strata of blood Pb levels comprised at least 55% of the study population and consequently the concentration-response relationships calculated for these lower strata likely are not outliers comprised a minimum 55% of the study population and consequently the concentration-response relationships calculated for these lower strata are not outliers or unrepresentative of the overall study population. 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 concurrent blood Pb levels <10 µg/dL. A linear model was found to be inadequate as the polynomial terms for concurrent blood Pb were statistically significant. These findings were corroborated in a separate analysis by Rothenberg and Rothenberg (2005) who found that the log-linear model fit the relationship between blood Pb level and IQ better than a linear model did.

A few studies demonstrated larger Pb-associated decreases in cognitive function with blood Pb levels < 5 µg/dL. Tellez-Rojo et al. (2006) estimated a larger decrement in IQ per unit increase in blood Pb level for children (age 2 years) with concurrent blood Pb levels <5 µg/dL compared with children with levels 5-10 µg/dL, and > 10 µg/dL (Figure 5-23 and Table 5-15). In an NHANES analysis 1989-1994, Lanphear et al. (2000) found larger decrements in reading and math skills and memory per unit increase in blood Pb level in children with concurrent blood Pb levels <2.5 µg/dL compared with children with levels <5 µg/dL, <7,5 µg/dL, <10 µg/dL, and all subjects. However, as the study population included adolescents, the oldest of whom were 16 years of age and born 1972-1978, higher Pb exposures earlier in childhood may have contributed to associations.



Note: Studies are presented in order of increasing mean blood Pb level. Strata refer to peak blood Pb level measured in child at any point during follow up. 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 the highest blood Pb stratum. Blue symbols represent effect estimates in lower blood Pb strata

Figure 5-23 Comparison of associations between blood Pb level and cognitive function among various blood Pb strata.

Table 5-15 Additional characteristics and quantitative results for studies presented in Figure 5-23

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)	4,853 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)
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 (-10, 9.2)[b] -1.04 (-1.8, -0.30)[b] -0.94 (-2.1, 0.2)[b] -1.71 (-3.0, -0.42)[b]
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 (-1.0, -0.2)[b] -1.56 (-2.9, -0.2)[b]
Canfield et al. (2003a)	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	-0.46 (-0.76, -0.15) -1.37 (-2.56, -0.17)
Lanphear et al. (2005)	1,333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts	Concurrent Median (5th-95th) 9.7 (2.5-33.2)	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)
Jusko et al. (2011)	194 children in Rochester, NY followed from age 6 mo (1994-1995) to age 6 yr.	Peak Mean (SD): 11.4 (7.3)	Linear regression model adjusted for sex, birth weight, transferrin saturation, maternal race, maternal IQ, maternal education, HOME score, family income, and maternal prenatal smoking	WPPSI-R at age 6 yr	All < 10	-0.19 (-0.46, 0.07) -1.66 (-3.1, -0.23)
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)
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 school-age	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]95% CIs calculated from reported p-value.

Several studies also found a nonlinear blood Pb-cognitive function relationship in nonparametric regression analyses using lowess with smoothing parameters or using splines (Min et al., 2009; Jusko et al., 2008; Schnaas et al., 2006). Min et al. (2009) performed a formal test of nonlinearity using nonparametric regression by testing the statistical significance of a restricted cubic spline term for blood Pb level. Although the

term was not statistically significant, the concurrent blood Pb level-FSIQ curve appeared to be more negative at blood Pb levels < 7 µg/dL. In a similar analysis in a Mexico City cohort, Schnaas et al. (2006) found a more negative blood Pb-FSIQ slope at concurrent blood Pb levels < 6 µg/dL and found a test of nonlinearity to be statistically significant. Among children in India ages 3-7 years, Palaniappan et al. (2011), most associations of concurrent blood Pb level with various indices of executive function in nonparametric regression analyses were found to be linear.

Studies of adults have not assessed widely whether the relationship between blood or bone Pb level and cognitive performance is described better with a linear or nonlinear function. In the various NHANES analyses, only log-linear models were used to fit the data (Krieg et al., 2010; Krieg and Butler, 2009; Krieg et al., 2009). Nonlinearity in the BMS and NAS cohorts was examined with the use of quadratic terms, penalized splines, or visual inspection of bivariate plots (Bandeen-Roche et al., 2009; Weisskopf et al., 2007a; Shih et al., 2006). There was some evidence for nonlinearity for some (Figure 5-12 and Figure 5-13) but not all cognitive tests or all subjects in the NAS cohort. For example, Wang et al. (2007a) found that among NAS men with an HFE variant, there was a steeper Pb-associated decline in MMSE score at higher tibia Pb levels (20-25 µg/g, Figure 5-13). In the BMS cohort, observations of a statistically nonsignificant quadratic term (Shih et al., 2006) or spline (Bandeen-Roche et al., 2009) for tibia Pb indicated that a linear model adequately fit the relationship between tibia Pb level and various tests of cognitive performance.

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, and saturation of biological mechanisms at higher levels; larger proportions of at-risk populations at lower exposure levels; and variations in other risk factors among exposure levels (Stayner et al., 2003). Other explanations for nonlinearity include differential activity of mechanisms 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 cognitive function in children has not been examined widely in epidemiologic studies to date. However, in several different populations, higher blood Pb levels have been measured in potentially at-risk 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 at-risk populations at lower blood Pb levels (Lanphear et al., 2005; Lanphear et al., 2000). 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 overwhelmingly larger effects of these other risk factors (Schwartz, 1994). Several studies found statistically 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 5-7). Few studies have compared blood Pb level effect estimates among groups in different sociodemographic strata, and the limited data are mixed. Greater Pb-associated neurocognitive deficits were reported in low-SES groups 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 level is associated with deficits in cognitive 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 observed nonlinear concentration-response relationship.

Rothenberg and Rothenberg (2005) formally assessed the influence of residual confounding on the nonlinear blood Pb-FSIQ 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.

Bowers and Beck (2006) postulated that a supralinear slope necessarily will be found when modeling a relationship between a log-normally distributed variable and a normally distributed variable. However, as discussed in the 2006 Pb AQCD, this modeling strategy was not employed in the epidemiologic analyses showing a supralinear concentration-response function. IQ scores generally were not forced into a normal distribution. Four of the seven studies included in the pooled analysis by Lanphear et al. (2005) did not use normalized IQ scores, and scores were not normalized in the pooled analysis (Hornung et al., 2006). Further, a log-linear model (a linear relationship between IQ and the log of blood Pb) provided the best fit of the pooled data.

In support of the nonlinear associations between blood Pb levels and cognitive function observed in children, toxicological studies provided some evidence of nonlinear relationships between Pb exposure and effects related to impaired learning and memory

in animals. Multiple studies showed U- or inverse U-shaped curves with lower exposures of Pb having different or often the opposite effect from higher exposures. Coherent with epidemiologic findings, results summarized across multiple studies in multiple species demonstrated that lower Pb exposures increased FI response rates relative to controls and higher Pb exposures decreased FI response rates (Cory-Slechta, 1994). Consistent with these findings, Rossi-George et al. (2011) found that 50 ppm gestational plus lactational Pb exposure when combined with stress increased FI responses of 2-month old rats whereas 150 ppm Pb exposure with stress did not affect FI responses. It is important to note that the larger effects observed with lower Pb exposures were less consistently observed with longer duration exposures (e.g., 8-11 months) (Rossi-George et al., 2011; Cory-Slechta, 1990). As discussed in Section 5.3.2.2, FI responses measure learning and memory by reflecting the ability of animals to learn to respond to one schedule of reinforcement and change responses according to a change in reinforcement.

Toxicological studies provided additional support for nonlinear relationships between Pb exposure and neurodevelopmental effects by finding that lower and higher Pb exposures differentially activate mechanisms underlying such effects. Gilbert et al. (1999) found reduced LTP with Pb exposures ranging between 0.1 and 0.5% but not 1.0%. LTP is one indication of synaptic plasticity (Section 5.3.8.4) and is considered to contribute to learning and memory. Likewise, glutamate release in the hippocampus was reduced in animals with blood Pb levels 27-40 µg/dL but not with blood Pb levels of 62-117 µg/dL (Lasley and Gilbert, 2002). Glutaminergic neurotransmission via its NMDA receptor has been implicated in learning and memory (Section 5.3.8.8).

Dopaminergic neurotransmission is involved in many CNS processes including cognition, behavior, and motor function. The shape of the Pb-DA concentration-response relationship varied among toxicological studies. Some studies found that lower Pb exposures (~ 50 ppm) did not affect or increased DA activity relative to controls and higher Pb exposure (109-250 ppm) (Leasure et al., 2008; Virgolini et al., 2005; Lewis and Pitts, 2004). However, compared with control and lower Pb exposures, higher Pb exposures (109 or 150 ppm) were found both to increase and impair DA activity (Leasure et al., 2008; Virgolini et al., 2005). These differential responses of DA may be related to the diverse actions of DA in different regions of the brain and on a range of CNS effects. For example, the increased dopamine turnover with 50 ppm Pb exposure may explain the greater spontaneous and amphetamine-induced motor activity in males induced by 50 ppm GLE (Leasure et al., 2008).

Lower and higher Pb exposures also were found to differentially affect calcineurin enzyme activity; activity was inhibited by higher Pb exposure and stimulated by lower Pb exposure (Kern and Audesirk, 2000). While calcineurin activity has been found to

modulate learning, LTP, and behavior in animals, studies have found lower calcineurin activity to be associated with both impaired and improved effects related to learning (Zeng et al., 2001). Thus, it is uncertain whether altered calcineurin activity contributes to the nonlinear relationships observed between Pb exposure and learning. Nonetheless, results indicated that lower and higher Pb exposures may have different modes of action. At lower concentrations, Pb may displace calcium at its binding sites on calmodulin and by acting as a calmodulin agonist at the catalytic A subunit of calcineurin, stimulate calcineurin activity. At higher Pb exposure, Pb may bind directly to a separate calcium-binding B subunit, override the calmodulin-dependent effect and turn off the activity of calcineurin. Similarly, Lasley and Gilbert (2002) also indicated that at higher concentrations (i.e., 1%), Pb stimulated gluatamate release by acting as a calcium mimetic.

While epidemiologic studies have not examined widely the shape of the concentration-response relationship for other nervous system effects, toxicological studies have found nonlinear relationships for diverse outcomes. U-shaped Pb concentration-response relationships were found for rotarod performance, amphetamine-induced motor activity, and latency to fall from rotarod (Leasure et al., 2008). Inverted U-shaped Pb concentration-response relationships were found for histological parameters such as the numbers of rod photoreceptors and bipolar cells, 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). 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 were significantly lower in the 50 ppm exposure group than in the control or 150 ppm Pb exposure group.

Sensory organ functions in animals also have shown to be differentially affected by lower versus higher Pb exposure (developmental Pb exposurefrom gestation to PND10, pup blood Pb levels 12, 24, and 46 µg/dL. Inverted U-shaped dose-response curves have been observed for rod photoreceptor numbers or neurogenesis (Giddabasappa et al., 2011) and retinal thickness (Fox et al., 2010). Thus, these dichotomous histological findings may give insight to the complex sensory organ findings that vary by exposure window and exposure dose (Section 5.3.4.3).

The supralinear concentration-response relationship widely documented for Pb is consistent with the lack of a threshold for Pb-associated neurodevelopmental 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 data from the Boston prospective cohort by regressing FSIQ 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 FSIQ 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 FSIQ 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, was the limited examination of associations in populations or subgroups 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. In the various NHANES analyses of children, a large proportion of children had blood Pb levels <1 µg/dL, for example, 50% of subjects in the 2001-2004 NHANES population (Braun et al., 2008).

More sensitive quantification methods have improved the detection limits, for example, from 0.6 µg/dL in 1999-2002 NHANES to 0.025 µg/dL in 2003-2004 NHANES. In analyses of children in multiple blood Pb quantiles below 1 µg/dL, 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). Despite the availability of large proportions of subjects at blood Pb levels below 1 µg/dL, the ability to discern a threshold for Pb-associated neurodevelopmental effects is limited due to the large proportions of adolescents in NHANES analyses who were born the 1970s and whose higher past exposures may have contributed to associations observed with concurrent blood Pb levels. Nonetheless, several recent studies reported associations between blood Pb levels and deficits in cognitive and behavioral endpoints in children ages 8-11 years with mean or quantile blood Pb levels <2 µg/dL (Cho et al., 2010; Kim et al., 2009b; Miranda et al., 2009). In comparisons of various quantiles of blood Pb, 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. Collectively, these new findings in children, do not provide evidence for a threshold for the neurodevelopmental effects of Pb in the range 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 and Smith, 1992), approximately 65-fold lower than that currently measured in U.S. populations and lower than the levels at which neurodevelopmental effects have been observed (1 µg/dL). Thus, the current evidence does not preclude the possibility of a threshold for neurodevelopmental effects in children existing in the large range of blood levels between 1 µg/dL and preindustrial "background" levels.

To conclude, several studies found a supralinear blood Pb-cognitive function concentration-response relationship in children based on comparisons of effect estimates in lower and higher strata of blood Pb level and nonparametric regression. Supporting evidence was provided by some toxicological studies that showed that lower Pb exposures induced learning and memory impairments in animals compared to control exposures or higher Pb exposures. While explanations for this supralinear relationship have not been well characterized by epidemiologic studies, recent toxicological studies that examined lower concentrations of experimental Pb exposure suggest that mechanisms may be differentially activated at lower and higher Pb exposures.

Nonlinear concentration-response relationships were found for effects such as LTP, hippocampal glutamate release, and calcineurin expression. Observations of associations between blood Pb levels and deficits in cognitive and behavioral endpoints in children (ages 8-11 years) with mean or range of blood Pb levels ≤ 2 µg/dL (Cho et al., 2010; Kim et al., 2009b; Miranda et al., 2009) do not provide evidence for a threshold for neurodevelopmental effects of Pb in the range of blood Pb levels examined to date.

5.3.11 Confounding in Epidemiologic Studies of Nervous System Effects

In addition to Pb exposure, many factors influence cognitive function and behavior in children, including parental IQ and education, SES of the family, quality of the caregiving environment, and other environmental exposures (Wasserman and Factor-Litvak, 2001). These other risk factors often are correlated with blood, tooth, and bone Pb levels, thus, a major challenge to observational studies examining associations of Pb biomarker levels with cognitive and behavioral function in children has been the assessment and control for potential confounding factors. By definition, a confounder is associated with both the exposure and the outcome and consequently has the potential to bias the association between the exposure of interest and the outcome. Epidemiologic studies of Pb biomarkers in children have most commonly examined potential confounding by quality of the caregiving environment (i.e., HOME score), parental IQ, and SES-related variables such as parental education, household income, and the Hollingshead Four-Factor Index of Social Position, which incorporates education and

income of both parents. A relatively smaller number of studies have considered confounding by other environmental exposures, measures of parental substance abuse, and psychopathology. Studies have varied with respect to the number of potential confounders examined, with some studies considering multiple SES-related variables and other studies focusing on a smaller set. It is important to note that the extent of confounding by a particular variable likely is study-specific, i.e., dependent on the population examined. Thus, the impact of adjustment for specific covariates on the Pb effect estimate also is likely to be study-specific.

Studies have used various methods to control for confounding, including examining a homogeneous population with respect to SES, examining populations in which factors are not correlated, conducting multivariate regression, characterizing the magnitude of change in the blood Pb level effect estimate with adjustment for a covariate, and examining associations in different strata of a covariate. While no single method is without limitation, the consistency of findings among different methodologies substantiates independent associations of blood Pb level with cognitive function decrements and behavioral problems in children. The evidence derived from each of these control strategies is discussed below.

In the Boston Prospective Study, potential confounding by SES was largely controlled for by study design. The study subjects were generally middle- to upper-middle-class children with married, college-educated parents. Hence, the potential for confounding by SES in this study was considerably less compared to other studies examining the same associations. Yet, in this cohort, blood Pb levels measured prenatally, at age 2 years, and integrated over ages 2 and 5 years were associated decrements in full-scale IQ and various measures of executive function (Stiles and Bellinger, 1993); (Bellinger et al., 1992; Bellinger et al., 1990). In some analyses of this cohort, larger effects were estimated as compared with other studies.

Studies also have demonstrated associations between blood Pb levels and cognitive function in populations in which blood Pb levels were not associated with SES-related variables (Factor-Litvak et al., 1999; Bellinger et al., 1987). In the Boston cohort, parental education, social class, and HOME score were similar among low (<3 µg/dL), medium (6-7 µg/dL), and high (≥ 10 µg/dL) cord blood Pb level groups. Nonetheless, adjusting for these and other demographic variables, Bellinger et al. (1987) found that children in the high cord blood Pb group had a 4.8-point lower Bayley MDI score at age 24 months than did children in the low cord blood Pb group. In the Yugoslavia cohort, blood Pb levels at age 4 years were higher in groups with higher maternal education, maternal IQ, and HOME score in one city and were lower in another city. Among all children, higher blood Pb level was associated with lower FSIQ and specific indices of

learning and memory and with greater depression and withdrawn behavior (Factor-Litvak et al., 1999).

The primary method used by epidemiologic studies to control for potential confounding has been multivariate regression. This was the main method employed in recent studies that examined children with blood Pb levels in the range of current U.S. levels. Some studies selected a set of covariates based on a priori knowledge, whereas others selected particular covariates based on their association with the outcome in a model with all potential covariates and/or a greater than 10% change in the blood Pb level effect estimate. Studies also varied in the number of variables included in models as potential confounders. Some included multiple SES-related variables, whereas others analyzed one or two variables. Regardless of the method used to select model covariates or the number of covariates included, studies consistently demonstrated associations of higher blood Pb level with lower cognitive function and greater behavioral problems. This consistency suggests that confounding by particular variables may be specific to the population examined and that no single measured variable or set of variables fully accounts for the associations observed with blood Pb levels and neurodevelopmental effects in children.

The consistency of associations across populations with different SES and across studies examining different covariates was reinforced in pooled and meta-analyses (Marcus et al., 2010; Lanphear et al., 2005; Schwartz, 1994). Pooling data from seven international longitudinal cohorts, Lanphear et al. (2005) found similar blood Pb-FSIQ effect estimates (-2.6 to 8.6% difference) among models, each with one study omitted. These results indicated that the pooled estimate is relatively stable despite between-study differences in population characteristics, including SES. In a meta-analysis, Schwartz (1994) found that individual study blood Pb-IQ effect estimates fell within a relatively narrow range despite large differences among studies in the correlation between blood Pb level and SES. A wider range of effect estimates would be expected if omitted SES factors confounded the blood Pb level association. A recent meta-analysis examined the association between blood Pb level and conduct problems in older and recent studies of children (Marcus et al., 2010). Among the studies included, adjustment for variables such as SES and home environment did little to attenuate the association between Pb and conduct problems.

Among studies that provided both unadjusted and adjusted effect estimates, most indicated statistically significant associations of blood Pb level with neurodevelopmental outcomes in children before and after adjusting for potential confounders. Although examples exist where blood Pb level was estimated to have a weaker, statistically nonsignificant effect after adjustment for potential confounders (Tong and Lu, 2001; Ernhart et al., 1989), most notably in multiple analyses of the Cleveland cohort, studies also reported stronger blood Pb level associations in covariate-adjusted models (Canfield

et al., 2003b; Factor-Litvak et al., 1999). In recent studies with population mean blood Pb levels between 5 and 10 µg/dL, associations between blood Pb levels and cognitive function (e.g., FSIQ, executive function, learning, and memory) remained statistically significant after adjusting for covariates (Min et al., 2009; Chiodo et al., 2007; Froehlich et al., 2007; Kordas et al., 2006; Schnaas et al., 2006). Although most effect estimates changed by 20-50% in multivariate models, they remained within the 95% CI of the unadjusted estimate. In particular, Schnaas et al. (2006) and Froehlich et al. (2007) found some adjusted effect estimates to be larger in magnitude compared with unadjusted estimates. Although information was not provided on the magnitude of change in effect estimates after adjustment for confounding, Chiodo et al. (2007) found statistically significant associations between blood Pb level and behavioral problems (e.g., inattention, hyperactivity, social problems, impulsivity) in models with blood Pb level alone and with covariates. These findings demonstrate that SES-related and demographic factors may partially account for but do not fully explain the associations observed between higher blood Pb level and neurodevelopmental impairments.

A challenge to separating the effects of Pb exposure from those related to SES and quality of the caregiving environment is the high correlation typically observed among these measures. In such cases, it is difficult to know how much variation in the outcome to attribute to each of the various risk factors (Needleman and Bellinger, 2001). For example, the high correlation between blood Pb level and SES may lead to an underestimation of the Pb effect when SES is added to the model. A reduction in the magnitude and statistical significance of the Pb effect estimate following adjustment for some measure of SES may be the result of the misattribution of the variance in outcome due to Pb to the variance due to SES. SES may be a proxy for Pb exposure rather than a confounder of the association of interest. This misattribution may be exacerbated when several correlated variables are included in the same model (i.e., overcontrol).

Instead of being a confounder, Pb may be on the causal pathway of the association between social class and IQ. Lower social class in urban children is closely linked to residence in older housing in poor condition that, in turn, increases exposure of children to environmental Pb and increases their risk of cognitive deficits (Clark et al., 1985). In such cases, statistical adjustment for SES will lead to the underestimation of the Pb effect (Bellinger, 2004a). One extreme example of overcontrol of this nature can be found in the New Zealand studies where investigators regularly adjusted for residence in older wooden housing, which is associated with higher exposure to Pb paint and accumulated dust and soil (Fergusson et al., 1988a, b). However, it is worth noting that, even in the models including this variable, Pb remained a statistically significant predictor of intellectual and academic under-attainment in the Christchurch Health Study. Variables related to SES have been shown to be effect modifiers in studies of Pb and child

development (Tong et al., 2000; Bellinger et al., 1990). That is, increases in blood Pb level have been associated with larger decreases in cognitive function among children from less advantaged (e.g., lower SES, lower HOME score, lower maternal IQ) household than among those from more advantaged households. Similarly, a larger decrease in cognitive function in association with higher blood Pb level was found among children with mothers with lower self-esteem, an indicator of stress and less connection with the child (Surkan et al., 2008).

In the collective body of epidemiologic evidence, higher blood Pb levels are consistently associated with cognitive function decrements and behavioral problems in children, with the weight of evidence indicating decrements in FSIQ and diverse measures of executive function and inattention and hyperactivity, respectively. These associations have been observed in diverse populations in the U.S., Mexico, Europe, Asia, and Australia. Associations have been observed across studies that use different methods of control for confounding and adjust for different potential confounders. While no single method is without limitation, the consistency of findings among different methodologies and sets of covariates substantiates independent associations of blood Pb level with cognitive function decrements and behavioral problems in children. In addition, Pb exposure has been extensively studied in animals that produce blood Pb levels in the range of those examined in children. Experimental animal studies are not vulnerable to confounding by such factors as social class and correlated environmental factors. Adding further support for the independent associations of blood Pb levels with neurodevelopmental outcomes in children is the coherence of findings in animal studies for Pb-induced impairments in tests of learning, inattention, and impulsivity, especially tests that are directly homologous to those in children, i.e., spatial memory, rule learning and reversal, and response inhibition. Further, an extensive body of toxicological evidence for Pb-induced changes in brain physiological processes that mediate cognition and behavior, including changes in neurogenesis, synaptic pruning, and neurotransmitter function in the frontal lobe and striatum of the brain provides biological plausibility for observations in children.

5.3.12 Public Health Significance of Associations between Lead Biomarkers and Neurodevelopmental Effects

As described in Section 5.3.2.1, most studies found that a 1 µg/dL increase in blood Pb level was associated with fractional decrements in FSIQ in school-aged children (Figure 5-2 and Table 5-3) and Bayley MDI in infants (Table 5-4). Similarly, a 1 µg/dL increase in blood Pb level typically was associated with decreases in specific cognitive abilities (Figure 5-5 and Table 5-5) and increases in inattention and hyperactivity (Section 5.3.3.1,

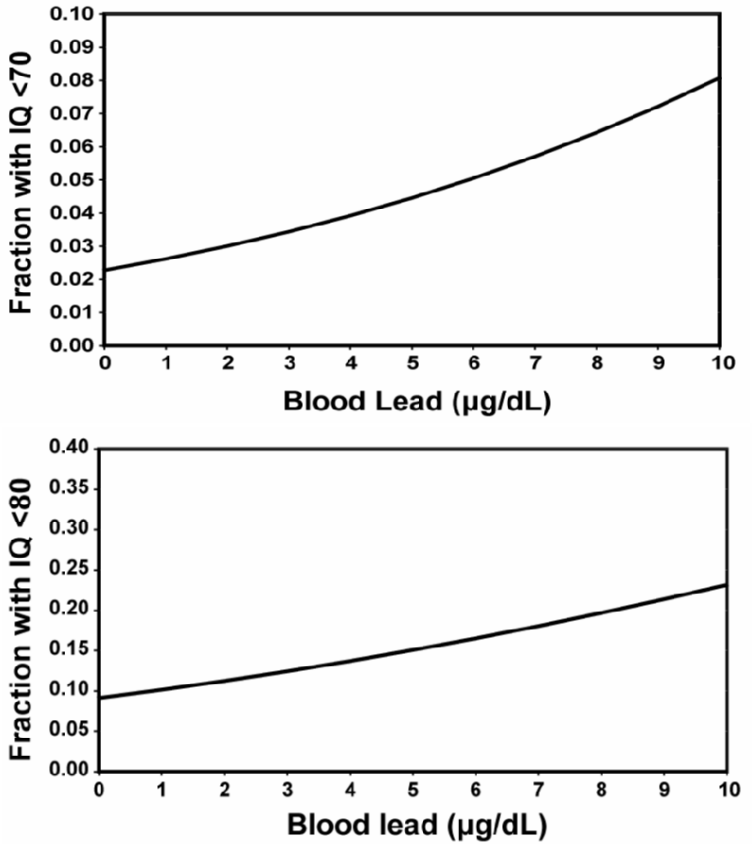
Figure 5-14 and Table 5-9) on the order of less than 1 standard deviation. These findings have generated discussion of the public significance of blood Pb level-associated changes in cognitive function and behavior in children, specifically, whether these fractional changes have consequences on the health and life-success of individuals. The World Health Organization definition of health is the following: "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (WHO, 1948). By this definition, even decrements in health status that are not severe enough to meet diagnostic criteria might be undesirable if they reflect a decrement in the well-being of an individual. Deficits in health indices or life-success may not be observable except in aggregate, at the population level. The American Thoracic Society discussed the need to consider the prevalence of exposures in the population and exposure to other risk factors in evaluating whether shifts in the population-level risk are adverse (ATS, 2000). It should also be noted that these deficits when measured in children may set affected children on trajectories more prone toward lower educational attainment and financial well-being. Thus, early deficits in children may have lifetime consequences.

It has been argued that fractional decrements in IQ points are meaningless given that these Pb-associated changes are within the 3- to 4-point standard error on a single test (i.e., the statistic that defines the range within which the true value of an individual is likely to lie) (Kaufman, 2001). However, this argument incorrectly assumes that conclusions drawn from individual-level data apply to populations. It is important to note that evidence does not indicate that the standard error is nonrandom, i.e., biased in one direction. In particular, evidence has not indicated that children with higher blood Pb levels systematically test lower than their true IQ value and that children with lower blood Pb levels test higher than their true IQ value. Thus, in a population of children, on a given assessment, some children will test lower than their true value and others will test higher than their true value. In such cases, between-group differences will be measureable on a population basis. Error in the measurement of IQ in an individual will contribute nondifferential error on a population-level and bias the association to the null.

The issue of individual-level versus population-level risk also pertains to the relevance of the magnitude of decrease in cognitive function or increase in behavioral problems per incremental increase in blood Pb level. Although fractional changes in IQ, memory, or inattention may not be consequential for an individual, they may be consequential on a population level, especially in the two tails of the distribution (Bellinger, 2007, 2004b). Weiss (1990) predicted, on purely statistical grounds, that a downward shift of five points in mean IQ, if the amount of dispersion in the distribution remained the same, should be accompanied by a doubling of the numbers of individuals with scores two or more standard deviations below the mean and a reduction by half of the number of individuals

with scores two or more standard deviations above the mean. Thus, for an individual functioning in the low range of the IQ distribution, a Pb-associated decline of several points might be sufficient to drop that individual into the range associated with increase risk of educational, vocational, and social failure.

This hypothesis is supported by several observations. In a recent study of fourth graders across the entire state of North Carolina, Miranda et al. (2009) found that higher blood Pb level measured once in each child between age 9 months and 3 years was associated with larger decreases in EOG scores in lower distribution of EOG scores (Figure 5-7). Needleman et al. (1982) found that a downward shift in the mean IQ value was associated not only with a substantial increase in the percentage of individuals achieving very low scores, but also with substantial decreases in percentages achieving very high scores. Based on the study by Bellinger et al. (1987) examining associations between blood Pb level and IQ scores in children, Weiss (1988) discussed the shift in the population distribution of IQ from a mean of 100 and a standard deviation of 15 to a mean of 95, a 5% reduction. When the mean IQ level is 100, 2.3% of the individuals in a given population would score above 130. However, with the population distribution shift and the resulting mean decline in IQ, only 0.99% of the individuals would score above 130. Weiss (1988) stated that the implication of such a loss transcends the current circumscribed definitions of risk. In a similar analysis presented in the 2006 Pb AQCD, using a blood Pb-IQ effect estimate of −0.9 points/µg/dL (based on the median of effect estimates for blood Pb levels <10 µg/dL), the fraction of the population with an IQ level less than 80 more than doubles from 9% with a blood Pb level of 0 µg/dL to 23% with a blood Pb level of 10 µg/dL (Figure 5-24). The proportion with an IQ level below 70, a level often requiring community support to live (WHO, 1992) increases from a little over 2% with a blood Pb level of 0 µg/dL to about 8% with a blood Pb level of 10 µg/dL (Figure 5-24) (U.S. EPA, 2006b).



Source: U.S. EPA (2006) Pb AQCD

Figure 5-24 Effect of blood Pb level on the proportion of the population with IQ levels <70 and <80 points.

Evidence also has demonstrated larger changes in neurodevelopmental outcomes in children with additional risk factors such as lower SES (Tong et al., 2000; Bellinger et al., 1990), mothers with low self-esteem (Surkan et al., 2008), or co-exposures to manganese (Kim et al., 2009a). Moreover, interventions that shift the population mean, in a beneficial direction, by an amount that is without clinical consequence for an individual have been shown to produce substantial decreases in the percentage of individuals with values that are clinically significant (Bellinger, 2007, 2004b).

Also supporting the public health significance of blood Pb level-associated changes in cognitive function and behavior are observations within the same cohorts that higher blood Pb level are associated with decrements in IQ and measures of executive function earlier in age and with lower academic performance, antisocial behavior, or delinquent behavior assessed later in adolescence or in early adulthood (Chandramouli et al., 2009; Wright et al., 2008; Kordas et al., 2006; Canfield et al., 2004; Kordas et al., 2004; Canfield et al., 2003b; Dietrich et al., 2001; Dietrich et al., 1993a; Bellinger et al., 1992).

Thus, the blood Pb-associated deficits measured in children may set affected children on trajectories more prone toward lower educational attainment and life success. Studies also found that higher blood Pb level is associated with measures of inattention and hyperactivity as rated by teachers or parents and by objective tests and with ADHD diagnosis or diagnostic indices (Nicolescu et al., 2010; Roy et al., 2009a; Nigg et al., 2008). These findings demonstrate that associations of blood Pb level with small changes in a health index can be markers or indicators of other changes that are likely to have occurred whose significance is more certain.

5.3.13 Summary and Causal Determination

Recent epidemiologic and toxicological studies substantiated the strong body of evidence presented in the 2006 Pb AQCD for the role of Pb in nervous system effects in two domains: cognitive function and behavior in children. Specifically, new studies of cognitive function focused on indices of learning, memory, and measures of executive function in children consistently showed Pb-related impairments. Coherence for these findings in children was provided by extensive toxicological evidence for Pb-induced impairments in homologous tests of learning and memory in juvenile rats and monkeys. New epidemiologic studies of behavior focused on and found evidence for Pb-related effects on inattention and impulsivity in children. These findings were supported by a large historical evidence base in juvenile animals. Consistent with these observations, new evidence demonstrated associations with ADHD in children. Recent studies of adults without current occupational Pb exposures continued to find associations between bone Pb levels, a biomarker of cumulative Pb exposure, and poorer cognitive function. Additional toxicological evidence for Pb-induced inhibition of neurotransmitter release, decline in synaptic plasticity, and decreases in the magnitude of LTP strengthened the biological plausibility for Pb exposure effects on decrements in both cognitive function and behavior in children. This section presents a summary of the collective body of evidence and identifies the new insights provided by recent studies.

5.3.13.1 Cognitive Function in Children

Epidemiologic studies provide robust evidence for higher blood Pb levels being associated with lower FSIQ in children ages 3-11 years (Figure 5-2 and Table 5-3), with the strongest evidence demonstrated by the consistency of association in prospective studies in diverse populations (e.g., varying distributions of blood Pb levels, SES, parental intelligence, and quality of caregiving) and the persistence of associations after adjustment for potential confounding by SES, parental intelligence, caregiving

environment, and other environmental exposures. Recent epidemiologic studies in children focused on and found associations of concurrent blood Pb levels with specific indices of cognitive function such as reading and verbal skills, memory, learning, and visuospatial processing. Several new studies shifted the weight of evidence for associations with cognitive performance to lower blood Pb levels (primarily concurrent), with populations means in the range of 2-7 µg/dL (2011; Kim et al., 2009b; Min et al., 2009; Miranda et al., 2009; Zailina et al., 2008; Chiodo et al., 2007). Evidence clearly indicates that prenatal cord and concurrent blood Pb levels are associated with lower cognitive function in younger children ages 6 months to 3 years (Table 5-4). Coherence for findings in children is derived from extensive evidence in animals that gestational and early postnatal Pb exposures resulted in impaired learning and memory, in particular, in homologous tests of spatial memory and rule learning and reversal. Reflecting the tendency to examine higher Pb exposures, toxicological studies most clearly demonstrated neurodevelopmental effects with blood Pb levels of 20-40 µg/dL in animals. However, several new studies added to the evidence for impaired learning and memory in animals with lower blood Pb levels, 8-17 µg/dL (Cory-Slechta et al., 2010; Li et al., 2009c; Niu et al., 2009; Virgolini et al., 2008a; Stangle et al., 2007).

The large body of evidence indicating associations between blood Pb levels and decrements in the diverse set of indices related to learning, memory, and other executive functions provides coherence with findings for FSIQ, a global measure of cognitive function that reflects the integration of these individual domains. Further, evidence for effects on various diverse measures of cognitive function provides biological plausibility for associations observed between blood Pb levels and factors that may be indicators of life success, including the level of educational attainment and academic performance.

5.3.13.2 Behavior in Children

Epidemiologic studies in children demonstrate associations of higher blood Pb levels with a range of behavioral problems, with the weight of evidence demonstrating associations with inattention and hyperactivity as rated by parents or teachers and as assessed using objective neuropsychological tests. Previous studies found associations with early childhood blood or tooth Pb level (i.e., ages 2-6 years), and recent studies expanded evidence to include associations with concurrent blood Pb level. Recent epidemiologic studies consistently found associations with inattention and hyperactivity in children ages 1 to 12 years with mean concurrent blood Pb levels of 2 to 5 µg/dL (Cho et al., 2010; Nicolescu et al., 2010; Plusquellec et al., 2010; Chiodo et al., 2007; Plusquellec et al., 2007), similar to those associated with cognitive function decrements. The epidemiologic findings are strengthened by observations in animals of Pb-induced

inattention and impulsivity in homologous tests of response inhibition. Such effects in animals are most clearly indicated with gestational and early postnatal Pb exposures that result in blood Pb levels of 10 to 40 µg/dL. Whereas previous evidence was inconsistent, several recent epidemiologic studies indicate associations between higher concurrent blood Pb level and higher prevalence or incidence of ADHD diagnosis and its contributing diagnostic indices in children ages 8–17 years (Cho et al., 2010; Nicolescu et al., 2010; Roy et al., 2009a; Nigg et al., 2008; Braun et al., 2006). The biological plausibility for associations with ADHD is strongly supported by the large epidemiologic and toxicological evidence base demonstrating Pb-associated increases in inattention and impulsivity, both of which are primary symptoms of ADHD. A smaller but equally consistent body of evidence indicated associations of concurrent and early childhood blood Pb levels with social misconduct in children and delinquent behaviors in adolescents and young adults (Chandramouli et al., 2009; Braun et al., 2008; Wright et al., 2008; Chiodo et al., 2007). Associations of blood Pb levels with ADHD, misconduct, and delinquency were observed in populations of children with a wide range of blood Pb levels, 1 to 11 µg/dL, all similar in the strength of evidence.

While the different behavioral indices are examined, Pb exposure also was found to affect behavior (decreased ability to escape predators or capture prey) in aquatic and terrestrial species (Sections 7.2 and 7.3).

5.3.13.3 Other Nervous System Effects in Children

A few new toxicological studies augmented the evidence for Pb-related effects to the visual system by demonstrating retinal changes in male rodent offspring in association with lower blood Pb levels (<15 µg/dL) than previously examined (20 to > 100 µg/dL) (Section 5.3.4.3). A small body of epidemiologic evidence together with a large historical base of toxicological evidence indicated associations of Pb biomarkers or exposure with impaired auditory function. Associations were found in children ranging from 4 to 19 years in age and with mean concurrent blood Pb levels of 7-12 µg/dL Section 5.3.4.1). While mood and emotional state have been examined less frequently compared with inattention and misconduct, several studies found associations of biomarkers of cumulative Pb exposure (i.e., tooth or childhood average blood Pb) and concurrent blood Pb levels with parental or teacher reports of withdrawn behavior or depression in children with mean blood Pb levels 8-28 µg/dL (Section 5.3.3.3). These findings in children are supported by a small body of toxicological studies in which prenatal plus lactational Pb exposure resulted in depression-like behavior in rodent models. Studies also reported associations of early childhood average and concurrent blood Pb levels with lower fine and gross motor function in children ages 3 to 17 years (Section 5.3.5). A common

observation across studies was finding that biomarkers of Pb exposure were associated with decrements in multiple neurodevelopmental outcomes, including cognitive function, externalizing behaviors, internalizing behaviors, and motor function, within the same population. These findings indicate that Pb exposure affects a broad spectrum of neurodevelopmental effects in children.

5.3.13.4 Factors that Modify Risk in Children

Several host and environmental factors were examined for their modification of associations between blood Pb levels and nervous system effects in children. Although each particular factor was examined in only one to two epidemiologic studies, it is important to note that several findings are supported by a larger base of toxicological evidence. Interactions of blood Pb levels with race/ethnicity and SES are not well characterized. Most investigation focused on sex-based differences. Cumulative epidemiologic evidence does not conclusively demonstrate increased risk of males or females for Pb-associated cognitive function decrements. Animal studies continue to demonstrate differential effects in males and females that vary depending on the endpoint.

The weight of evidence from animal studies continues to support interactions between developmental Pb exposure and stress. New animal studies find a potentiating effect of stress, whereby lower concentration Pb exposures impact behavior and memory with co-exposures to stress than with Pb exposures alone. In comparison, epidemiologic evidence for such interactions is sparse; however, a recent study indicated that among children with a positive social environment, as characterized by maternal self-esteem, blood Pb level is not associated with a decrease in cognitive function (Surkan et al., 2008). This finding was consistent with a previous study in rats, in which Pb-exposed animals reared in cages with enriched environments (i.e., toys) performed better in a test of spatial learning and memory than did their Pb-exposed littermates reared in traditional caging,

Studies have examined modification of Pb-associated cognitive function by genes that affect Pb toxicokinetics and/or function in neurophysiological and neurochemical processes that mediate cognition. Most investigation has focused on ALAD variants but has not consistently found to be in a consistent direction. Inconsistencies also have been observed for VDR and dopamine receptor variants. In addition to host factors, recent studies suggested that associations between blood Pb levels and cognitive function in children are greater with co-exposures to environmental tobacco smoke (Froehlich et al., 2007) and manganese (Claus Henn et al.; Kim et al., 2009b)..

5.3.13.5 Nervous System Effects in Adults

Among adults, associations of blood Pb level with the spectrum of nervous system effects (e.g., impairments in memory, attention, mood, balance, motor function) were most consistently observed in occupationally-exposed adults with blood Pb levels ≥ 14 µg/dL. Evidence in adults without occupational Pb exposures is derived primarily from cross-sectional analyses in a few different cohorts, thus the important magnitude, timing, frequency, and duration of Pb exposures contributing to the observed associations are uncertain. The weight of evidence demonstrated associations of bone Pb levels and not blood Pb levels with poorer cognitive performance, including declines in function over time (Bandeen-Roche et al., 2009; Weisskopf et al., 2007a). These findings point to a stronger effect of cumulative Pb exposure, including likely higher past exposures. One explanation for the overall weaker body of evidence in adults 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 observed for higher tibia Pb levels, representing higher long-term or cumulative Pb exposure.

Based on a smaller body of epidemiologic studies, blood and bone Pb levels were associated with essential tremor and PD, respectively, in adults (Section 5.3.7.1). However, in these case-control studies, it is difficult to establish temporality between Pb exposure and disease. Support for epidemiologic findings for PD is provided by toxicological evidence for Pb-induced decreased dopaminergic cell activity in the substantia nigra, which contributes to the primary symptoms of Parkinson's disease. Whereas evidence for association with Alzheimer's Disease in adults is weak, developmentalbut not adult-only Pb exposures of monkeys (early postnatal, PND 1-400) and rats (lactational) has been shown to induce formation of amyloid plaques, pathology that underlies Alzheimer's Disease (Section 5.3.7.2). Thus, epidemiologic studies that examined concurrent bone or brain Pb levels or occupational Pb exposure may not have examined the etiologically relevant exposure period.

Rather than examining externalizing behaviors and criminal behavior, a small body of studies of behavior in adults examined and found associations of blood (Bouchard et al., 2009) and tibia (Rajan et al., 2008) Pb levels with depression and anxiety symptoms. It is not surprising that Pb exposure may increase the risk of different nervous system endpoints in children and adults given the predominance of different neurophysiological processes operating at different ages, for example, neurogenesis and brain development in children and neurodegeneration in adults.

5.3.13.6 Neurophysiological and Neurochemical Changes

Extensive evidence from toxicological studies clearly provides the coherence and biological plausibility for effects observed in epidemiologic and toxicological studies on cognitive function, behavior, mood, and neurodegenerative conditions by characterizing underlying mechanisms (Section 5.3.8). Dopamine plays a key role in cognitive functions mediated by the prefrontal cortex and also motor functions mediated by the substantia nigra. Thus, extensive evidence for Pb-induced dopaminergic changes in animals provides mechanistic support for associations in humans between blood Pb levels and cognitive 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 brains of animals. Pb has been shown to induce 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 and are dynamic in nature. Pb exposure affects NMDA receptors, which may explain findings in animals and humans for Pb-associated symptoms of depression and withdrawn behavior. Recent toxicological studies continue to document Pb exposure effects on synapse formation, adhesion molecules, and nitrosative stress. A new study of epigenetics details that Pb exposure affects methylation patterns in rodent brains, which may provide a mechanism by which prenatal Pb exposure leads to impaired neurodevelopmental function later in life. Biological plausibility also is provided by a small body of evidence in young adults in which childhood blood Pb levels are associated with altered structure and activity in regions of the brain (assessed by MRI or MRS) that mediate cognitive processes and behavior.

5.3.13.7 Lifestages and Duration of Lead Exposure

Toxicological studies clearly demonstrate that in utero and early postnatal exposure to Pb results in impaired learning, memory, and behavior. This evidence is well supported by knowledge that processes such as neurogenesis, synaptogenesis, and synaptic pruning are most active during this developmental period. In epidemiologic studies reviewed in the 2006 Pb AQCD, biomarkers of prenatal, early life, concurrent, and cumulative Pb exposures were associated with decrements in neurodevelopmental function in children, with no clear indication that blood Pb levels measured at a particular lifestage was more strongly associated with neurodevelopmental effects (Section 5.3.9). Distinguishing among the effects of Pb exposures at different lifestages is difficult in epidemiologic studies due to the high correlations among blood Pb levels within children over time. Recent studies in children primarily examined concurrent blood Pb levels but also found association with prenatal cord (Pilsner et al., 2010; Jedrychowski et al., 2009b;

Plusquellec et al., 2007) and early childhood blood Pb levels (Chandramouli et al., 2009; Min et al., 2009; Miranda et al., 2009). Thus, while biomarkers of Pb exposure across lifestages were associated with neurodevelopmental decrements in children, the weight of cumulative evidence demonstrated associations with concurrent blood Pb levels. In adults, the weight of evidence supported associations of cumulative Pb exposure (i.e., tibia Pb) with poorer cognitive performance.

With regards to duration of exposure, several epidemiologic studies indicated that Pb exposures of less than 1 year, represented by blood Pb levels prenatally (maternal or cord) and at age 6, 12, or 24 months, were associated with lower cognitive function in children ages 6 months to 3 years (Table 5-4). Toxicological studies supported these observations with effects observed with gestational and early postnatal Pb exposures.

5.3.13.8 The Lead Concentration-Response Relationship

In the 2006 Pb AQCD, several individual epidemiologic 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 or more negative slope among children in lower strata of blood Pb levels compared with children in higher strata of blood Pb levels (Figure 5-23 and Table 5-15). Although a majority of epidemiologic evidence used a blood Pb level of 10 μg/dL to define lower and higher blood Pb levels, some found that among children less than age 10 years with mean concurrent blood Pb levels of 3 μg/dL, the blood Pb-cognitive function slope was more negative with blood Pb levels <7.5 (Lanphear et al., 2005) or <5 μg/dL (Tellez-Rojo et al., 2006).

While explanations for this supralinear relationship have not been well characterized by epidemiologic studies, recent toxicological studies that examined lower concentrations of experimental Pb exposure suggest that mechanisms may be differentially activated at lower and higher Pb exposures. Multiple studies showed U- or inverse U-shaped curves with lower Pb exposures having different levels or often the opposite effect from higher exposures. Nonlinear concentration-response relationships were found for effects such as learning (Section 5.3.2.2), adult forebrain dopamine levels (Leasure et al., 2008), and neurogenesis (Fox et al., 2008). Sensory organ parameters in animals, namely, the numbers of rod photoreceptors and bipolar cells and ERG wave amplitudes also show vastly different changes with low versus higher Pb exposure (Table 5-12).

The examination of populations of children with large proportions of subjects at very low blood Pb levels has improved the ability to discern a threshold for Pb-associated effects on cognitive function and behavior. Several recent epidemiologic studies reported

associations between blood Pb levels and deficits in cognitive and behavioral endpoints in children (ages 8-11 years) with mean or range of blood Pb levels ≤ 2 µg/dL (Cho et al., 2010; Kim et al., 2009b; Miranda et al., 2009). Collectively, these new findings in children do not provide evidence for a threshold for neurodevelopmental effects of Pb in the ranges of blood Pb levels examined to date.

5.3.13.9 Evidence that Forms the Basis of the Causal Determination

In summary, recent findings strengthen epidemiologic and toxicological evidence indicating that Pb exposure is associated with nervous system effects. The weight of epidemiologic and toxicological evidence clearly supports associations of higher blood Pb levels with lower cognitive function in children, i.e., full-scale IQ and various measures of learning and memory. In epidemiologic studies, these associations were substantiated in children ages 1 to 11 years and in populations with mean blood Pb levels between 2 and 7 µg/dL. Observation of a supralinear concentration-response relationship and associations with mean (or quantile) blood Pb levels < 2 µg/dL do not provide evidence for a threshold for the neurodevelopmental effects of Pb exposure. Epidemiologic and toxicological evidence clearly demonstrates Pb-associated increases in behavioral problems, in particular, inattention and impulsivity. Associations are substantiated in children ages 1 to 12 years with mean concurrent blood Pb levels of 2 to 5 µg/dL. In animals, the weight of evidence demonstrates effects on cognition and behavior with prenatal and early postnatal Pb exposures that resulted in blood Pb levels of 10 to 40 µg/dL. In epidemiologic studies, associations with cognitive function and behavior were observed after adjustment for a range of potential confounding variables, but most commonly, parental IQ, parental education, and other SES-related variables. In children, the weight of evidence supports cognitive function decrements and behavioral problems in association with concurrent blood Pb levels. Associations also are observed with prenatal, early childhood, and childhood average blood Pb levels, thus uncertainty remains regarding the lifestage of exposure within childhood that is associated with the greatest risk. The weight of toxicological evidence demonstrates neurodevelopmental effects with prenatal and early postnatal Pb exposures that can have effects persisting to adulthood. The biological plausibility for epidemiologic and toxicological findings for effects on cognitive function and behavior is provided by evidence characterizing underlying mechanisms, including Pb-induced changes in neurogenesis, synaptogenesis and synaptic pruning, long term potentiation, and neurotransmitter function. In adults, the timing, level, frequency, and duration of Pb exposure implicated in nervous system effects remain uncertain. Among occupationally-exposed adults, a spectrum of nervous system effects is associated with concurrent blood Pb level (≥ 14 µg/dL), which reflects

both current and cumulative exposure. However, in adults without occupational exposure, cognitive performance is more strongly associated with tibia Pb levels than blood Pb levels, which indicates an effect of long-term, cumulative Pb exposures. Based most heavily on cognitive function decrements and inattention in children, the collective body of evidence integrated across epidemiologic and toxicological studies is sufficient to conclude that there is a causal relationship between Pb exposures and nervous system effects.

5.4 Cardiovascular Effects

5.4.1 Introduction

The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that both epidemiologic and animal toxicological studies support the relationship between increased Pb exposure and increased cardiovascular effects, in particular, increased BP and increased incidence of arterial hypertension. Although fewer in number, epidemiologic studies demonstrated associations of blood and bone Pb levels with other cardiovascular diseases (CVDs) in adults, such as ischemic heart disease, cerebrovascular disease, peripheral vascular disease, and CVD-related mortality. As the cardiovascular and renal systems are intimately linked, cardiovascular effects can arise secondarily to Pb-induced renal injury (Section 5.5). Toxicological studies also provided compelling evidence supporting the biological plausibility for Pb-associated cardiovascular effects by characterizing a number of the underlying mechanisms by which Pb exposure can lead to human cardiovascular health effects. Such studies demonstrated that the Pb content in heart tissue of animals reflects the increases in blood Pb levels (Lal et al., 1991), indicating that the cardiovascular morbidity associated with blood Pb levels may represent the effects of the bioavailable Pb in the target tissue. The strongest evidence supported the role of oxidative stress in the pathogenesis of Pb-induced hypertension. Additionally, several toxicological studies characterized other pathways or cellular, molecular, and tissue events promoting the Pb-induced increase in BP. These mechanisms included inflammation, adrenergic and sympathetic activation, renin-angiotensin-aldosterone system (RAAS) activation, vasomodulator imbalance, and vascular cell dysfunction.

With regard to the concentration-response relationship, meta-analysis of human studies found that each doubling of blood Pb level (between 1 and >40 μg/dL measured concurrently in most studies) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP (Nawrot et al., 2002). On a population-wide basis, the estimated effect size could translate into a clinically significant increase in the

segment of the population with the highest BP. In a moderately-sized population, a relatively small effect size thus has important health consequences for the risk of sequelae of increased BP, such as stroke, myocardial infarction, and sudden death. It was also noted that most of the reviewed studies examining bone Pb levels, biomarkers of cumulative Pb exposure, also showed increased BP (Cheng et al., 2001; Hu et al., 1996b) or increased hypertension with increasing bone Pb level (Lee et al., 2001a). Across studies, over a range of bone Pb concentrations (<1.0 to 96 μg/g), every 10 μg/g increase in bone Pb was associated with increased odds ratios of hypertension between 1.28 and 1.86. Studies observed an average increase in systolic BP of ~0.75 mmHg for every 10 μg/g increase in bone Pb concentration over a range of <1 to 52 μg/g.

With regard to etiologically-relevant timing of Pb exposure, the weight of toxicological evidence demonstrated increases in BP after long-term (> 4 weeks) Pb exposure. In epidemiologic studies, as cardiovascular outcomes were most often examined in cross-sectional studies with one or a limited number of Pb biomarker measurements, uncertainty exists as to the specific Pb exposure level, timing, frequency, and duration that contributed to the observed associations. While associations of adult bone Pb (particularly tibia Pb) with health outcomes in adults are indicative of effects related to past or cumulative exposures, interpretation of similar associations involving adult blood Pb levels, especially those measured concurrently with outcomes, are complicated by the generally higher past exposures common in this population. Detailed interpretation of Pb in blood and bone are provided in Sections 4.3 and 4.7.3. Briefly, higher past Pb exposures in adults increased their bone Pb stores which contribute to current blood Pb levels through the normal process of bone remodeling, as well as during periods of increased bone remodeling and loss (e.g., osteoporosis and pregnancy). Due to the long latency period for the development of increased BP and CVD, associations of cardiovascular effects with low concurrent blood Pb levels (e.g., population means 1.6-4 μg/dL) in adults may be influenced by higher past Pb exposures (Section 4.4.1).

This section reviews the published studies pertaining to the cardiovascular effects of Pb exposure in humans, experimental animals, isolated vascular tissues, and cultured vascular cells. With the large and strong existing body of evidence serving as the foundation, emphasis was placed on studies published since the 2006 Pb AQCD (U.S. EPA, 2006b). Epidemiologic and toxicological studies continued to augment the evidence for increases in BP and hypertension development associated with long-term Pb exposure and expanded the evidence for the biological pathways of these effects. Epidemiologic studies strengthened the evidence for associations between Pb biomarkers and cardiovascular effects after adjusting for potential confounding factors such as age, diet, alcohol use, BMI, comorbidities, and smoking. The epidemiologic evidence was

substantiated with results from several available prospective studies indicating associations between Pb biomarkers and the incidence of cardiovascular health effects.

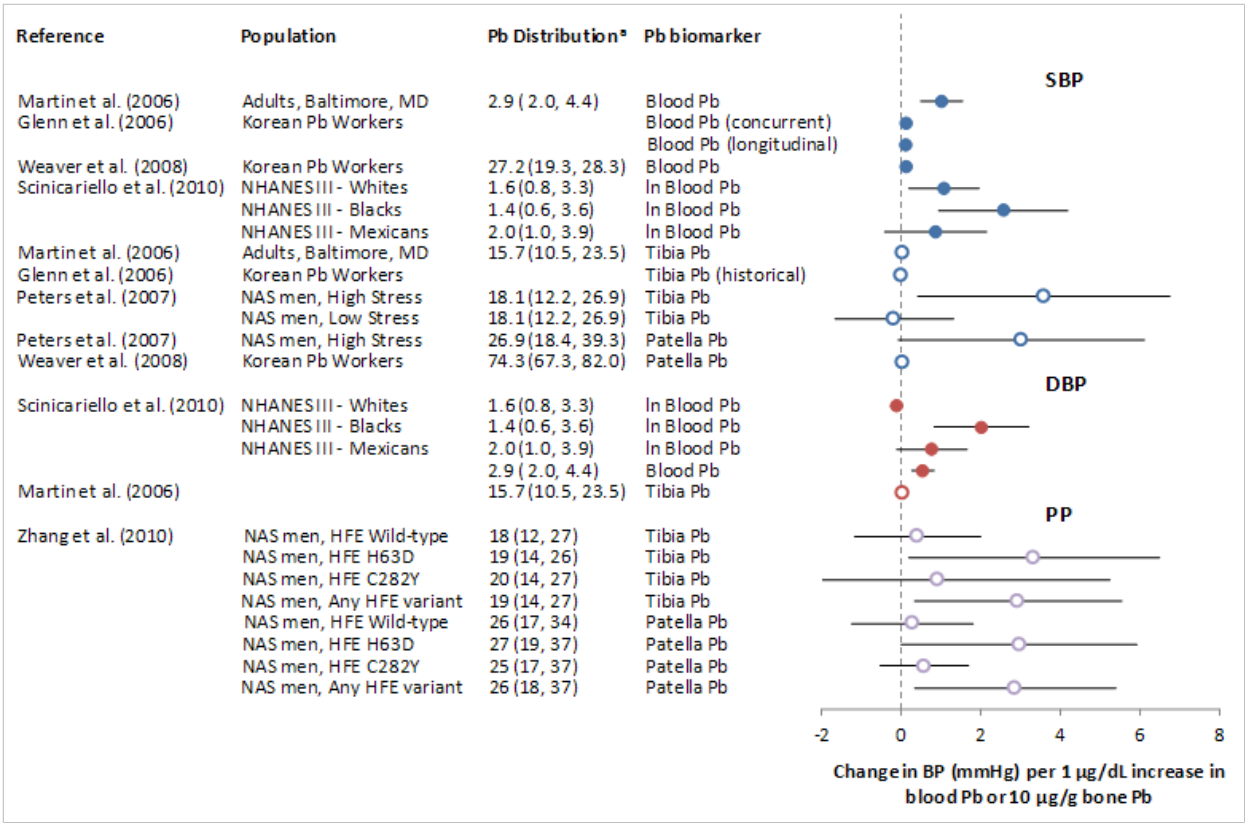
5.4.2 Blood Pressure and Hypertension

5.4.2.1 Epidemiology

The most commonly used indicator of cardiovascular morbidity was increased BP and its derived index, hypertension. Hypertension in these studies was defined as diastolic and/or systolic BP above certain cut-points or use of anti-hypertensive medicines. The BP cut-points employed have been historically established by reference to informed medical opinion, and as medical knowledge has improved BP cut-points defining hypertension have been lowered over time. Consequently, different studies using "hypertension" as a cardiovascular outcome may have assigned different cut-points, depending on the year and location of the study and the individual investigator. All of the new studies in the current review used the same criteria for hypertension (e.g., systolic BP at or above 140, diastolic BP at or above 90, or use of anti-hypertensive medications). Studies in the medical literature show that elevated BP is associated with increased risk of CVD including coronary disease, stroke, peripheral artery disease, and cardiac failure. Coronary disease (i.e., myocardial infarction, angina pectoris, sudden death) is the most lethal sequela of hypertension (Ingelsson et al., 2008; Chobanian et al., 2003; Pastor-Barriuso et al., 2003; Prospective Studies Collaboration, 2002; Kannel, 2000a, b; Neaton et al., 1995). Several recent general population and occupational cohort and cross-sectional studies strengthened the evidence that blood and bone Pb level were associated consistently with measures of BP (Figure 5-25 and Table 5-16) as well as with the prevalence and incidence of hypertension (Figure 5-26 and Table 5-17). Further, recent studies expanded evidence by finding differences in association among racial/ethnic groups, perceived stress, diet, and genetic variants and thus, identified populations potentially at increased risk of Pb-associated cardiovascular effects.

In a cross-sectional analysis, Martin et al. (2006) examined the associations of concurrent blood and tibia Pb levels with BP and hypertension in a community-based study of older adults (n = 964, age ranging from 50 to 70 years) in Baltimore, MD. A key strength of this study was the extensive consideration of potential confounding variables. Four models evaluated associations for BP and hypertension. The base model included age, sex, BMI, sodium intake, potassium intake, total cholesterol, time of day, testing technician, and hypertensive medication use. Other models added SES, race/ethnicity, or both as covariates. Blood Pb but not tibia Pb level was a strong predictor of BP in all

models; a 1 µg/dL increase in concurrent blood Pb level was associated with an approximately 1 mmHg increase in systolic BP and an approximately 0.5 mmHg increase in diastolic BP. Tibia Pb but not blood Pb was associated with hypertension in logistic regression models. The authors applied propensity analysis to their models to better account for the effect of other risk factors for hypertension such as race/ethnicity, age, and SES that were strongly associated with tibia Pb level. The propensity score analysis and model adjustment did not substantially change the numerical findings and conclusions (e.g., tibia Pb and hypertension were positively associated independently of race/ethnicity and SES), indicating that neither SES nor race/ethnicity confounded the association between tibia Pb level and hypertension. No evidence for effect modification by race/ethnicity was found either. Martin et al. (2006) concluded that Pb in blood has an acute effect on BP and that Pb contributes to hypertension risk as a function of cumulative, chronic exposure (as represented as bone Pb in this population). While different aspects of Pb exposure may contribute differentially to increases in BP and hypertension, it is important to note that concurrent blood Pb levels in adults also reflect cumulative Pb exposure. Thus, its association with BP may not reflect an acute effect but may also reflect an effect of cumulative Pb exposure.



[a]Pb distribution presents the median (IQR) that were estimated from the mean and SD assuming a normal distribution.

[b]Effect estimates were standardized to 1 µg/dL blood Pb or 10 µg/g bone Pb.

Note: In general, results are categorized by specific BP parameter, then by Pb biomarker. For categories with multiple studies, the order of the studies follows the order of discussion in the text. For associations of a 1 µg/dL increase in blood Pb level (closed circles) or 10 µg/g increase in bone Pb (open circles) with systolic BP (SBP; blue), diastolic BP (DBP; red), and pulse pressure (PP; purple) in adults.

Figure 5-25 Concentration-response relationships (95% CI).

Table 5-16 Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 5-25

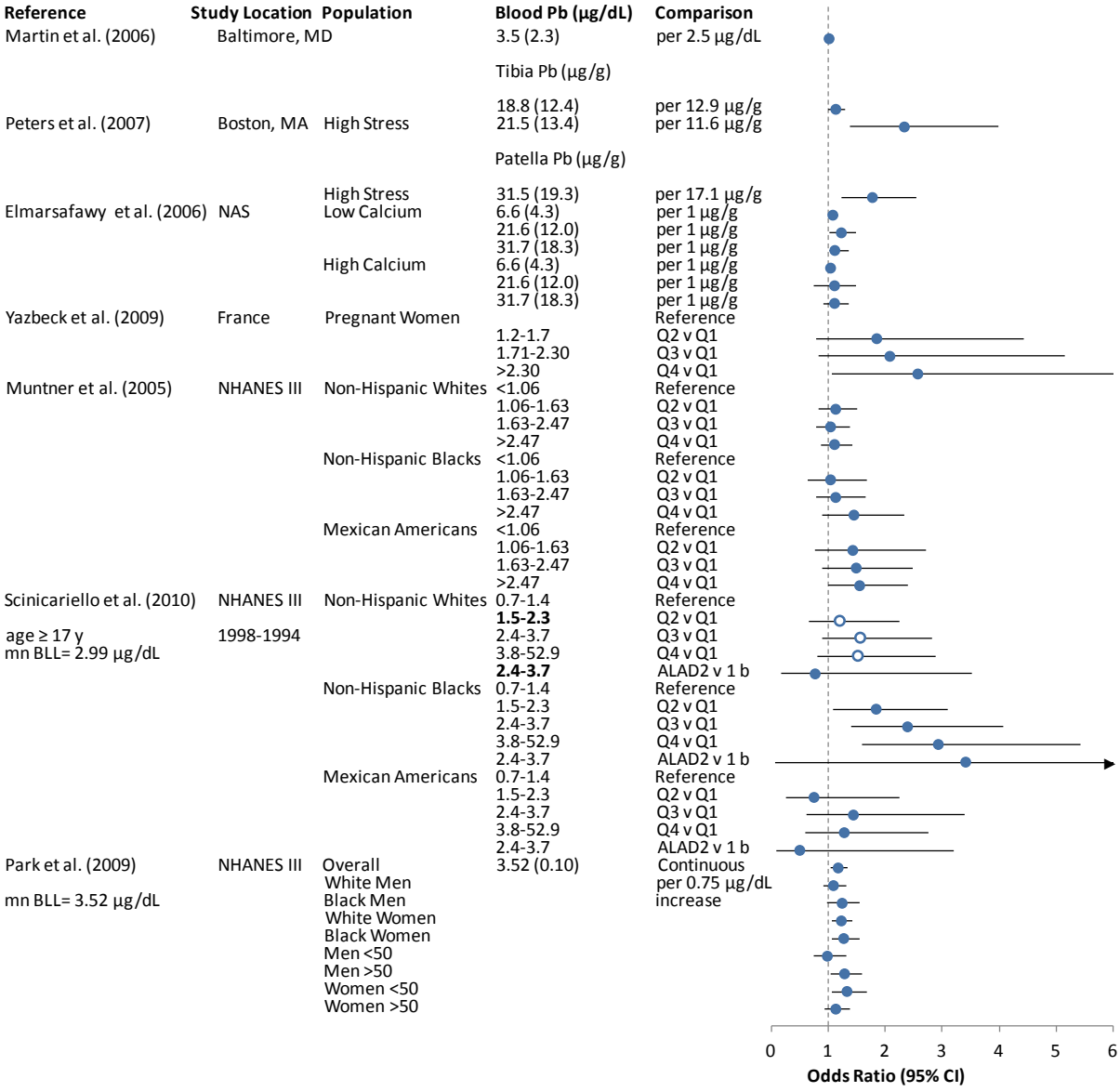
Study	Population /Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Martin et al. (2006)	964 men and women, 50-70 yr, 40% African American, 55% White, 5% other, in Baltimore, MD	BP	Concurrent Mean Blood Pb: Mean (SD): 3.5 (2.3) µg/dL African American: 3.4 (2.3) White: 3.5 (2.4) 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 5-25 and tabulated here.)	Blood Pb SBP: 1.05 (0.53,1.58) DBP; 0.53 (0.25, 0.81) Tibia Pb: SBP: 0.07 (-0.05, 0.14) DBP: 0.05 (-0.02, 0.08) mmHg per µg/dL blood Pb mmHg per µg/g bone Pb
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 0.08 (-0.01, 0.16) Blood Pb (longitudinal) 0.09 (0.01, 0.16) Model 4: short and longer-term) Blood Pb concurrent 0.10 (0.01, 0.19) Blood Pb longitudinal: 0.09 (0.01, 0.16) mmHg per 10 µg/dL blood Pb
Weaver et al. (2008)	652 current and former Pb workers in South Korea (12/1999-6/2001)	BP	Concurrent Blood Pb: Mean (SD): 30.9 (16.7) µg/dL Concurrent Patella Pb: Mean (SD): 75.1 (101.1) µg/g	Linear regression model adjusted for age, gender, BMI, diabetes, antihypertensive and analgesic medication use, Pb job duration, work status, tobacco and alcohol use	SBP Patella Pb 0.0059 (-0.008, 0.02)[a] Blood Pb 0.1007 (0.02, 0.18)[a] mmHg per 1 µg/dL blood Pb or 1 µg/g patella Pb Interaction between blood Pb/patella Pb with ALAD and vitamin D receptor polymorphisms not significant.
Peters et al. (2007)	513 elderly men (mean 67 y) from NAS in Greater Boston, MA area	BP	Tibia Pb: mean (SD): 21.5 (13.4) µg/g Patella Pb: Mean (SD): 31.5 (19.3) µg/g	Logistic and linear regression models adjusted for age, age squared, sodium, potassium, and calcium intake, family history of hypertension, BMI, educational level, pack-years of smoking, alcohol consumption, and physical activity	SBP Tibia Pb/ High Stress: 3.57 (0.39, 6.75) Low Stress: 0.21 (-1.70, 1.29) per SD increase in tibia Pb Patella Pb/ High Stress: 2.98 (-0.12, 6.08) per SD increase in tibia Pb Patella Pb/ Low Stress: NR

Study	Population /Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Scinicariello et al. (2010)	6,016 NHANES III (1988-1994) participants ≥ 17 yr	BP	Concurrent 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	SBP Non-Hispanic whites: 1.05 (0.32, 1.78) Non-Hispanic blacks: 2.55 (1.59, 3.51) Mexican Americans: 0.84 (-0.06, 1.74) DBP Non-Hispanic whites: -0.14 (-1.1, 0.82) Non-Hispanic blacks: 1.99 (1.13, 2.85) Mexican Americans: 0.74 (-0.005, 1.48) mmHg per unit increase in ln Blood Pb Significant interactions with blood Pb and ALAD observed in relation to SBP for non-Hispanic whites and non-Hispanic blacks
Zhang et al. (2010a)	619 older adult males (mean 67 yr) enrolled in the NAS in Greater Boston, MA area	PP	Wild type HFE Tibia Pb: Median (IQR):8(12-27) µg/g Patella Pb: Median (IQR):26(17-37) µg/g C282Y HFE Tibia Pb: Median (IQR):20 (14-27) µg/g Patella Pb: Median (IQR):25(17-37) µg/g H63D HFE Tibia Pb: Median (IQR):19(14-26) µg/g Patella Pb: Median (IQR):27(19-37)µg/g	Linear mixed effects regression models with repeated measurements adjusted for age; education; alcohol intake; smoking; daily intakes of 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 mmHg per 13 µg/g Tibia Pb: Wild Type HFE: 0.38 (0,1.96) H63D HFE: 3.30 (0.16, 6.46) C282Y HFE: 0.89 (0, 5.24) Any HFE variant: 2.90 (0.31, 5.51) mmHg per 19 µg/g Patella Pb: Wild Type HFE: 0.26 (0, 1.78) H63D HFE: 2.95 (0, 5.92) C282Y HFE: 0.55 (0, 1.66) Any HFE variant: 2.83 (0.32,5.37)
Perlstein et al. (2007)	593 predominantly white men from 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	PP 4.2 (1.9, 6.5) mmHg higher in men with tibia Pb > 19 µg/g (median) compared with men with tibia Pb < µg/g Blood Pb (mean difference): Q5: -1.49 (–4.93, 1.94) Q4: -1.39 (–4.94, 2.15), Q3: -2.56 (–5.78, 0.67) Q2: -4.37 (–7.88,–0.86) Q1: Referent group Tibia Pb (mean difference): Q5: 2.58 (–1.15, 6.33) Q4: 2.64 (–0.93, 6.21) Q3: -0.73 (–4.27, 2.82) Q2: -3.02 (–6.48, 0.44) Q1: Referent group

Study	Population /Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (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	BP Pooled Estimates mmHg per 10 µg/g Tibia Pb Prospective/SBP 0.33 (-0.44, 1.11) X-sectional SBP 0.26 (0.02, 0.50) X-sectional DBP 0.02 (-0.15, 0.19) Hypertension per 10 µg/g patella Pb x-Sectional hypertension OR: 1.04 (1.01, 1.07) Pooled Estimate hypertension OR: 1.04 (0.96, 1.12)
Yazbeck et al. (2009) [b]	971 pregnant women, age 18-45 yr, in France	BP	Midpregnancy Blood Pb: PIH group mean (SD): 2.2 (1.4) No PIH group mean (SD): 1.9 (1.2)	Multivariable logistic regression models adjusted for maternal age; 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) [b]	471 elderly men (mean 67 yr) from NAS in Greater Boston, MA area	BP	Blood Pb: Mean (SD): 6.6 (4.3) µg/dL Tibia Pb: Mean (SD): 21.6 (12.0) µg/g Patella Pb: Mean (SD): 31.7 (18.3) µg/g	Linear regression models adjusted for age, BMI, family history of hypertension, history of smoking, dietary sodium intake, and cumulative alcohol ingestion	Tibia Pb High calcium group (>800 mg/d): SBP: 0.40.(0.11, 0.70) Low calcium group (<800 mg/d): SBP: 0.19 (0.01, 0.37) mmHg per µg/g tibia Pb

[a]95% CIs estimated from given p-value.

[b]References not included in Figure 5-25.



Note: Studies are categorized by Pb biomarker. Within each category, studies generally are presented in order of discussion in the text. (a) The outcomes plotted are hypertension prevalence with the exception of Yazbeck et al. (2009) which measured pregnancy induced hypertension and Peters et al. (2007) which measured hypertension incidence. (b) ALAD2 vs. 1 indicates comparison between ALAD 2 carriers (e.g., ALAD1-2 and ALAD2-2) and ALAD 1 homozygotes (e.g., ALAD1-1). (c) Effect estimates were standardized to a 1 µg/dL increase in blood Pb. (d) Effect estimates were standardized to a 10 µg/g increase in bone Pb.

Figure 5-26 Odds ratios (95% CI) for associations of blood and bone Pb with hypertension prevalence and incidence.

Table 5-17 Additional characteristics and quantitative data for associations of blood and bone Pb with hypertension measures for results presented in Figure 5-26

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
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/dL blood Pb mmHg per µg/g bone Pb
Weaver et al. (2008) [a]	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)
Peters et al. (2007)	513 elderly men (mean 67 y) from NAS in Greater Boston, MA area	Hypertension (mean SBP >140 mmHg, DBP >90 mmHg; or physician diagnosis)	Tibia Pb: mean (SD): 21.5 (13.4) µg/g Patella Pb: Mean (SD): 31.5 (19.3) µg/g	Cox proportional hazards models adjusted for age, age squared, sodium, potassium, and 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
Elmarsafawy et al. (2006)	471 elderly men (mean 67 y) from NAS in Greater Boston, MA area	Hypertension (mean SBP ≥ 160 mmHg, DBP ≥ 95 mmHg; and/or physician diagnosis with current use of antihypertensive medications)	Blood Pb: Mean (SD): 6.6 (4.3) µg/dL Tibia Pb: Mean (SD): 21.6 (12.0) µg/g Patella Pb: Mean (SD): 31.7 (18.3) µg/g	Logistic regression models adjusted for age, BMI, family history of hypertension, history of smoking, dietary sodium intake, and cumulative alcohol ingestion	Low 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) Per µg/dL blood Pb or µg/g tibia or 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 Blood Pb OR=3.29 (1.11, 9.74) per 1 unit increase in log maternal blood Pb level Q1: Reference group Q2: OR 1.84 (0.77, 4.41) Q3: OR=2.07 (0.83, 5.13) Q4: OR=2.56 (1.05, 6.22)

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)	Concurrent 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 Non-Hispanic white: Q1: Reference group Q2 OR=1.12 (0.83, 1.50) Q3 OR=1.03 (0.78, 1.37) Q4 OR=1.10 (0.87, 1.41) Non-Hispanic black Q1: Reference group Q2 OR=1.03 (0.63, 1.67) Q3 OR=1.12 (0.77, 1.64) Q4 OR=1.44 (0.89, 2.32) Mexican American Q1: Reference group Q2 OR=1.42 (0.75, 2.71) Q2 OR=1.48 (0.89, 2.48) Q3 OR=1.54 (0.99, 2.39) Significant trend (p=0.04)
Scinicariello et al. (2010)	6,016 NHANES III (1988-1994) participants ≥ 17 y	Hypertension (current use of antihypertensive medication, SBP ≥ 140 mmHg, or DBP ≥ 90 mmHg)	Concurrent 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 group Q2 POR=1.21 (0.66, 2.24) Q3 POR=1.57 (0.88, 2.80) Q4 POR=1.52 (0.80, 2.88) ALAD1-2/2-2: POR= 0.76 (0.17, 3.50) ALAD-1: Reference group Non-Hispanic blacks: Q1 Reference Q2 POR=1.83 (1.08, 3.09) Q3 POR=2.38 (1.40, 4.06) Q4 POR=2.92 (1.58, 5.41) ALAD1-2/2-2: POR= 3.40 (0.05, 219.03) ALAD-1: Reference group Mexican Americans: Q1 Reference Q2 POR=0.74 (0.24, 2.23) Q3 POR=1.43 (0.61, 3.38) Q4 POR=1.27 (0.59, 2.75) ALAD1-2/2-2: POR= 0.49 (0.08, 3.20) ALAD-1: Reference group POR for hypertension with *ALAD2* carriers across quartiles of blood Pb level also reported. *ALAD2* carriers associated with hypertension in non-Hispanic whites.

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Park et al. (2009a)	12,500 NHANES III (1988-1994) participants	Hypertension	NHANES III Concurrent 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 per SD (0.75 µg/dL) in log blood Pb: Overall: 1.12 (1.03, 1.23). White men: 1.06 (0.92, 1.22) Black men: 1.17 (0.98, 1.38) White women:1.16 (1.04, 1.29) Black women: 1.19 (1.04, 1.38) Men <50 yr: 0.98 (0.80, 1.22) Men >50 yr: 1.20 (1.02, 1.41), Women <50 yr: 1.23 (1.04, 1.46), Women >50 yr:1.09 (0.94, 1.26).

[a]Not included in Figure 5-26 because OR data were not reported.

In an occupational cohort in South Korea, Glenn et al. (2006) simultaneously modeled multiple Pb dose measures of individuals collected repeatedly over four years of follow up. Thus, through the assessment of cross-sectional and longitudinal relationships with BP, this study provided key insight on potentially important time periods of Pb exposure. The initial blood Pb level was used as a baseline covariate and the difference in blood Pb level between visits was computed for each subsequent visit. The bone Pb measures were used to indicate historical exposure and cumulative dose. Four models were specified: Model 1 was conceptualized to reflect short-term changes in BP associated with recent dose; Model 2 to reflect longer-term changes associated with cumulative dose controlling for association of baseline BP with recent dose; Model 3 to reflect longer-term changes associated with cumulative dose controlling for cross-sectional influence of cumulative dose on baseline BP; and Model 4 to reflect both short-term change with recent dose and longer-term change with cumulative dose. Concurrent blood Pb and increases in blood Pb between visits were associated with increases in systolic BP in Model 1 (short-term dose) and Model 4 (short- and longer-term dose). No association was observed between BP and tibia Pb at baseline while higher tibia Pb was associated with a decrease in systolic BP in each of the models.

These results indicate that circulating Pb (e.g., blood Pb) may act continuously on systolic BP and reduction in blood Pb may contribute to reductions in BP, while cumulative Pb exposure (represented by bone Pb in this study) may contribute to hypertension incidence by different mechanisms over longer time periods and in older subjects. This analysis in relatively young subjects with a low prevalence of hypertension suggests that at least one of the biological pathways that influences how systolic BP

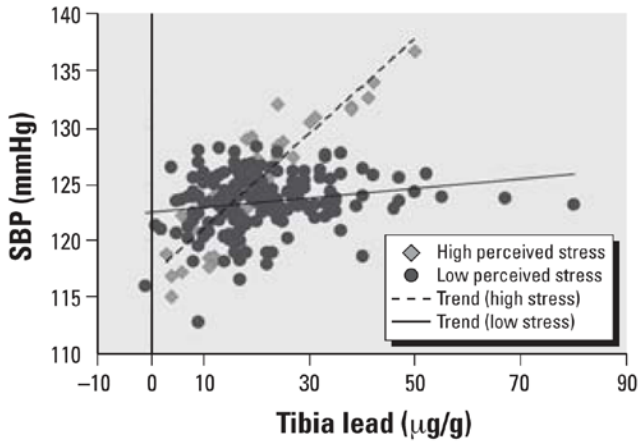
responds to Pb operates over a relatively rapid timeframe and may reflect an immediate response to Pb at a biochemical site of action as a consequence of the biologically available Pb circulating in blood. A persistent effect of cumulative doses over a lifetime may occur via other mechanisms. This study was strengthened by the analysis of associations between changes in blood Pb and changes in BP over time within individual subjects. Bone Pb level may exert influence on blood Pb levels and consequently on BP in an aging population with prolonged Pb exposure. Thus, there is strength in the conclusions drawn from this study that inform various short and long-term exposure relationships with increases in BP and hypertension. It is important to acknowledge the uncertainty regarding the applicability of these findings regarding short-term and long-term effects in Pb workers with relatively high current Pb exposures contributing to blood Pb levels (mean blood Pb levels over time: 20-37 µg/dL) to adults in the U.S. general population whose concurrent blood Pb levels are influenced more by Pb mobilized from bone stores.

In a separate analysis of the same occupationally exposed group in year three of follow-up, Weaver et al. (2008) examined cross-sectionally associations of concurrent patella Pb and blood Pb level with systolic BP, diastolic BP, and hypertension and effect modification by ALAD and vitamin D receptor (VDR) polymorphisms. None of the Pb biomarkers were associated with diastolic BP. Patella Pb alone was not significantly associated with systolic BP, while blood Pb, either alone or with patella Pb was significantly associated with higher systolic BP. The patella Pb-age and blood Pb-age interactions were not statistically significant. There were no significant associations of blood Pb or patella Pb with hypertension status or effect modification by age or sex. Further, interactions between polymorphisms of the VDR and of ALAD with blood Pb and patella Pb on systolic BP were not statistically significant. Mean blood Pb level was high (30.9 µg/dL) compared to non-occupational groups.

Weaver et al. (2010) provided the results of further analysis of this Korean worker cohort, with a focus on determining the functional form of the concentration-response relationships. In a log linear model, the coefficient indicated that every doubling of blood Pb level was associated with a systolic BP increase of 1.76 mmHg. The J test, a statistical test for determining which, if either, of two functional forms of the same variable provides a superior fit to data in non-nested models (Davidson and MacKinnon, 1981), returned a p-value of 0.013 in favor of the natural log blood Pb level over the linear blood Pb level specification. This analysis indicates that the systolic BP increase in this cohort is better described as a logarithmic function of blood Pb level within the blood Pb level range of the study than by a linear function.

Several analyses in the NAS cohort of predominantly white older men in the greater Boston area found associations of blood and bone Pb level with BP and hypertension and indicated effect modification by calcium intake, perceived stress, and HFE gene variants. In a cross-sectional analysis, Perlstein et al. (2007) found a statistically significant association between blood Pb and diastolic BP in adjusted models. The subjects in this study had at least one bone Pb measurement during the years 1991-1997 and were not on antihypertensive medication at the time of the measurement. While tibia Pb was not significantly associated with BP, it was associated with pulse pressure (PP). Men with tibia Pb above the median (19 μg/g) had a higher mean PP (4.2 mmHg [95%CI: 1.9, 6.5]) compared to men with tibia Pb below the median. The trend toward increasing PP with increasing quintile of tibia Pb was statistically significant although none of the confidence intervals for PP referenced to the lowest quintile of tibia Pb (< 7.4 μg/g) excluded the null value.

Peters et al. (2007) examined cross-sectionally the modification of the associations of tibia and patella Pb with BP and hypertension by self-reported stress (assessed by questionnaire) in NAS men. High stress also has been linked with higher BP, potentially via activation of sympathetic pathways, ROS, and the HPA axis. Among all subjects, higher bone Pb level was associated (statistically nonsignificant) with greater odds of hypertension status and higher systolic BP. As indicated in Figure 5-27, the association between systolic BP and tibia Pb differed between those with high and low self-reported stress (β for tibia Pb x stress interaction = 3.77 [CI: 0.46, 7.09]) per SD increase in tibia Pb. Stress also was found to modify the patella Pb-BP association (β for patella Pb x stress interaction = 2.60 [CI: -0.95, 6.15] per SD increase in patella Pb). Neither bone, self-reported stress, nor their interaction was associated significantly with diastolic BP. Peters et al. (2007) also used Cox proportional hazards models to assess the interaction of stress and bone Pb level in the development of hypertension among those free of hypertension at baseline. The results of this analysis showed that increasing tibia and patella Pb were associated with greater risk of developing hypertension among those with high stress compared with those with lower perceived stress (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 results provide evidence supporting adults with higher stress as a population at increased risk of Pb-associated cardiovascular effects.



Source: Peters et al. (2007)

Figure 5-27 The relationship between tibia Pb and estimated systolic BP (SBP) for those with high self-reported stress versus those with low self-reported stress.

Elmarsafawy et al. (2006) examined the modification of the relationship between Pb and hypertension risk by dietary calcium, with 467 subjects from the NAS. Responses on a semi-quantitative dietary frequency questionnaire with one-year recall were used to estimate calcium intake. Effect modification by calcium intake (dichotomized at 800 mg/day) was examined using interaction terms in logistic regression models and by conducting analyses stratified on the calcium variable. Increasing bone and blood Pb increased the risk of hypertension, particularly among subjects with low dietary calcium.

Zhang et al. (2010a) examined the effect of polymorphisms of the hemochromatosis gene (HFE) on the relationship of bone Pb with PP in NAS men. Subjects had up to three PP measurements during the 10 year study period. The overall results demonstrated a strong relationship between bone Pb and PP in this study, similar to an earlier cross-sectional PP study of many of the same subjects (Perlstein et al., 2007). Zhang et al. (2010a) extended these findings by demonstrating larger increases in PP per unit increase in tibia and patella Pb level among those with the H63D variant compared to those with the wild-type or the C282Y variant.

A small number of cross-sectional studies examined and found that blood Pb level was associated with hypertension in pregnancy. Yazbeck et al. (2009) examined a community-based group of pregnant women in France and unlike most other studies, adjusted for potential confounding by blood concentrations of cadmium, manganese, and selenium. Pregnancy induced hypertension (PIH) was defined as systolic BP >140 mmHg and/or diastolic BP >90 mmHg during at least two clinic visits after week 22 of gestation. Patients with pre-existing chronic hypertension were excluded. The mean (SD) blood Pb

levels measured during pregnancy were 2.2 (1.4 µg/dL) in PIH cases and 1.9 (1.2) µg/dL in normotensive women. An association between blood Pb and PIH was observed (OR 3.29 [95% CI: 1.11, 9.74] per unit increase in log-transformed blood Pb level). 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. Investigators observed no significant interactions among blood Pb level, any of the other elements, and maternal characteristics in predicting the risk of PIH. Interaction between blood selenium and Pb concentrations was not significant, and the putative protection effects of selenium through antioxidative properties were not found in this study.

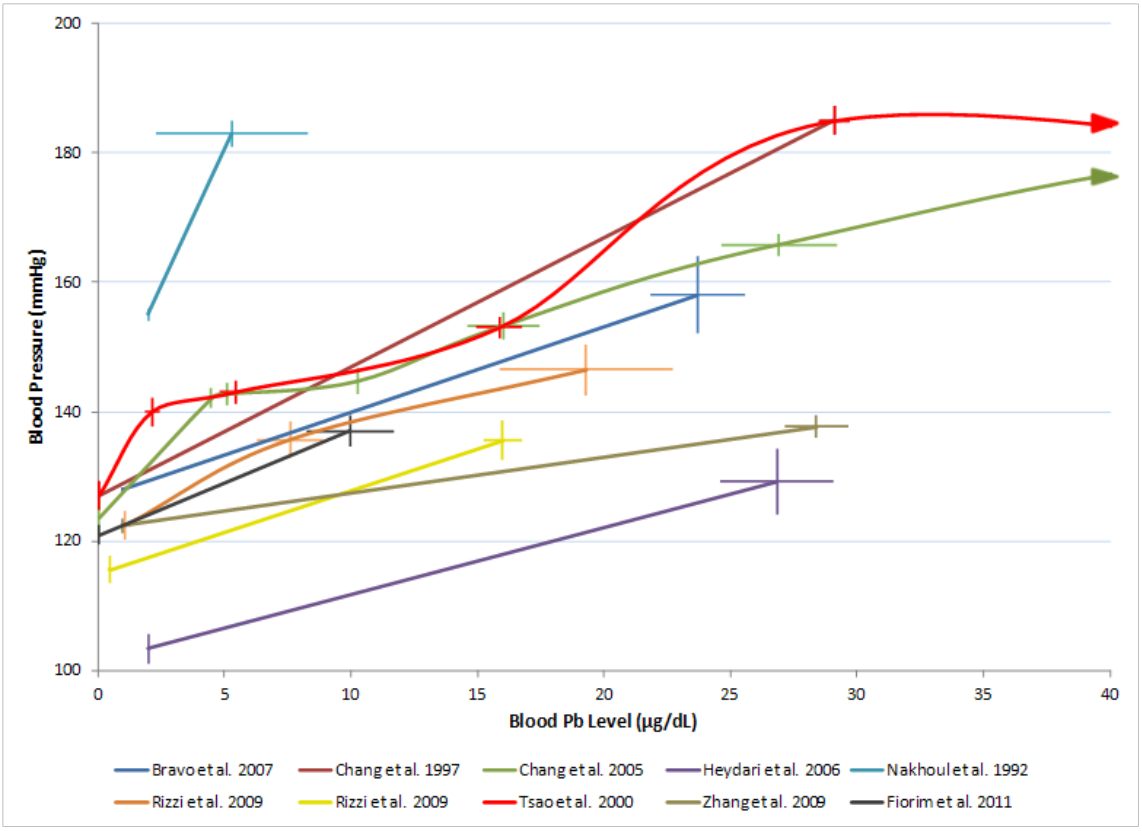
Wells et al. (2011b) measured the relationship of cord blood Pb with BP in 285 women at admission to the Johns Hopkins Hospital in Baltimore, MD, during labor and delivery. Women with cord blood Pb levels in the highest quartile for the study group (>0.96 µg/dL) had significantly higher systolic and diastolic BP (upon admission and for maximum BP) compared to women in the first quartile (<0.46 µg/dL). The authors used Benchmark Dose Software V2.1, developed by the EPA, to estimate the blood Pb level (benchmark dose or BMD) and the associated lower confidence limit (BMDL) that was associated with one standard deviation (SD) increase in BP. In this study group, one SD is approximately equivalent to a 10% increase above the mean for the first quartile blood Pb reference group. The BMD approach was used only as a means of quantifying the relationship of blood Pb with BP in this population. This analysis indicated that the 95% lower bound confidence limit on the maternal blood Pb level (estimated from cord blood Pb levels) that was associated with a 1 SD increase in all blood pressure outcomes was about 1.4 µg/dL. These reported results are similar to those reported in the 2006 Pb AQCD as well as those found 25 years ago but with blood Pb levels in the more recent study an order of magnitude lower.

New analyses using NHANES data continued to indicate associations of Pb biomarkers with BP and hypertension. Muntner et al. (2005) previously used the NHANES 1999-2002 data to indicate that concurrent blood Pb levels were associated with hypertension, peripheral artery disease (PAD), and chronic kidney disease. The PAD results are discussed later in Section 5.4.3.4, and chronic kidney disease results are discussed in Section 5.5.2.2. Blood Pb increased regularly with age (geometric means [95% CIs]: 1.28 µg/dL [1.23, 1.33] in the 18-39 age group to 2.32 µg/dL [2.20, 2.44] in the 75 and older age group). Associations were observed between concurrent blood Pb level and hypertension across race/ethnicity groups with significant trends observed for non-Hispanic blacks and Mexican Americans.

In the NHANES III 1988-1994 population, Scinicariello et al. (2010) found a gene-environment interaction between blood Pb level and ALAD genotype in relation to SBP and DBP in a cross-sectional analysis. These interactions varied across race/ethnicity strata. The strongest associations were observed among non-Hispanic blacks (Figure 5-25, Table 5-16). A statistically significant interaction was observed between concurrent blood Pb level and ALAD1-2/2-2b among non-Hispanic whites and non-Hispanic blacks. Scinicariello et al. (2010) also found an interaction between ALAD genotype and blood Pb level in the association with hypertension. Statistically significant associations between concurrent blood Pb level and hypertension were observed among non-Hispanic blacks and nonsignificant increases were observed among non-Hispanic whites and Mexican Americans (with the exception of Q2 association for Mexican Americans) (Figure 5-26, Table 5-17). In addition, non-Hispanic white ALAD2 carriers in the highest blood Pb level quartile 3.8-52.9 µg/dL) had a significantly higher association with hypertension compared with ALAD1 homozygous individuals in the highest quartile of blood Pb. In the same NHANES population, Park et al. (2009a) predicted bone Pb levels using a model developed with NAS data. Concurrent blood Pb was associated with hypertension overall in the NHANES population, with larger associations observed among black men and women as well as older adults Figure 5-26, Table 5-17). Associations also were observed with estimated bone Pb.

5.4.2.2 Toxicology

Studies on the effect of Pb (as blood Pb level) on systolic BP in unanesthetized adult rats consistently reported an increase in BP with increasing blood Pb level as shown in Figure 5-28 (results summarized in Table 5-18). An array of studies have provided evidence that long-term Pb exposure (> 4 weeks), resulting in blood Pb levels below 10 µg/dL can result in the onset of hypertension (after a latency period) in experimental animals that persists long after the cessation of Pb exposure (U.S. EPA, 2006b). Tsao et al. (2000) presented evidence for increased systolic and diastolic BP in rats with blood Pb levels relevant to those in humans (mean [SD]: 2.15 [0.92] µg/dL blood Pb; 140 [7] mmHg systolic BP, 98 [7] mmHg diastolic BP) compared to untreated controls (mean [SD]: 0.05 [0.05] µg/dL blood Pb; 127 [7] mmHg systolic BP, 88 [7] mmHg diastolic BP). As this was the lowest Pb level tested, no evidence of a threshold was evident. Further, a test for linear trend revealed a statistically significant, positive trend for increasing BP with increasing blood Pb levels up to 56 µg/dL (e.g., mean [SD]: 5.47 [2.1] µg/dL blood Pb; 143 [6] mmHg systolic BP, 97 [8] mmHg diastolic BP), with the effect leveling off at higher blood Pb levels.



Note: Crosses represent standard error for blood Pb and BP measurements. If no crossbar is present, error results were not reported. Arrows represent higher doses tested.

Figure 5-28 Changes in BP after Pb exposure (represented as blood Pb level) in unanesthetized adult rats across studies.

Table 5-18 Characteristics of studies of blood Pb with BP measures in animals presented in Figure 5-28

Reference[a]	Lifestage; Sex	Exposure Duration	Exposure Level; Route	Mean [SEM][b] Blood Pb Level (µg/dL)	n	ΔSBP (mmHg; lowest blood Pb level compared with control)[c]	Comments
Fiorim et al. (2011)	Adult; M	7 days	4 µg/100 g followed by 0.05 µg/100 g daily; intramuscular	9.98 [1.7]	12	16	
Nakhoul et al. (1992)	Adult; M	8 weeks	100 ppm; drinking water	5.3 [3]	7	28	Spontaneously hypertensive rat model
Chang et al. (2005)	Adult; M	8 weeks	2% (20,000 ppm) then removal and measurements 1-7 mo after; drinking water	Range: 4.5 – 83	5	13.8	
Tsao et al. (2000)	Adult	8 weeks	0.01 - 2% (100 - 20,000 ppm); drinking water	Range of means: 2.15 [0.29] – 85.76 [1.29]a	10	13	
Rizzi et al. (2009)	Adult; M	8 weeks	30 - 90 ppm; drinking water	7.6 [1.3], 19.3 [3.4]	11	13.3	
Chang et al. (1997)	Adult; M	8 weeks	0.5% (500 ppm); drinking water	29.1 [0.6][a]	10	58	
Heydari et al. (2006)	Adult; M	12 weeks	100 ppm; drinking water	26.8 [2.2]	6	25.8	
Bravo et al. (2007)	Adult; M	14 weeks	100 ppm; drinking water	23.7 [1.9][a]	12	30	
Zhang et al. (2009a)	Adult; M	40 weeks	100 ppm; drinking water	28.4 [1.1][a]	8-10	15.3	

[a]Studies are presented in order of increasing duration of exposure.
[b]Standard deviation converted to SEM.
[c]Difference in systolic BP (SBP) between group means not within one exposure group.

Experimental animal studies continued to provide evidence that long-term Pb exposure results in sustained arterial hypertension after a latency period. Systolic BP increased in rats after exposure to 90-10,000 ppm Pb (as Pb-acetate in drinking water) for various time periods that resulted in blood Pb levels between 19.3-240 µg/dL (Mohammad et al., 2010; Zhang et al., 2009a; Badavi et al., 2008; Grizzo and Cordellini, 2008; Reza et al., 2008; Bravo et al., 2007; Vargas-Robles et al., 2007; Heydari et al., 2006; Bagchi and Preuss, 2005). Past studies have shown statistically significant elevations in BP in rats with lower blood Pb levels. For example, long-term Pb exposure to spontaneously hypertensive rats (resulting in mean [SEM] blood Pb level: 5.3 [3] µg/dL) led to increased BP (Nakhoul et al., 1992). Consistent with measurements of systolic BP by tail-cuff plethysmography, Pb exposure (100 ppm for 14 weeks; mean blood Pb level: 24 µg/dL) also caused an increase in intra-aortic mean arterial pressure (Bravo et al., 2007). In a study that tested low levels of Pb exposure (30 ppm; mean blood Pb level: 7.6 µg/dL), a statistically significant increase in systolic BP was not observed despite elevated blood Pb level after 8 weeks of treatment. Nonetheless, there was a trend of higher BP with higher blood Pb levels (Rizzi et al., 2009).

Studies found that Pb-induced increased BP persisted long after cessation of Pb exposure. Bagchi and Preuss (2005) found that elevated systolic BP was maintained for 210 days after Pb exposure cessation. However, chelation therapy using $Na_2CaEDTA$ returned systolic BP to levels comparable to those in rats not treated with Pb (Bagchi and Preuss, 2005). Chang et al. (2005) reported a partial reversibility of effect after cessation of Pb exposure, where Pb-induced elevated BP decreased but did not return to control levels 7 months post Pb exposure. After Pb exposure was removed, blood, heart, aorta, and kidney Pb levels decreased quickly within the first three months (Chang et al., 2005). Pb-induced elevated systolic BP persisted for one month following Pb exposure cessation, followed by obvious decreases in BP until 4 months after Pb exposure cessation. Between 4 and 7 months after Pb exposure cessation, the still-elevated BP did not decrease further, thus never returning to control BP levels. Decreases in BP were strongly correlated with decreases in blood Pb level after exposure cessation.

The aforementioned studies all assessed the relationship between long-term exposure (> 4 weeks) of rats to Pb and measures of BP. However, recent research also investigated BP elevation occurring after short-term treatment with Pb (< 4 weeks). Studies found increased systolic BP after 7 days of Pb treatment (daily injections resulting in mean [SEM] blood Pb levels of 9.98 [1.7] µg/dL) (Fiorim et al., 2011) and after 2 weeks of Pb exposure (100 ppm via drinking water) (Sharifi et al., 2004). A study utilizing intra-arterial pressure measurements found that a single high-dose Pb injection (resulting in mean [SEM] blood Pb levels of 37 [1.7] µg/dL) increased systolic arterial pressure after only 60 minutes (Simões et al., 2011). These studies suggest that there is the potential for increase in BP following short-term Pb treatment. It is possible that the increases in BP following short- and long-term Pb exposures are occurring through separate mechanisms; however, studies using both short- and longer-term Pb exposure have correlated increased BP with an activation of the renin-angiotensin system (i.e., increase in angiotensin converting enzyme (ACE) activity) (Section 5.4.2.3). Several of these aforementioned studies used the injection route of Pb administration, and the relevance of these bolus doses over short periods of time to human routes of short-term exposure is uncertain. However, it is important to acknowledge that the results were similar to those from the study that examined short-term exposure to Pb via drinking water,

5.4.2.3 Hypertension Modes of Action

The 2006 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. As described below, recent studies

in experimental animals and cells further supported roles for these potential mechanisms in mediating hypertension from Pb exposure.

Oxidative Stress Response - Reactive Oxygen Species and Nitric Oxide

Several studies discussed in the 2006 Pb AQCD demonstrated a role for oxidative stress in the pathogenesis of Pb-induced hypertension, mediated by the inactivation of nitric oxide ($^{\bullet}$NO) and downregulation of soluble guanylate cyclase (sGC) (Dursun et al., 2005; Attri et al., 2003; Gonick et al., 1997; Vaziri et al., 1997; Khalil-Manesh et al., 1994; Khalil-Manesh et al., 1993b). Pb-induced reduction of biologically active $^{\bullet}$NO was found not to be due to a reduction in $^{\bullet}$NO-production capacity (Vaziri and Ding, 2001; Vaziri et al., 1999b); instead it was found to result from inactivation and sequestration of $^{\bullet}$NO by ROS (Malvezzi et al., 2001; Vaziri et al., 1999a). Oxidative stress from Pb exposure in animals may be due to upregulation of NAD(P)H oxidase (Ni et al., 2004; Vaziri et al., 2003), induction of Fenton and Haber-Weiss reactions (Ding et al., 2001; Ding et al., 2000), and failure of the antioxidant enzymes, CAT and GPx, to compensate for the increased ROS (Farmand et al., 2005; Vaziri et al., 2003). Many biological actions of $^{\bullet}$NO, such as vasorelaxation, are mediated by cGMP, which is produced by sGC from the substrate GTP. Oxidative stress also has been found to play a role in Pb-induced downregulation of sGC (Farmand et al., 2005; Courtois et al., 2003; Marques et al., 2001). The reduction of the vasodilator $^{\bullet}$NO leads to increased vasoconstriction and BP.

Pb-induced oxidative stress also has been found to induce renal tubulointerstitial inflammation which plays a crucial role in models of hypertension (Rodriguez-Iturbe et al., 2005; Rodriguez-Iturbe et al., 2004). Tubulointerstitial inflammation from treatment with Pb has been coupled with activation of the redox sensitive NF-κB (Ramesh et al., 2001). Pb-induced hypertension, inflammation, and NF-κB activation can be ameliorated by antioxidant therapy (Rodriguez-Iturbe et al., 2004). There is mixed evidence to suggest that Pb-induced hypertension may also be promoted by activation of PKC leading to enhanced vascular contractility (Valencia et al., 2001; Watts et al., 1995).

Recent studies continued to provide evidence for the role of ROS and $^{\bullet}$NO metabolism in Pb-induced hypertension and vascular disease. Increased systolic BP after Pb exposure was 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, a measure of lipid peroxidation) (Bravo et al., 2007), and increased 3-nitrotyrosine (Vargas-Robles et al., 2007). Inhibition of NAD(P)H oxidase, an enzyme that generates $O_2^{\cdot-}$ and hydrogen peroxide, was able to block Pb-induced (1 ppm) aortic contraction to 5-hydroxytryptamine (5-HT) (Zhang et al., 2005). Increases in systolic BP, intra-aortic mean arterial pressure, and MDA after Pb exposure (100 ppm; mean blood Pb level:

23.7 µg/dL) were also prevented by treatment with the immunosuppressant, mycophenolate mofetil (MMF) (mean blood Pb level in MMF-treated animals: 27 µg/dL) (Bravo et al., 2007). MMF has been shown to inhibit endothelial NAD(P)H oxidase, which could explain how it decreases Pb-induced increases in oxidative stress and BP. MMF was not found to alter blood Pb levels of animals. Red grape seed extract and ascorbic acid supplementation were also able to protect rats from Pb-induced (100 ppm) increased BP and heart rate, perhaps through the antioxidant properties of the extract (Badavi et al., 2008) and vitamin (Mohammad et al., 2010). Red grape seed extract did not alter the accumulation of Pb in blood, indicating that its protective effect was not mediated through altered Pb toxicokinetics; however, internal doses of Pb were not measured in the vitamin C study to clarify the mechanism of action of vitamin C. Another study found that the antioxidant, anti-inflammatory chemical, curcumin, as well as physical exercise training reversed Pb-induced increases in serum creatinine kinase-MB (CK-MB), low density lipoprotein (LDL), heart high-sensitivity C-reactive protein (hs-CRP), and MDA, and Pb-induced decreases in serum total antioxidant capacity, high density lipoprotein (HDL), and heart glutathione peroxidase (GPx); however, internal doses of Pb were not measured to clarify the mechanism of action in this study (Roshan et al., 2011).

Exposure to Pb can also affect the activity and levels of antioxidant enzymes. Male (♂) and female (♀) rats exposed to Pb for 18 weeks (100-1,000 ppm) had altered responses in antioxidant enzymes in heart tissue (Sobekova et al., 2009; Alghazal et al., 2008a). Pb exposure in female rats increased the activity of cardiac SOD, GST, GR, and GPx (> 100 ppm) and increased cardiac thiobarbituric acid reactive substances (TBARS, measure of lipid peroxidation) (1,000 ppm). Pb exposure in male rats did not affect the activity of SOD or production of TBARS, however decreased the activity of GST and GR (>100 ppm). Male and female rats also accumulated different amounts of Pb in the cardiac tissue after similar exposure (♂ 100 ppm: 205% of control, 1,000 ppm: 379%; ♀ 100 ppm: 246%, 1,000 ppm: 775%), which could explain the sex differences observed in antioxidant enzyme responses.

Oxidative stress can trigger a cascade of events that promote cellular stress, renal inflammation, and hypertension. As was shown previously (Rodriguez-Iturbe et al., 2005), Pb exposure can increase renal NF-κB, which was associated with tubulointerstitial damage and infiltration of lymphocytes and macrophages (Bravo et al., 2007). These events could also be ablated by MMF treatment, likely due to its anti-inflammatory and antioxidant properties. Pb also was found to induce inflammation in human endothelial cells as a model for vessel intima hyperplasia (Zeller et al., 2010). The pro-inflammatory cytokine, interleukin (IL)-8 protein and mRNA were increased, concentration- and time-dependently, after in vitro Pb exposure (5-50 µM). Enhanced IL-

8 production was mediated through activation of the transcription factor Nrf2 (but not NF-κB, hypoxia inducible factor-1, or aryl hydrocarbon receptor), as shown through increased nuclear translocation and Nrf2 cellular knockdown experiments. Additionally, measures of endothelial stress, NQO1 and HO-1 protein, were induced by Pb exposure (Zeller et al., 2010). Pb treatment (20 ppm, i.p., 3 days/week, 8 weeks) increased the inflammatory markers hs-CRP and CK-MB in rat hearts (Roshan et al., 2011).

Oxidative stress affects vascular reactivity and tone through inactivation and sequestration of •NO, causing a reduction in biologically active •NO. Recent studies affirmed past conclusions on the interplay of ROS and •NO metabolism in the cardiovascular effects of Pb. Elevated systolic BP and altered vasorelaxation after Pb exposure was accompanied by a decrease in total nitrates and nitrites (NO_X) (Mohammad et al., 2010; Zhang et al., 2007a; Heydari et al., 2006). Serum NO_X levels in Pb-treated rats remained depressed for 8 weeks and then reversed after 12 weeks, despite continued elevation in systolic BP (Heydari et al., 2006). This return of serum NO_X levels to levels similar in controls could be a result of compensatory increases in endothelial NOS (eNOS) attempting to replenish an over-sequestered •NO supply. With this in mind, studies showed increased eNOS protein expression after long-term Pb exposure in kidney (Zhang et al., 2007a) and isolated cultured aorta (Vargas-Robles et al., 2007). No change in inducible NOS was observed in isolated cultured aorta after 1 ppm Pb exposure (Zhang et al., 2007a). In contrast to long-term exposure, Pb treatment over a short time period (daily injections resulting in mean [SEM] blood Pb levels of 9.98 [1.7] µg/dL) was found to increase iNOS and phosphorylated eNOS protein (Fiorim et al., 2011) which may cause an increase in •NO production and a short-term increase in •NO bioavailability. This increase in •NO bioavailability early after Pb exposure could be the immediate compensatory mechanism against the elevation in BP.

•NO, also known as endothelium-derived relaxing factor, is a potent endogenous vasodilator. Toxicological studies continued to investigate the effects of Pb on •NO-dependent vascular reactivity by using •NO stimulating vasodilators, such as acetylcholine (ACh) and sodium nitroprusside (SNP), and •NO inhibiting vasoconstrictors, such as L-NAME. Studies provided mixed evidence; however, results suggested that Pb disrupts the vasorelaxant response to •NO in the aorta due to damage to the endothelium. Pb exposure (1 ppm and 100 µM, 1 hour) decreased ACh-induced vasorelaxation, which triggers the release of •NO from the endothelial cell, in isolated rat tail artery, suggesting damage to the endothelium (Silveira et al., 2010; Zhang et al., 2007a). In aortic rings of perinatally exposed rats (1,000 ppm through pregnancy and lactation, mean blood Pb level: 58.7 µg/dL), blocking NOS with L-NAME abolished the relaxant response evoked by ACh (Grizzo and Cordellini, 2008). However, there was no change observed in the relaxation response to ACh by Pb alone (Fiorim et al., 2011; Rizzi

et al., 2009; Grizzo and Cordellini, 2008). Conversely, Skoczynska and Stojek (2005) found that Pb exposure (50 ppm; blood Pb level 11.2 µg/dL) enhanced •NO-mediated vasodilation by ACh in rat mesenteric arteries, and NOS inhibition enhanced the ACh relaxant response. A number of studies found that Pb exposure did not affect smooth muscle integrity since SNP-induced vasorelaxation, which is endothelium independent, was unchanged (Fiorim et al., 2011; Silveira et al., 2010; Rizzi et al., 2009; Grizzo and Cordellini, 2008).

•NO also was found to play a role in the interaction between Pb and the vasoconstrictor response. Blocking NOS with L-NAME or inhibiting iNOS specifically, which decreases •NO production, increased the contraction of aortic rings to the vasoconstrictor phenylephrine (PHE) and Pb exposure potentiated this response (Fiorim et al., 2011). Also, L-NAME increased the Pb pressor response to PHE after perinatal Pb exposure (1,000 ppm through pregnancy and lactation, blood Pb level 58.7 µg/dL) (Grizzo and Cordellini, 2008). Conversely, in rat renal interlobar arteries, Pb exposure blunted the increase in renal angiotensin II (AngII)-mediated contraction from NOS inhibition by L-NAME (Vargas-Robles et al., 2007). Treatment with the SOD mimetic tempol, which would increase •NO bioavailability, decreased, but did not eliminate, the Pb pressor response (Silveira et al., 2010).

In summary, recent studies continued to provide evidence for the role of ROS in Pb-induced hypertension and vascular disease by indicating Pb-induced increases in ROS and modulation of cardiovascular responses by antioxidant substances. Additionally, recent studies continued to show that Pb-induced hypertension and vascular responses are mediated primarily via inactivation of •NO not via inhibition of •NO production.

Vascular Reactivity

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 levels of norepinephrine (NE) and other catecholamines (Carmignani et al., 2000; Chang et al., 1997), and decreased β adrenergic receptor density and β agonist-stimulated cAMP production in the aorta and heart (Tsao et al., 2000; Chang et al., 1997). These stimulatory effects on the SNS paralleled the effects of Pb on BP, cardiac contractility, and carotid blood flow. Pb-induced elevations in arterial pressure and heart rate were abrogated by ganglionic blockade (Simões et al., 2011; Lai et al., 2002). Arterial pressure and heart rate gradually decreased 7 months after Pb exposure cessation as did the Pb-induced SNS alterations (Chang et al., 2005).

Increases in BP can be caused by activation of the SNS, which can lead to vascular narrowing, in turn, resulting in increased total peripheral resistance. In this neural mechanism, activation of the SNS leads to vasoconstriction, whereas inhibition leads to vasodilation. It has been suggested that Pb leads to increased vascular reactivity to catecholamines (i.e., epinephrine, NE, and dopamine), hormones of the SNS. Indeed, the isolated mesenteric vessel bed from Pb-treated rats (50 ppm with blood Pb level: 11.2 µg/dL, but not 100 ppm with blood Pb level: 17.3 µg/dL) exhibited increased reactivity to NE (Skoczynska and Stojek, 2005). However, in another study, 100 ppm Pb did not affect the NE-induced contractile response after 10 months of exposure (Zhang et al., 2009a), suggesting a small range of Pb doses affects pressor response to NE. Catecholamines act primarily through the adrenergic and dopaminergic receptors. Antagonists of α1-adrenergic, α2-adrenergic, β-adrenergic, and dopamine D1 receptors were found to abolish Pb-induced aortic contraction (Fazli-Tabaei et al., 2006; Heydari et al., 2006). However, the α1-adrenergic receptor agonist PHE induced aortic contractions and these were enhanced by treatment with Pb (100 ppm; blood Pb level: 26.8 µg/dL), indicating a specific role for the α1-adrenergic receptor (Silveira et al., 2010; Grizzo and Cordellini, 2008; Heydari et al., 2006). Removal of the endothelium blunted the PHE-induced contraction. Conversely, short-term Pb exposure (7 days) decreased the contractile response induced by PHE in rat aortas resulting in a decreased vascular reactivity (Fiorim et al., 2011). This decrease may be playing a compensatory role in attempting to correct the Pb-induced BP elevation. Additionally, Pb blunted the isoproterenol-induced relaxation, supporting a role for the β-adrenoceptors (Vassallo et al., 2008; Heydari et al., 2006).

Recently, there was mixed evidence for Pb disrupting vascular reactivity to other pressor agents. Pb (1 ppm) treatment of isolated rat thoracic aorta increased 5-HT induced contraction, which was endothelium dependent, but not due to 5-HT2B receptor expression (Zhang et al., 2005). Follow-up of this study in whole animals found, on the contrary, that Pb (100 ppm; blood Pb level: 28.4 µg/dL) decreased the maximum contractile response to 5-HT, but did not affect 5-HT plasma levels or 5-HT2B receptor expression (Zhang et al., 2009a). In addition, Pb exposure (100 ppm, 12 weeks) increased the renal vascular response to AngII in isolated perfused kidneys from Pb-exposed rats (Vargas-Robles et al., 2007).

Studies continued to investigate the effects of Pb on $^{\bullet}$NO-dependent vascular reactivity by using $^{\bullet}$NO stimulating vasodilators, such as ACh and SNP, and $^{\bullet}$NO inhibiting vasoconstrictors, such as L-NAME. These studies were discussed in the preceding section (Oxidative Stress Response).

Renin-Angiotensin-Aldosterone and Kininergic Systems

The adrenergic system also affects the renin-angiotensin-aldosterone system (RAAS), which is responsible for fluid homeostasis and BP regulation, and has been shown to be affected by Pb exposure. A meta-analysis found that Pb exposure (resulting in blood Pb levels: 30-40 µg/dL) increased plasma renin activity and renal tissue renin in young but not old rats (Vander, 1988). Exposure of experimental animals to Pb also induced increases in plasma, aorta, heart, and kidney angiotensin converting enzyme (ACE) activity; plasma kininase II, kininase I, and kallkrein activities; and renal AngII positive cells (Rodriguez-Iturbe et al., 2005; Sharifi et al., 2004; Carmignani et al., 1999). ACE activity declined over time while arterial pressure stayed elevated, suggesting that the RAAS may be involved in the induction, but not the maintenance of Pb-induced hypertension in rats.

Recent studies continued to implicate the RAAS in the development of Pb-induced hypertension, especially during early exposure in young animals. AngII, a main player in the RAAS, induces arteriolar vasoconstriction leading to increased BP. Pb exposure increased the vascular reactivity to AngII (Vargas-Robles et al., 2007). Acute (60 minutes) or short-term (7 days) exposure of Pb to rats increased the plasma ACE activity (Fiorim et al., 2011; Simões et al., 2011), and Fiorim et al. (2011) additionally found this increase to be correlated with the Pb-induced increase in systolic BP. However, at these short time points there were no changes in the AngII receptors 1 or 2 protein levels or expression. Treatment with the AngII receptor (AT_1R) blocker, losartan, or the ACE inhibitor, enalapril, blocked the Pb-induced systolic BP increase (Simões et al., 2011) and decreased the PHE-induced vasoconstrictor response in Pb-treated aortas (Fiorim et al., 2011). Similarly, treatment with Losartan resulted in a greater decrease in systolic BP in highly Pb exposed rats (1% Pb, 40 days; blood Pb level >240 µg/dL after exposure, 12-13 µg/dL after chelation after 1 year) compared to control rats that continued into later periods of follow-up (day 283) (Bagchi and Preuss, 2005). Increased systolic BP after early exposure to Pb corresponded with increased water intake, urine output, potassium excretion, and decreased urinary sodium and urine osmolality. These functional changes in renal behavior are consistent with the actions of a stimulated RAAS. Lower level Pb (100 ppm, 14 weeks; range of blood Pb levels: 23.7-27 µg/dL) exposure increased renal cortical AngII content and the number of tubulointerstitial AngII-positive cells (Bravo et al., 2007). This heightened intrarenal angiotensin corresponded with sodium retention and increased systolic BP and was ablated by the anti-inflammatory antioxidant, MMF. Sodium reabsorption is important for the maintenance of BP, and Na^+ transporters play a key role in this process. In other studies, Pb exposure increased activity and levels of the α-1 subunit protein of $Na^{+/}K^{+-}$ATPase, which plays a major role in Na^+ reabsorption and is regulated by the RAAS (Fiorim et al.,

2011; Simões et al., 2011). These studies point to the activation of the RAAS in the course of Pb-induced hypertension, particularly in the early stages of elevated BP.

Vasomodulators

The balance between production of vasodilators and vasoconstrictors is important in the regulation of BP and cardiovascular function. The 2006 Pb AQCD reported that Pb did not affect all vasomodulators in the same way. Urinary excretion of the vasoconstrictor, thromboxane (TXB_2), and the vasodilatory prostaglandin, 6-keto-PGF1α, 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 (VSMCs) via activation of phospholipase A_2 (Dorman and Freeman, 2002). Plasma concentration and urinary excretion of the vasoconstrictive peptide, endothelin (ET) 3 was increased after low (100 ppm), but not high-level (5,000 ppm) Pb exposure in rats (Gonick et al., 1997; Khalil-Manesh et al., 1994; Khalil-Manesh et al., 1993b). Antagonism of the ET receptor A blunted the downregulation of sGC and cGMP production by Pb in isolated rat artery segments, suggesting that some of the hypertensive effects of Pb exposure may be mediated through ET (Courtois et al., 2003). Additionally, Pb-exposed animals exhibited fluid retention and a concentration-dependent decline in the vasodilator, atrial natriuretic factor (ANF) (Giridhar and Isom, 1990). Results from these studies suggest that Pb may interfere with the balance between vasodilators and vasoconstrictors that contribute to the complex hormonal regulation of vascular contraction and BP.

The imbalance in vasomodulators is one explanation for the concentration-dependent vasoconstriction observed in animals after Pb exposure (Valencia et al., 2001; Watts et al., 1995; Piccinini et al., 1977). Vasoconstriction after Pb exposure was not reported in all studies (Shelkovnikov and Gonick, 2001) and is likely varied depending on the type of vessel used, the Pb concentration employed, and the animal species being studied. Studies have reported Pb-induced attenuation of ACh- and $^{\bullet}NO$-mediated vasodilation (Marques et al., 2001; Oishi et al., 1996) in some, but not all vascular tissues and in some, but not all studies (Purdy et al., 1997). These effects have been variably attributed to Pb-mediated activation of PKC and direct action on the VSMCs through the Ca^{2+} mimetic properties of Pb among other possibilities (Valencia et al., 2001; Watts et al., 1995; Piccinini et al., 1977).

A recent study investigated the role of the endothelial-derived vasoconstrictor, ET-1, in Pb-induced hypertension. ET-1 from the endothelium acts on the ET_A-type receptors located on the vascular smooth muscle layer and may be involved in vascular reactivity by $^{\bullet}NO$ 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 decreased the Pb-induced pressor response to ACh (Grizzo and 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.

In summary, a small number of available recent studies continued to show that Pb exposure affect vasomodulatory pathways that are important for the maintenance of vascular tone; however, results indicated that not all vascular cell types are similarly affected by Pb exposure. Further, effects appeared to vary according to the concentration of Pb exposure. Pb exposure has been shown to interrupt baseline or endogenous •NO-mediated vasodilation of vessels via alterations in PKC, sGC, VSMC, endothelial cells, NADPH oxidase, and Ca^{+2} levels. Recent studies indicated that Pb exposure may affect vascular reactivity by increasing COX-2 and COX-2-dependent vasoconstrictors. Also, the vasoconstrictor endothelin may contribute to Pb-induced vasomodulation via similar pathways as •NO including sGC and COX-2.

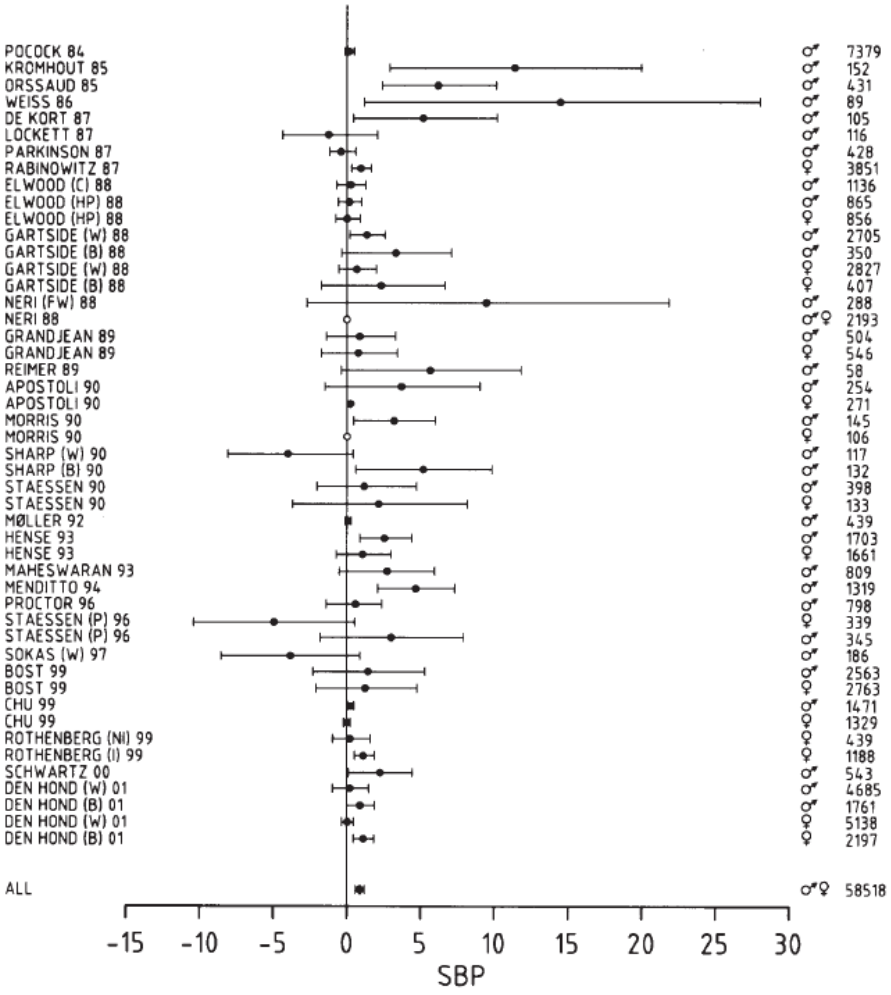
5.4.2.4 Summary of Blood Pressure and Hypertension

The 2006 Pb AQCD reported a clear association between higher blood Pb levels and higher BP. The effect was modest, but robust, as determined by a meta-analysis (Nawrot et al., 2002) of over 30 studies comprising over 40,000 adults (Figure 5-29). In the meta-analysis, each doubling of concurrent blood Pb was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP. Recent epidemiologic studies supported this conclusion at lower concurrent blood Pb levels (in populations with mean blood Pb levels < 2 µg/dL) and added to the evidence base regarding populations potentially at increased risk (i.e., high stress, genetic variants) and regarding associations of bone Pb levels with BP and hypertension in populations with mean bone Pb levels less than 20 µg/g. As these studies were mostly cross-sectional in design and were conducted

in adults whose concurrent blood Pb levels are influenced both by current Pb exposures and past Pb exposures mobilized from bone, uncertainty exists over the Pb exposure conditions that contributed to the associations observed between concurrent blood Pb level with increased BP and hypertension (Sections 4.3 and 4.7.3).

A recent study in an ethnically diverse community-based cohort of women and men aged 50-70 years of age found associations of both blood and tibia Pb levels with BP (Martin et al., 2006). This study also affirmed findings from other studies by demonstrating that with each increase of 1 µg/dL concurrent blood Pb level, systolic BP increased 1 mmHg and diastolic BP increased 0.5 mmHg and strengthened the evidence for an independent association with blood Pb level through the extensive examination of potential confounding. Additionally, recent epidemiologic studies provided evidence for associations in an adult cohort between blood Pb and BP and hypertension with relatively low blood Pb levels; a positive relationship was found in the NHANES adult data (1999-2002) with a geometric mean blood Pb level of 1.64 µg/dL (Muntner et al., 2005). However, as noted above, in adults, uncertainty exists regarding the magnitude, timing, frequency, and duration of Pb exposure that contribute to the associations observed with concurrent blood Pb levels. A new prospective study in Pb workers found independent associations of both baseline blood Pb level and subsequent changes in blood Pb over follow-up with changes in BP over follow-up and bone Pb level with hypertension (Glenn et al., 2006). Although these Pb workers had higher current Pb exposure compared with nonoccupationally-exposed adults, the results indicated that different mechanisms may mediate shorter-term Pb-associated increases in BP and longer-term Pb-associated development of hypertension.

In concordance with epidemiologic evidence, collectively, the animal toxicological studies providing blood Pb level and BP measurements reported higher BP with higher blood Pb levels (Figure 5-28). While the contribution of low concurrent blood Pb levels to the findings is difficult to ascertain in adults, animal toxicological studies provide support for low blood Pb level effects with increases in BP observed in groups of animals with blood Pb levels as low as 2 µg/dL (Rizzi et al., 2009; Tsao et al., 2000; Nakhoul et al., 1992). However, the majority of animal toxicological studies showing Pb-induced hypertension were conducted at higher Pb exposure levels that result in blood Pb levels > 10 µg/dL. In addition, new animal evidence suggests the potential for increased BP following short-term (4 weeks) Pb treatment that included injected boluses (Fiorim et al., 2011; Simões et al., 2011; Sharifi et al., 2004). New studies also demonstrated partial reversibility (not to levels in controls) of Pb-induced elevations in BP following Pb exposure cessation or chelation.



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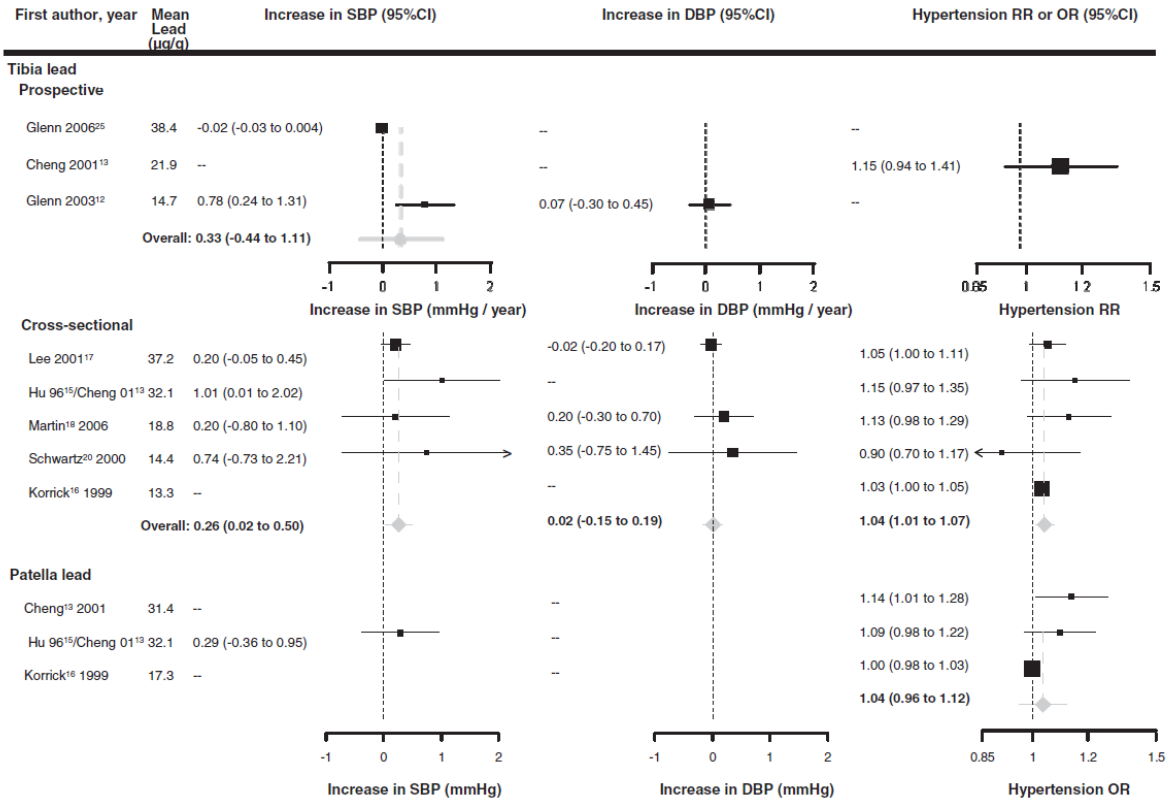
Study Key: C - Caerphilly Study; HP - Welsh Heart Program; W - Whites; B - Blacks; NI - Non-immigrants; I - Immigrants; FW - Foundry Workers; CS - Civil Servants; P - PheeCad (Public Health and Environmental Exposure to Cadmium) Study.
Note: Circles represent individual groups and squares represent the combined association sizes. Open circles denote a nonsignificant association size that was assumed to be zero.

Figure 5-29 Change in systolic BP (SBP), in mmHg with 95% CI, associated with a doubling in the blood Pb concentration.

Epidemiologic studies continued to investigate the relationship between bone Pb and BP. A recently published meta-analysis (Figure 5-30) (Navas-Acien et al., 2008) included several studies (three prospective, five cross-sectional) which individually showed that bone Pb level was associated with systolic BP but not diastolic BP. In the cross-sectional studies, a pooled estimate indicated an increase in systolic BP of 0.26 mmHg (95% CI: 0.02, 0.50) per 10 µg/g tibia Pb. In the longitudinal studies, a 0.33 mmHg (95% CI: -0.44, 1.11) increase was estimated per 10 µg/g bone Pb. Most studies also reported associations of bone Pb with hypertension. Pooled 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.



Source: Reprinted with permission of Elsevier Publishers, Navas-Acien et al. (2008)

In the Normative Aging Study, Hu et al. (1996b) reported the cross-sectional association between bone Pb levels and the prevalence of hypertension and Cheng et al. (2001) reported the cross-sectional association between bone Pb levels and systolic BP in study participants free of hypertension at baseline.

Note: The studies are ordered by increasing mean bone Pb levels. The area of each square is proportional to the inverse of the variance of the estimated change or log relative risk. Horizontal lines represent 95% confidence intervals. Diamonds represent summary estimates from inverse-variance weighted random effects models. Because of the small number of studies, summary estimates are presented primarily for descriptive purposes. RR indicates risk ratio.

Figure 5-30 Prospective and cross-sectional increase in systolic BP (SBP) and diastolic BP (DBP) and relative risk of hypertension per 10 µg/g increase in bone Pb levels.

A few recent epidemiologic studies also emphasized the potential interaction between measures of long-term Pb exposure, i.e., bone Pb levels, and factors such as chronic stress and HFE genetic variants to moderate or modify the relationship of BP and hypertension with Pb. For example, among NAS men, tibia Pb level was associated with a larger risk of developing hypertension in an originally nonhypertensive group among men with higher self-reported stress (Peters et al., 2007).

In addition to stress, recent epidemiologic studies investigated effect modification by race/ethnicity and genetic variants. In the NHANES 1988-1994 population of adults, the association of concurrent blood Pb with systolic BP was higher among Mexican Americans. In the same NHANES population, the association between blood Pb level and hypertension was higher among non-hispanic Blacks with the ALAD2 allele (Figure 5-25 and Figure 5-26 for results) (Scinicariello et al., 2010). Additionally, the association between blood Pb and PP was larger among NAS men with the HFE H63D variant (Figure 5-25). PP represents a good predictor of cardiovascular morbidity and mortality and an indicator of arterial stiffness (Zhang et al., 2010a). The aforementioned genes are related to iron metabolism and have been linked with differences in Pb distribution in blood and bone. Park et al. (2009b) provided further evidence of variants in iron metabolism genes impacting the association of bone Pb levels with QT interval changes (Table 5-19 for results).

Animal toxicological evidence continued to build on the evidence characterizing the mechanisms leading to these Pb-induced cardiovascular alterations. Biological plausibility for the consistent associations observed between blood and bone Pb and cardiovascular effects is provided by enhanced understanding of Pb-induced oxidative stress including $^{\bullet}$NO inactivation, endothelial dysfunction leading to altered vascular reactivity, activation of the RAAS, and vasomodulator imbalance.

5.4.3 Vascular Effects and Cardiotoxicity

Not only has Pb been shown to increase BP and alter vascular reactivity, but Pb can alter cardiac function, initiate atherosclerosis, and increase cardiovascular mortality. Past toxicological studies have reported that Pb can increase atheromatous plaque formation in pigeons, increase arterial pressure, decrease heart rate and blood flow, and alter cardiac energy metabolism and conduction (Prentice and Kopp, 1985; Revis et al., 1981). A limited number of available epidemiologic studies discussed in the 2006 Pb AQCD provided evidence of associations of blood Pb level with ischemic heart disease (IHD) and peripheral artery disease (PAD).

5.4.3.1 Effects on Vascular Cell Types

The endothelial layer is an important constituent of the blood vessel wall, which regulates macromolecular permeability, VSMC tone, tissue perfusion, and blood fluidity. Damage to the endothelium is an initiating step in development of atherosclerosis, thrombosis, and tissue injury. Given that epidemiologic and toxicological evidence suggests that long-

term Pb exposure is associated with a number of these conditions, numerous toxicological studies have investigated and found an effect of Pb on endothelial dysfunction. A recent occupational study found that endothelial function assessed by flow-mediated dilatation was impaired in highly Pb exposed workers (mean blood Pb levels: 24.1 in workers versus 7.8 µg/dL in unexposed controls) (Poręba et al., 2010).

The endothelial layer makes up only a small part of the vascular anatomy; the majority of the vessel wall is composed of VSMCs, which work in concert with the endothelial cells (EC) in contraction and relaxation of the vessel, local BP regulation, and atherosclerotic plaque development. Since Pb has been shown repeatedly to result in hypertension and vascular disease in experimental animals, studies continued to investigate and find an effect of Pb on VSMCs.

In in vitro assays, Pb (50 µM, 2 weeks) stimulated VSMC invasiveness in isolated human arteries leading to the invasion of medial VSMC into the vessel intima and development of intimal hyperplasia, a key step in atherosclerotic progression (Zeller et al., 2010). In addition, treatment with Pb (50 µM, 12 hours) promoted VSMC elastin expression and increased arterial extracellular matrix in isolated human arteries. VSMC invasiveness was also increased in culture by treatment with supernatant of Pb-treated human EC (50 µM), suggesting that Pb-exposed ECs secrete an activating compound. This compound was confirmed to be IL-8. Pb exposure (5-50 µM) was able to, in a concentration-dependent manner, increase IL-8 synthesis and secretion in human umbilical vein EC cultures through activation of the transcription factor Nrf2. Neutralization of IL-8 could block VSMC invasion and arterial intima thickening (Zeller et al., 2010). This study provides evidence that Pb exposure stimulates ECs to secrete IL-8 in an Nrf2-dependent manner that stimulates VSMC invasion from the vessel media to intima leading to a vascular thickening and possibly atherogenesis.

A number of CVDs, including atherosclerosis, are characterized by increased inflammatory processes. Numerous studies have shown that Pb exposure is associated with an inflammatory environment in vascular tissues of humans and animals as indicated by higher levels of inflammatory mediators like prostaglandin E_2 (PGE_2). Human aortic VSMCs treated with Pb (1 µM, 1-12 hours) exhibited increased secretion of PGE_2 time-dependently through enhanced gene transcription (Chang et al., 2011). This was preceded by a Pb-induced increase in the gene expression of 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 PGE_2 secretion and activation of $cPLA_2$ and COX. A follow-up to this study found that Pb treatment (1µM) of a human epithelial cell line increased COX-2 gene expression, promoter activity, and protein (Chou et al., 2011). Inhibition of NF-κB decreased the Pb-induced COX-2 activation; whereas EGFR inhibition blocked COX-2 upregulation and NF-κB nuclear translocation. Overall these studies suggest that Pb can induce pro-inflammatory events in VSMC in the form of increased PGE_2 secretion and $cPLA_2$ and COX-2 expression through activation of EGFR via ERK1/2 and NF-κB pathways.

Damage to the endothelium is a hallmark event in the development of atherosclerosis. Past studies have shown that Pb exposure results in de-endothelialization, impaired proliferation, and inhibition of endothelium repair processes after injury (Fujiwara et al., 1997; Ueda et al., 1997; Kaji et al., 1995; Kishimoto et al., 1995). However, Pb exposure was not found to lead to nonspecific cytotoxicity at low exposure levels (2-25 µM) as shown by the lack of release of lactate dehydrogenase (LDH) from Pb-treated bovine aortic EC (Shinkai et al., 2010). Instead, Pb induced specific cytotoxicity (caspase3/7 activation) through endoplasmic reticulum (ER) stress that was protected against by the ER chaperones glucose-regulated protein 78 (GRP78) and glucose-regulated protein 94 (GRP94). GRP78 and GRP94 play key roles in the adaptive unfolded protein response that serves as a marker of and acts to alleviate ER stress. Exposure of Pb to ECs induced *GRP78* and *GRP94* gene (2-25 µM) and protein (GRP78 [5-25 µM] and GRP94 [10-25 µM]) expression through activation of the IRE1-JNK-AP-1 pathways (Shinkai et al., 2010). This 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 et al., 2005a; Bener et al., 2001a; Kristal-Boneh et al., 1999) examining higher than current adult blood Pb levels reported higher total cholesterol levels related to Pb exposure, but mixed results for HDL, LDL, and triglycerides. More recently, Poreba et al. (2010), in an occupational study, reported no significant differences in parameters of lipid metabolism between Pb exposed workers (mean blood Pb level: 25 µg/dL) and unexposed individuals. Conversely, Kamal et al. (2011) reported that occupational Pb exposure (mean blood Pb level: >40 µg/dL) was associated with higher levels of triglycerides, total cholesterol, and LDL, and decreased HDL-C. Other Pb studies adjusted models for total cholesterol to control for this coronary heart disease risk factor. Higher mean total cholesterol with higher blood Pb levels has been reported (Menke et al., 2006). In developing models to predict bone Pb levels, Park et al. (2009a)

noted that total and HDL cholesterol were selected as 2 of 18 predictors for the bone Pb level model. Their findings suggested that higher Pb exposure may be associated with higher total and HDL cholesterols. A recent study reported increased LDL and decreased HDL in rats treated with Pb (20 ppm, i.p., 3 days/week, 8 weeks) (Roshan et al., 2011). The major risk factor that lipids represent for heart disease make relating lipid levels to Pb exposures an interesting but challenging hypothesis to test.

5.4.3.3 Heart Rate Variability

Pb has been shown not only to affect vascular contractility in animals, but also is associated with cardiac contractility. Eum et al. (2011) and Park et al. (2009b) followed up a previous NAS report (Cheng et al., 1998), which found increasing duration of corrected QT interval (QTc) with increasing bone Pb levels in men <65 years, but not in men ≥ 65 years. Eum et al. (2011) prospectively examined the association between blood and bone Pb levels and the development of electrocardiographic (ECG) conduction abnormalities among 600 men who were free of ECG abnormalities at the baseline assessment. A second ECG was obtained for 496 men 8.1 (SD = 3.1) years later on average. Baseline Pb concentrations in blood (mean [SD]: 5.8 [3.6] µg/dL), patella bone (mean [SD]: 30.3 [17.7] µg/g), and tibia bone (mean [SD]: 21.6 [12.0] µg/g) were similar to those found in other samples from the general U.S. adult population and much lower than those reported in occupationally exposed groups. Higher tibia Pb was associated with increases in QTc interval and QRSc duration. Compared with those in the lowest tertile of baseline tibia Pb (< 16 µg/g), participants in the highest tertile (> 23 µg/g) had a 7.94 msec (95% CI: 1.42, 14.45) greater increase in QTc interval and a 5.94 msec (95% CI: 1.66, 10.22) greater increase in QRSc duration over 8 years after adjusting for covariates. There were no statistically significant associations with patella or blood Pb levels. These associations were observed in men with relatively low blood and bone Pb concentrations who were free of cardiac conduction abnormalities at baseline and were examined prospectively. Thus, they indicate that long-term cumulative Pb exposure may increase the risk of developing cardiac abnormalities. Uncertainty exists as to the specific Pb exposure level, timing, frequency, and duration contributing these associations observed for tibia Pb levels. A recent occupational study reported lower HRV and abnormal parameters of heart rate turbulence in Pb-exposed workers (mean blood Pb levels: ~25 µg/dL) compared to control subjects (Poręba et al., 2011a).

Park et al. (2009b) examined whether polymorphisms in genes known to alter iron metabolism (HFE, transferrin [TF] C2, heme oxygenase-1 [HMOX-1]) modify the association between Pb biomarker levels and the QT interval. Investigators examined associations in data stratified on polymorphisms in the three genes. They also analyzed

interaction models with cross-product terms for genotype and the Pb biomarker. The distributions of all genotypes but the HFE variant, H63D, were in Hardy-Weinberg equilibrium. Subjects homozygous for the other HFE variant, C282Y, had higher bone Pb levels and those homozygous for H63D and heterozygous for both C282Y and H63D had lower bone Pb levels. The HMOX-1 L variant (longer repeats of GT, associated with lower enzyme inducibility) alone, compared to the wild type, showed a statistically significant interaction with tibia Pb (11.35 msec longer QTc interval for each 13 µg/g increase in bone Pb in L-allele variants). No other gene variant alone showed different Pb-associated QTc intervals from those in wild types, either for tibia and patella Pb or for (linear) concurrent blood Pb. Lengthening of QTc with higher tibia and blood Pb was more pronounced with an increase in the total number of gene variants, driven by a joint effect between HFE variant and HMOX-1 L allele. There was a trend observed with blood and tibia Pb-associated QTc interval increasing with increasing number of gene variants from 0 to 3. This study provided further evidence of gene variants modifying associations of Pb biomarkers with cardiovascular effects.

The interaction of key markers of the metabolic syndrome with bone Pb levels in affecting HRV was investigated in a group of 413 older adults with patella Pb measurements in the NAS (Park et al., 2006). Metabolic syndrome was defined to include three or more of the following: waist circumference >102 cm, hypertriglyceridemia (>150 mg/dL), low HDL cholesterol (<40 mg/dL in men), high BP >130/85 mmHg, and high fasting glucose (>110 mg/dL). Men using antihypertensive medication or diabetes medications were counted as high BP or high fasting glucose, respectively. The strongest relationships between patella Pb levels and lower HRV were observed among those with three or more metabolic abnormalities. A trend was observed for larger patella Pb-associated decreases in HRV with increasing number of metabolic abnormalities. These results suggest multiplicative effects of cumulative Pb exposure and metabolic abnormalities on key predictors of CVD. Park et al. (2006) also reported the penalized spline fits to bone Pb in models assessing only main effects of bone Pb. The optimal degree of smoothing determined by the generalized cross-validation criterion for all HRV measures was 1, which indicated that the associations were nearly linear. The spline fits and associated statistics showed that the bone Pb main effects on HRV measures were linear. However, the relationship with LF/HF was linear with log(LF/HF).

Increased incidence of arrhythmia and atrioventricular conduction block was found in rats after 12 weeks of Pb exposure (100 ppm; mean blood Pb level 26.8 µg/dL) (Reza et al., 2008). Also, Pb exposure for 8 weeks increased heart rate and systolic BP. These increases corresponded with increased cardiac contractile force and prolonged ST interval, without alteration in QRS duration or coronary flow. In contrast, another study found that Pb (100 µM) exposure, in a concentration-dependent manner, 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 changed the response to inotropic agents and blunted the force produced during contraction. Conversely, past studies have found that Pb exposure increases intracellular Ca^{2+} content (Lal et al., 1991; Favalli et al., 1977; Piccinini et al., 1977), which could result in increased cardiac output and hypertension.

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 for adults were discussed previously, also examined the association of blood Pb with PAD. The authors observed an increasing trend in the odds of PAD with increasing concurrent blood Pb level. The OR for PAD comparing the fourth quartile of blood Pb (> 2.47 µg/dL) to the first quartile of blood Pb (< 1.06 µg/dL) was 1.92 (95% CI: 1.02, 3.61). These results are consistent with those from a previous NHANES analysis conducted by Navas-Acien et al. (2004).

Navas-Acien et al. (2004) reported a trend of increasing OR for PAD with increasing quartile of concurrent blood Pb or Cd in adults who were 40 years of age in the 1999-2000 NHANES population. These authors tested both Pb and Cd in separate models, tested the metals simultaneously, and tested the interaction between the metals. The correlation coefficient between natural log Pb and natural log Cd was 0.32 ($p < 0.001$). Although the interaction was not statistically significant, when blood Pb and blood Cd were in the same model, the ORs were diminished slightly but both showed statistically significant trends of increasing OR with increasing quartile of the metal. These results indicate that blood Cd levels did not confound the association between blood Pb level and PAD. In a subsequent analysis, Navas-Acien et al. (2005) used the same 1999-2000 NHANES dataset, but constructed PAD models using a suite of urine metal concentrations. Power was reduced in this study because only 659-736 subjects (compared to 2,125) had spot urine metal tests in the data set. Urinary Cd, but not urinary Pb, was consistently associated with PAD in all models. Associations also were observed with urinary antimony and tungsten. Spot urine Pb measurements are less reliable compared to blood Pb measurements. In Navas-Acien et al. (2005), the urinary Pb level association with PAD was sensitive to adjustment for urinary creatinine, indicating that spot urine Pb measurements are affected by differences in urine dilution. This finding

illustrates the limited reliability of spot urine Pb measurements compared to blood Pb measurements.

5.4.3.5 Ischemic Heart Disease

A few studies discussed in the 2006 Pb AQCD indicated associations between Pb biomarker levels and increased risk of cardiovascular outcomes associated with IHD, including left ventricular hypertrophy (Schwartz, 1991) and myocardial infarction (Gustavsson et al., 2001). Recently, Jain et al. (2007) reported on the incidence of IHD (physician confirmed MI, angina pectoris) among older adult males enrolled in the NAS and followed 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. Baseline blood, tibia, and patella Pb levels were log-transformed. Blood Pb level and patella Pb level were associated with increased risk of IHD over the 10-year follow-up period. When blood Pb and patella Pb were included simultaneously in the model, each of their HRs was only moderately attenuated (HR: 1.24 [95% CI: 0.80, 1.93] per SD increase in blood Pb and HR: 2.62 [95% CI: 0.99, 6.93] per SD increase in patella Pb). When blood Pb and tibia Pb were included simultaneously in the model, their risk estimates were only moderately attenuated (HR: 1.38 [95% CI: 0.89, 2.13] per SD increase in blood Pb and HR: 1.55 [95% CI: 0.44, 5.53] per SD increase in tibia Pb). These findings indicate that both blood and bone Pb levels contribute independently to IHD incidence.

IHD, characterized by reduced blood supply to the heart, may result from increased thrombosis. A recent animal study suggested that Pb exposure promotes a procoagulant state that could contribute to thrombus formation (Shin et al., 2007). In a rat model of venous thrombosis, Pb treatment (i.v. 25 mg/kg) resulted in increased thrombus formation. Additionally, Pb treatment to human erythrocytes (red blood cells, RBCs) increased coagulation at a dose of 5 µM and thrombin generation in a concentration-dependent manner at doses from 2-5µM. This enhanced procoagulant activity in Pb-treated RBCs was the result of increased outer cell membrane phosphatidylserine (PS) surfacing (human RBCs: 2-5 µM; rat RBCs: 5 µM). Similar to these in vitro results, PS externalization on erythrocytes was increased in Pb-treated rats (i.v. 50-100 mg/kg, not 25 mg/kg). Increased PS externalization was likely the result of increased intracellular calcium (5 µM Pb), enhanced scramblase activity (5-10 µM Pb), inhibited flippase activity (5-10 µM Pb), and ATP depletion (1-5 µM Pb) after Pb exposure (Shin et al., 2007).

5.4.3.6 Atherosclerosis

Epidemiologic and toxicological studies provide evidence for increased atherosclerosis and intimal medial thickening (IMT) due to Pb exposure. The association between stroke subtypes and severity of cerebral atherosclerosis was examined in relation to a single concurrent blood Pb level and total 72-hour urinary Pb level (body Pb store-EDTA mobilization test) in a cross-sectional study of 153 patients (mean age 63.7 years) receiving digital subtraction angiography in Chang Gung Memorial Hospital in Taiwan from 2002 to 2005 (Lee et al., 2009). In an analysis adjusted for age, sex, hypertension, diabetes, triglyceride, uric acid, smoking, and alcohol consumption, a 1 µg increase in urine Pb was associated with ≥ 50% stenosis in the intracranial carotid system with an OR (95% CI) of 1.02 (1.00, 1.03). Urine Pb was associated with greater stenosis in the extracranial or vertebrobasilar systems. Blood Pb level was not associated with greater stenosis in any region. As the development of atherosclerosis is a lifelong process, body Pb stores, analyzed by total 72-hour urine Pb amount, may more strongly be associated with atherosclerosis than are single blood Pb measurements.

A recent study correlated greater carotid artery IMT with higher concurrent serum Pb levels (mean [SD] 0.41 [0.38] µg/dL) in hemodialysis patients (Ari et al.). A few available recent occupational studies also presented evidence for increased measures of atherosclerosis in highly Pb exposed adult populations with mean blood Pb levels around 25 µg/dL. Poręba et al. (2011b) reported increased local arterial stiffness and more frequent left ventricular diastolic dysfunction in Pb-exposed workers with hypertension compared to nonexposed controls with hypertension. Occupational exposure to Pb (mean blood Pb levels: 24 µg/dL in workers, 8.3 µg/dL in nonexposed group) was also associated with greater IMT and atherosclerotic plaque presentation, analyzed by Doppler ultrasound (Poreba et al., 2011).

Zeller et al. (2010) examined human radial and internal mammary arteries exposed to Pb in culture and reported a concentration-dependent increase in arterial intimal thickness (statistically nonsignificant at 5 µM Pb, significant at 50 µM Pb, 2 week treatment) and intimal extracellular matrix accumulation (50 µM). Also, Pb promoted EC proliferation (5 and 50 µM, 72 hours) and VSMC elastin expression (50 µM, 12 hours), as discussed above (Section 5.4.3.1) (Zeller et al., 2010). Another study showed that Pb exposure (100 ppm in drinking water for 10 months; mean blood Pb level 28.4 µg/dL) of rats also increased the aortic media thickness, media-lumen ratio, and medial collagen content (Zhang et al., 2009a). These morphological changes to the vessel due to Pb exposure indicate initiation of arteriosclerosis and could be the cause of decreased contractile response of the vessel due to altered visco-dynamic vessel properties. Alternatively, these vascular changes could be an effect of Pb-induced hypertension.

Table 5-19 Characteristics and quantitative data for associations of blood and bone Pb with other CVD measures in epidemiologic studies ordered as they appear in the text

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)[a]
Heart rate variability					
Eum et al. (2011)	600 men free of electrographic abnormalities at the time of baseline ECG from NAS in Greater Boston, MA area (496 with follow-up ECG 8 years later)	ECG conduction[b] (QTc, QRSc, JTc, QT prolongation, JT prolongation, IVCD[c], AVCD, Arrhythmia)	Baseline Blood Pb: Mean (SD): 5.8 (3.6) µg/dL Baseline Patella Pb: Mean (SD): 30.3 (17.7) µg/g Baseline Tibia Pb: Mean (SD): 21.6 (12.0) µg/g Q1: <16 µg/g (n = 191) Q2: 16.0 - 23 µg/g (n = 208) Q3: >23 µg/g (n = 195)	Repeated measures linear regression adjusted for age, education, smoking, BMI, albumin-adjusted serum calcium, and diabetes status at baseline, and years between ECG tests and QT-prolongation drugs at the time of ECG measurement.	Tibia Pb: Adjusted 8-year change (95% CI): QTc: Q2 vs. Q1 (reference): 7.49 (1.22, 13.75) msec, Q3 vs. Q1: 7.94 (1.42, 14.45) msec p for trend = 0.03 QRSc: Q2 vs. Q1: 0.52 (-3.60, 4.65) msec Q3 vs. Q1: 5.94 (1.66, 10.22) msec p for trend = 0.005 No associations with patella or blood Pb
Park et al. (2009b)	613 men from NAS in Greater Boston, MA area (8/1991 - 12/1995)	QTc[b] interval	Baseline Blood Pb: Median (IQR): 5 (4-7) µg/dL Baseline Patella Pb: Median (IQR): 26 (18-37) µg/g Baseline Tibia Pb: Median (IQR): 19 (14-27) µg/g	Linear regression models adjusted for age, BMI, smoking status, serum calcium, and diabetes.	Per IQR (3 µg/dL) increase in blood Pb 1.3 (-0.76, 3.36) msec after 8-year follow up Per IQR (19 µg/g) increase in patella Pb 2.64 (0.13, 5.15) msec Per IQR (13 µg/g) increase in tibia Pb 2.85 (0.29, 5.40) msec
Park et al. (2006)	413 men from NAS in Greater Boston, MA area (11/14/2000 - 12/22/2004)	HRV (SDNN, HF, HF_{norm}, LF, LF_{norm}, LF/HF)	Baseline Patella Pb (measured within 6 mo of HRV: Median (IQR): 23.0 (15-34) µg/g Estimated[a]: Median (IQR): 16.3 (10.4-25.8) µg/g Baseline Tibia Pb: Median (IQR): 19.0 (11-28) µg/g	Log linear regression models adjusted for age, cigarette smoking, alcohol consumption, room temperature, season (model 2) BMI, fasting blood glucose, HDL cholesterol, triglyceride, use of β-blockers, calcium channel blockers, and/or ACE inhibitors	Tibia Pb: Model 2 Change (95%CI) HF: –0.9 (–3.8, 2.1) normalized units (nu) LF: 0.9 (–2.0, 3.9) nu Log LF/HF: 3.3 (–10.7, 19.5) (%) Per 17 µg/g tibia Pb Patella Pb: Model 2 Change (95%CI) HF: –0.6 (–3.1, 1.9) nu LF: 0.6 (–1.9, 3.1) nu Log LF/HF: 3.0 (–8.7, 16.2) (%) Per 15.4 µg/g patella Pb Effect estimates were more pronounced among those with metabolic syndrome.
Peripheral artery disease					

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)[a]
Muntner et al. (2005)	9,961 NHANES (1999-2002) participants	PAD	Range Concurrent Blood Pb: Q1: <1.06 µg/dL, Q2: 1.06-1.63 µg/dL Q3: 1.63-2.47 µg/dL Q4: >2.47 µg/dL	Logistic regression models adjusted for age, race/ethnicity, sex, diabetes mellitus, BMI, cigarette smoking, alcohol consumption, high school education, health insurance status	OR (95% CI): Q1: 1.00 (Reference), Q2: 1.00 (0.45, 2.22), Q3: 1.21 (0.66, 2.23), Q4: 1.92 (1.02, 3.61)
Navas-Acien et al. (2005)	790 participants, age ≥ 40 yr, from NHANES (1999-2000)	PAD	Concurrent urinary 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 in urinary Pb Array of metals in urine also evaluated.
Ischemic Heart Disease					
Jain et al. (2007)	837 men from NAS in Greater Boston, MA area (1991-2001)	IHD (MI or angina pectoris)	Baseline Blood Pb Mean (SD): Non-cases 6.2 (4.3) µg/dL; Cases 7.0 (3.8) µg/dL Baseline Patella Pb Mean (SD): Non-cases 30.6 (19.7) µg/dL; Cases 36.8 (20.8) µg/dL Baseline Tibia Pb Mean (SD): Non-Cases 21.4 (13.6) µg/g; Cases 24.2 (15.9) µg/g Cases: Blood Pb range: 1.0 to 20.0 µg/dL Patella Pb range: 5.0 to 101 µg/g Tibia Pb range: -5 to 75 µg/g	Cox proportional hazards models adjusted for age, BMI, education, race, smoking status, pack-years smoked, alcohol intake, history of diabetes mellitus and hypertension, family history of hypertension, DBP, SBP, serum triglycerides, serum HDL, and total serum cholesterol	Blood Pb level ≥ 5 µg/dL OR over 10-year follow-up: 1.73 (1.05, 2.87) Ln blood Pb OR: 1.45 (1.01, 2.06) Ln patella Pb level OR: 2.64 (1.09, 6.37) Ln tibia Pb level OR: 1.84 (0.57, 5.90) Per 1 SD increase in Pb biomarker

[a]Estimated patella Pb accounts for declining trend in patella Pb levels between analysis of bone Pb and HRV.

[b]Heart-rate-corrected QT interval calculated by Bazett's formula

[c]IVCD, intraventricular conduction defect; AVCD, atrioventricular conduction defect

5.4.3.7 Summary of Vascular Effects and Cardiotoxicity

There are a limited number of studies that investigate the associations between Pb biomarkers and cardiovascular effects other than BP or hypertension (Table 5-19). As presented in Table 5-19, these studies demonstrated associations between various biomarkers of Pb exposure and clinical cardiovascular outcomes such as atherosclerosis, IHD, PAD, and HRV occurrence in adult populations after adjusting for potential confounding by variables such as age, sex, BMI, smoking, alcohol consumption, and diabetes. In a limited body of studies, mixed evidence of association between occupational exposure to Pb and altered cholesterol was reported. Studies of Pb-induced endothelial dysfunction, VMSC invasiveness, and inflammation in isolated vascular tissues and cells provide mechanistic evidence to support the biological plausibility of these other vascular effects and cardiotoxicity. A recent study provided evidence for the

interaction between biomarkers of Pb exposure and the HFE C282Y and HMOX-1 L variant on the prolonged QT interval in nonoccupationally-exposed older men (Park et al., 2009b). Also, in the NAS population, bone Pb levels were associated with larger decreases in HRV parameters among subjects identified as having metabolic abnormalities (Park et al., 2006). These metabolic abnormalities, abdominal obesity, hypertriglyceridemia, low HDL cholesterol, high BP/medication use, or high fasting glucose, have been shown to be associated with increased risk of cardiovascular events.

A prospective NAS study reported that higher baseline tibia Pb was associated with increases in QTc interval and QRSc duration over an 8-year follow-up period (Eum et al., 2011). Concurrent blood Pb levels (population means >2.5 µg/dL) were associated with greater odds of PAD in adults in NHANES analyses (Muntner et al., 2005; Navas-Acien et al., 2004). In addition, in the 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 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 ECs, which was responsible for stimulating VSMC invasion into the vessel intimal layer. Pb treatment also increased extracellular matrix and elastin, primary sites for lipid deposition in the vessel wall. Overall, the relatively available few studies provide support for associations between Pb biomarkers and other cardiovascular conditions, yet further research is warranted to understand these relationships.

5.4.4 Cardiovascular Function and Blood Pressure in Children

5.4.4.1 Introduction

The study of cardiovascular function effects in relation to blood Pb levels in children potentially offers unique information on several topics. First, by examining endpoints predictive of future cardiovascular pathology, these studies may offer information on the potential cardiovascular effects of Pb exposure in an understudied population. Second, examination of cardiovascular changes that are antecedent to increased BP and changes in other CVD-related endpoints at later lifestages may inform uncertainties in regards to the time course of cardiovascular changes associated with Pb exposure. Finally, these studies address gaps in knowledge regarding Pb exposure effects in populations with mean blood Pb levels in the range of < 5 to 10 µg/dL.

An advantage of examining the literature about the association between cardiovascular effects and blood Pb levels in children is that the blood Pb levels of children in new studies may better reflect their relatively recent Pb exposure and its effect on CVD than blood Pb levels do in adults because of the uncertainties related to the effects of earlier higher Pb exposure levels. A possible disadvantage of studying the associations of blood Pb levels and cardiovascular effects in children is the lower prevalence of such conditions in children. For example, the prevalence for hypertension in children (9 to 10 years old) ranges from to 2 to 5 percent (Daniels, 2011; Steinthorsdottir et al., 2011). The prevalence of hypertension increases with age. More than half of people aged 60 to 69 years have hypertension (Chobanian et al., 2003). Further, compensatory mechanisms in children may be more active than in adults, and the cardiovascular tissue of the young may be less susceptible to damage than that of adults. The lower disease prevalence may make it more difficult to find a relationship between blood Pb level and cardiovascular effects in child studies. Although studies in children may be limited with regard to study power in assessing associations with present CVD pathology, they provide for the examination of early function changes that are associated with subsequent CVD.

The limited numbers of studies published on children examined endpoints such as total peripheral resistance (TPR), BP, and autonomic nervous system activation. These new and earlier studies are presented in Table 5-20. Multiple studies in New York State evaluated two child cohorts born in the 1990s after Pb was removed from gasoline in the U.S. with mean blood Pb levels of 4.62 and 1.01 µg/dL (Gump et al., 2011; Gump et al., 2009; Gump et al., 2007; Gump et al., 2005). Zhang et al. (In Press) examined children in Mexico City born from 1994 to 2003, when Pb was being taken out of gasoline in Mexico (Martinez et al., 2007). The geometric mean cord and concurrent blood Pb levels of the children in the Mexico City cohort were 4.67 and 2.56 µg/dL.

Table 5-20 Studies of Children Cardiovascular Endpoints and Pb Biomarkers Ordered as They Appear in the Text

Study	Population/ Location	Parameters	Blood Pb Dataa	Statistical Analysis	Effect Estimates/Results
Gump et al. (2005)	122 children age 9.5 yr in Oswego, NY (born at a single hospital in New York from 1991-94)	SBP, TPR (total peripheral vascular resistance)	Cord blood Pb: GM (GSD): 2.56 µg/dL (1.16) Childhood (mean age of measurement: 2.6 yr) blood Pb: GM (GSD): 4.06 µg/dL (1.14)	Multivariate linear regression models examined the relationship of blood Pb with change in z-score for outcome (post- and pre-stress). Potential confounders considered: HOME score, SES, birth weight, child BMI, child sex.	Per 1 µg/dL increase in childhood blood Pb level, 0.088 (95% CI: 0.023, 0.153) dyne-s/cm^5 change in TPR Per 1 µg/dL increase in cord blood Pb level, 12.16 (95% CI: 2.44, 21.88) mmHg higher SBP
Gump et al. (2007)	122 children age 9.5 yr in Oswego, NY	SBP, TPR	Childhood (mean age of measurement: 2.6 yr) blood Pb: GM (GSD): 4.06 µg/dL (1.14)	Linear regression models adjusting for the same covariates as in Gump et al. (2005). Separate models testing whether Pb is a mediator of SES associations,(Sobel test) and whether Pb moderates SES associations (Pb-SES interaction).	Blood Pb was a mediator of the SES-TPR relationship SES alone: -0.62 dyne-s/cm^5 ($p < 0.05$) SES with Blood Pb: -0.40 dyne-s/cm^5 ($p > 0.10$), change in R^2 attributable to SES: -55.3% Blood Pb was a potential moderator of the SES-TPR relationship. Blood Pb × SES interaction: p = 0.07 . Blood Pb was a moderator of SES-SBP relationship Pb × SES interaction: p = 0.007 At blood Pb levels > 4 µg/dL, SES not significantly associated with SBP
Gump et al. (2009)	122 children age 9.5 yr in Oswego, NY	Salivary cortisol	Cord blood Pb: GM (GSD): 2.56 µg/dL (1.16) Childhood (mean age of measurement: 2.6 yr) blood Pb: GM (GSD): 4.06 µg/dL (1.14)	Linear regression to examine whether blood Pb level mediates or moderates the relationship between SES and salivary cortisol as in Gump et al. (2007)	Blood Pb was a mediator of the SES-cortisol association. SES was no longer significantly associated with cortisol after adjusting for blood Pb level. R^2 for SES decreased by 40, 33, 50% for cortisol measured at 21, 40, and 60 min. Blood Pb was not a significant moderator of SES-cortisol association. Blood Pb × SES interaction term was not statistically significant
Gump et al. (2011)	140 children ages 9-11 yr	SBP, TPR, HRV (heart rate variability) in response to acute stress (mirror tracing task)	Concurrent blood Pb: GM: 1.01 µg/dL Quartiles: Q1: 0.14-0.68 µg/dL Q2: 0.69-0.93 µg/dL Q3: 0.94-1.20 µg/dL Q4: 1.21-3.76 µg/dL	Outcomes were analyzed as continuous variables for the pre-stress values or the change post- and pre-stress. Regression models were adjusted for sex, SES, BMI, and age.	Blood Pb levels associated with autonomic and cardiovascular dysregulation in response to stress – greater vascular resistance, reduced stroke volume, and cardiac output Change in SBP (mmHg) across quartiles: Q1: 5.30, Q2: 7.33, Q3: 7.07, Q4: 7.23, p for trend = 0.31 Change in TPR (%) across quartiles: Q1: 2.91, Q2: 8.18, Q3: 9.55, Q4: 9.51, p for trend = 0.03 Change in Stroke Volume (%) across quartiles: Q1: 2.23, Q2: 0.91, Q3: -3.47, Q4: -0.89, p for trend = 0.04

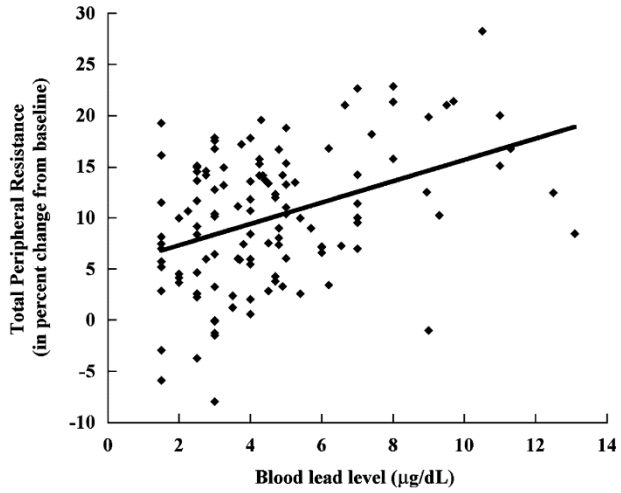
Study	Population/ Location	Parameters	Blood Pb Dataa	Statistical Analysis	Effect Estimates/Results
Zhang et al. (In Press)	457 mother child pairs in a birth cohort, born 1994 to 2003 in Mexico City. Children were evaluated 2008-2010 at ages 7-15 yr	SBP	Cord blood Pb: GM (GSD): 4.67 µg/dL (1.18) (N=323) Concurrent blood Pb: GM (GSD): 2.56 µg/dL (1.16) (N=367) Maternal post-partum bone Pb: Median (IQR): Tibia Pb: 9.3 (3.3, 16.1) µg/g Patella Pb: 11.6 (4.5, 19.9) µg/g	Multiple regression models and generalized estimating equations (log linear for cord blood, linear for concurrent blood and maternal bone). The base model considered maternal education, birth weight, BMI, sex, and child concurrent age as covariates.	Prenatal Pb exposure may be associated with higher BP in female offspring. Among girls, an IQR (13 µg/g) increase in maternal tibia Pb was associated with a 2.11 (95% CI: 0.69, 3.52) mmHg increase in SBP IQR (16 µg/g) increase in maternal patella Pb was associated with a 0.87 (95% CI: -0.75, 2.49) mmHg increase in SBP IQR (4 µg/dL) increase in cord blood Pb was associated with a 0.75 (95% CI: -1.13, 2.63) mmHg increase in SBP
Factor-Litvak et al. (1999; 1996)	260 children ages 5.5 years old in K. Mitrovica and Pristina, Yugoslavia	SBP	Concurrent blood Pb range: 4.1 to 76.4 µg/dL	Linear regression analysis. Potential confounders considered: gender, maternal education, birth weight, HOME score, and BMI.	Per 1 µg/dL increase in concurrent blood Pb level, 0.05 (95% CI: -0.02, 0.13) mmHg higher SBP Blood Pb level at birth and cumulative blood Pb level were not as strongly associated with SBP at age 5.5 yr.
Gerr et al. (2002)	Young adults age 19-29 years, born 1965-1975, male and female; half of the subjects had grown up around an active Pb smelter in Silver Valley, Idaho	BP	While the concurrent mean blood Pb level was 3.15 µg/dL for the highest bone Pb category (> 10 µg/g), early childhood mean blood Pb levels in this group were substantially elevated for all bone Pb level categories and were highest among participants in the highest bone Pb level category. The mean blood Pb level was 65 µg/dL among participants with bone Pb level >10 µg/g.	Multiple linear regression models always included age, sex, height, BMI, current smoking status, frequency of alcohol consumption, current use of birth-control medication, hemoglobin level, serum albumin, and income, regardless of significance levels. Both blood Pb (as a linear term) and bone Pb (a four category ordinal variable from <1 µg/g to >10 µg/g) were tested together.	Group in highest quartile of tibia Pb level (> 10 µg/g) had 4.26 (95% CI: 1.36, 7.16) mmHg higher SBP and 2.80 (95% CI: 0.35, 5.25) mmHg higher DBP compared to the lowest tibia Pb group (< 1 µg/g).

[a]Blood Pb data are estimates of geometric mean (GM) and geometric standard deviation (GSD) using the arithmetic mean and SD.

5.4.4.2 Cardiovascular Functioning in Children

The relationship between cardiovascular functioning (TPR, BP, stroke volume, and cardiac output,) and blood Pb levels was examined by Gump et al. (2007; 2005) in a cohort born at a single New York hospital. Higher early childhood Pb levels (average age 2.6 years) were associated with greater TPR response to acute stress induced by mirror tracing on a computer at age 9.5 years as shown in Figure 5-31. Testing blood Pb with linear, quadratic, and cubic terms did not produce significantly different Pb-TPR associations which the authors suggested showed effects that were concentration dependent and, notably were not emergent at a specific exposure threshold. TPR increased with increasing quartile of blood Pb level. A mediational analysis indicated that Pb was a significant mediator of the SES-TPR reactivity association; some evidence also suggested moderation, whereby the inclusion of blood Pb into the model reduced the effect estimate for SES. Biological plausibility for these observations in children is

provided by observations that Pb exposures increases TRP in toxicological studies and by mechanistic evidence indicating that Pb-induced changes in SNS activity may mediate such effects (Section 5.4.2.3). Additionally, higher blood Pb level measured at age 2.6 years was associated with a smaller stroke volume and cardiac output responses to acute stress at age 9.5 years. In a further analysis in this cohort, Gump et al. (2009) examined the possibility that Pb may mediate an association between SES and cortical responses to acute stress. Elevated cortisol has been associated with hypertension (Whitworth et al., 2000). Gump et al. (2009) found that lower family income was associated with greater cortisol levels following an acute stress task and that blood Pb was a mediator of this association.



Source: Reprinted with permission of Elsevier (Gump et al., 2005)

Figure 5-31 Children's adjusted total peripheral resistance (dyn-s/cm^5) responses to acute stress tasks, as a function of childhood Pb levels.

In a different cohort of 140 children 9 to 11 years of age recruited from local pediatrician offices and from mailings to homes with children in this age group, Gump et al. (2011) used a similar acute stress-producing paradigm to that used in the previous studies to examine the associations of concurrent blood Pb with cardiovascular responses. TPR significantly increased in a concentration-dependent relationship with blood Pb, with most of the increase occurring between the first quartile blood Pb (0.14-0.68 µg/dL) and the second quartile blood Pb (0.69-0.93 µg/dL). This result is consistent with those of Gump et al. (2005). Also, these new findings provided evidence of associations with concurrent blood Pb levels and with lower blood Pb levels (Gump et al., 2011) than were previously examined in Gump et al. (2005).

Studies examining HRV in adults and animal toxicology are discussed in Section 5.4.3.3. In Gump et al. (2011), cardiac autonomic regulation decreased in a concentration-dependent manner with increasing concurrent blood Pb quartile, with the largest change relative to the first quartile (0.14-0.68 µg/dL) measured in the highest blood Pb quartile (1.21-3.76 µg/dL). Also, high frequency HRV, decreased more with acute stress in the highest Pb quartile group (1.21-3.76 µg/dL). In the earlier cohort, early childhood (mean age at collection: 2.6 years) blood Pb level was associated with reduced stroke volume and cardiac output (Gump et al., 2007; Gump et al., 2005). In this new study, Gump et al. (2011) found the same but for concurrent blood Pb level and at lower blood Pb levels.

5.4.4.3 Blood Pressure in Children

Zhang et al. (In Press) conducted a longitudinal study that examined changes in BP in 323 girls and boys aged 7 to 15 years old in a Mexico City cohort for a relationship with maternal bone Pb measured one month post-partum (a measure of cumulative exposure that could expose fetuses to Pb through accelerated mobilization of bone Pb during pregnancy), and cord blood Pb at delivery. This was the first study to examine the association of maternal bone Pb, as a marker of prenatal exposure, with offspring BP. The model including both girls and boys (without adjustment for concurrent blood Pb) showed no statistically significant association overall of any Pb biomarker with child BP. A significant interaction was found between maternal tibia Pb and sex, and in models stratified by sex, maternal tibia Pb was associated with adjusted systolic and diastolic BP in females, but not males. Maternal post-partum median tibia Pb was 9.3 µg/g (IQR: 3.3, 16.1 µg/g) with no significant differences between mothers of male and female offspring. Suboptimal growth in utero is associated with accelerated weight gain in offspring during childhood and greater risk of later hypertension (Barker and Bagby, 2005; te Velde et al., 2004; Barker et al., 1989). These may represent biologically plausible mechanisms by which prenatal Pb exposure may result in increased BP later in childhood as was demonstrated in female offspring. The relationship between birth weight and Pb biomarkers is discussed in Section 5.8.7.

Gump et al. (2011; 2005) examined the relationship of blood Pb level with BP in their two cohorts of contemporary children around age 10 years in New York State. Gump et al. (2005) reported an association of cord blood levels with systolic BP (12.16 mmHg [95% CI: 2.44, 21.88] increase per 1 µg/dL increase in cord blood Pb level). Gump et al. (2011) found that with acute stress, children in higher quartiles of concurrent blood Pb level (>0.69 µg/dL) had larger increases in systolic BP. For example, children with blood Pb levels between 1.21 and 3.76 µg/dL had a 7.23 mmHg change, and children with

blood Pb levels between 0.14 and 0.68 µg/dL had a 5.30 mmHg change. A linear trend was not observed across quartiles. An interaction between long-term perceived stress and bone Pb levels in association with BP and hypertension also was reported in a study of adults (Peters et al., 2007) (described in Section 5.4.2.1). An earlier study (Factor-Litvak et al., 1999; Factor-Litvak et al., 1996), of children with higher blood Pb levels ranging from 4.1 to 76.4 µg/dL found that a 1 µg/dL increase in concurrent blood Pb was associated with a 0.05 (95% CI: -0.02, 0.13) mmHg increase in systolic BP. An additional study (Gerr et al., 2002) reported that young adults (ages 19-29 years) in the highest category of bone Pb levels (greater than 10 µg/g), in whom the mean concurrent blood Pb level was 65 µg/dL, systolic BP was 4.26 mmHg higher compared with young adults with bone Pb levels < 1 µg/dL.

The pathogenesis of CVD has been hypothesized to begin in childhood (Kapuku et al., 2006). Early markers observable in youth include increased blood pressure during stress, reduced heart rate variability, increased IMT, and vascular endothelium dysfunction. Kapuku et al. (2006) state that endothelial dysfunction is the center of the CVD paradigm. The factors measured in childhood or as a cumulative burden since childhood are predictors of outcomes in young adults who are still too young to experience coronary events (Li et al., 2003), and early-life exposures may induce changes in arteries that contribute to the development of atherosclerosis (Raitakari et al., 2003). Berenson et al. (2002) observed that the effects of multiple risk factors on coronary atherosclerosis support evaluation of cardiovascular risk in young people. Thus, evidence relating levels of biomarkers of Pb exposure of children to cardiovascular function in the groups of studies presented in the preceding text when combined with the evidence for the potential pathogenesis of CVD starting in childhood that yield effects in adulthood provides coherence with evidence in adults supporting the effects of long-term, cumulative Pb exposures in the development of cardiovascular effects.

Few animal studies have examined the effect of Pb exposure during pregnancy and lactation on BP in offspring and those that have used high levels of exposure. Recently, pups of Pb-exposed dams (1,000 ppm through pregnancy and lactation) exhibited increased blood Pb level (mean blood Pb level: 58.7 µg/dL) and increased arterial systolic BP after weaning (Grizzo and Cordellini, 2008) suggesting a role for childhood Pb exposure leading to adult disease.

5.4.4.4 Summary of Child Cardiovascular Studies

Studies have reported antecedent cardiovascular changes such as TPR responses to acute stress tasks as a function of childhood blood Pb levels. Also, a study reported associations

with acute stress-induced autonomic and cardiovascular dysregulation responses. Biomarkers of prenatal Pb exposure (maternal post-partum patella and tibia) were related to later higher BP. This may be related to intrauterine growth restriction and subsequent accelerated weight gain in childhood and may indicate greater risk of hypertension later in life. The results are not uniform with respect to the important lifestages of Pb exposure and can differ by sex and other factors. Uncertainties in these studies may be related to sample size, single measures of BP, variation of age of onset of puberty, and cross-sectional design. Some of these uncertainties result in random error, however, and result in the attenuation of observed associations rather than the generation of spurious associations. These study findings indicate that in children with mean blood Pb levels in the range of < 5 to 10 µg/dL, increasing blood Pb level may be associated with small increases in BP and changes in the cardiovascular system that may be related to later development of CVD.

Factors may limit the ability of studies to detect statistically significant Pb-associated changes with BP. The relatively young age of the subjects may have limited the ability of these studies to detect significant BP effects (as opposed to early function effects) if longer duration Pb exposure is necessary to produce the cardiovascular changes considering the lower prevalence and strength of compensatory mechanisms in children. There is uncertainty in the shape of the concentration-response relationship to cardiovascular endpoints at lower blood Pb levels since most studies modeled a linear relationship. Several studies that compared linear and nonlinear relationships between blood Pb level and decrements in cognitive function found a better fit for the nonlinear relationship (See Section 5.3.10).

Cardiovascular endpoints other than baseline BP may be more sensitive outcomes by which to measure Pb-associated cardiovascular effects in very young children. The series of studies by Gump et al. (2011; 2009; 2007; 2005) evaluating much smaller samples than did the adult studies, was able to demonstrate statistically significant relationships of blood Pb levels with cardiovascular outcomes such as TPR related to acute stress. It suggests that the stress paradigm may be useful to detect associations of blood Pb levels with effects on the cardiovascular system of children. Selection of the appropriate cardiovascular outcome in children is an important factor to consider in the design of studies. Rather than using indicators of already present cardiovascular problems, such as BP, informative evaluation of cardiovascular changes that are antecedent to increased BP and changes in other CVD-related endpoints at later lifestages may reduce uncertainties regarding the time course of cardiovascular changes associated with early Pb exposure where the relevance and persistence of these endpoints has been shown to be associated with future pathology.

Overall this body of evidence, based on different cohorts, locations, and study designs, begins to form a literature base that indicates there is a relationship between biomarkers of Pb exposure and cardiovascular effects in children. One longitudinal study ties in maternal bone Pb level, and cord and concurrent blood Pb level for the children. Limitations exist in the studies. While blood pressure increases are more prevalent in older adults than in children, BP increases have been related to higher blood Pb level in older studies of children and young adults (Gerr et al., 2002; Factor-Litvak et al., 1999; Factor-Litvak et al., 1996). The newer children studies provide information in populations with mean blood Pb levels in the range of < 5 to 10 µg/dL for BP and antecedents for CVD such as increases in TPR and changes in cardiac autonomic regulation.

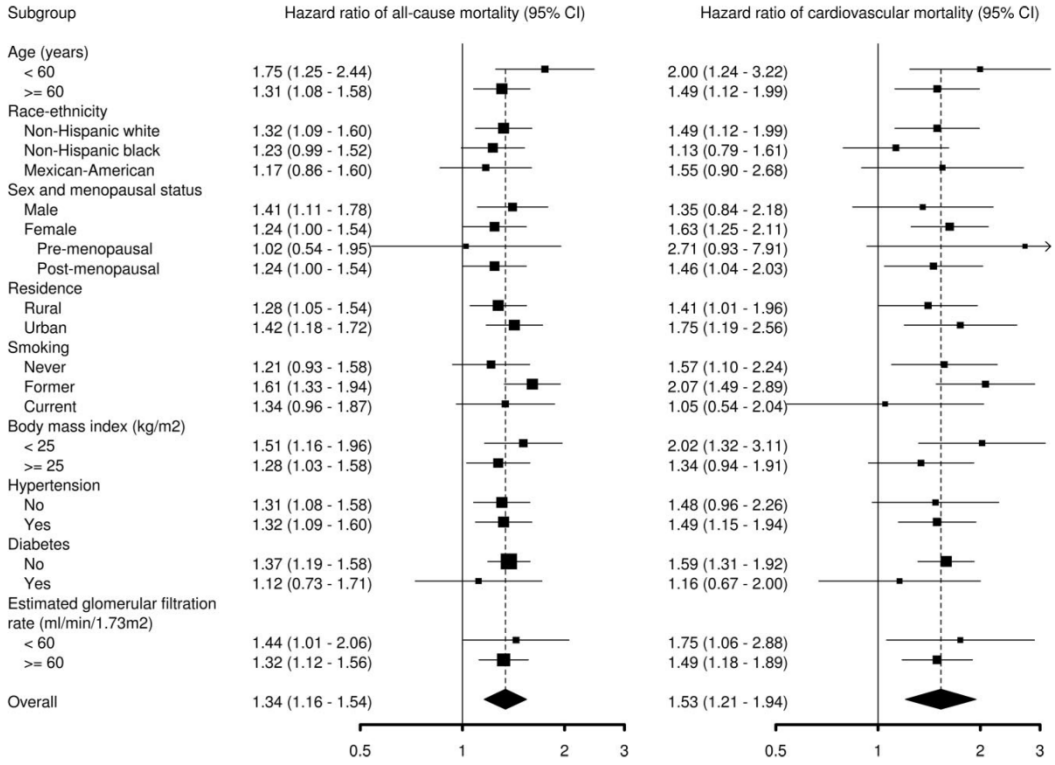
5.4.5 Mortality

The 2006 Pb AQCD stated that collectively the then available evidence suggested an effect of Pb on cardiovascular mortality in the general U.S. population but cautioned that these findings should be replicated before these estimates for Pb-induced cardiovascular mortality could be used for quantitative risk assessment purposes (U.S. EPA, 2006b). Previous results involved NHANES II and III analyses that examined the association of adult concurrent blood Pb with all cause and cause-specific mortality (Schober et al., 2006; Lustberg and Silbergeld, 2002). As blood Pb levels in adults reflect contributions from both recent Pb exposure and mobilization of historic Pb from bone, it is unclear to what extent recent, past, or cumulative Pb exposures contributed to the observed associations. Given the decline in ambient air Pb concentrations and population blood Pb levels, it is likely that study subjects had a much higher Pb exposure in their past than during the study period. Using NHANES II (1976-1980) data, Lustberg and Silbergeld (2002) found significant increases in all-cause, circulatory and cancer mortality, comparing adults with blood Pb levels (measured 12-16 years before ascertainment of vital status) of 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 increased all-cause mortality, cardiovascular, and cancer mortality comparing adults with blood Pb levels (measured a median of 8.8 years before ascertainment of vital status) from 5-9 µg/dL and above 10 µg/dL to those with blood Pb levels less than 5 µg/dL.

Several new studies substantially strengthen the evidence base for Pb-associated mortality. A further analysis of the NHANES III database by a different research group using different methods addressed uncertainties from other earlier analyses by considering a more extensive number of potential confounding factors and by characterizing concentration-response relationships. A few longitudinal prospective

studies in different cohorts conducted by different researchers with different methods in different parts of the U.S. provide coherence with the evidence base for blood Pb and add new evidence for mortality associated with bone Pb levels.

Menke et al. (2006) examined all-cause and cause-specific mortality using NHANES III data. Subjects at least 18 years of age were followed up to 12 years after their blood Pb was measured, and 1,661 deaths were identified. Those with baseline blood Pb levels from 3.63 to 10 µg/dL had significantly higher risks of all-cause (HR: 1.25 [95% CI: 1.04, 1.51]), cardiovascular (HR: 1.55 [95% CI: 1.08, 2.24]), MI (HR: 1.89 [95% CI: 1.04, 3.43]), and stroke (HR: 2.51 [95% CI: 1.20, 2.26]) mortality compared to those with baseline blood Pb levels less than 1.93 µg/dL and increased risk of cancer mortality (HR: 1.10 [95% CI: 0.82, 1.47]). Effect estimates adjusted for demographic characteristics were robust to the additional adjustment for factors such as smoking, alcohol consumption, diabetes, BMI, hypertension, and level of kidney function. The consistency of HRs across models with a varying number of control variables indicated little residual confounding. Hazard ratios were not higher comparing adults with blood Pb levels from 1.94 to 3.62 µg/dL to those with blood Pb levels <1.93 µg/dL. However, tests for linear trend were statistically significant for all mortality outcomes except for cancer mortality. Menke et al. (2006) evaluated several of the model covariates (e.g., diabetes, hypertension, and glomerular filtration rate [GFR]) in a subgroup analysis (Figure 5-32). The authors reported that there were no interactions between blood Pb and other adjusted variables. 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 = 9686) 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 increased HRs comparing adults with blood Pb levels ≥ 10 µg/dL to those with blood Pb levels <5 µg/dL for all-cause (HR: 1.59 [95% CI: 1.28, 1.98]), CVD (HR: 1.55 [95% CI: 1.16, 2.07]), and cancer (HR: 1.69 [95% CI: 1.14, 2.52]) mortality and generally statistically nonsignificant higher HRs comparing adults with blood Pb levels from 5-9 µg/dL to those with blood Pb levels <5 µg/dL. The median follow-up time between measurement of blood Pb and death ascertainment was 8.55 years.



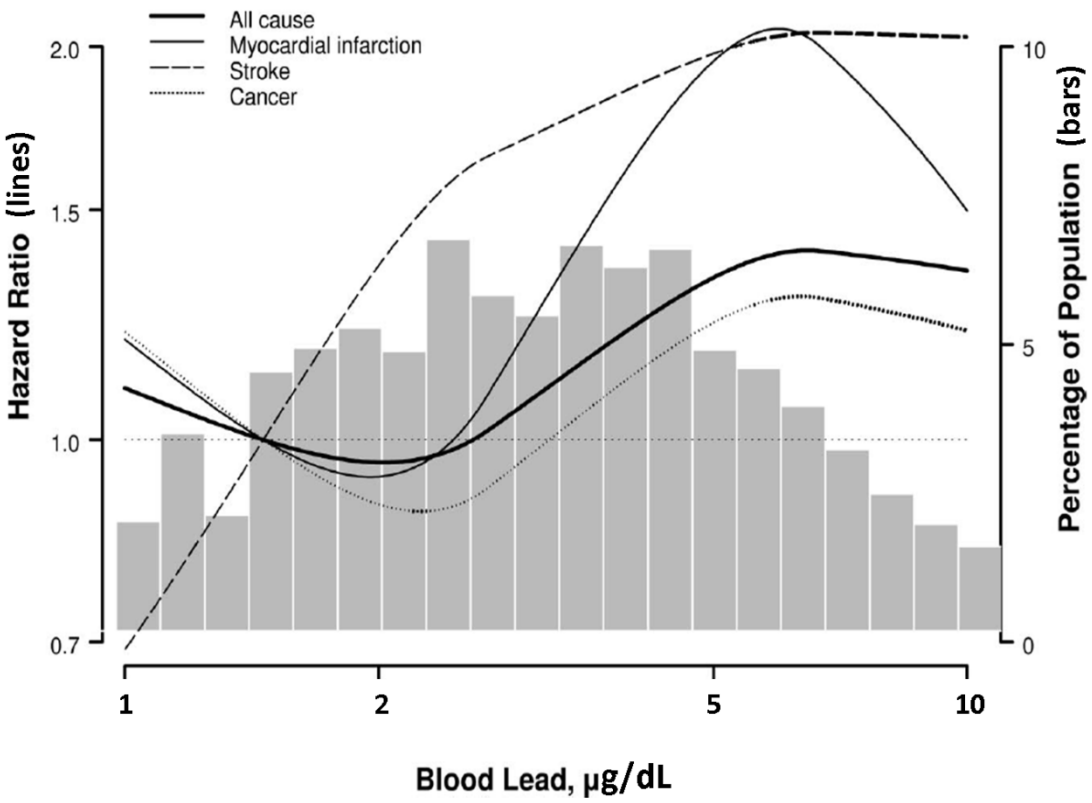
Source: Reprinted with permission of Lippincott Williams & Wilkins, Menke et al. (2006)

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 5-32 Multivariate adjusted relative hazards of all-cause and cardiovascular mortality.

Both Menke et al. (2006) and Schober et al. (2006) presented mortality curves that plot the HRs against blood Pb level. Figure 5-33 shows the mortality hazard ratio curves (not absolute cases of mortality) for both stroke and MI reported by Menke et al. (2006). Nonlinear associations were modeled. The curves were fitted using predetermined restricted quadratic splines with knots at the 10th percentile (1.00 µg/dL), the 50th percentile (2.67 µg/dL), and the 80th percentile (5.98 µg/dL) blood Pb levels. The authors did not explain the shape of the blood Pb-mortality curves in detail; however, the knots corresponded with the inflection points in the curve. In the tails of the blood Pb distribution, hazard ratios decreased with increasing blood Pb level. However, hazard ratios remained above 1 over most of the blood Pb distribution, and in the most heavily populated portion of the blood Pb distribution, hazard ratios increased with increasing blood Pb level. Hazard ratios peaked for all outcomes at a blood Pb level of approximately 6 µg/dL. Lower concentration-response functions at higher blood Pb

levels also have been found for blood Pb-cognitive function relationships in children (Section 5.3.10).

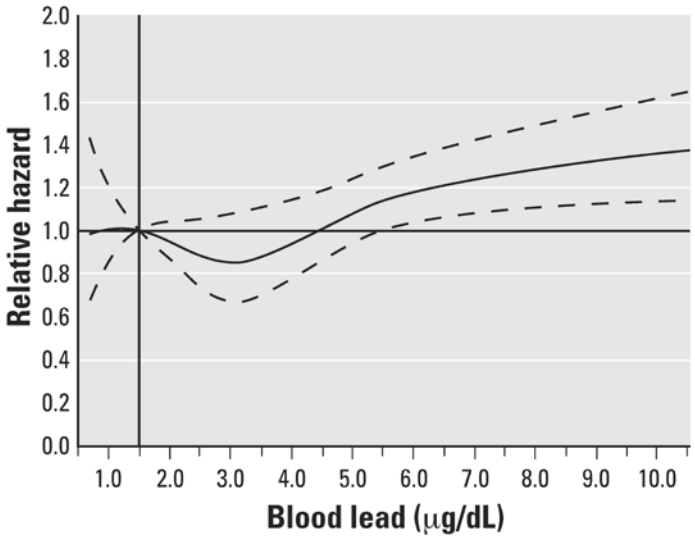


Source: Reprinted with permission of Lippincott Williams & Wilkins, Menke et al. (2006)
Note: A histogram of blood Pb levels is superimposed in the background and displayed on the right axis.

Figure 5-33 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 reported). A statistically significant increasing linear trend for mortality was observed across blood Pb tertiles. The results of the spline fit of the continuous blood Pb level term to relative hazard of all cardiovascular diseases reported by Schober et al. (2006) are shown in Figure 5-34. In contrast to the curve presented by Menke et al. (2006), Schober et al. (2006) found the relative hazard axis and the blood Pb axis largely to be linear (solid line). Dashed lines are 95% CIs. The hazard ratio was fixed at 1.0 for the referent blood Pb level of 1.5 µg/dL. In this study, hazard ratios were less than one in the lower range of blood Pb levels. Despite differences in the

age groups included, follow-up time, categorization of blood Pb levels, and differences in hazard ratio across the blood Pb range, results reported by Menke et al. (2006) and Schober et al. (2006) are consistent in finding associations between higher blood Pb and increased CVD mortality.



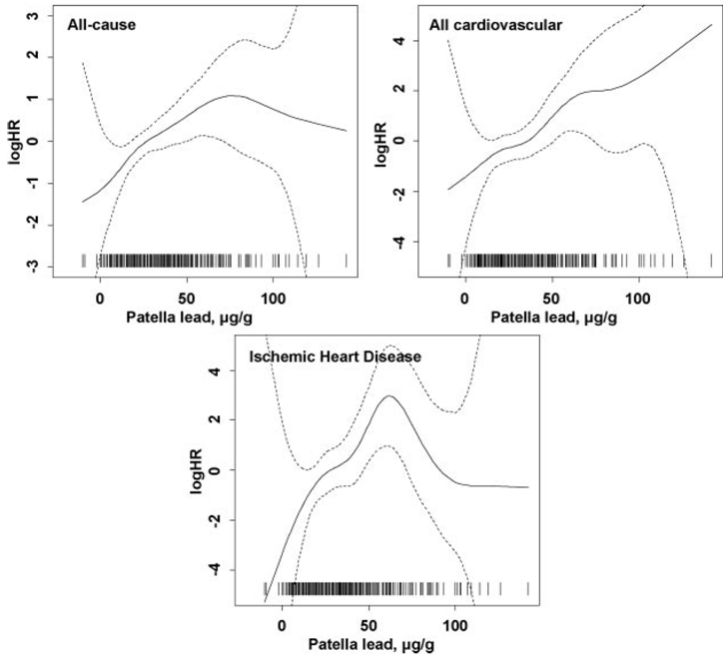
Source: Schober et al. (2006)
Note: The solid line shows the fitted five-knot spline relationship; the dashed lines are the point-wise upper and lower 95% CIs.

Figure 5-34 Relative risk of all cause mortality for different blood Pb levels compared with referent level of 1.5 μg/dL (12.5th percentile).

In addition to the NHANES analyses described above, studies of older adult males (Weisskopf et al., 2009) and older adult females (Khalil, 2010; Khalil et al., 2009a) were conducted recently. Weisskopf et al. (2009) used data from the NAS to determine the associations of blood, tibia, and patella Pb with mortality. The authors identified 241 deaths over an average observation period of 8.9 years (7,673 person-years). The strongest associations were observed between mortality and baseline patella Pb concentration. Baseline tibia Pb levels were more weakly associated with CVD mortality. Tibia bone Pb level is thought to reflect a longer cumulative exposure period than is patella bone Pb level because the residence time of Pb in trabecular bone is shorter than that in cortical bone. IHD contributed most to the relationship between patella Pb and all CVD death with an individual HR of 2.69 (95% CI: 1.42, 5.08). Although there was high correlation between tibia and patella Pb (Pearson r = 0.77), compared with cortical bone Pb, trabecular bone Pb may have more influence on circulating blood Pb level, and thus local organ concentration of Pb, because of its shorter residence time in bone. In contrast

to the NHANES analyses, baseline blood Pb was not significantly related to cardiovascular mortality in the NAS 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 duration of follow-up was similar across studies. 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, who were 18 and 40 years, respectively. Further, the blood Pb tertile analysis of Weisskopf et al. (2009) could have been affected if the majority of a hypothesized nonlinear association was contained largely in the lowest (reference) blood Pb tertile.

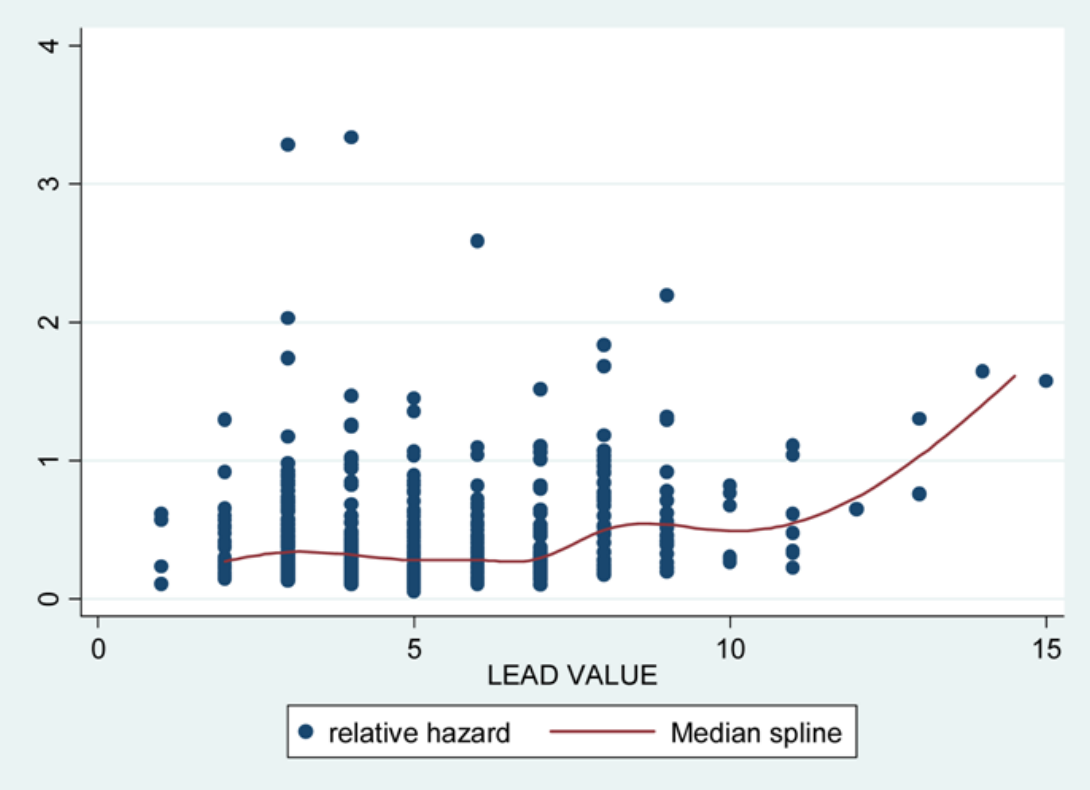
Weisskopf et al. (2009) also conducted a concentration-response analysis. A linear trend was observed for increasing HR across tertiles of both tibia and patella Pb levels. The linear relationship using tertile patella Pb was confirmed in other models in which continuous patella Pb and nonlinear penalized spline terms (higher order terms) were not statistically significant. The number of knots and their placement within the Pb variable, which can influence these results, were determined by an iterative best fit procedure. Concentration-response relationships shown in Figure 5-35 were approximately linear for patella Pb on the log HR scale for all CVD, but appeared nonlinear for IHD ($p < 0.10$). The peak HR is shown around 60 µg/g, beyond which the HR tends to decrease. It is important to note the wide confidence limits, which increase uncertainty at the lower and upper bounds of patella Pb levels.



Source: Reprinted with permission of Lippincott Williams & 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 abscissa. Adjusted for age, education, smoking status, and pack-years of smoking among participants without ischemic heart disease at baseline.

Figure 5-35 Associations between patella bone Pb level and the log of HR (logHR) for all-cause, cardiovascular, and ischemic heart disease.

The association of adult blood Pb with mortality has also been examined among women enrolled in the Study of Osteoporotic Fractures (SOF) (Khalil et al., 2009a). This prospective cohort (N = 533) enrolled female volunteers from two U.S. locations, Baltimore, MD and Monongahela Valley, PA and followed women for an average of 12 years after blood Pb measurement. All-cause mortality comparing women with blood Pb levels >8 µg/dL to those with blood Pb levels <8 µg/dL was significantly increased (HR: 1.59 [95% CI: 1.02, 2.49]). Combined cardiovascular disease mortality (HR: 1.78 [95% CI: 0.92, 3.45]), coronary heart disease mortality (HR: 3.08 [95% CI: 1.23, 7.70]), but not stroke mortality (HR: 1.13 [95% CI: 0.34, 3.81]) HR was increased among the women enrolled in this study with blood Pb levels >8 µg/dL. In addition, analyses of blood Pb tertiles and quintiles indicated that blood Pb-mortality HRs were consistently elevated in groups with blood Pb levels > 7 µg/dL (Khalil, 2010). The findings for elevated mortality HRs with the highest blood Pb levels are reinforced by the results displayed in Figure 5-36. The HR curve for all-cause mortality is relatively flat over most of population blood Pb distribution (represented by the blue dots) and increases only in the upper tail of the blood Pb distribution where there are relatively few subjects (i.e., fewer dots).



Source: Khalil et al. (2010)

Figure 5-36 Multivariate adjusted relative hazard (left axis) of mortality as a function of blood Pb levels between 1 µg/dL and 15 µg/dL.

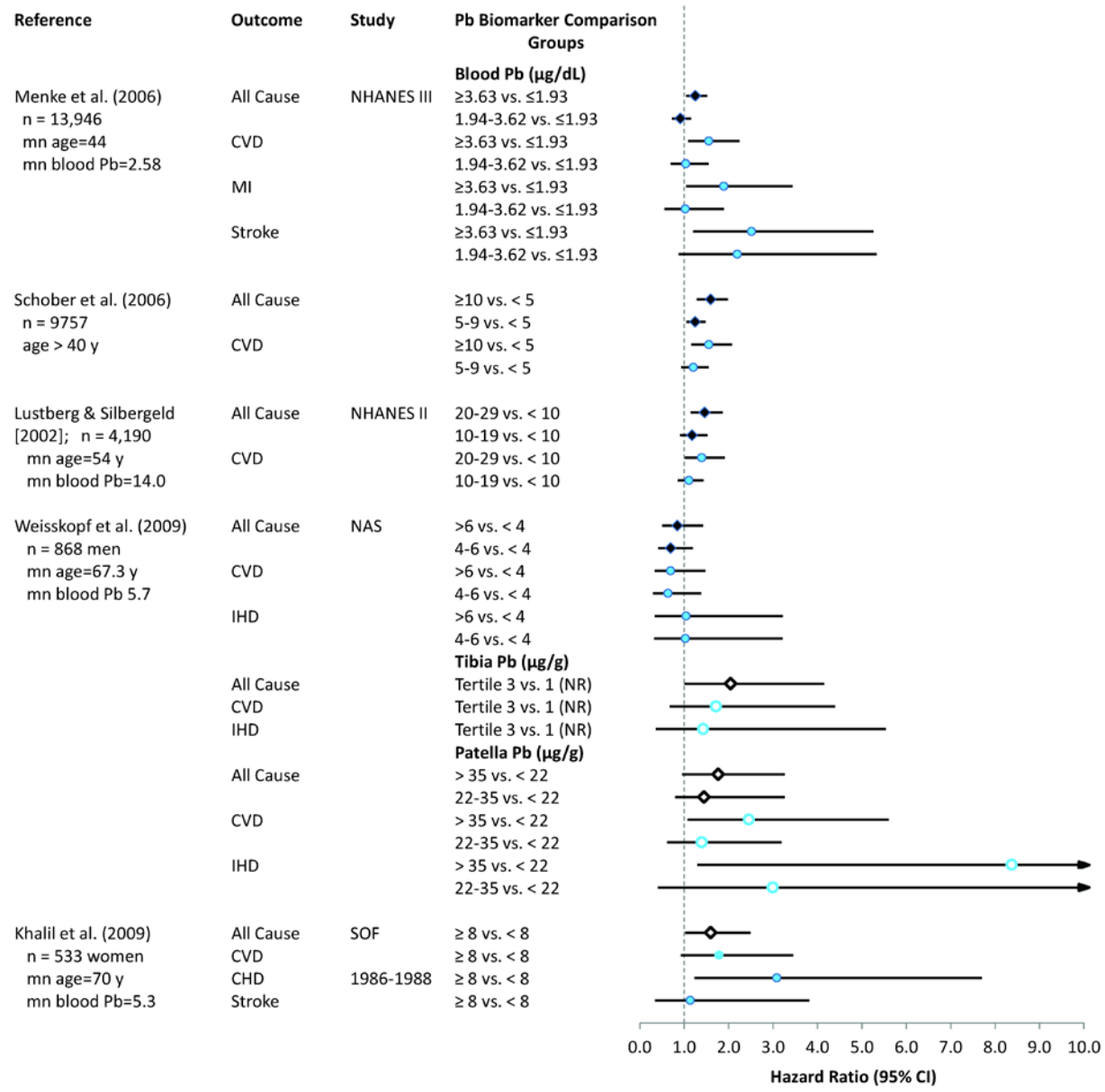
5.4.5.1 Summary of Mortality

The mortality results in this review supported and expanded upon findings from the 2006 Pb AQCD (U.S. EPA, 2006b), which included a few NHANES mortality studies (Schober et al., 2006; Lustberg and Silbergeld, 2002). 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 concentration-response relationships, extensive model evaluations, and examination of mortality from specific CVDs. Further, an association with increased mortality was observed at lower mean population blood Pb levels. The mean blood Pb level of the NHANES III population was 2.58 µg/dL. The Pb attributable risk of increased cardiovascular mortality in the NHANES III analysis of Menke et al. (2006) increased with increasing blood Pb level over the most heavily population portion of the blood Pb distribution and reached its maximum at blood Pb levels between of 6 and 7 µg/dL. It is important to note that the relative contributions of

recent, past, and cumulative Pb exposure to associations observed with the baseline blood Pb levels is uncertain. In addition, the first evidence that bone Pb, a metric of cumulative Pb exposure, is associated with increased mortality was reported recently among NAS men (Weisskopf et al., 2009).

Quantitative differences in Pb-associated hazard for death between studies may be influenced by age range of the study groups, follow up time to death, variation in model adjustment, central tendency and range of the Pb biomarker levels, assumptions of linearity in relationship with Pb biomarkers, and choice of Pb biomarker. Quantitative differences in Pb-associated mortality across NHANES II and NHANES III studies or between different NHANES III analyses may be explained by the use of continuous or ordered blood Pb terms and different data selection strategies. Further, studies using ordered categories of blood Pb level may obtain different results, as the range of blood Pb level represented in the reference category will affect the calculated coefficients of the remaining percentiles or groups.

Specifically, Menke et al. (2006) is the strongest study presently published for estimating the effects of Pb on cardiovascular disease-related mortality. The study uses the nationally representative NHANES III (1988-1994) sample of men and women. The results provide confirmation of earlier published NHANES studies but address some of the key weaknesses noted in those studies. For example, Menke et al. (2006) examined potential confounding by a large number of factors, including hypertension and kidney function. Weisskopf et al. (2009) is the first published mortality study using bone Pb as an exposure index. The study is a prospective study with nearly 100% successful follow-up of deaths. This rigorous study found increased cardiovascular disease mortality in association with patella bone Pb. The Khalil et al. (2010; 2009a) SOF provides supporting results in a different study cohort consisting of white females aged 65-87 years. Further, a number of prior studies have found association between accumulated Pb reflected in bone Pb measurements and higher CVD morbidity (Sections 5.4.2.1 and 5.4.3). This evidence base is augmented with new findings indicating that biomarkers of longer-term cumulative Pb exposure increases CVD mortality. The NAS and SOF examine only men and women, respectively. However, the consistency of findings between the two studies indicates that the results of either study may be applicable widely. Despite the differences in design and methods across studies, with few exceptions associations between higher levels of Pb biomarkers and higher risk of mortality were consistently observed (Figure 5-37 and Table 5-21). In studies that examined mortality from specific CVD causes, MI, stroke, and IHD mortality, which are causes related to higher BP and hypertension were all significantly elevated with higher Pb biomarker levels.



Note: Studies are presented in order of strength of study design and follow the order of discussion in the preceding text. Hazard ratios represent the hazard in the higher blood or bone Pb group relative to that in the lowest blood or bone Pb group (reference).

Figure 5-37 Hazard ratios for associations of blood Pb (closed markers) and bone Pb (open markers) with all-cause mortality (black diamonds) and cardiovascular mortality (blue circles).

Table 5-21 Additional characteristics and quantitative data for associations of blood and bone Pb with CVD mortality for studies presented in Figure 5-37

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Hazard Ratio or SMR (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).	Baseline Blood Pb (measured an average of 12 yr before mortality): Mean: 2.58 µg/dL Tertiles: <1.93 µg/dL, 1.94-3.62 µg/dL, ≥ 3.63 µg/dL	Survey-design adjusted Cox proportional hazard regression analysis (up to 12 yr follow-up) adjusted for Model 1: age, race/ethnicity, sex, Model 2: 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)	9,686 adult participants of NHANES III, ≥ 40 yr	All cause and cause-specific mortality	Ordered categorical blood Pb level, measured a median of 8.55 yr prior to death <5 µg/dL 5-9 µg/dL ≥ 10 µg/dL	Survey-design adjusted Cox proportional hazard adjusted for sex, age, race/ethnicity, smoking, education level	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)
Lustberg and Silbergeld (2002)	4,190 adult participants of NHANES III, yr (1976-1980)	All cause and cause-specific mortality	Categorical blood Pb level Mean: 14.0 (5.1) Median: 13 µg/dL <10 µg/dL (Reference) 10-19µg/dL 20-29 µg/dL	Crude mortality RRs adjusted for age, sex, location, education, race, income, smoking, BMI, exercise	All-cause (2nd vs. 1st): 1.40 (1.16-1.69) All-cause (3rd vs. 1st): 2.02 (1.62-2.52) Circulatory (2nd vs. 1st): 1.27 (0.97-1.57) Circulatory (3rd vs. 1st): 1.74 (1.25-2.40) Cancer (2nd vs. 1st): 1.95 (1.28-2.98) Cancer (3rd vs. 1st): 2.89 (1.79-4.64)

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Hazard Ratio or SMR (95% CI)
Weisskopf et al. (2009)	868 men, >55 yr, 95% white, from NAS in Greater Boston area, MA	All cause and cause-specific mortality	Pb biomarkers collected an average of 8.9 years before death Blood Pb: Mean (SD): 5.6 (3.4) µg/dL Patella Pb: Mean (SD): 31.2 (19.4) µg/g Tertiles: <22 µg/g, 22-35 µg/g, >35 µg/g Tibia Pb: Mean (SD): 21.8 (13.6) µg/g	Cox proportional hazard regression analysis adjusted for age, smoking, education. Additional models adjusted for alcohol intake, physical activity, BMI, total cholesterol, serum HDL, diabetes mellitus, race, and hypertension	All-cause (3rd vs. 1st patella Pb tertile): 1.76 (0.95, 3.26) All CVD (3rd vs. 1st tertile): 2.45 (1.07, 5.60) IHD (3rd vs. 1st): 8.37 (1.29, 54.4) Cancer (3rd vs. 1st): 0.59 (0.21, 1.67) After excluding 154 subjects with CVD and stroke at baseline: All-cause (3rd vs. 1st): 2.52 (1.17-5.41) All CVD (3rd vs. 1st): 5.63 (1.73, 18.3) All-cause (3rd vs. 1st blood Pb tertile): 0.93 (0.59, 1.45) All CVD (3rd vs. 1st): 0.99 (0.55, 1.78) IHD (3rd vs. 1st): 1.30 (0.54, 3.17)
Khalil et al. (2009a)	533 women, 65-87 yr, from Study of Osteoporotic Fractures cohort in Baltimore, MD and Monongahela Valley, PA	All cause and cause-specific mortality	Blood Pb measured an average 12 (SD; 3) yr before death: Mean (SD; range): 5.3 (2.3; 1-21) µg/dL	Cox proportional hazards regression analysis adjusted for age, clinic, BMI, education, smoking, alcohol intake, estrogen use, hypertension, total hip BMD, walking for exercise, and diabetes	≥ 8 µg/dL vs. <8 µg/dL All cause: 1.59 (1.02, 2.49) CVD: 1.78 (0.92, 3.45) Coronary Heart Disease: 3.08 (1.23, 7.70) Stroke: 1.13 (0.34, 3.81) Cancer: 1.64 (0.73, 3.71)

Study	Population/ Location	Parameter	Pb Data	Statistical Analysis	Hazard Ratio or SMR (95% CI)
[a]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 (SMR) based on 2000 U.S. Census data	Heart disease: Both sexes: 114.1 (113.1, 115.2) Men: 118 (116.4, 119.6) Women: 111 (109.5, 112.5) Stroke: Both sexes: 121.6 (119.2, 123.9) Men: 146.7 (107.4, 195.7) Women: 106.5 (80.2, 138.6)
[a]Cocco et al. (2007)	933 male Pb smelter workers from Sardinia, Italy (1973-2003)	All cause and cause-specific mortality	Not reported	SMR	All cause: 56 (46, 68) CVD: 37 (25, 55)

[a]These references not included in Figure 5-37 because they reported standardized mortality ratios.

5.4.6 Air Lead-Particulate Matter Studies

5.4.6.1 Cardiovascular Morbidity

A relatively small number of studies used Pb measured in PM_{10} and $PM_{2.5}$ ambient air samples to represent Pb exposures. However, given that size distribution data for Pb-PM are fairly limited, it is difficult to assess the representativeness of these concentrations to population exposure (Section 3.5.3). Moreover, data illustrating the relationships of Pb-PM_{10} and Pb-$PM_{2.5}$ with blood Pb levels are lacking. A few available studies exposed rats, dogs, or humans to concentrated ambient particles (CAPS) in which Pb and several other components were measured. Consistent with epidemiologic studies of blood and bone Pb and with studies of animals exposed to Pb, exposure to Pb-containing CAPS resulted in various changes related to increased vasoconstriction (Urch et al., 2004; Wellenius et al., 2003; Batalha et al., 2002). Whereas studies of Pb biomarkers primarily found cardiovascular effects with indicators of long-term Pb exposure, studies of Pb-containing CAPS provide evidence for cardiovascular effects with short-term exposure (2-6 hours over multiple days) It is important to note that Urch et al. (2004) estimated the Pb effect on brachial artery diameter based only on the ambient concentrations of Pb but did not directly expose their young (mean age: 35 years, SD: 10), healthy adult participants to Pb isolated from CAPS.

A U.S. time-series study of almost 3 million pregnant women found that increases in ambient Pb-TSP concentrations were associated with increased odds of PIH assessed at delivery (Chen et al., 2006c). In contrast, epidemiologic studies provide weak evidence for an association between short-term changes (daily average) in ambient air concentrations of Pb- $PM_{2.5}$ and cardiovascular morbidity in adults. Some of these time-series studies analyzed Pb individually, whereas others applied source apportionment techniques to analyze Pb as part of a group of correlated components. In a time-series study of 106 U.S. counties, Bell et al. (2009) found that an increase in lag 0 Pb- $PM_{2.5}$ was associated with an increased risk of cardiovascular hospital admissions among adults ages 65 years and older. Quantitative results were not presented; however, the 95% CI was wide and included the null value. In this study, statistically significant associations were observed for other PM metal components such as nickel, vanadium, and zinc. In the absence of detailed data on correlations among components or results adjusted for copollutants, it is difficult to exclude confounding by ambient air exposures to these other components or copollutants.

To address correlations among PM chemical components, some studies applied source apportionment techniques to group components into common source categories. In these source-factor studies, it is not possible to attribute the observed association (Sarnat et al., 2008) or lack of association (Andersen et al., 2007) specifically to Pb.

5.4.6.2 Mortality

Time-series epidemiologic studies of ambient air Pb- $PM_{2.5}$ reported positive associations with mortality. Although limited in number, these studies indicated associations in multiple cities across the U.S. In the Harvard Six Cities Study, Laden et al. (2000) found a 1.16% (95% CI: 0.20, 2.9%) increased risk in all-cause mortality per 461.4 ng/m^3 (5th-95th percentile) increase in Pb- $PM_{2.5}$. In six California counties, Ostro et al. (2007) found that a 5 ng/m^3 (interquartile range) increase in Pb- $PM_{2.5}$ was associated with a 1.89% (95% CI: -0.57, 4.40%) increased risk of cardiovascular mortality and a 1.74% (95% CI: 0.24, 3.26%) increased risk of all-cause mortality during the cool season. The limitations of air-Pb studies were described in Section 5.4.6.1 above and also are relevant to the interpretation of these findings for mortality.

5.4.7 Summary and Causal Determination

The 2006 Pb AQCD concluded that there was a relationship between higher blood Pb and bone Pb and cardiovascular effects in adults, in particular increased BP and increased

incidence of hypertension (U.S. EPA, 2006b). This conclusion was substantiated by the coherence observed between epidemiologic and toxicological findings. The large evidence base comprising epidemiologic studies conducted in diverse populations using different designs clearly demonstrated a positive association between blood Pb level and BP. Meta-analysis of these studies found that each doubling of concurrent blood Pb level (between 1 and >40 µg/dL) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP (Nawrot et al., 2002). In addition, most of the reviewed studies using bone Pb levels also showed an association with BP, pointing to an effect of cumulative Pb exposure. Similarly, toxicological studies provided evidence that long-term exposure to Pb (> 4 weeks) results 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 Pb-induced oxidative stress, activation of RAAS, altered sympathetic activity, and vasomodulator imbalance. Studies in the 2006 Pb AQCD also found associations between Pb biomarkers and other cardiovascular diseases such as IHD, cerebrovascular disease, peripheral vascular disease, and cardiovascular disease related mortality; however, the available evidence was limited.

Building on the strong body of evidence presented in the 2006 Pb AQCD, recent epidemiologic and toxicological studies strengthened the evidence that long-term Pb exposure is associated with cardiovascular effects in adults with the largest body of evidence demonstrating associations of Pb with increased BP and hypertension. Recent epidemiologic studies addressed past uncertainties, including the potential for confounding. A recent study in an ethnically diverse community-based cohort of women and men aged 50-70 years found hypertension risk to be associated with blood and tibia Pb levels (Martin et al., 2006). These findings are consistent with those of other studies, demonstrating that with each increase of 1 µg/dL concurrent blood Pb level, systolic BP increased 1 mmHg and diastolic BP increased 0.5 mmHg. Recent epidemiologic studies in adults found associations with hypertension in populations with relatively low mean blood Pb levels. For example, a positive relationship was found in the nationally representative NHANES III (1988-1994) where the geometric mean blood Pb level of the population was 1.64 µg/dL (Muntner et al., 2005). Despite the extensive evidence for relatively low concurrent blood Pb levels, as these cardiovascular outcomes were most often examined in adults that have been exposed to higher levels of Pb earlier in life, uncertainty remains concerning the Pb exposure level, timing, frequency, and duration contributing to the observed associations. A new prospective study in Pb workers found independent associations of both baseline blood Pb level and subsequent changes in blood Pb over follow-up with changes in BP over follow-up and bone Pb level with hypertension (Glenn et al., 2006). The results indicated that different mechanisms may

mediate shorter-term Pb-associated increases in BP and longer-term Pb-associated development of hypertension.

Collectively, all animal toxicological studies providing blood Pb level and BP measurements reported increases in BP with increasing blood Pb level (Figure 5-29). Importantly, an animal toxicological study provided support for increased BP following long-term Pb exposure resulting in low blood Pb levels with mean blood Pb levels as low as 2 µg/dL (Tsao et al., 2000). However, a majority of studies examined Pb exposures that resulted in mean blood Pb levels > 10 µg/dL. Thus, the weight of the evidence demonstrated such effects in animals with blood Pb levels >10 µg/dL. New studies also demonstrated only partial reversibility of Pb-induced increased BP following Pb exposure cessation or chelation and the possibility for short-term Pb exposure-induced increases in BP.

The epidemiologic evidence for a relationship between Pb exposure and cardiovascular disease is strengthened by observations of cardiovascular effects in association with both blood and bone Pb level after adjusting for multiple potential confounding factors, including age, sex, BMI, antihypertensive medication, SES, race/ethnicity, alcohol consumption, serum total cholesterol, smoking, educational level, diabetes, and measures of renal function. The epidemiologic evidence also was substantiated by several available prospective studies that found associations between biomarkers of Pb and cardiovascular health endpoints. These studies inform the temporality of these relationship between biomarkers of Pb exposure and cardiovascular morbidity (e.g., HRV, IHD, BP, hypertension) (Eum et al., 2011; Jain et al., 2007; Peters et al., 2007; Glenn et al., 2006) and mortality (Khalil et al., 2009a; Weisskopf et al., 2009; Menke et al., 2006; Schober et al., 2006).

Epidemiologic studies continued to demonstrate a relationship between bone Pb, which is a metric of cumulative Pb exposure, and BP in adults. Studies that examined both blood and bone Pb levels did not conclusively demonstrate a stronger association for either blood or bone Pb (Perlstein et al., 2007; Glenn et al., 2006; Martin et al., 2006). In a recent meta-analysis, Navas-Acien et al. (2008) found that studies passing the detailed inclusion criteria all showed a relationship between higher bone Pb levels and higher BP. Also, all but one that characterized hypertension showed higher relative risks or odds ratios associated with higher bone Pb levels. Recent epidemiologic studies also emphasized the interaction between bone Pb levels and factors that modify the association with BP or hypertension, such as race/ethnicity, chronic stress and metabolic syndrome. Bone Pb coupled with high perceived stress was associated with an increased risk of developing hypertension in an originally nonhypertensive group of adults (Peters et al., 2007). Also, bone Pb level was associated with larger decreases in HRV (which has

been associated with increased cardiovascular events) among adults with metabolic syndrome, also associated with increased risk of cardiovascular events (Park et al., 2006). The extensive epidemiologic evidence for bone Pb levels in concert with the animal toxicological evidence for long-term Pb exposure, support an effect of long-term, cumulative Pb exposure on cardiovascular morbidity.

Recent epidemiologic studies of adults investigated the interaction of Pb biomarkers with genetic variants in associations with cardiovascular effects. Evidence was presented for a larger blood Pb-associated increase in BP in carriers of the ALAD2 allele, which is associated with greater binding affinity for Pb in the bloodstream (Figure 5-26 for results) (Scinicariello et al., 2010). Additionally, bone Pb concentration was associated with larger increases in PP, which represents as a good predictor of cardiovascular morbidity and mortality and an indicator of arterial stiffness, among adults with the HFE H63D and/or C282Y variant (Zhang et al., 2010a) (Figure 5-26 for results). Park et al. (2009b) provided further evidence of HFE and transferrin gene variants, related to iron metabolism, impacting the associations of bone Pb levels with cardiovascular effects, evaluated by QT interval changes.

Epidemiologic and toxicological evidence indicates that Pb exposure not only increases BP and hypertension, but can contribute to the development of other cardiovascular diseases in adults. However, fewer studies have been published compared to studies of BP and hypertension. Both recent epidemiologic and toxicological studies provide evidence in adults for blood Pb-associated increased atherosclerosis, thrombosis, IHD, PAD, arrhythmia, and cardiac contractility in populations with mean blood Pb levels >2.5 µg/dL (Table 5-19). Further, animal toxicological evidence continued to build on the evidence supporting the mechanisms leading to these cardiovascular system responses, as well as Pb-induced changes in BP and hypertension. Enhanced understanding of Pb-induced oxidative stress including $^{\bullet}$NO inactivation, endothelial dysfunction leading to altered vascular reactivity, activation of the RAAS, and vasomodulator imbalance provides biological plausibility for the consistent associations observed between higher blood and bone Pb levels and greater cardiovascular effects.

Several studies in children reported associations of childhood (concurrent and measured at an average age of 2.6 years) blood Pb levels with cardiovascular changes such as TPR and autonomic and cardiovascular dysregulation in response to acute stress tasks measured between ages 5 to 15 years (Gump et al., 2011; 2009; Gump et al., 2007). Also, maternal bone and cord blood Pb levels, biomarkers of prenatal Pb exposure, were related to higher BP in children (Gump et al., 2005; Zhang et al., In Press). These study findings of children add evidence that Pb exposure is associated with small increases in BP, and

that changes in the cardiovascular system precedent to later CVD are potentially impacted by early-life Pb exposure.

New evidence extended the potential continuum of Pb-related cardiovascular effects by demonstrating associations between Pb biomarkers and both cardiovascular and all-cause mortality with follow-up periods ranging between 8 and 12 years. All-cause mortality was increased with increasing blood Pb level. A recent analysis of the NHANES III sample reported associations of adult blood Pb level with cardiovascular mortality, with stronger associations observed 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 higher effect estimates were observed for mortality from cardiovascular disease and coronary heart disease (Khalil et al., 2009a). Weisskopf et al. (2009) published the first mortality study using bone Pb as an exposure index. This prospective study found that patella bone Pb levels were associated with increased mortality from cardiovascular disease and IHD with hazard ratios of 5.6 and 8.4, respectively.

Changes in BP that have been associated with biomarkers of Pb exposure indicate a modest change for an individual; however, these modest changes can have a substantial implication at the population level. The reported effects represent a central tendency of Pb-induced cardiovascular effects among individuals; some individuals may differ in risk and manifest effects that are greater in magnitude. For example, a small increase in BP may shift the population distribution and result in considerable increases in the percentages of individuals with BP values that are clinically significant, i.e., an indication of hypertension and medication use. Studies in the medical literature show that increasing BP, even within the nonhypertensive range, is associated with increased rates of death and cardiovascular disease, including coronary disease, stroke, PAD, and cardiac failure (Ingelsson et al., 2008; Chobanian et al., 2003; Pastor-Barriuso et al., 2003; Prospective Studies Collaboration, 2002; Kannel, 2000a, b; Neaton et al., 1995).

In summary, new studies evaluated in the current review supported and expanded upon the strong body of evidence presented in the 2006 Pb AQCD that Pb exposure is associated with cardiovascular health effects. The weight of both epidemiologic and toxicological evidence continues to support a consistent relationship between Pb exposure and increased BP or hypertension development in adults. The epidemiologic evidence is strengthened by several prospective studies that find associations between biomarkers of Pb and BP and hypertension and by effect estimates that are observed after adjustment for multiple potential confounding factors. The weight of epidemiologic evidence supported associations in adults with mean concurrent blood Pb levels less than 5 µg/dL. As these outcomes in epidemiologic studies were most often observed in adults

with likely higher past than current Pb exposures, uncertainty exists as to the Pb exposure level, timing, frequency, and duration contributing to the observed associations. Recent epidemiologic studies found that bone Pb level, a metric of cumulative exposure, is strongly related to hypertension risk in adults with mean bone Pb levels greater than 20 µg/g. However, uncertainties also exist as to the specific Pb exposure conditions that contributed to the associations. The weight of animal evidence also demonstrates an increase in BP after long-term (i.e., greater than 4 weeks) exposure to Pb. Whereas the majority of studies examined and found increases in BP in animals with mean blood Pb levels greater than 10 µg/dL, a recent studies found elevated BP in animals with a mean blood Pb level of 2 µg/dL. By demonstrating Pb-induced oxidative stress including $^{\bullet}$NO inactivation, endothelial dysfunction leading to altered vascular reactivity, activation of the RAAS, and vasomodulator imbalance, toxicological studies have characterized the modes of action of Pb and provided biological plausibility for the consistent associations observed in epidemiologic studies between blood and bone Pb and cardiovascular effects. These associations of Pb with cardiovascular morbidity observed in both epidemiologic and toxicological studies support recent epidemiologic findings of increased Pb-associated cardiovascular mortality. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of other cardiovascular endpoints examined 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. Findings summarized across epidemiologic and toxicological studies indicate that chronic Pb exposure is associated with pathological changes in the renal system such as proximal tubule (PT) cytomegaly, renal cell apoptosis, mitochondrial dysfunction, aminoaciduria, increased electrolyte excretion, ATPase dysfunction, oxidant redox imbalance, altered glomerular filtration rate (GFR), chronic kidney disease (CKD) development, and altered $^{\bullet}$NO homeostasis with ensuing elevated BP. As several of these outcomes are most often observed in adults with likely higher past Pb exposures, uncertainty exists as to the Pb exposure level, timing, frequency, and duration contributing to the associations observed with blood or bone Pb levels.

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 interactions between these systems can lead to further exacerbation of vascular and kidney dysfunction following Pb exposure. As kidney dysfunction can increase BP and increased BP can lead to further damage to the kidneys, Pb-induced damage to both systems may result in a cycle of further increased severity of disease.

In general, associations between bone Pb (particularly in the tibia) and health outcomes in adults indicate chronic effects of cumulative Pb exposure. In adults without current occupational Pb exposure, blood Pb level represents both recent and cumulative Pb exposure. In particular, blood Pb level may represent cumulative exposure in physiological circumstances of increased bone remodeling or loss (e.g., osteoporosis and pregnancy) when Pb from bone of adults contributes substantially to blood Pb concentrations. Blood Pb in children is also influenced by Pb stored in bone due to rapid growth-related bone turnover in children relative to adults. Thus, blood Pb in children is also reflective of cumulative dose. Additional details on the interpretation of Pb in blood and bone are provided in Section 4.3.5. The toxicokinetics of Pb in blood and bone are important considerations in making inferences about etiologically-relevant Pb exposures that contributed to associations observed between blood and bone Pb levels and health outcomes.

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 epidemiologic studies and clinical settings. Creatinine is the most commonly measured endogenous compound; blood urea nitrogen (BUN) has also been examined. Increased serum concentration or decreased kidney clearance of these markers both indicate decreased kidney function. The main limitation of endogenous compounds identified to date is that non-kidney factors impact their serum levels. Specifically, since creatinine is 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), that are unrelated to kidney function. Measured creatinine clearance, involving measurement and comparison of creatinine in both serum and urine, can address this problem. However, measured creatinine clearance utilizes timed urine collections, traditionally over a 24-hour period, and the challenge of complete urine collection over an extended time period makes compliance difficult.

Therefore equations to estimate kidney filtration that utilize serum creatinine but also incorporate age, sex, race, and, in some, weight (in an attempt to adjust for differences in muscle mass), have been developed. Although these are imperfect surrogates for muscle mass, such equations are currently the preferred outcome assessment method. Traditionally, the Cockcroft-Gault equation (Cockcroft and Gault, 1976), which estimates creatinine clearance, a GFR surrogate, has been used. In the last decade, the abbreviated Modification of Diet in Kidney Disease (MDRD) Study equation (Levey et al., 2000; Levey et al., 1999), which estimates GFR, has become the standard in the kidney epidemiologic and clinical communities. With widespread use of the MDRD equation, it became clear that the equation underestimates GFR at levels in the normal range. Therefore, the CKD-Epidemiology Collaboration (CKD-EPI) equation was recently developed to be more accurate in this range (Levey et al., 2009). This is a decided advantage in nephrotoxicant research since most participants in occupational and many even in general population studies have GFRs in a range that is underestimated by the MDRD equation.

Both the MDRD and CKD-EPI equations use serum creatinine. Due to the inability to adjust serum creatinine levels for muscle mass, alternative serum biomarkers have been

evaluated such as cystatin C, a cysteine protease inhibitor that is filtered, reabsorbed, and catabolized in the kidney (Fried, 2009). It is produced and secreted by all nucleated cells thus avoiding the muscle mass confounding that exists with serum creatinine (Fried, 2009). However, recent research indicates that serum cystatin C varies by age, sex, and race (Kottgen et al., 2008). Thus, a cystatin C-based eGFR equation was recently developed that includes age, sex, and race (Stevens et al., 2008).

Most of the kidney outcome measures discussed above were developed for use in the clinical setting. 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 and Russo, 2009). Kidney EBE markers are a major recent focus for research in patients with acute kidney injury (AKI) and markers such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (Kim-1), developed in AKI research, may prove useful for chronic nephrotoxicant work as well (Ferguson et al., 2008; Devarajan, 2007).

5.5.2 Nephrotoxicity and Renal Pathology

5.5.2.1 Toxicology

Figure 5-38 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. In animals, Pb has been found to induce changes in a wide range of indicators of renal function. Most studies examined Pb exposure concentrations that resulted in higher blood Pb levels (> 20 µg/dL) than those in the current U.S. general population. As indicated in Figure 5-38 and Table 5-22, toxicological information on renal dysfunction with blood Pb levels < 10 µg/dL) generally is not available.

Dysfunction in kidney function measures, including urinary flow, ALP, microalbumin, and NAG, was observed at blood Pb concentrations above 20 µg/dL (Wang et al., 2010d).

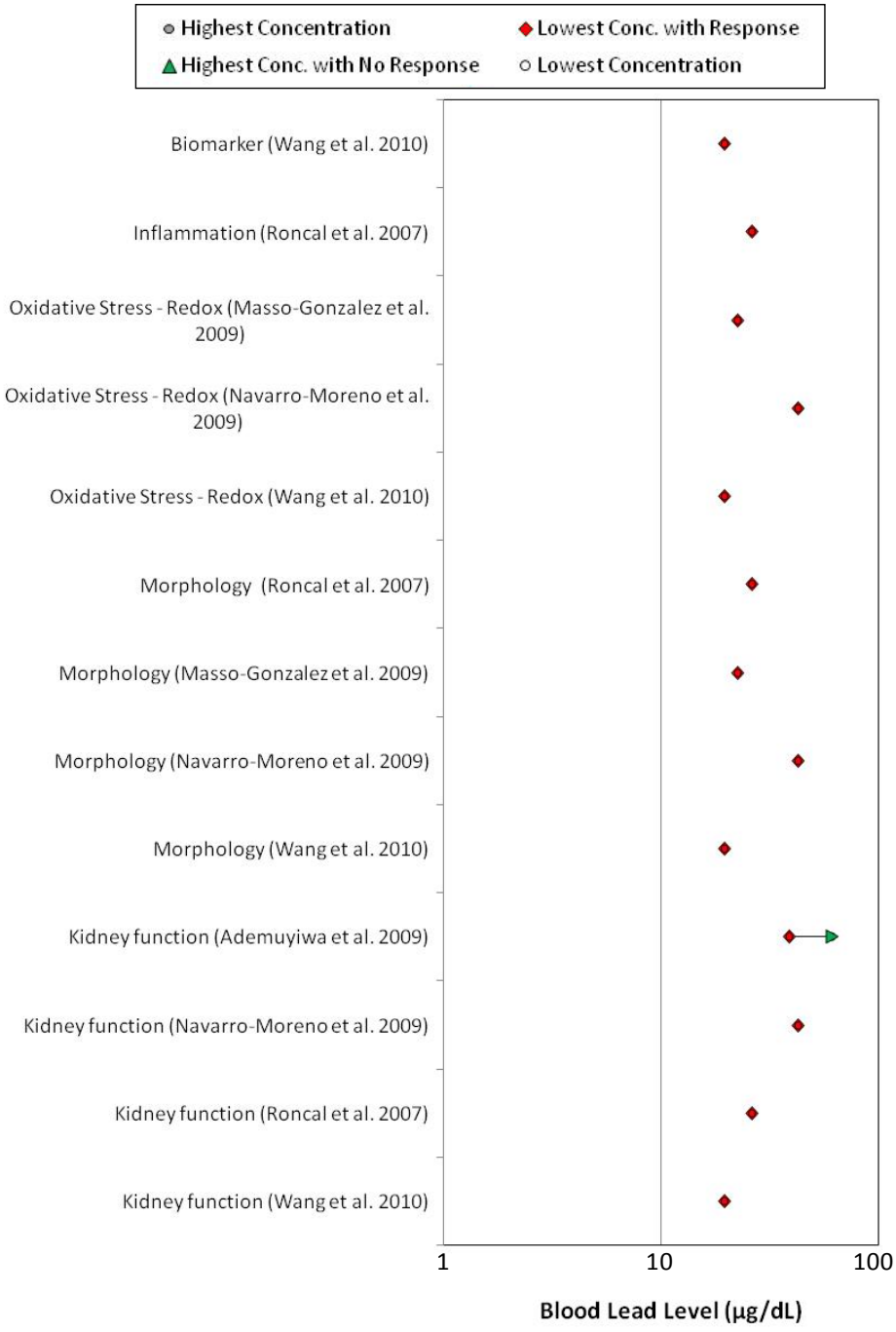


Figure 5-38 Concentration-response representation of the effect of Pb on renal outcomes in animal toxicology studies.

Table 5-22 Additional characteristics for results of toxicological studies presented in Figure 5-38

Reference	Species; Lifestage; Sex	Pb Dose; Exposure Duration	Blood Pb Level with Response (µg/dL)	Outcome
Wang et al. (2010d)	Rat; Adult; Female	300 ppm Pb-acetate in drinking water; 8 weeks	20	Biomarker - Aberrant NAG, GGT, β2-microgobulin expression
Roncal et al. (2007)	Rat; Adult; Male	150 ppm Pb-acetate in drinking water; 16 weeks with remnant kidney surgery at week 4	26	Inflammation - Elevation in number of macrophages & marker MCP-1 in Pb exposed kidneys with remnant kidney surgery.
Navarro-Moreno et al. (2009)	Rat; Adult; Male	500 ppm Pb-acetate in drinking water; 28 weeks	43	Oxidative stress - Increased kidney lipid peroxidation (i.e., TBARS)
Wang et al. (2010d)	Rat; Adult; Female	300 ppm Pb-acetate in drinking water; 8 weeks	20	Oxidative Stress - Pb caused increased lipid peroxidation (i.e., MDA production), elevated kidney antioxidant enzymes (SOD, GPx, CAT), and depleted GSH
Massó-González et al. (2009)	Rat; Weanling pups	300 ppm Pb-acetate in drinking water; GD1 to PND21	23	Oxidative stress - Elevated TBARS and catalase activity
Roncal et al. (2007)	Rat; Adult; Male	150 ppm Pb-acetate in drinking water; 16 weeks with remnant kidney surgery at week 4	26	Morphology - Pb induced pre-glomerular vascular disease of kidney (i.e., sclerosis, fibrosis, peritubular capillary loss)
Navarro-Moreno et al. (2009)	Rat; Adult; Male	500 ppm Pb-acetate in drinking water; 28 weeks	43	Morphology - Electron micrography showed lumen reduction, microvilli loss, brush border loss, and mitochondrial damage
Wang et al. (2010d)	Rat; Adult; Female	300 ppm Pb-acetate in drinking water; 8 weeks	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)	Rat; Weanling pups	300 ppm Pb-acetate in drinking water; GD1 to PND21	23	Morphology - Pb elevated relative kidney weight at PND21
Navarro-Moreno et al. (2009)	Rat; Adult; Male	500 ppm Pb-acetate in drinking water; 28 weeks	43	Kidney function - Pb exposed males had elevated urinary pH and protein, and glucose and blood in the urine.
Roncal et al. (2007)	Rat; Adult; Male	150 ppm Pb-acetate in drinking water; 16 weeks with remnant kidney surgery at week 4	26	Kidney function - Remnant kidney surgery and Pb exposure induced decreased creatinine clearance and proteinuria.
Wang et al. (2010d)	Rat; Adult; Female	300 ppm Pb-acetate in drinking water; 8 weeks	20	Kidney function - Elevated urinary total protein, urinary albumin, and serum urea nitrogen.
Ademuyiwa et al. (2009)	Rat; Adult	200, 300, and 400 ppm Pb-acetate in drinking water; 12 weeks	39 and 61	Kidney Function - Renal phospholipidosis and depletion of renal cholesterol after gestational Pb exposure.

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 100 ppm [lower dose] or 5,000 ppm [higher 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 et al., 1993a; Khalil-Manesh et al., 1992a; Khalil-Manesh et al., 1992b). At 6 months of exposure, GFR decreased in the high dose animals, albuminuria was present, and pathology ensued with focal tubular atrophy and

interstitial fibrosis formation. This pathology was persistent out to 12 months, and at 12 months glomeruli developed focal and segmental sclerosis. Similarly, GFR remained decreased after 12 months of exposure in the high dose group. The toxicological evidence for differences in GFR according to duration of Pb exposure, i.e., hyperfiltration with 3-month exposure versus decreased GFR with 6- or 12-month exposure, provide biological plausibility for epidemiological studies that observed a similar phenomenon by age in adults in association with Pb biomarker levels. These duration-dependent dichotomous changes in GFR are consistent between the toxicological and epidemiologic 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 renal biomarker since renal ALAD is protected by the kidney antioxidant GSH. Urinary NAG and GST levels were found to increase in rats after 3 months of high dose Pb exposure (resulting in blood Pb level of 125 µg/dL) in rats (Khalil-Manesh et al., 1992a), whereas only urinary NAG was increased following low dose Pb exposure (resulting in blood Pb level of 29 µg/dL) in rats (Khalil-Manesh et al., 1993a). An in vitro study also found an increase in NAG with high-level Pb exposure (50 µg/dL) (Dehpour et al., 1999). Occupational studies found that urinary NAG correlated best with recent blood Pb changes.

The renal effects of chronic Pb exposure as detailed above were partially rescued in rats with chelation therapy such as DMSA (Khalil-Manesh et al., 1992b). 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 greater detail in the antioxidant section (Section 5.5.5), produced similar findings. There was a functional 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 corroborated the previously observed increase in serum creatinine following Pb exposure in rats. Abdel Moneim et al. (2011b) reported Pb-induced (i.p. 20 ppm, 5 days) increased serum creatinine accompanied by histological alterations in rat kidneys. 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-induced renal dysfunction are listed in Table 5-23

below. The elevated serum creatinine in the Pb-exposed animals compared to controls suggests that animals exposed to low dosage (i.e., 50 ppm) Pb from birth may develop renal abnormalities. However, the lack of measurements of GFR or renal pathology weakens the conclusions. Other investigators have also shown that chronic Pb exposure has detrimental 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).

Table 5-23 Indicators of renal damage in male rats exposed to 50 ppm Pb for 40 and 65 days, starting at parturition

Biomarker (Mean±SD)	PND40 Control	PND40 Pb	PND65 Control	PND65 Pb
Blood Pb level (µg/dL)	1.8±0.33	12.7±1.7	2.1±0.35	7.5±0.78
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: Modified with permission of John Wiley & Sons, Berrahal et al. (2011)

Qiao et al. (2006) measured the effect of Pb on the expression of the renal fibrosis-related nuclear factor-kappa B (NF-κB), 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 NF-κB increased progressively at each time period. These changes were hypothetically related to the development of Pb-induced renal fibrosis in rats, but no histology was performed.

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, then subjected to remnant kidney surgery (left kidney mass reduced by 2/3 and right kidney removed), and subsequently exposed to Pb for an additional 12 weeks. Pb-treated rats had higher systolic BP, lower creatinine clearance, and higher proteinuria than did controls. Most striking was development of worse arteriolar disease, peritubular capillary loss, tubulointerstitial damage, and macrophage infiltration. Pb treatment was associated with significant worsening of pre-glomerular vascular disease, as characterized by an increase in the media-to-lumen ratio. There was also a higher percentage of

segmental sclerosis within glomeruli and a tendency for a higher number of sclerotic glomeruli. Additionally, a loss of peritubular capillaries, as reflected by a reduction in thrombomodulin staining, was observed. This was associated with worse tubular injury (osteopontin staining) due to more interstitial fibrosis (type III collagen staining) and a greater macrophage infiltration in the interstitium. The increase in macrophages was associated with higher renal MCP-1 mRNA. As a whole, these findings indicate that Pb exposure concomitant with existing renal insufficiency due to surgical kidney resection accelerated vascular disease and glomerular pathology. These findings are consistent with the previous work of Bagchi and Preuss (2005) also showing that Pb-exposed animals with non-Pb-related CKD (remnant surgery) had kidney dysfunction including impairment of the renin-angiotensin system (Losartan challenge), elevated systolic BP, and alterations in renal excretion of Pb, K^+, and Na^+. Thus, this model shows that low Pb exposure may exacerbate pre-existing underlying kidney disease.

Histological Changes

Historical studies discussed in previous Pb AQCDs have identified Pb-related renal damage by the presence of dense intranuclear inclusion bodies, which are capable of sequestering Pb (Goyer et al., 1970a). Chelators such as $CaNa_2EDTA$ 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 found in the kidney with acute (i.e., < 4 weeks) 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 et al., 1970b). Symptoms similar to the PT transport-associated Fanconi syndrome appear with Pb exposure, albeit often at high doses of Pb, i.e., Pb-poisoning. These symptoms, which include increased urinary electrolyte excretion (zinc), decreased Na-K-ATPase activity, mitochondrial aberrations, and aminoaciduria, also have been associated with blood Pb levels in 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. Jabeen et al. (2010) exposed pregnant albino BALB/c mice to a daily oral dose of Pb-acetate (10 mg/kg body weight, daily throughout pregnancy) until GD18, at which point the fetal kidneys were processed for histological examination. Histology revealed Pb exposure induced decreased kidney cortical thickness, decreased diameter of renal corpuscles, and increased renal tubular atrophy with desquamated epithelium and degenerated nuclei in

the distal and proximal tubules. Blood Pb levels were not reported in this study. Nonetheless, these data show that in utero Pb exposure had significant histological effects on the fetal kidney, which could contribute to altered renal function including clearance of waste products, electrolyte balance, and vasoregulation. Massanyi et al. (2007) reported on Pb-induced alterations in male Wistar rat kidneys after single i.p. doses of Pb-acetate (50, 25, and 12.5 mg/kg); kidneys were removed and analyzed 48 hours after Pb administration. Qualitative microscopic analysis detected dilated Bowman's capsules and dilated blood vessels in the interstitium with evident hemorrhagic alterations. Quantitative histomorphometric analysis revealed increased relative volume of interstitium and increased relative volume of tubules in the experimental groups. The diameter of renal corpuscles and the diameter of glomeruli and Bowman's capsule were significantly increased. Measurement of tubular diameter showed dilatation of the tubule with a significant decrease of the height of tubular epithelium compatible with degenerative renal alterations. These findings extend the observations of Fowler et al. (1980) and Khalil-Manesh et al. (1992a; 1992b); in particular, the enlarged glomeruli are consistent with the early hyperfiltration caused by Pb.

Abdel Moneim et al. (2011b) reported histological evidence of inflammation after Pb treatment in rats (i.p. 20 ppm, 5 days). This evidence included increased inflammatory cellular infiltrations, cytoplasmic vacuolation, and dilatation of some kidney tubules. Inflammation was accompanied by an increase in apoptotic cells and increased oxidative stress.

A recent study has also reported inclusion body formation in the nuclei, cytoplasm, and mitochondria of PT cells of Pb-treated rats (50 mg Pb/kg bw i.p., every 48 hours for 14 days) (Navarro-Moreno et al., 2009). These inclusion bodies were not observed in chronically Pb-exposed rats (500 ppm Pb in drinking water, 7 months). However, chronic Pb exposure resulted in morphological alterations including loss of PT apical membrane brush border, collapse and closure of the PT lumen, and formation of abnormal intercellular junctions.

Vogetseder et al. (2008) examined the proliferative capacity of the renal PT (particularly the S3 segment) following i.v. administration of Pb to juvenile and adult male Wistar rats. Proliferation induction was examined by detection of Bromo-2'-deoxyuridine (BrdU), Ki-67 (labels S, G2, and M phase cells), and cyclin D1 (an essential cell cycle progression protein). The cycling marker Ki-67 revealed a much higher proliferation rate in the S3 segment in control juvenile rats (4.8 ± 0.3%) compared with control adult rats (0.4 ± 0.1%). Pb administration (3.8 mg/100 g bw) increased the proportion of Ki-67-positive cells to 26.1 ± 0.3% in juvenile rats and 31.9 ± 0.3% in adult rats. Thus, the increased proliferation caused by Pb was age independent. The proliferation induction

caused by Pb administration may be a result of reduced cell cycle inhibition by $p27^{kip-1}$. Acute Pb treatment increased the incidence of cyclin D1 labeling in the BrdU-positive cells suggesting Pb was able to accelerate re-entry of cells into the cell cycle and cause proliferation in the PT. Pb-induced cellular proliferation has also been reported in the retina with gestational and early postnatal rodent Pb exposure (Giddabasappa et al., 2011).

Ademuyiwa et al. (2009) examined Pb-induced phospholipidosis and cholesterogenesis in rat tissues. Sprague-Dawley rats were exposed to 200, 300 and 400 ppm Pb-acetate for 12 weeks. The Pb exposure resulted in induction of phospholipidosis in kidney tissue, accompanied by depletion of renal cholesterol. The authors suggested that induction of cholesterogenesis and phospholipidosis in kidney may be responsible for some of the subtle and insidious cellular effects found with Pb-mediated nephrotoxicity. Drug-induced PT phospholipidosis is seen clinically with use of the potentially nephrotoxic aminoglycoside drugs, including gentamicin (Baronas et al., 2007).

Various antioxidants have been shown to attenuate Pb-induced histopathological changes to the kidney. Ozsoy et al. (2010) found L-carnitine to be protective in a model of experimental Pb toxicity in female rats. Markers of histopathological change in the kidney, including tubule dilatation, degeneration, necrosis, and interstitial inflammation were rescued by L-carnitine treatment in females. Male rats exposed to Pb (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 to Pb and extract produced histology indiscernible from that in controls. Post treatment with extract partially rescued the Pb-induced histopathology. El-Neweshy and El-Sayed (2011) studied the influence of vitamin C supplementation (20 mg/kg pretreatment every other day) on histopathological alterations in Pb-exposed male rats (20 mg/kg by intragastric feeding once daily for 60 days). Control rats showed normal histology, while Pb-treated rats exhibited karyomegaly with eosinophilic intranuclear inclusion bodies in the epithelial cells of the proximal tubules. Glomerular damage and tubular necrosis with invading inflammatory cells were also found. Rats treated with Pb-acetate plus vitamin C exhibited relatively mild or no karyomegaly with eosinophilic intranuclear inclusion bodies in the proximal tubules. Normal glomeruli were noted in animals exposed to Pb and vitamin C. These findings are presented in more detail in Section 5.5.5 but they consistently show that some

antioxidants are capable of preventing or rescuing Pb-induced renal 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 can 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, $^{\bullet}$NO and ET-1, are found to be affected by Pb exposure. Antioxidants attenuated Pb-related oxidative/nitrosative stress in the kidney and abrogated the Pb-induced increased BP (Vaziri et al., 1999b). Administration of the vasoconstrictor endothelin-1 (ET-1) affected mean arterial pressure (MAP) and decreased GFR (Novak and Banks, 1995). Acute high-dose Pb exposure (24 nmol/min for 15 or 30 minutes) completely blocked this ET-1-mediated GFR decrease but had no effect on MAP. Depletion of the endogenous antioxidant glutathione using the drug buthionine sulfoximine, a GSH synthase inhibitor, increased BP and increased kidney nitrotyrosine formation without Pb exposure, demonstrating the importance of GSH in maintenance of BP (Vaziri et al., 2000). Multiple studies have shown that Pb exposure depletes GSH stores. Catecholamines are vascular moderators that are also affected by Pb exposure (Carmignani et al., 2000). The effect on BP with Pb exposure is especially relevant to the kidney because it is both a target of Pb deposition and a mitigator of BP. These 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 the nitric oxide synthase inhibitor L-NAME in both Pb-treated and control animals. Pb exposure significantly increased BP (134 ± 3 versus 100 ± 6 mmHg), eNOS protein expression, oxidative stress, and vascular reactivity to angiotensin II. L-NAME potentiated the vascular response to angiotensin II in the control group, but had no effect on the Pb-treated group. Conversely, passive microvessel distensibility, measured after deactivation of myogenic tone by papaverine, was significantly lower in the arteries of Pb-exposed rats. Nitrites released from the kidney under the influence of angiotensin II in the Pb group were lower as compared to the control group whereas 3-nitrotyrosine was higher in the Pb group. The authors concluded that Pb exposure increases vascular tone through nitric oxide-dependent and -independent mechanisms, increasing renal vascular sensitivity to vasoconstrictors.

Simões et al. (2011) reported that acute Pb treatment (Pb-acetate i.v. bolus dose of 320 µg/kg bw, blood Pb of 37 µg/dL at 120 minutes after Pb administration) in adult male Wistar rats increased systolic arterial pressure 60 minutes after treatment without affecting diastolic arterial pressure or heart rate. With this single injection, serum angiotensin converting enzyme (ACE) activity was significantly elevated. The Pb-induced altered systolic BP was under the control of the renin-angiotensin system as evidenced by attenuation of the effects of Pb in Losartan (Ang II receptor blocker) or Enalapril (ACE inhibitor) co-treated animals (Simões et al., 2011). These data agree with earlier reports of Pb-related increases in ACE activity in young rats exposed to Pb for 2-8 weeks (Sharifi et al., 2004) and adult rats exposed to Pb for 10 months (Carmignani et al., 1999).

Apoptosis and/or Ischemic Necrosis of Tubules and Glomeruli

Apoptosis or programmed cell death in excess can cause cell atrophy while an insufficiency can lead to uncontrolled cell proliferation, such as cancer. Pb exposure has been shown to cause morphological changes to the kidney structure. Some of these Pb-induced changes are a result of cellular apoptosis or necrosis. Past studies have shown Pb-induced necrosis in proximal tubule cells (Fowler et al., 1980). Pb-induced apoptosis is known to act through the mitochondria (Rana, 2008). Pb-induced calcium overload may depolarize the mitochondria, resulting in cytochrome *c* release, caspase activation, and apoptosis. The apoptosis is mediated by Bax translocation to the mitochondria and can be blocked by overexpression of Bcl-xl. Also, Pb-induced ALA accumulation can generate ROS, which may damage DNA leading to apoptosis.

Mitochondria are targets of Pb toxicity and often involved in apoptosis. Pb can induce uncoupling of oxidative phosphorylation, decreased substrate utilization, and modification of mitochondrial ion transport. ATP energetics are affected when ATP-Pb chelates are formed and ATPase activity is decreased. ROS formation can contribute to these mitochondrial changes and to other changes within the kidney. Antioxidant supplementation after Pb exposure can remedy some changes. All of these outcomes, in conjunction with Pb-related depletion of antioxidants (e.g., GSH) and elevation of lipid peroxidation point to possible susceptibility of the kidney to apoptosis or necrosis.

Rodriguez-Iturbe et al. (2005) reported that chronic exposure to low doses of Pb (100 ppm in drinking water for 14 weeks) results in renal infiltration of immune cells, apoptosis, NF-κB activation and overexpression of tubulointerstitial Ang(II). Similarly, higher level Pb treatment in rats (i.p. 20 mg/kg, 5 days) induced inflammatory cellular infiltrations and an increase in apoptotic cells, accompanied by more pronounced BAX staining in kidney tubule epithelial cells (Abdel Moneim et al., 2011b). Pb treatment (0.5-

1 µM) of isolated rat proximal tubular cells increased cell death by apoptosis and necrosis
2 in a concentration- and time-dependent manner (Wang et al., 2011b). This was
3 accompanied by increased morphological changes typical of apoptosis such as
4 fragmented chromatin, condensed chromatin, and shrunken nuclei. These cells also
5 exhibited decreased mitochondrial membrane potential, decreased intracellular pH,
6 inhibition of Na+-K+ ATPase and Ca2+-ATPase activity, and increased intracellular
7 Ca2+ following Pb treatment.

8 Navarro-Moreno et al. (2009) examined the effect of 500 ppm Pb in drinking water over
9 7 months on the structure (including intercellular junctions), function, and biochemical
10 properties of PT cells of Wistar rats. Pb effects in epithelial cells consisted of an early
11 loss of the apical microvilli, followed by a decrement of the luminal space and the
12 respective apposition and proximity of apical membranes, resulting in the formation of
13 atypical intercellular contacts and adhesion structures. Inclusion bodies were found in
14 nuclei, cytoplasm, and mitochondria. Lipid peroxidation (TBARS measurement) was
15 increased in the Pb-treated animals as compared to controls. Calcium uptake was
16 diminished and neither proline nor serine incorporation that was present in controls was
17 noted in the PT of Pb-exposed animals. The authors speculated that Pb may compete with
18 calcium in the establishment and maintenance of intercellular junctions.

19 Tubular necrosis was also observed in rats treated with Pb-acetate (100 ppm s.c.) for 30
20 days (El-Sokkary et al., 2005). Histological sections of kidneys from Pb-treated rats
21 showed tubular degeneration with some necrotic cells. Similarly, El-Neweshy and El-
22 Sayed reported glomerular damage and tubular necrosis with invading inflammatory
23 cells after Pb treatment (20 mg/kg by intragastric feeding once daily for 60 days) to male
24 rats. The incidence of necrosis was decreased in both of these studies by pretreatment
25 with either melatonin or vitamin C. Pretreatment with melatonin (10 mg/kg), an
26 efficacious free radical scavenger and indirect antioxidant, resulted in a near normal
27 tubular structure. The authors concluded that melatonin protected the liver and kidneys
28 from the damaging effects of exposure to Pb through inhibition of lipid peroxidation and
29 stimulation of endogenous antioxidative defense systems (El-Sokkary et al., 2005).
30 Vitamin C supplementation (20 mg/kg pretreatment every other day) protected the renal
31 architecture and histology (El-Neweshy and El-Sayed, 2011).

32 Wang et al. (2009c) examined the effect of Pb-acetate (0.25, 0.5 and 1 µM) on cell death
33 in cultured rat primary PT cells. A progressive loss in cell viability, due to both apoptosis
34 and necrosis, was observed in cells exposed to Pb. Apoptosis predominated and could be
35 ameliorated with concomitant N-acetylcysteine exposure, whereas necrosis was
36 unaffected. Elevation of ROS levels and intercellular calcium, depletion of mitochondrial
37 membrane potential, and intracellular glutathione levels was observed during Pb

exposure. Pb-induced apoptosis was demonstrated morphologically (Hoechst 33258 staining) with condensed/fragmented chromatin and apoptotic body formation. CAT and SOD activities were significantly elevated, reflecting the response to accumulation of ROS.

Table 5-24 presents the acute and chronic renal effects of Pb exposure observed in recent and past animal toxicology studies.

Table 5-24 Effects of Pb on the kidney/renal system related to exposure duration– evidence from animal toxicology studies

Effects with less than 3 months of exposure	Effects with 6 or 12 months of exposure
Mitochondrial dysfunction	Mitochondrial dysfunction
Renal cell apoptosis	Renal cell apoptosis
Nuclear Inclusion Body Formation	Oxidant redox imbalance
Proximal Tubule Cytomegaly	Altered NO homeostasis
Glomerular Hypertrophy	ATPase dysfunction
Increased GFR	Aminoaciduria
	Increased electrolyte excretion
	Elevated blood pressure
	Decreased GFR

5.5.2.2 Epidemiology in Adults

A number of advances in research on the impact of Pb on the kidney in the 20 years following the 1986 Pb AQCD (U.S. EPA, 1986a) were noted in the 2006 Pb AQCD (U.S. EPA, 2006b). These included research in general and CKD patient populations at much lower blood Pb levels (5-10 µg/dL) at the time of evaluation than were previously studied. These advances contributed to the understanding of the effects of Pb exposure on kidney dysfunction overall in the population. Pb, at much lower doses than those causing chronic Pb nephropathy, may act as a cofactor with other more established kidney risks to increase the risk for CKD and disease progression in susceptible patients. Maric and Hall (2011) note that data from basic and clinical studies suggest that obesity, hypertension, hyperglycemia, hyperlipedemia, and other elements of the metabolic syndrome are highly interrelated and contribute to the development and progression of diabetic nephropathy and thus represent populations potentially at increased risk for kidney dysfunction.

In the 2006 Pb AQCD (U.S. EPA, 2006b), several key issues could not be completely resolved based on the Pb-kidney literature published to date. These included characterizing the lowest Pb dose at which altered kidney function effects occur, the

impact of higher past exposures on associations with current Pb biomarker levels, 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 addressing several of these challenges have been published.

General Population Studies

The 2006 Pb AQCD reported studies that examined associations between indicators of Pb exposure and 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. As illustrated in Figure 5-39 and Table 5-25, studies consistently demonstrate associations between higher blood Pb level and lower renal function in adults. 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. However, because blood Pb level in nonoccupationally-exposed adults reflects both recent and past Pb exposures, the magnitude, timing, frequency, and duration of Pb exposure contributing to the observed associations was uncertain. The evidence of Pb-associated renal effects in general population studies was substantiated by results that were adjusted for multiple potential confounding factors including age, race, sex, education, household income, smoking, alcohol use, cadmium exposure, and various health indicators such as diabetes, SBP, BMI, and history of cardiovascular disease.

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 concurrent blood Pb was 11.4 μg/dL (range 2.3-72.5) and 7.5 μg/dL (range 1.7-60.3) in men and women, respectively. After adjustment, 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.

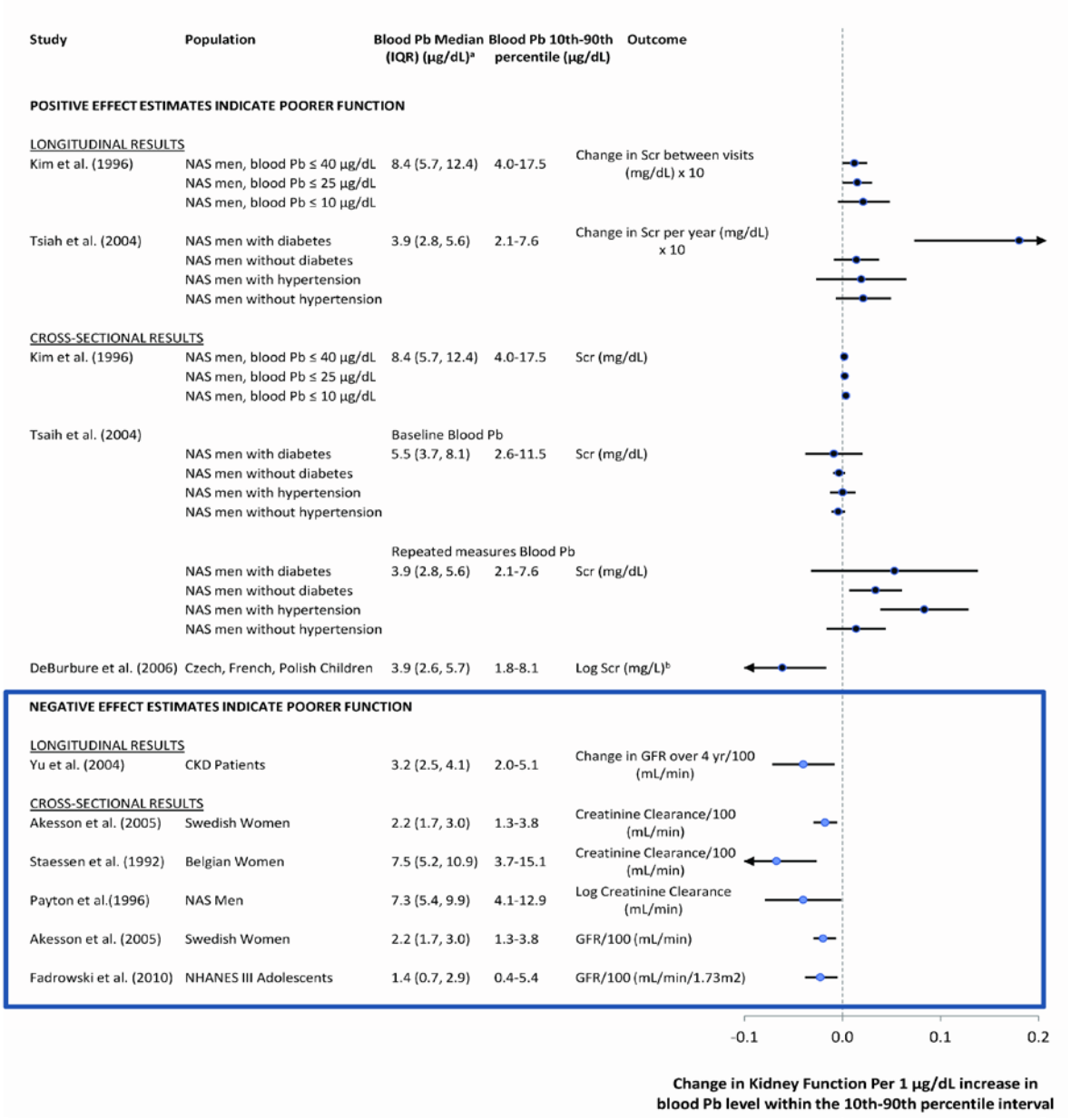
Multiple analyses assessing the kidney impact of Pb exposure have been conducted in the NAS population (Tsaih et al., 2004; Wu et al., 2003a; Kim et al., 1996; Payton et al., 1994). Participants in this study were originally recruited in the 1960s in the Greater Boston area. Inclusion criteria included male sex, age 21 to 80 years, and absence of chronic medical conditions. Longitudinal data contained in 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 (Kim et al., 1996). Participants were randomly selected to be representative of the entire NAS population in terms of age and follow-up. Kidney function was assessed with serum creatinine. Data from four evaluations were available for the majority of participants. At baseline, 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 with repeated measures, ln-transformed blood Pb was associated with an increase in serum creatinine from the previous to current follow-up period in the 428 participants whose highest blood Pb level was ≤ 25 μg/dL (β= 0.027 mg/dL [95% CI: 0.0, 0.054] per unit increase in ln blood Pb); effect estimates in the entire group and subsets with different peak blood Pb levels (≤ 10 or 40 μg/dL) also were positive (and larger for blood Pb levels ≤ 10 μg/dL) but 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 statistically significant positive association between blood Pb and concurrent serum creatinine remained in a cross-sectional analysis. 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 (0.7-2.1 mg/dL), 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 NAS between 1991 and 1995. Associations between baseline blood, tibia, and patella Pb and change in serum creatinine over an average of 6 years in 448 men were reported 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. Baseline blood Pb level 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. Per unit increase in ln blood Pb, the increase in serum creatinine between follow-up periods was substantially stronger in diabetics (β = 0.076 mg/dL [95% CI: 0.031, 0.121]) compared to non-diabetics (β = 0.006 mg/dL [95% CI: -0.004, 0.016]). A similar relationship was observed for tibia Pb. An interaction was also observed between tibia Pb and hypertension, although it is possible that many of the 26 diabetics were also included in the hypertensive group and

were influential there as well. 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.



[a]Blood Pb data are presented as median and (IQR) in µg/dL for blood Pb. For uniform presentation, median and IQR were estimated from the given distributional statistics by assuming normal distributions.

[b]The cross product of logged blood Pb and ranked urine Hg was included in the regression to model the interaction between these two variates. The significant hyperfilrative effect to these children could be due to a biphasic time course sometimes seen in early exposure.

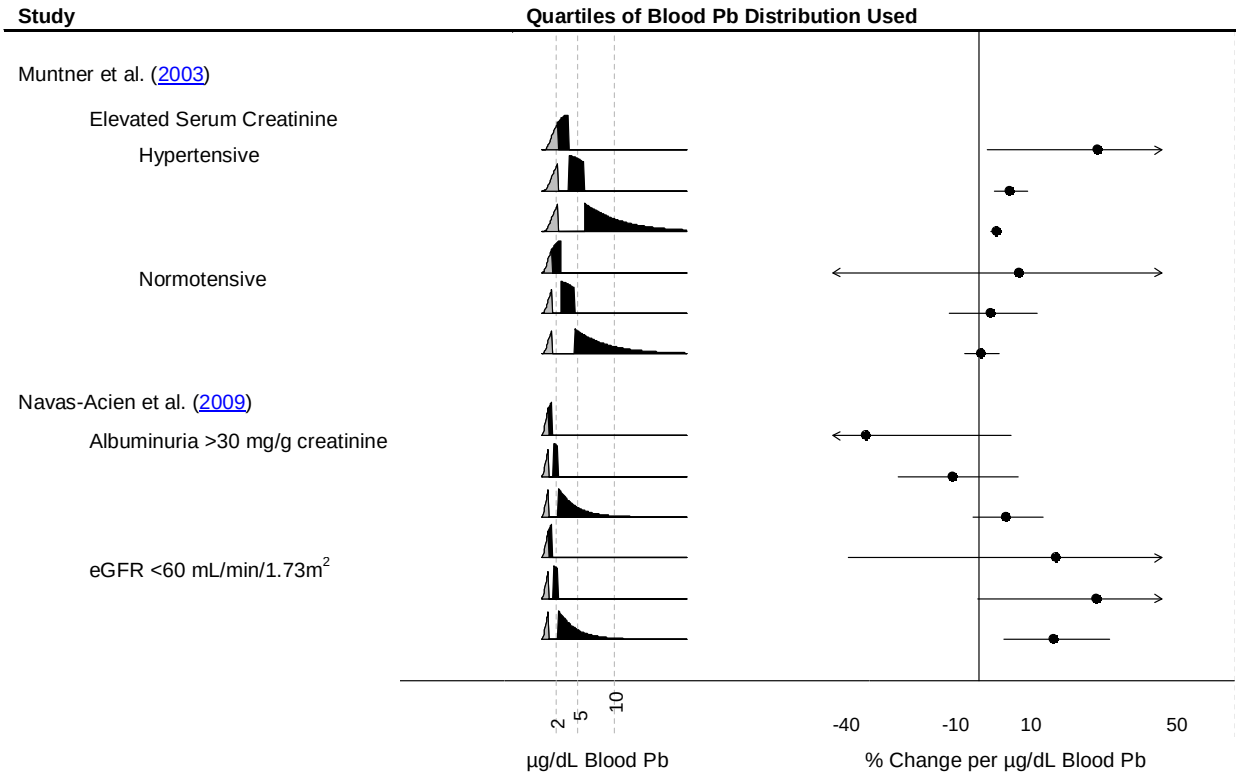
Note: Results are presented first for kidney function tests where an increase is considered impaired function (black circles) then for tests where a decrease is considered impaired function (blue circles, outlined in box). Within a category, results are presented first for longitudinal analyses followed by cross-sectional analyses. To compare results for linear and nonlinear modeling, effect estimates were standardized to a 1 µg/dL increase in blood Pb level within the 10th-90th percentile interval. Magnitudes of the effect should not be compared among different kidney metrics.

Figure 5-39 Kidney metric slopes for blood Pb or bone Pb.

The impact of Pb on the kidney has been examined in multiple NHANES datasets obtained over the last few decades (Figure 5-40 and Table 5-25). 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. The results, covering different time frames, have been consistent in providing support for Pb as a CKD risk factor, including NHANES III, conducted from 1988-1994, in which adults with hypertension and diabetes were observed to be susceptible populations (Muntner et al., 2003) and NHANES 1999-2002 (Muntner et al., 2005). However, because the various NHANES analyses were cross-sectional in design, examining associations between concurrent measures of kidney function and blood Pb levels, a common limitation is the uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations.

A recent publication examined NHANES data collected from 1999 through 2006 (Navas-Acien et al., 2009). The geometric mean concurrent blood Pb level was 1.58 μg/dL in 14,778 adults aged ≥ 20 years. After adjustment for survey year, sociodemographic factors, CKD risk factors, and blood 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 (> 2.4 μg/dL) to the lowest (≤ 1.1 μg/dL) blood Pb quartiles. Thus, in the subset of the population with the most severe kidney disease (both reduced eGFR and albuminuria), the magnitude of association with concurrent blood Pb was greater. When blood cadmium was included as a covariate, blood 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 in Section 5.5.4.1).

An important contribution of all NHANES publications is that they provide evidence that blood Pb remains associated with reduced kidney function (<60 mL/min/1.73 m^2 as estimated with the MDRD equation cross-sectionally) despite steadily declining blood Pb levels during the time periods covered. Additional studies in this category have also reported worse kidney function related to blood Pb levels (Lai et al., 2008a; Hernandez-Serrato et al., 2006; Goswami et al., 2005).



Note: These articles reported ORs of kidney function measures by grouping the population into quartiles of blood Pb and then comparing each group to the quartile with the lowest blood Pb (reference group). The blood Pb distribution of the examined group is shaded black and the reference group is shaded gray. To express these odds ratios in terms of blood Pb concentration, a log normal distribution was fit to the statistics presented and then the medians of each group were determined. The adjusted OR was the exponentiated quantity (log(OR) divided by the difference in the medians of the groups compared). The resulting odds ratio is presented in terms of percent change=100*(OR-1).

Figure 5-40 Percent change for kidney outcomes associated with blood Pb.

Table 5-25 Additional characteristics and quantitative data for associations of blood and bone Pb with kidney outcomes for results presented in Figure 5-39 and Figure 5-40

Reference	Population	Study Location; Time Period	N	Pb Biomarker Data	Outcome	Statistical Analysis	Effect Estimate (95% CI)
FIGURE 5-39: Positive Effect Estimates Indicate Poorer Function							**Per 1 µg/dL increase in blood Pb within the 10th-90th percentile interval**
Longitudinal Results							
Kim et al. (1996)	Adult males	Boston, MA; Multiple examinations 1979-1994	459	Median baseline blood = 8.6 µg/dL 10th-90th percentile: 4.0-17.5	Change in serum creatinine between visits x 10 (mg/dL)	Random-effects modeling adjusted for baseline age, time since initial visit, BMI, smoking status, alcohol ingestion, education level, hypertension, baseline serum creatinine, and time between visits	Peak blood Pb ≤ 40 µg/dL: 0.012 (-0.0001, 0.025) Peak blood Pb ≤ 25 µg/dL: 0.015 (0.0002, 0.03) Peak blood Pb ≤ 10 µg/dL: 0.021 (-0.005, 0.048)
Tsaih et al. (2004)	Adult males	Boston, MA; 8/1991-1995 with mean 6 year follow-up	448	Mean (SD) Baseline Blood Pb = 6.5 (4.2) µg/dL 10th-90th percentile: 2.1-7.6 Tibia Pb = 21.5 (13.5) µg/g Patella Pb = 32.4 (20.5) µg/g	Change in serum creatinine per year x 10 (mg/dL)	Log linear regression adjusted for age, age squared, BMI, hypertension, diabetes, smoking status, alcohol consumption, analgesic use, baseline serum creatinine, serum creatinine squared	With diabetes: 0.18 (0.07, 0.29) Without diabetes: 0.014 (-0.009, 0.037) With hypertension: 0.019 (-0.027, 0.065) Without hypertension: 0.021 (-0.007, 0.049) Per unit increase in ln-transformed tibia Pb With diabetes: 0.082 (0.03, 0.14) Without diabetes: 0.005 (-0.01, 0.02) With hypertension: 0.023 (0.003, 0.04) Without hypertension: 0.0004 (-0.01, 0.01)
Cross-Sectional Results							
Kim et al. (1996)	Adult males	Boston, MA; Multiple examinations 1979-1994	459	Median baseline blood = 8.6 µg/dL 10th-90th percentile: 4.0-17.5	Serum creatinine (mg/dL)	Random-effects modeling adjusted for baseline age, time since initial visit, BMI, smoking status, alcohol ingestion, education level, hypertension.	Peak blood Pb ≤ 40 µg/dL: 0.0017 (0.0005, 0.003) Peak blood Pb ≤ 25 µg/dL: 0.0021 (0.0007, 0.0035) Peak blood Pb ≤ 10 µg/dL: 0.0033 (0.0012, 0.0053)

Reference	Population	Study Location; Time Period	N	Pb Biomarker Data	Outcome	Statistical Analysis	Effect Estimate (95% CI)
Tsaih et al. (2004)	Adult males	Boston, MA; 8/1991-1995 with mean 6 yr follow-up	448	Mean (SD) baseline Blood Pb = 6.5 (4.2) µg/dL 10th-90th percentile: 2.6-11.5 Repeated measures 10th-90th percentile: 2.1-7.6 Tibia Pb = 21.5 (13.5) µg/g Patella Pb = 32.4 (20.5) µg/g	Serum creatinine (mg/dL)	Log linear regression adjusted for age, age squared, BMI, hypertension, diabetes, smoking status, alcohol consumption, analgesic use	Baseline blood Pb With diabetes: -0.009 (-0.038, 0.020) Without diabetes: -0.004 (-0.010, 0.003) With hypertension: 0 (-0.013, 0.013) Without hypertension: -0.005 (-0.011, 0.002) Follow-up blood Pb With diabetes: 0.053 (-0.032, 0.138) Without diabetes: 0.034 (0.007, 0.061) With hypertension: 0.083 (0.038, 0.128) Without hypertension: 0.014 (-0.016, 0.044)
De Burbure et al. (2006)	Children, mean age = 10 years, age range = 8.5-12.3 years	France, Czech Republic, and Poland; dates not provided	804	Concurrent Blood Pb Median (IQR) = 3.9 (2.6, 5.7) µg/dL 10th-90th percentile: 1.8-8.1	Log-transformed serum creatinine, cystatin C, and β2-microglobulin	Log linear regression adjusted for cadmium, urinary creatinine, urinary mercury	Log serum creatinine (mg/L): -0.062 (-0.106, -0.017)[a] Log Cystatin C: -1.3 (-2.4, -0.21)[a] Log β2-microglobulin: -2.2 (-4.0, -0.54)[a]
FIGURE 5-39: Negative Effect Estimates Indicate Poorer Function							**Per 1 µg/dL increase in blood Pb within the 10th-90th percentile interval**
Longitudinal Results:							
Yu et al. (2004)	Adult CKD patients	Taipei, Taiwan; 48 month longitudinal study period	121	Mean (SD) Baseline blood = 4.2 (2.2) µg/dL 10th-90th percentile: 2.0-5.1	Change in MDRD eGFR over 4 yr/100 (mL/min/1.73 m² body surface area)	Generalized estimating equations adjusted for age, sex, BMI, hyperlipidemia, hypertension, smoking, use of ACE inhibitor, baseline serum creatinine, daily protein excretion, daily protein intake, underlying kidney disease	-0.040 (-0.072, -0.008)[a]
Cross-Sectional Results:							
Akesson et al. (2005)	WHILA, adult women	Sweden; 6/1999-1/2000	820	Median (5-95%) concurrent blood = 2.2 (1.1, 4.6) µg/dL 10th-90th percentile: 1.3-3.8	Creatinine clearance/100 (mL/min)	Linear regression adjusted for age, BMI, diabetes, hypertension, regular use of nephrotoxic drug, smoking status	-0.018 (-0.03, -0.006)
					Cystatin C-based eGFR (Larsson et al. 2004)/100 (mL/min)		-0.02 (-0.03, 0.007)

Reference	Population	Study Location; Time Period	N	Pb Biomarker Data	Outcome	Statistical Analysis	Effect Estimate (95% CI)
Staessen et al. (1992)	Adults	Belgium; 1985-1989	1,981	Concurrent Blood Pb Mean (SD) Males: 11.4 µg/dL Females: 7.5 µg/dL 10th-90th percentile: 3.7-15.1	Creatinine clearance/100 (mL/min)	Log linear regression adjusted for age, age squared, sex, BMI, BP, ferritin level, smoking status, alcohol ingestion, rural/urban residence, analgesic and diuretic use, blood and urinary cadmium, diabetes, occupational exposure to heavy metals, and gamma glutamyl transpeptidase	Females: -0.067 (-0.108, -0.027)[a] Males: -0.051 (-0.097, -0.047)[a]
Payton et al. (1994)	Adult males	Boston, MA; 1988-1991	744	Mean (SD) concurrent blood = 8.1 (3.9) µg/dL 10th-90th percentile: 4.1-12.9	Log-transformed creatinine clearance (mL/min)	Log linear regression adjusted for age, BMI, analgesic and diuretic use, alcohol consumption, smoking status, SBP, DBP	-0.040 (-0.079, -0.0015)
Fadrowski et al. (2010)	NHANES, adolescents	U.S.; 1988 1994	769	Median concurrent blood = 1.5 µg/dL 10th-90th percentile: 0.4-5.4 Q1: <1.0 Q2: 1.0 to 1.5 Q3: 1.6 to 2.9 Q4: >2.9	Cystatin C-based eGFR/100 (mL/min/1.73 m^2; calculated using the Filler and Lepage equation)	Log linear regression adjusted for age, sex, race/ethnicity, urban/rural residence, smoking, obesity, household income, education level of family reference person, BP, lipid levels, glucose levels	-0.022 (-0.038, -0.0054) Q1: Referent Q2: -1.4 (-7.4, 4.5) Q3: -2.6 (-7.3, 2.2) Q4: -6.6 (-12.6, -0.07)
FIGURE 5-40: Analysis of Blood Pb Quartiles:							**%change in kidney outcome**
Muntner et al. (2003)	NHANES III, adults	U.S.; 1988-1994	4813	Mean (SD) concurrent blood Pb With Hypertension: 4.2 (0.14) µg/dL Q1: 0.7 to 2.4 Q2: 2.5 to 3.8 Q3: 3.9 to 5.9 Q4: 6.0 to 56.0	Elevated Serum Creatinine (99th percentile of each race-sex specific distribution for healthy young adults)	Logistic regression adjusted for age, race, sex, diabetes, SBP, smoking, history of CVD, BMI, alcohol consumption, household income, education level, marital status, health insurance	Q1: Referent With hypertension Q2: 47% (3, 110) Q3: 80% (34, 142) Q4: 141% (46, 297) Without hypertension Q2: 11% (-44, 121) Q3: 19% (-38, 125) Q4: 9% (-47, 122)
				Without Hypertension: 3.3 (0.10) µg/dL Q1: 0.7 to 1.6 Q2: 1.7 to 2.8 Q3: 2.9 to 4.6 Q4: 4.7 to 52.9	CKD		With hypertension Q2: 44% (0, 109) Q3: 85% (32, 159) Q4: 160% (52, 345) Without hypertension Q2: -10% (-63, 116) Q3: 0% (-55, 122) Q4: 9% (-59, 189)
Navas-Acien et al. (2009)	NHANES III, adults	U.S.; 1999-2006	14,778	Geometric concurrent blood 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 adjusted for survey year, age, sex, race/ethnicity, BMI, education, smoking, cotinine, alcohol intake, hypertension, diabetes, menopausal status	Q1: Referent Q2: 10% (-20, 51) Q3: 36% (-1, 85) Q4: 56% (17, 108)
					Albuminuria and eGFR <60 mL/minute/1.73 m^2		Q2: 53% (-15, 177) Q3: 57% (-17, 198) Q4: 139% (31, 337)

[a]95% CI estimated from given p-value.

Patient Population Studies

CKD as defined by the National Kidney Foundation - 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 criterion and markers of kidney damage are not required for the second criterion.

Several key studies in CKD patients provide prospective data to evaluate the impact of baseline blood Pb level on CKD progression (kidney function decline) in patient populations (Table 5-26). Yu et al. (2004), discussed in the 2006 Pb AQCD, followed 121 patients over a four year period. Eligibility required well-controlled CKD with serum creatinine between 1.5 and 3.9 mg/dL. Importantly, EDTA-chelatable Pb <600 μg/72 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 did those in the lower EDTA chelatable Pb levels (<80 μg Pb/72 hours) group (2/58). Associations between baseline chelatable or blood Pb level and change in serial measurements of eGFR (estimated by the MDRD equation (Levey et al., 1999) were modeled separately using generalized estimating equations. Based on these models, a 10 μg higher chelatable Pb level or 1 μg/dL higher blood Pb level reduced the GFR by 1.3 and 4.0 mL/min/1.73 m^2, respectively, during the 4-year study period. Recent studies expanded the CKD patient populations in which this effect was observed to those with diabetic nephropathy (Lin et al., 2006a) and with the lowest blood Pb levels studied to date (Lin et al., 2006b). Results of these observational studies have been summarized (Weaver and Jaar, 2010).

Table 5-26 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. (2006a)	87	6.5 (3.4)	108.5 (53.8)	35.1 (9.0)	1	3.87	Type II diabetics with nephropathy
Lin et al. (2006b)	108	2.9 (1.4)[a]	40.2 (21.2) (all <80)	47.6 (9.8)	2	1.1	Lowest Pb exposed CKD patients

[a]Notably, mean blood Pb level in this study was below that observed in a recent large general population study of 50- to 70-year olds in Baltimore, MD (Martin et al., 2006).

Source: Reprinted with permission of UpToDate.com, Weaver and Jaar (2010)

A recent population-based case-control study examined occupational Pb exposure as a risk factor for severe CKD (Evans et al., 2010). The study included 926 cases with first time elevations of serum creatinine >3.4 mg/dL for men and >2.8 mg/dL for women and 998 population-based controls. Occupational Pb exposure was assessed using an expert rating method based on job histories. 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 U.S. OSHA limit is 50 μg/m^3). In multivariable logistic regression modeling, the OR for CKD (adjusted for age, sex, smoking, alcohol consumption, diabetes, education, and BMI) was 0.97 (95% CI: 0.68, 1.38) in Pb-exposed compared to non-exposed participants. In analyses comparing low (≥ 3 to <10% of occupational exposure limit), medium (≥ 10 to < 30% of limit), and high (≥ 30% of limit) exposure groups, although ORs were elevated (statistically nonsignificant) in the medium exposure group compared to the never exposed group for average and lifetime cumulative exposure metrics, a monotonic increase in OR across exposure groups was not observed. In the low and high exposure groups, odds of CKD tended to be lower compared with the never exposed group. 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, the results overall did not provide strong evidence that Pb exposure was associated with renal effects.

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. Other case-control studies examining occupational risk factors for CKD found Pb exposure to be a risk factor (Nuyts et al., 1995; Steenland et al., 1990). Nuyts et al. (1995) found adults with history of occupational Pb exposure to have elevated odds of CKD (OR for ever- versus never-exposed: 2.11 [95% CI: 1.23, 4.36]). The association was weaker in Steenland et al. (1990) (OR for ever- versus never-exposed: 1.73 [95% CI: 0.82, 3.65]). Regular moonshine consumption, also a potential source of Pb exposure, was a stronger risk factor for CKD (OR: 2.42 [95% CI: 1.10, 5.36]).

The prospective observational aspect of Evans et al. (2010) is similar in design to the work of Lin and colleagues but differs in several important respects. In Evans et al. (2010), only occupational Pb exposure was considered whereas the work in Taiwan excluded occupational exposure and used blood and chelatable Pb measures. In the past in developed countries, environmental exposures were substantial. For example, mean tibia Pb levels were 21.5 and 16.7 μg/g bone mineral, in environmentally-exposed 50- to 70-year-old African-Americans and whites, respectively, in Baltimore (Martin et al., 2006). In Korean Pb workers, mean baseline tibia Pb level was only twofold higher (35.0 μg/g) (Weaver et al., 2003a) which illustrates the substantial body burden in middle- and older-aged Americans from lifetime Pb exposure. Declines in blood Pb levels in Sweden have been reported and attributed to the leaded gasoline phase-out (Strömberg et al., 1995; Elinder et al., 1986), although blood Pb levels were lower than those noted during the U.S. phase-out. Finally, the severe degree of CKD among subjects in Evans et al. (2010) creates a survivor bias at enrollment and limits the eGFR decline possible during follow-up, thus limiting the ability to identify factors that influence that decline.

ESRD Patient Studies

End stage renal disease (ESRD) is a well-established public health concern, and is characterized by the use of dialysis to perform the normal functions of the kidney. Incidence and prevalence in the U.S. continue to increase resulting in rates that are the third highest among nations reporting such data (U.S. Renal Data System, 2009). Studies in patients with CKD requiring chronic hemodialysis (ESRD) have also been published in the past five years. A study of 271 adult patients on regular thrice weekly dialysis

reported much higher blood Pb levels than had been appreciated by the treating clinicians (Davenport et al., 2009). Blood Pb levels ranged from 3 to 36.9 μg/dL; 25.5% had levels >20 μg/dL, 59% had values of 10-20 μg/dL, and 15.5% were <10 μg/dL. Few details on the statistical analysis were provided which complicates interpretation of the findings. However, blood Pb was positively correlated with hemodialysis vintage (months on dialysis; Spearman r = 0.38, p <0.001); negatively correlated with urine output (r = -0.44, p <0.001) and higher in patients using single carbon filter and reverse osmosis water purification devices. Another recent publication reported higher Pb in dialysate than in the tap water used in its preparation (Chen et al., 2009a). A systematic review of a wide range of trace elements in hemodialysis patients reported higher Pb levels in patients compared to controls although the difference was not large (Tonelli et al., 2009). These data suggest that blood Pb monitoring in dialysis patients may be useful.

Interpretation of blood and bone Pb in patients on dialysis is challenging for several reasons. First, renal osteodystrophy, the bone disease related to kidney disease, may result in increased release of Pb from bone stores. Thus, interpretation of blood and even bone Pb levels may require adjustment with one or more of a range of osteoporosis variables. Secondly, as observed above (Davenport et al., 2009), residual kidney function may have a substantial impact on blood Pb levels in populations with such minimal excretion. Third, as illustrated in the studies cited above (Chen et al., 2009a; Davenport et al., 2009), water and concentrates used in dialysis may be variable sources of Pb. A recent study reported decreased blood Pb in post-dialysis compared to pre-dialysis samples (Kazi et al., 2008). Thus, substantial fluctuations in blood Pb are possible while on dialysis. Finally, anemia is common in CKD and Pb is stored in red blood cells. Thus, measurement of blood Pb in anemia may require adjustment for hemoglobin; no standardized approach to this currently exists.

Given these caveats, a pilot study observed higher median blood Pb levels in 55 African-American dialysis patients compared to 53 age- and sex-matched controls (6 and 3 μg/dL respectively; p <0.001) (Muntner et al., 2007). 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 compared by levels of serum parathyroid hormone, calcium, phosphorus, and albumin and were not found to be significantly different (Ghosh-Narang et al., 2007). A study of 211 diabetic patients on hemodialysis (Lin et al., 2008) found parathyroid hormone and serum creatinine to be associated with blood Pb level in crude but not adjusted associations. In contrast, a study of 315 patients on chronic peritoneal dialysis observed parathyroid hormone to be positively correlated and residual renal function to be negatively

correlated with logarithmic-transformed blood Pb levels after adjustment (Lin et al., 2010). In the prospective portion of this study, blood Pb levels at baseline were categorized by tertile (range of 0.1 to 29.9 μg/dL with cut points of 5.62 and 8.66 μg/dL). Cox multivariate analysis, after adjustment for parathyroid hormone level, residual renal function, and 20 other variables, showed increased all-cause mortality in the middle (5.62-8.66 μg/dL) and highest (> 8.66 μg/dL) compared to the lowest (< 5.62 μg/dL) tertiles after 18 months of follow-up (hazard ratio= 2.1 [95% CI: 2.0, 2.2] and 3.3 [95% CI: 1.3, 13.5], respectively). A recent publication of an 18-month follow-up of 927 patients on maintenance hemodialysis also reported increased hazard ratios for all-cause (4.7 [95% CI: 1.9, 11.5]), cardiovascular-cause (9.7 [95% CI: 2.1, 23.3]), and infection-cause (5.4 [95% CI: 1.4, 20.8]) 18-month mortality in the highest (> 12.64 μg/dL) compared to the lowest tertile (< 8.51 μg/dL) of baseline blood Pb level, after adjustment for sex, urban residence, hemodialysis vintage, hemoglobin, serum albumin, and ferritin (Lin et al., 2011). Given other recent publications in hemodialysis patients by this group, it would be valuable to examine these risks after adjustment for hemoglobin A1C (Lin-Tan et al., 2007a), and blood cadmium (Yen et al., 2011; Hsu et al., 2009a).

Clinical Trials in Chronic Kidney Disease Patients

Randomized chelation trials in CKD patients, uncommon in nephrotoxicant research, provide unique information on the 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 5-27 below.

Table 5-27 Clinical randomized chelation trials in chronic kidney disease patients

Reference	Group	n	Baseline mean (SD) blood Pb (µg/dL)	Baseline mean (SD) chelatable Pb (µg/72 hr)	Baseline mean (SD) eGFR (mL/min/1.73 m^2)	Months of treatment/ follow-up	Change in eGFR per yr (mL/min/1.73 m^2)	Comments
Lin et al. (2003)	Chelated	32	6.1 (2.5)	150.9 (62.4)	32.0 (12.1)	27	+ 1.07	
	Control	32	5.9 (3.0)	144.5 (87.9)	31.5 (9.0)		- 2.7	
Lin et al. (2006a)	Chelated	15	7.5 (4.6)	148.0 (88.6)	22.4 (4.4)	15	-3.5	Subjects with Type II diabetes and nephropathy
	Control	15	5.9 (2.2)	131.4 (77.4)	26.3 (6.2)		-10.6	
Lin et al. (2006b)	Chelated	16	2.6 (1.0)[a]	43.1 (13.7)	41.2 (11.2)	27	+3.0	Lowest Pb exposed and treated range Body Lead Burden (72 h urinary Pb excretion) ≥ 20-<80 µg
	Control	16	3.0 (1.1)	47.1 (15.8)	42.6 (9.7)		-2.0	
Lin-Tan et al. (2007b)	Chelated	58	5.0 (2.2)	164.1 (111.1)	36.8 (12.7)	51	-0.3	Subjects without diabetes
	Control	58	5.1 (2.6)	151.5 (92.6)	36.0 (11.2)		-2.9	

[a]Notably, mean blood Pb level in this study was below that observed in a recent large general population study of 50- to 70-year olds in Baltimore, MD (Martin et al., 2006).

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 (Saxena and Flora, 2004; Jacobsen et al., 2001). EDTA benefits in a Pb rodent model appeared to occur via reduced oxidation (Saxena and 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 nonPb-exposed rat model (Gonick et al., 1996). Benefits from chelation reported in rodent models of Pb-related nephrotoxicity (Sanchez-Fructuoso et al., 2002a; Sanchez-Fructuoso et al., 2002b; Khalil-Manesh et al., 1992b) did not appear to occur via reversal of structural damage (Khalil-Manesh et al., 1992b); again suggesting that improved hemodynamics from reduction of reactive oxidant species, which could be due to reduced Pb and/or directly to the chelating agent, may be a mechanism (Gonick et al., 1996). However, the most parsimonious explanation for the combination of the observational

and experimental chelation work of Lin and colleagues is that reduced Pb is the underlying reason.

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 assessment that includes estimates of the bioavailable dose, longitudinal statistical analysis, and control for multiple kidney risk factors. However, the generalizability of the results to broader populations is unknown. In addition, the association observed between Pb dose and decline in GFR has been variable; the annual decline in eGFR per standard deviation (SD) higher Pb dose at baseline was much lower in the 2003 study than in subsequent publications (Table 5-27 above). Small sample sizes and differences in renal diagnoses between groups may be factors in this variability. Additional research in large populations at multiple centers with assessment of neuropsychological as well as kidney outcomes is needed.

Occupational Studies

The vast majority of studies in the literature on the impact of Pb on the kidney have been conducted in the occupational setting. In general, study size and extent of statistical analysis are much more limited than those in general population studies. Publications in few populations have reported adjusted results in occupationally exposed workers in the five years since the 2006 Pb AQCD. 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 (Weaver et al., 2009). Tibia Pb was evaluated at the beginning of each follow-up period (yearly on average) and Pb biomarker levels were adjusted for baseline levels and other covariates. In males, serum creatinine 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 at the beginning of the follow-up interval. In females, decreasing serum creatinine was associated with declining blood Pb (as in males); however, increasing blood Pb was associated with a concurrent increase in serum creatinine. Women (25.9% of the study population) were older and more likely to be former Pb workers than were men which may have been important factors in the effect modification observed by sex.

Chia and colleagues observed a significant, positive association between concurrent blood Pb and urine NAG in linear regression models after adjustment for age, sex, race, exposure duration, ALAD G177C polymorphism and the interaction between ALAD genotype and blood Pb (Chia et al., 2006). Similar positive associations were observed between blood Pb and a wider range of EBE markers in models that adjusted for age, sex,

race, exposure duration, and the HpyCH4 ALAD polymorphism (Chia et al., 2005). Other studies published in the last 5 years also focused on ALAD polymorphisms but did not find effect modification to be in a consistent direction (Gao et al., 2010a; Wang et al., 2009a; Weaver et al., 2006; Weaver et al., 2005b). In adults with the ALAD2 genotype, Pb has been associated with better and poorer renal function in separate cohorts of Pb workers.

A study of 155 male workers reported significant, positive correlations between blood and urine Pb and urine NAG and albumin after controlling for age and job duration (Sun et al., 2008a). An important additional study that analyzed occupational Pb exposure is discussed below under patient population studies (Evans et al., 2010).

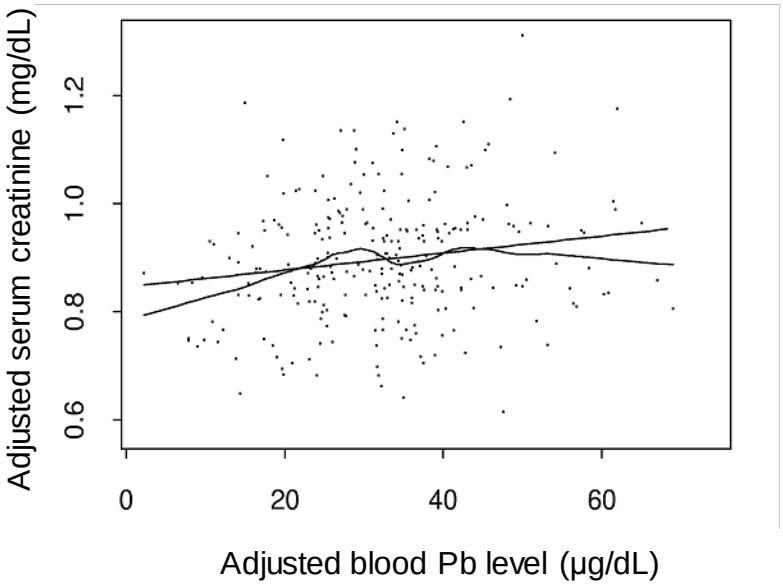
A few studies of occupationally-exposed adults have performed benchmark dose calculations for the effect of Pb on the kidney. Both used only EBE markers and found NAG to be the most sensitive outcome; reported lower confidence limits on the benchmark doses were 10.1 μg/dL (Sun et al., 2008a), and 25.3 μg/dL (Lin and Tai-yi, 2007).

A number of other publications in the five years since the 2006 Pb AQCD have reported significantly worse kidney outcomes in unadjusted analyses in occupationally-exposed adults compared to unexposed controls (Onuegbu et al., 2011; Patil et al., 2007) and/or significant correlations between higher levels of Pb biomarkers and worse kidney function (Alasia et al., 2010; Khan et al., 2008; Sun et al., 2008a; Garcon et al., 2007; Lin and Tai-yi, 2007; Alinovi et al., 2005). One small study found no significant differences (Orisakwe et al., 2007). In a study of 108 Pb workers with mean blood Pb level of 36.2 μg/dL, no significant correlations were observed between blood Pb concentration and GFR, creatinine clearance, uric acid clearance or uric acid excretion fraction (Karimooy et al., 2010). However, interpretation of this study is limited by the fact that "only 30 subjects had a correct 24 hours urine volume" and no methods are described for kidney outcome measurement or analysis.

Overall, the occupational literature published in the last five years on the kidney impact of Pb exposure has been more consistent in reporting statistically significant associations than were data reviewed for the 2006 Pb AQCD. This may reflect increased reliance on EBE markers as more sensitive outcome measures, publication bias, or multiple comparisons due to a greater number of outcomes assessed.

A small number of publications that include concentration-response information provides evidence of Pb-related nephrotoxicity in the occupational setting across the blood Pb ranges analyzed (Weaver et al., 2003a; Ehrlich et al., 1998). Data in 267 Korean Pb workers in the oldest age tertile (mean age = 52 years) did not provide evidence of a

threshold for a Pb effect on serum creatinine levels (added variable plot shown in Figure 5-41) (Weaver et al., 2003a). It is important to note the uncertainty regarding whether the concentration-response information provided in these studies applies to lower blood Pb levels or to populations with lower current environmental Pb exposures.



Source: Reprinted with permission of the BMJ Publishing Group, Weaver et al. (2003a)

Note: Both the adjusted regression line (straight line) and the line estimated by the smoothing method of the S-PLUS statistical software function lowess (line with curves) are displayed. Both have been adjusted for covariates. For ease of interpretation, axes have been scaled, so that the plotted residuals are centered on the means, rather than zero.

Figure 5-41 Added variable plot of association between serum creatinine and blood Pb in 267 Korean Pb workers in the oldest age tertile.

A major challenge in interpretation of the occupational literature is the potential for Pb-related hyperfiltration. Hyperfiltration involves an initial increase in glomerular hypertension which results in increased GFR. If persistent, the risk for subsequent CKD increases. This pattern has been observed in diabetes, hypertension, and obesity (Nenov et al., 2000). As discussed in the 2006 Pb AQCD, findings consistent with hyperfiltration have been observed in occupational populations (Weaver et al., 2003a; Hsiao et al., 2001; Roels et al., 1994), a study of adults who were Pb poisoned as children (Hu, 1991), and a study in European children (De Burbure et al., 2006). Longitudinal data in Pb-exposed rodents provide evidence of a hyperfiltration pattern of increased, followed by decreased GFR, associated with Pb exposure and are critical in interpretation of the human Pb-kidney literature (Khalil-Manesh et al., 1992a). Pb could induce glomerular hypertension resulting in hyperfiltration by several mechanisms including increased ROS, changes in

eicosanoid levels, and/or an impact on the renin-angiotensin system (Vaziri, 2008b; Roels et al., 1994). Whether hyperfiltration contributes to pathology in humans is unclear; longitudinal studies are needed.

Regardless, significant findings could be obscured if opposite direction associations are present in different segments of the study population and interaction models are not performed to address this. In the Korean Pb workers (Weaver et al., 2003a; Weaver et al., 2003b), significant associations in opposite directions were observed only when relevant effect modifiers such as age or genetic variants in ALAD, VDR, and NOS were included in the model. This is a valid concern for risk assessment, since the factors involved in these inverse associations in Pb-exposed workers are not well defined at present.

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 (Coria et al., 2009). Chelation was raised as a potential explanation for this discrepancy in the 2006 Pb AQCD.

With declining Pb exposure levels, recent work has focused on studies in children with much lower blood Pb levels. However, 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). The few studies included the 2006 Pb AQCD that analyzed clinical kidney outcomes in children found associations with indicators of Pb exposure that were inconsistent in direction. Fels et al. (1998) found no difference in mean serum creatinine between 62 children living near Pb-producing factories and 50 control children living in communities without Pb emission sources. In a study of 200 Belgian adolescents aged 17 years, higher concurrent blood Pb level was associated with higher serum cystatin-C in 200 (De Burbure et al., 2006); however, among 300-600 European children (n varied by

outcome), higher concurrent blood Pb level was associated with lower serum creatinine and cystatin C (Staessen et al., 2001).

Recent studies of children with elevated Pb exposure did not consistently indicate that Pb exposure was associated with reduced kidney function. A study in 123 children of workers in Pakistani Pb smelters and battery recycling plants and 123 control children, ages 1-6 years, reported elevated blood Pb levels, serum creatinine and urea in children of Pb-exposed workers compared to controls (medians: 8.1 versus 6.7 µg/dL; 56 versus 52 µM; and 4,500 versus 4,300 µM, respectively ($p \leq 0.01$ for all) in unadjusted analyses (Khan et al., 2010a). Blood Pb levels were correlated with serum creatinine (Spearman r = 0.13; p = 0.05). However, a study of 77 participants, ages 10-25 years, who were previously Pb poisoned through contaminated flour and chelated, reported no difference in renal effects between children with blood Pb levels > 48 µg/dL and < 43 µg/dL although lower IQ was observed in the subset who were exposed before the age of six years (Coria et al., 2009).

One of the key gaps identified in the 2006 Pb AQCD was limited data in children and adolescents particularly with respect to GFR measures and in populations without the elevated Pb exposure associated with Pb poisoning, living near a Pb source, or having parents with occupational Pb exposures. A recently published NHANES analysis in adolescents begins to fill this gap (Fadrowski et al., 2010). Associations between concurrent blood Pb and kidney function were investigated in 769 adolescents aged 12-20 years in the U.S. NHANES III, conducted 1988-1994. Kidney function was assessed with two eGFR equations. One utilized serum cystatin C and the other used the more traditional marker, serum creatinine. Median concurrent blood Pb and cystatin C-based eGFR levels were 1.5 µg/dL and 112.9 mL/min/1.73 m^2, respectively. Cystatin C-based eGFR was lower (-6.6 mL/min/1.73 m^2 [95% CI: -0.7, -12.6]) in participants with blood Pb levels in the highest quartile (≥ 3.0 µg/dL) compared with those in the lowest (<1 µg/dL). A doubling of blood Pb level was associated with a -2.9 mL/min/1.73 m^2 (95% CI: -0.7, -5.0) lower eGFR. In contrast, the association between blood Pb and creatinine-based eGFR, although in the same direction, was not statistically significant. As these children were born between 1968 and 1982, some likely had higher Pb exposures in earlier childhood, although notably, not as high or as long in duration as did older adults examined in aforementioned studies. Nonetheless, in this study of NHANES adolescents, there also is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations. Additional research in children is warranted, in particular studies with longitudinal follow-up, multiple outcome assessment methods, and examination of children born after Pb was banned from gasoline.

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 concurrent 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 of cadmium dose measures were stronger for the cystatin C based outcome. Staessen et al. (2001) found a statistically significant association between concurrent blood Pb level and serum cystatin C in a cross-sectional study of adolescents; creatinine-based measures were not reported. However, in a cross-sectional study of European children, higher concurrent blood Pb levels were associated with lower serum cystatin C and creatinine; these inverse associations were attributed to hyperfiltration (De Burbure et al., 2006). A 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 3,941 adults who participated in the 1999-2002 NHANES cystatin C subsample (Spector et al., 2011). Similar to the NHANES adolescent analysis, associations with the cystatin C outcomes were stronger. After multivariable adjustment, differences in mean eGFR for a doubling blood Pb were -1.9 (95% CI: -3.2, -0.7), -1.7 (95% CI: -3.0, -0.5), and -1.4 (95% CI: -2.3, -0.5) mL/min/1.73 m^2, using the cystatin C single variable, multivariable and combined creatinine/cystatin C equations, respectively, reflecting lower eGFR with increased blood Pb. The corresponding differences were -0.9 (95% CI: -1.9, 0.02) and -0.9 (95% CI: -1.8, 0.01) using the creatinine-based CKD-EPI and MDRD equations, respectively.

5.5.2.5 Reverse Causality

As discussed briefly above, reverse causality has been considered as an alternative hypothesis to explain associations observed between indicators of Pb exposure and renal dysfunction in adults. The reverse causality hypothesis attributes increased blood and bone Pb levels to reduced Pb excretion from nonPb-related causes rather then implicating Pb-related renal dysfunction as a contributing factor to CKD. The 2006 Pb AQCD

concluded that "reverse causality is not likely to be a major explanatory factor accounting for observed associations between Pb and renal dysfunction" (U.S. EPA, 2006b). The strongest evidence against reverse causality was provided by longitudinal studies in which associations between baseline measurements of Pb biomarkers and subsequent changes in renal function were demonstrated. In the NAS, baseline blood Pb levels were associated with subsequent declines in renal function over follow-up periods ranging from 3 to 6 years after adjustment for baseline renal function (Tsaih et al., 2004; Kim et al., 1996). Similar results were reported in studies of patients with renal disease (Yu et al., 2004; Lin et al., 2003). The longitudinal analyses in NAS studies also indicated that associations between blood Pb level and serum creatinine persisted in the lower range of serum creatinine levels, including those in the normal range (Tsaih et al., 2004; Kim et al., 1996). Thus, the association was not limited to the segment of the population with potentially clinically significant renal dysfunction in whom reduced Pb excretion would be more likely.

Additional support against reverse causality was provided by findings in Swedish women that both higher blood and urinary Pb were associated with lower creatinine clearance (Akesson et al., 2006; Akesson et al., 2005). If reverse causality were the more likely hypothesis for these associations, lower creatinine clearance would be associated with lower urinary Pb, which it is not. Among adults with chronic kidney disease, renal failure was not associated with increases in blood or bone Pb levels or chelatable Pb levels (Van De Vyver et al., 1988). Batuman et al. (1983) found that chelatable Pb levels were similar in adults with renal disease of unknown and known nonPb-related causes. Study had bone Pb levels (group means: 18 and 19 µg/g) in the range of those measured in recent epidemiologic studies. In summary, evidence that higher blood Pb levels are associated with subsequent declines in renal function from baseline levels, that associations persist among adults with normal renal function, and that renal failure does not increase Pb biomarker levels collectively do not support reverse causality and thus demonstrate that reverse causality does not provide a more suitable alternative hypothesis to explain the associations consistently observed between Pb biomarker levels and renal dysfunction.

5.5.3 Modes of Action for Lead-Induced Nephrotoxicity

5.5.3.1 Altered Uric Acid

Higher occupational Pb exposure or blood Pb levels have been linked to increased risk for both gout and kidney disease (Shadick et al., 2000; Batuman, 1993). Pb is thought to increase serum uric acid by decreasing its kidney excretion (Emmerson and Ravenscroft,

1975; Ball and Sorensen, 1969; Emmerson, 1965). Research during the past decade indicates that uric acid is nephrotoxic at lower levels than previously recognized (Johnson et al., 2003). Therefore, the 2006 Pb AQCD reviewed literature implicating increased uric acid as a mechanism for Pb-related nephrotoxicity (Weaver et al., 2005a; Shadick et al., 2000). However, this does not appear to be the only mechanism, since associations between blood Pb and serum creatinine have remained significant even after adjustment for uric acid (Weaver et al., 2005a). 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 (Lin et al., 2001).

Alterations in serum uric acid have been studied in animal models exposed to Pb. Conterato et al. (2007) followed various parameters of kidney function after single or multiple Pb injections in rats. The single dosing regimen consisted of a single i.p. injection of 25 or 50 mg/kg Pb-acetate, while the multiple injections involved once daily i.p. injection of either vehicle or Pb-acetate (5 or 25 mg/kg) for 30 days. Single and multiple injections at both dose levels increased plasma uric acid levels. Similarly, Abdel Moneim et al. (2011b) reported increased serum uric acid and urea levels after 5 days of Pb-acetate treatment (i.p. 20 mg/kg). In male rats exposed to Pb in drinking water from lactation to puberty (40 days) or post-puberty (65 days), Berrahal et al. (2011) found that plasma urea levels increased after 40 days of exposure (puberty blood Pb level of 12.7 µg/dL) but decreased after 65 days of Pb exposure (post-puberty blood Pb level of 7.5 µg/dL) (Table 5-23).

5.5.3.2 Oxidative Damage

A role for ROS in the pathogenesis of experimental Pb-induced hypertension and renal disease has been well characterized (Vaziri, 2008a, b; Vaziri and Khan, 2007). The production of oxidative stress following Pb exposure is detailed in respect to modes of action of Pb (Section 5.2.4). Past studies have shown that Pb treatment (single or three daily i.p. injections) can elevate kidney GST levels, affecting glutathione metabolism (Daggett et al., 1998; Moser et al., 1995; Oberley et al., 1995).

Animal studies continue to provide evidence for increased oxidative stress playing a role in the pathogenesis of Pb-induced renal toxicity. Increased ROS, serum NO, and renal NO were observed after Pb injections in rats (i.p. 20 mg/kg, 5 days) (Abdel Moneim et al., 2011b). Pb exposure to rat proximal tubular cells (0.5-1 µM) also increased ROS production, in a concentration-dependent manner (Wang et al., 2011b). Oxidative stress

was also demonstrated by increased lipid peroxidation (i.e., MDA) in serum and renal tissue after Pb exposure (Abdel Moneim et al., 2011b; Lodi et al., 2011; Wang et al., 2011b). Berrahal et al. (2011) reported increased MDA in Pb-exposed (50 ppm Pb-acetate pre- and post-natally) rat kidney relative to controls at both 40 (puberty; blood Pb 12.7 µg/dL) and 65 (post-puberty; blood Pb 7.5 µg/dL) days of age. In addition, total sulfhydryl groups were significantly decreased at 65 days. These increases in oxidative stress were accompanied by age-dependent Pb nephrotoxicity in male rats (Table 5-24).

Alterations in endogenous antioxidants and antioxidant enzymes that may lead to oxidative stress have also been reported after Pb exposure. Pb treatment decreased the activity of the renal antioxidant enzymes, CAT, SOD, GST, GPx, and GR (Abdel Moneim et al., 2011b) and protein levels of CAT and GSH (Lodi et al., 2011). Additionally, proteomic analysis of high-level Pb treated (1,500 ppm, 5 weeks; resulting in blood Pb level of 53.4 µg/dL) rat kidney identified decreased abundance of a rate-limiting enzyme in the synthesis of GSH (glutamate cysteine ligase) (Chen et al., 2011).

Conterato et al. (2007) examined the effect of Pb-acetate on the cytosolic thioredoxin reductase activity and oxidative stress parameters in rat kidneys. A single injection of Pb-acetate consisted of a single i.p. injection of 25 or 50 mg/kg Pb-acetate, while repeated injections consisted of one daily i.p. injection of Pb-acetate (5 or 25 mg/kg) for 30 days. Measured were thioredoxin reductase-1, a selenoprotein involved in many cellular redox processes, SOD, δ-ALAD, GST, GPx, non protein thiol groups (NPSH), CAT, as well as plasma creatinine, uric acid, and inorganic phosphate levels. The single injection at the 25 mg Pb dose level resulted in increased SOD and thioredoxin reductase-1 activity, while the 50 mg dose level increased CAT activity and inhibited δ-ALAD activity in the kidney. Repeated injections at the 5 mg dose level of Pb inhibited δ-ALAD and increased GST, NPSH, CAT, and thioredoxin reductase-1. Repeated injections at the 25-mg dose level reduced δ-ALAD but increased GST, NPSH, and plasma uric acid levels. No changes were observed in TBARS, GPx, creatinine or inorganic phosphate levels after either single or repeated injection dosing. As both dosing regimens increased thioredoxin reductase-1 activity, the authors suggest that this enzyme may be a sensitive indicator of renal changes with low dose Pb treatment.

Jurczuk et al. (2006) published a study of the involvement of some low molecular weight thiols in the peroxidative mechanisms of action of Pb in the rat kidney. Wistar rats were fed a diet containing 500 ppm Pb-acetate for a period of 12 weeks and were compared to a control group receiving distilled water for the same time period. GSH, metallothionein (MT), total and nonprotein SH groups (TSH and NPSH) were measured, as were the blood activity and urinary concentration of δ-ALA. The concentrations of GSH and NPSH were decreased by Pb administration, while MT concentration was unchanged. δ-

ALAD in blood was decreased, whereas urinary δ-ALA was increased by Pb administration. Negative correlations were found between the kidney GSH concentrations and previously reported concentrations of Pb and MDA in kidneys of these rats. It is apparent from graphical presentation of the data that GSH was reduced by more than 50% following Pb administration, while TSH was reduced by approximately 15%. No values for either blood or kidney Pb levels or kidney MDA were reported in this article. In 2007, the same authors (Jurczuk et al., 2007) reported on the renal concentrations of the antioxidants, vitamins C and E, in the kidneys of the same Pb-treated and control rats. Exposure to Pb significantly decreased vitamin E concentration by 13% and vitamin C concentration by 26%. The kidney concentration of vitamin C negatively correlated with MDA concentration. The authors concluded that vitamins E and C were involved in the mechanism of peroxidative action of Pb in the kidney, and their protective effect may be related to scavenging of free radicals.

Studies have used antioxidant compounds to investigate the role of oxidative stress in Pb-induced nephrotoxicity. Abdel Moneim et al. (2011b) reported that flaxseed oil treatment protected rats from Pb-induced (i.p. 20 mg/kg, 5 days) oxidative stress, inflammation, and apoptosis. However, the flaxseed oil also decreased the accumulation of Pb in renal tissue making it difficult to ascertain whether the protection was due to decreased oxidative stress or to altered Pb uptake kinetics.

El-Neweshy and El-Sayed (2011) studied the influence of vitamin C supplementation on Pb-induced histopathological alterations in male rats. Rats were given Pb-acetate, 20 mg/kg by intragastric feeding once daily for 60 days. Control rats were given 15 mg of sodium acetate per kg once daily, and an additional group was given Pb-acetate plus vitamin C (20 mg/kg every other day) 30 minutes before Pb feeding. Control rats showed normal histology, while Pb-treated rats exhibited karyomegaly with eosinophilic intranuclear inclusion bodies in the epithelial cells of the proximal tubules. Glomerular damage and tubular necrosis with invading inflammatory cells were also seen in Pb-treated animals. Among rats treated with Pb-acetate plus vitamin C, five exhibited relatively mild karyomegaly and eosinophilic intranuclear inclusion bodies of proximal tubules and an additional five rats were normal. Normal glomeruli were noted in all. Thus, vitamin C was shown to ameliorate the renal histopathological effects of Pb intoxication, however no measures of Pb accumulation were provided to clarify the mechanism of action of vitamin C.

Masso-Gonzalez and Antonio-Garcia (2009) studied the protective effect of natural antioxidants (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 were analyzed. Pb-exposed pups had decreased body weights. Blood Pb levels

were 1.43 µg/dL in the control group, 22.8 µg/dL in the Pb group, 21.2 µg/dL in the Pb plus zinc plus vitamins group, and 0.98 µg/dL in the zinc plus vitamin group. The kidney TBARS were significantly elevated in Pb exposed pups, while treatment with vitamins and zinc returned TBARS to control levels. Kidney CAT activity was significantly increased above control with Pb treatment; however supplementation with zinc and vitamins reduced CAT activity toward 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 was effective in attenuating the redox imbalance induced by developmental, chronic low-level Pb exposure, likely through the alteration of Pb accumulation.

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 conducted in male rats, a couple of studies have compared the response of male rats with female rats (Sobekova et al., 2009; Alghazal et al., 2008a). Sobekova et al. (2009) contrasted the activity response to Pb on the antioxidant enzymes, GPx and GR, and on TBARS in both male and female Wistar rats of equal age. Males weighing 412 ± 47 g and females weighing 290 ± 19 g were fed diets containing either 100 ppm or 1,000 ppm Pb-acetate for 18 weeks. In the male rats, kidney Pb content increased by 492% on the 100 ppm Pb diet and by 7,000% on the 1,000 ppm Pb diet. In the female rats, kidney Pb content increased by 410% on the 100 ppm Pb diet and by 23,000% on the 1,000 ppm Pb diet. There was virtually no change in GPx in the kidney of male rats given the 100 ppm Pb diet but there was a significant reduction in GPx in the female rats on both the 100 ppm diet and 1,000 ppm diet. In male rats, GR was increased from 182 units/gram of protein in control kidneys to 220 units on the 100 ppm Pb diet and 350 units on the 1,000 ppm diet. In female rats, kidney GR decreased from 242 units in control animals to 164 units in animals on the 100 ppm Pb diet and 190 units in animals on the 1,000 ppm diet. In male rats, kidney TBARS content increased from 7.5 units/gram protein to 10.0 units (1,000 ppm Pb diet group). In female rats, there was a reduction in TBARS from 14.4 units per gram protein to 10.0 units in rats on the 100 ppm Pb diet and to 11 units in rats on the 1,000 ppm Pb diet.

Alghazal et al. (2008a) compared the activity responses of the antioxidant enzyme, SOD and the detoxifying enzyme, GST, of the same rats exposed to 100 ppm or 1,000 ppm Pb-acetate for 18 weeks. Similar to the previous study, kidney TBARS were increased

only in male rats given the higher dose of Pb. Kidney SOD activity, on the other hand, was increased in both males and females at the higher dose of Pb, while GST activity was increased in kidney of males at the higher dose of Pb and decreased at the lower dose, but was decreased at both doses of Pb in females. Thus there were significant differences in the responses of male and female rats to Pb exposure. Differences may be accounted for in part due to the greater deposition of Pb in female rat kidneys. Another explanation, offered by the authors, is that male rats are known to metabolize some foreign compounds faster than do females, so the biological half-life of xenobiotics in the females may be longer.

5.5.3.3 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. Aguilar et al. (2008) studied changes in renal gangliosides following Pb exposure (600 ppm Pb-acetate in drinking water for 4 months) in adult male Wistar rats. Pb exposure caused an increase in blood Pb from 2.1 to 35.9 µg/dL. There was no change in serum creatinine or in hemoglobin, but there was an increase in urinary δ-ALA. The following renal gangliosides were measured by immunohistochemistry and by thin layer chromatography: GM1, GM2, GM4, and 9-O-acetylated modified form of the GD3 ganglioside (9-O-Ac-GD3). The ganglioside pattern was mainly characterized by a decrease in the GM1 ganglioside as well as by a mild increase in GM4 and GM2 gangliosides, while the strongest alteration was observed in the 9-O-Ac-GD3, which was overexpressed. The latter was observed only in the glomerular zone. This was associated with a decrease in apoptotic glomerular cells, as assessed by the TUNEL assay. The authors hypothesized that the increase in GD3-O-acetylation could represent a strategy to attenuate the normal renal apoptotic process and therefore contribute to cell survival during Pb exposure.

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 appears

to exert a dual effect on MT expression in the kidney: enhancement of MT gene transcription but suppression of MT mRNA translation.

Zuo et al. (2009) examined 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.

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 have been shown to be significantly affected by Pb exposure. The disposition of Pb in the soft tissues (kidney and spleen) can change with exposure to Pb and other compounds. Pb plus Cd exposure changed Pb disposition with increased blood Pb (versus Pb alone group) and decreased metal concentration in the kidney and liver (versus Pb alone). An iron deficient diet significantly increased Pb deposition in adult animals (Hashmi et al., 1989), pregnant dams, and maternally-exposed fetuses (Singh et al., 1991). Dietary thiamine plus zinc slightly reduced blood and kidney Pb in exposed animals (Flora et al., 1989). Selenium, a cofactor for GPx, attenuated Pb-induced lipid peroxidation and abrogated the Pb-induced attenuation of GR and SOD. Concomitant exposure to the cations aluminum and Pb protected animals from ensuing nephropathy (Shakoor et al., 2000). In summary, Pb has been shown to affect processes mediated by endogenous divalent cations. In addition,

exposure to other metals or divalent cations can modulate Pb disposition and its effects in the body.

5.5.4.1 Lead and Cadmium

Cd shares many similarities with Pb; it has been shown to be a ubiquitous PT nephrotoxicant and accumulates in the body. Despite the similarities, few studies have evaluated associations between Cd exposure and CKD or the impact of joint exposure of Pb and Cd or other metals on CKD. As discussed in the 2006 Pb AQCD, environmental exposure to Cd, at levels common in the U.S. and other developed countries, has been shown to impact substantially associations between indicators of Pb exposure and the kidney EBE marker, NAG, even in the presence of occupational level Pb exposure. In an occupational study, mean NAG, although higher in the Pb-exposed worker group compared to controls, was correlated with urine Cd but not blood or tibia Pb (Roels et al., 1994). In another occupational population where both metals were significantly associated with NAG, a 0.5 μg/g creatinine increase in Cd had the same effect on NAG as did a 66.9 μg/g bone mineral increase in tibia Pb (Weaver et al., 2003a).

The 2006 Pb AQCD noted that data examining the concentration-response 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 concurrent blood Cd level was 0.41 μg/L in 14,778 adults aged ≥ 20 years. After adjustment for survey year, sociodemographic factors, CKD risk factors, and blood Pb, the ORs for albuminuria (≥ 30 mg/g creatinine), reduced eGFR (<60 mL/min/1.73 m^2), and both albuminuria and reduced eGFR were 1.92 (95% CI: 1.53, 2.43), 1.32 (95% CI: 1.04, 1.68), and 2.91 (95% CI: 1.76, 4.81), respectively, comparing the highest with the lowest blood Cd quartiles. Both Pb and Cd remained significantly associated after adjustment for the other. Effect modification was not observed; however, ORs were higher for adults in the highest quartiles of both metals compared with the ORs for the highest quartiles of concurrent blood Cd or Pb alone (Table 5-25). Compared with adults with blood Cd levels ≤ 0.2 μg/L and blood Pb levels ≤ 1.1 μg/dL, adults with blood Cd levels > 0.6 μg/L and blood Pb levels > 2.4 μg/dL had ORs (95% CIs) of 2.34 (95% CI: 1.72, 3.18) for albuminuria, 1.98 (95% CI: 1.27, 3.10) for reduced eGFR, and 4.10 (95% CI: 1.58, 10.65) for albuminuria and reduced eGFR together. These findings are consistent with other recent publications (Akesson et al., 2005; Hellstrom et al., 2001), support consideration of both metals as independent CKD risk factors in the general

population, and provide novel evidence of increased risk in those with higher environmental exposure to both metals.

However, a very recent study suggests that interpretation of Cd associations with GFR measures may be much more complex. Conducted in Pb workers to address the fact that few studies have examined the impact of environmental Cd exposure in workers who are occupationally exposed to other nephrotoxicants such as Pb, the study assessed Cd dose with urine Cd, which is widely considered the optimal dose metric of cumulative Cd exposure. In 712 Pb workers, mean (SD) blood and tibia Pb, urine Cd, and eGFR using the MDRD equation were 23.1 (14.1) μg/dL, 26.6 (28.9) μg/g, 1.15 (0.66) μg/g creatinine, and 97.4 (19.2) mL/min/1.73m^2, respectively (Weaver et al., 2011). After adjustment for age, sex, BMI, urine creatinine, smoking, alcohol use, education, annual income, diastolic BP, current or former Pb worker job status, new or returning study participant, and blood and tibia Pb, higher urine Cd was associated with higher calculated creatinine clearance, eGFR (β = 8.7 mL/min/1.73 m^2 [95% CI: 5.4, 12.1] per unit increase in ln-transformed urine Cd) and ln-NAG, but lower serum creatinine. These unexpected paradoxical associations have been reported in a few other publications (De Burbure et al., 2006; Hotz et al., 1999) and have been observed in other populations. Potential explanations for these paradoxical results included a normal physiologic response in which urine Cd levels reflect renal filtration; the impact of adjustment for urine dilution with creatinine in models of kidney outcomes; and Cd-related hyperfiltration.

Wang et al. (2009c) studied the effects of Pb and/or Cd on oxidative damage to rat kidney cortex mitochondria. In this study young female Sprague Dawley rats were fed for 8 weeks with either Pb-acetate (300 ppm), Cd chloride (50 ppm), or Pb and Cd together in the same dosage. Lipid peroxidation was assessed as MDA content. Renal cortex pieces were also processed for ultrastructural analysis and for quantitative rtPCR to identify the mitochondrial damage and to quantify the relative expression levels of cytochrome oxidase subunits (COX-I/II/III). Cytochrome oxidase is the marker enzyme of mitochondrial function, and COX-I, II, and III are the three largest mitochondrially-encoded subunits which constitute the catalytic functional core of the COX holoenzyme. Mitochondria were altered by either Pb or Cd administration, but more strikingly by Pb plus Cd administration, as indicated by disruption and loss of mitochondrion cristae. Kidney cortex MDA levels were increased significantly by either Pb or Cd, given individually, but more so by Pb plus Cd. COX-I/II/III were all reduced by either Pb or Cd administration, but more prominently by Pb plus Cd administration. This study adds to knowledge of the 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 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 addition to Pb or Pb and As showed an additive increase in the urinary excretion of porphyrins, indicating that, although measured tissue burdens of Pb were reduced, the biologically available fraction of Pb is actually increased (Mahaffey et al., 1981; Mahaffey and Fowler, 1977).

Stress proteins were examined after exposure to mixtures of Pb and other metals. Induction of MT was strongest in groups with Cd treatment. However, co-exposure to Pb and As induced higher levels of MT protein than did either Pb or As exposure alone in kidney tubule cells. Heat shock proteins (Hsps) are commonly altered with exposure to metal mixtures. A study found in vitro (low dose) and in vivo that Pb induced Hsps in a metal/metalloid-, concentration- and time-specific manner (Wang et al., 2005). Additive or more than additive interactions occurred 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. Co-treatment of Pb with zinc (10 mg/kg i.p.) or ascorbic acid (10, 20 and 30 mg/kg i.p.) showed a moderate therapeutic effect when administered individually, but more pronounced protective effects after combined therapy (Upadhyay et al., 2009).

Jamieson et al. (2008) studied the effect of dietary 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 (30 mg Zn/kg), or supplemental zinc (SZn, 300 mg Zn/kg) groups, with or without Pb-acetate (200 ppm for 3 weeks). Pb exposure did not result in nephromegaly or histological alterations. The MZ rats had higher renal Pb (35%) and lower renal zinc (16%) concentrations than did CT rats. On the other hand, SZn was more protective than the CT diet was against renal Pb accumulation (33% lower). Standard procedures for indirect immunoperoxidase staining were used to determine MT localization in the kidney. Pb had no effect on MT staining

intensity, distribution, or relative protein amounts. Western blot analysis confirmed that MT levels were responsive to dietary 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 Hsps, glucose-regulating proteins and MTs in a rat proximal tubular cell line (NRK-52E) exposed to subcytotoxic doses of inorganic mercury ($HgCl_2$, 1-40 μM) and Pb ($PbCl_2$, 2-500 μM) were analyzed. ROS and reactive nitrogen species (RNS) were detected by flow cytometric analysis. Endogenous total GSH content and the enzymatic activity of GST were determined in cell homogenates. Western blot analysis and immunohistochemistry were used for quantification of hsps and MTs. Reverse transcription PCR was used for quantification of metallothionein. The higher doses of 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 ROS and RNS, maximal RNS at 24 hours, and maximal ROS at 48 hours. Pb (60 or 300 μM) did not cause an increase in ROS or RNS beyond the levels measured in control cells. Total GSH significantly increased in cells grown in the presence of Pb; the effect was concentration-dependent and GSH reached its maximal value at a dose of 300 μM Pb. The effect of mercury was biphasic: 10 μM significantly enhanced GSH by 600%, while the amount of GSH detected after 20 μM mercury only increased by 50% compared to control levels. GST activity was enhanced by both Pb and mercury. 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 MT mRNA expression, which reached its maximal value 3 hours after beginning treatment and reverted to control values at 24 hours. With Pb, on the other hand, mRNA transcription was concentration- and time-dependent. The transcripts remained overexpressed compared to controls up to 72 hours. The results of this study with regard to the Pb effect on MT synthesis clearly differ from those of Jamieson et al. (2008), which found no increase in MT following Pb exposure. This discrepancy remains to be clarified.

5.5.5 Impact of Treatment with Antioxidants on Renal Lead Accumulation and Pathology

5.5.5.1 Treatment with Antioxidants

Wang et al. (2010d) 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 toward 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 toward normal by Pb plus NAC treatment (MDA increased and the remainder decreased). Thus NAC was 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 for their capability to reduce Pb concentration in blood and soft tissues and to recover Pb-induced oxidative stress. Male Wistar rats were exposed to Pb-acetate (0.1% in drinking water) for 20 weeks. Rats were then treated orally once daily for five days with DMSA or its two analogues at doses up to 100 mg/kg. 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 synthetase (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) examined the effect of Pb 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 at a dose of 5 mg/kg daily for 6 weeks. No blood Pb levels were reported. TBARS were elevated above levels in 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 (mean [SE]: respectively, 48.6 [5.6] versus 95.5 [10.1] nmol/g tissue for oxidized form and 35.4 [3.0] versus 61.4 [5.1] nmol/g tissue for reduced form). On the other hand, levels of oxidized and reduced coenzyme Q10 were unchanged. Thus, the reduced levels of coenzyme Q attributable to Pb exposure may be a factor 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 and 4-hydroxyalkenals (4-HAD). Pb increased kidney lipid peroxidation products, but these were reduced toward normal with melatonin co-treatment. 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 concluded that melatonin protected the liver and kidneys from the damaging effects of exposure to Pb through inhibition of lipid peroxidation and stimulation of endogenous antioxidative defense systems.

Ozsoy et al. (2010) 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 plus L-carnitine group, serum creatinine was reduced to control values and the histopathological changes were reversed. Immunological staining indicated Cu/Zn-SOD stimulation by Pb feeding alone and reduction by L-carnitine co-treatment. The authors attributed the beneficial effects of L-carnitine to its antioxidant effect.

Reddy et al. (2010) treated Sprague-Dawley rats 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.

The effectiveness of various plant or bacterial extracts as antioxidants in the kidney was examined in a few studies. Kharoubi et al. (2008a) described the prophylactic effects of Wormwood (*Artemisia absinthium L.*) plant extracts on kidney function in 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 (e.g., 55.6 µg/dL blood Pb versus 22.3 µg/dL, respectively). 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 toward 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.

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 200 ppm Pb-acetate and/or 100 ppm of an extract of *A. vulgaris* 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 indicated 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 treated with 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 subsequent increased renal blood flow by enhancing the delivery of NO. This question arose because of the observations of Lin et al. (2006b) that repeated injections of $CaNa_2EDTA$ lead to improvement in kidney function in patients with chronic renal failure, even in individuals with very low body Pb stores as indicated by EDTA mobilization tests (i.e., < 80 μg 72 hour urinary Pb excretion). 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 suggested 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. (2006a) 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) on δ-ALAD activity 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 conjectured that the chelators may deplete the cells of zinc, an essential element for δ-ALAD activity. These observed effects with chelators were supported by Bradberry and Vale (2009), Hamidinia et al. (2006), and 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 over follow-up of 4 to 15 years in association with higher baseline blood and bone Pb levels (U.S. EPA, 2006b). Data in general and patient populations of adults provided consistent evidence of Pb-associated lower renal function in populations with mean concurrent or baseline blood Pb levels of 2-10 μg/dL (Akesson et al., 2005; Tsaih et al., 2004; Yu et al., 2004; Kim et al., 1996); associations with lower eGFR were observed in adults with hypertension with a mean concurrent blood Pb level of 4.2 μg/dL (Muntner et al., 2003). The conclusion from the 2006 Pb AQCD was substantiated by the coherence of effects observed across epidemiologic and toxicological studies. Both human and animal studies observed Pb-associated 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, exposure for 6 or 12 months resulted in decreased GFR, interstitial fibrosis, and kidney dysfunction. Additionally, toxicological studies found that early effects of Pb on tubular cells were generally reversible, but continued exposure resulted in chronic irreversible damage. Toxicological studies provided mechanistic evidence to support the biological plausibility of Pb-induced renal effects, including oxidative stress leading to $^{\bullet}$NO inactivation. Despite the strong body of evidence presented in the 2006 Pb AQCD, uncertainty remained on the contribution of past Pb exposures to associations observed in adults, the impact in children, and the implication of hyperfiltration.

Recent epidemiologic studies in adult general and patient populations continue to support Pb-related nephrotoxicity with consistently observed associations of blood and bone Pb levels with worse kidney function. These studies benefit from a number of strengths that vary by study but include comprehensive assessment of Pb dose with measurements of blood Pb, bioavailable Pb, and bone Pb as a biomarker of cumulative exposure; prospective study design; and statistical approaches that utilize a range of exposure and outcome measures, while adjusting for numerous potential confounding factors including age, race, sex, education, household income, smoking, alcohol use, cadmium exposure, and various health indicators such as diabetes, SBP, BMI, and history of cardiovascular disease. Large sample sizes provide strength to the general population studies. Re-examination of a study from the 2006 Pb AQCD provided data to conclude that a 10-fold increase in concurrent blood Pb (e.g., from 1 to 10 μg/dL) was associated with an 18 mL/min decrease in estimated creatinine clearance or a 25% decrease from the mean, and that an increase in blood Pb from the 5th to the 95th percentile (3.5 μg/dL) had the same negative impact on eGFR as did an increase of 4.7 years in age or 7 kg/m^2 in body mass

index (Akesson et al., 2005). At current blood Pb levels in the U.S. adult population, 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. For example, in adults with mean (concurrent or baseline measured 4-6 years before kidney function tests) blood Pb levels that are comparable to that of the general U.S. population (1.6 to 4.2 μg/dL), higher blood Pb level was found to be associated with clinically-relevant effects (e.g., eGFR < 60 mL/min/1.73 m^2, doubling of serum creatinine) (Fadrowski et al., 2010; Yu et al., 2004) and larger magnitudes of effect in potentially at-risk populations with cormorbidities for CKD such as diabetes mellitus (Tsaih et al., 2004) and hypertension (Tsaih et al., 2004; Muntner et al., 2003) or higher co-exposure to other environmental nephrotoxicants such as cadmium (Navas-Acien et al., 2009).

Recent NHANES analyses added to the evidence for Pb-associated lower renal function in populations with low concurrent mean blood Pb levels (< 2 μg/dL) (Fadrowski et al., 2010; Navas-Acien et al., 2009). However, because of uncertainties concerning the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations, it is difficult to assess whether a threshold exists for Pb-related renal effects.

Research in the occupational setting has traditionally been far less consistent than that in environmentally exposed populations (Section 5.5.2.2). 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 reflects limitations inherent in the study design. 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. For these reasons, nonsignificant associations or paradoxical associations in the occupational setting cannot be used as a rationale for discounting Pb-related nephrotoxicity at lower environmental levels.

Important data on the effects of Pb on the kidney in children were reported in a recent NHANES analysis in adolescents, ages 12-20 years, which observed an association between higher concurrent blood Pb (mean: 1.5 μg/dL) and lower cystatin C-based eGFR (Fadrowski et al., 2010). These findings are consistent with results from a rodent model study 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). This recent epidemiologic study along with several previous studies that included children with higher Pb exposures (due to residence near sources, Pb poisoning, or parental occupational exposure) provide evidence that renal function in children may be affected by Pb exposure; however, additional research is warranted. It is important to recognize that the NHANES adolescents from Fadrowski et al. (2010) likely had higher Pb exposures earlier in childhood, thus, the magnitude, timing, frequency, and duration of Pb exposure contributing to the observed association is uncertain.

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 was associated with increased glomerular sclerosis, tubulointerstitial injury, increased collagen staining, and an increase in macrophages associated with higher levels of MCP-1 mRNA. 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 modes of action for Pb exposure leading to renal alterations, including the influence of Pb-induced oxidative stress. The mode of action of Pb in the kidneys has been extended to the field of immunology, evidenced by observations that Pb exposure resulted in infiltration of lymphocytes and macrophages associated with increased expression of NF-κB in proximal tubules and infiltrating cells (Roncal et al., 2007). Additionally, recent findings expand 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. As indicated in Figure 5-38 and Table 5-22, studies found dysfunction in various kidney function measures, including urinary flow, ALP, microalbumin, and NAG in animals with blood Pb levels > 20 µg/dL (Wang et al., 2010d). Lower concentration Pb exposures and lower blood Pb levels in animals have not been examined widely.

In summary, new epidemiologic and toxicological studies evaluated in the current review support or expand upon the strong body of evidence presented in the 2006 Pb AQCD indicating that Pb exposure is associated with renal effects. The weight of epidemiologic evidence demonstrates consistently a relationship between higher blood Pb level and

kidney dysfunction (e.g., lower creatinine clearance, higher serum creatinine, and lower GFR) in nonoccupationally-exposed adults with mean concurrent or baseline blood Pb levels of 2-10 µg/dL. A few analyses find higher blood Pb levels to be associated with a greater longitudinal decrease in kidney function over time (4-15 years), suggesting that past Pb exposures may contribute to ongoing renal effects. The epidemiologic evidence is strengthened by associations between Pb biomarker levels and renal function that were observed after adjustment for multiple potential confounding factors such as age, sex, comorbid cardiovascular conditions, BMI, smoking, and alcohol use. Similar findings among adults with CKD indicate that in populations with pre-existing renal disease, Pb exposure may be associated with greater progression of disease. Because blood Pb level in nonoccupationally-exposed adults reflects both recent and past Pb exposures, the magnitude, timing, frequency, and duration of Pb exposure contributing to the observed associations is uncertain. Coherence for epidemiologic findings is provided by observations in animal models that Pb exposure for greater than 6 months decreases GFR and increases serum creatinine. The weight of evidence in animal studies indicates Pb-induced histopathological changes, including tubular atrophy and sclerosis. Overall, reduced renal function and increased kidney damage in animals are observed with chronic Pb (> 4 weeks) exposure that result in blood Pb levels > 20 µg/dL. By demonstrating Pb-induced renal oxidative stress, inflammation, mitochondrial dysfunction, apoptosis, and glomerular hypertrophy, toxicological studies provide biological plausibility for the associations observed in epidemiologic studies between blood Pb levels and kidney dysfunction. Collectively, the evidence integrated across epidemiologic and toxicological studies as well as across the spectrum of renal outcomes 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, the immune effects of Pb exposure have been extensively examined over several decades. Animal studies of the effects of Pb exposure on host resistance date back to the 1960s while those focusing on Pb-induced immune functional alterations, including developmental immunotoxicity, were first conducted during the 1970s. Despite this long history of research, Pb-associated immune effects in animals with blood Pb levels in the range of current U.S. population levels (i.e., <10 µg/dL), particularly early in life, have been observed only relatively recently within the last 10-15 years (Dietert and McCabe, 2007).

Over the last 10-15 years, advances in the understanding of Pb-associated changes in immunological parameters in humans have substantiated the immune effects of Pb.

The 2006 Pb AQCD presented consistent evidence for immune system effects associated with Pb exposure (U.S. EPA, 2006b). Rather than producing overt cytotoxicity or pathology, Pb exposure was found to be associated with alterations in several subclinical parameters related to cellular and humoral immunity (Figure 5-42). These conclusions were based most heavily on toxicological evidence in animals and in vitro models for the direct effects of Pb exposure in inducing changes in a wide spectrum of immune outcomes, but principally, a shift away from T-derived lymphocyte helper (Th)1 cytokines toward Th2 cytokines, suppression of Th1-dependent delayed type hypersensitivity (DTH), elevation in Th2-driven immunoglobulin E (IgE), and modulation of macrophages into a hyperinflammatory phenotype. Relatively fewer studies were available in humans and for fewer immune-related endpoints. A majority of studies in humans were conducted in Pb-exposed male workers, and while a larger number of immune outcomes were examined, the most consistent evidence comprised effects on neutrophil functionality. In a smaller body of studies of humans without occupational exposures, the weight of evidence indicated associations of higher blood Pb levels with lower abundance of T lymphocyte cells and, in concordance with toxicological studies, higher IgE levels in children. Due to limited examination, the immune effects of Pb exposure in adults without occupational exposures were not well characterized.

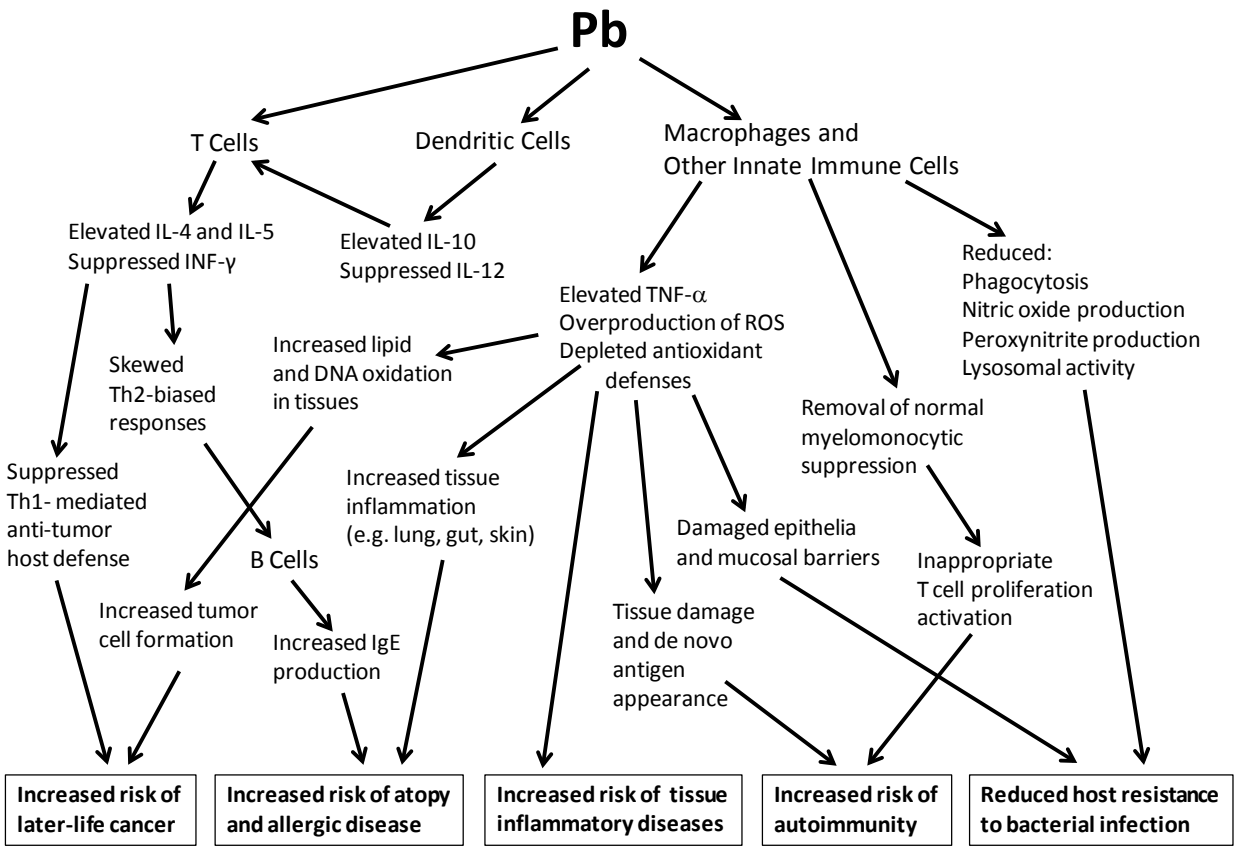


Figure 5-42 Immunological pathways by which Pb exposure may increase risk of immune-related diseases.

The pathways by which Pb exposure may alter immune cell function and consequently increase the risk of immune-related diseases are presented in Figure 5-42. Both toxicological and epidemiologic studies of children indicated Pb exposure effects on T cells. A large body of toxicological evidence demonstrated Pb-induced effects on macrophages. Neutrophil functionality was found to be reduced with higher Pb exposure, based on studies of Pb-exposed workers with mean blood Pb levels > 40 µg/dL. Alterations in immune cells can lead to changes in cell-to-cell interactions, multiple signaling pathways, and inflammation that affect both innate and acquired immunity, that in turn, influence the risk of developing infectious, allergic and autoimmune diseases as well as exacerbating inflammatory responses in other organ systems. Studies conducted in animal and in vitro models provided consistent evidence for Pb exposure inducing effects on the range of immune effects presented in this continuum. Among the hallmarks reported for Pb-induced changes in functional pathways were: (1) a suppression of T-derived lymphocyte helper (Th)1-driven cell-mediated immunity (as measured by a DTH response); (2) an increase in Th2-driven IgE antibody and Th2 cytokine production; and

(3) a pro-inflammatory shift in macrophage function. The latter was characterized by increased production of reactive oxygen species (ROS), prostaglandin E_2 (PGE_2), and inflammatory cytokines such as tumor necrosis factor-*αlpha* (TNF-α) and interleukin (IL)-6 and decreased production of IL-12 and nitric oxide (NO). While epidemiologic studies did not provide evidence for this full spectrum of immune effects, a shift to a Th2 phenotype was indicated by associations observed between higher blood Pb level and higher serum IgE levels in children.

Reflecting an inhibition of Th1 activity, toxicological evidence presented in the 2006 Pb AQCD linked Pb exposure of animals to impaired host resistance to bacteria (U.S. EPA, 2006b). Indicating a hyperinflammatory state and local tissue damage, a few available toxicological studies found Pb exposure-induced generation of autoantibodies, suggesting an elevated risk of autoimmune reactions. Additionally, the shift toward a Th2 response suggested that Pb could elevate the risk of atopy and allergic responses. While toxicological evidence for Pb-induced Th2 activity, elevated IgE, and inflammation supported the biological plausibility of such effects, epidemiologic evidence was too sparse to draw conclusions regarding associations between blood Pb levels and these broader indicators of immune dysfunction in humans.

Changes in the spectrum of immune endpoints were associated with a wide range of blood Pb levels. Several toxicological studies found blood Pb levels in the range of 7-100 µg/dL to be associated with juvenile and/or adult immune effects (e.g., suppressed DTH, elevated IgE, changes in cytokine levels). Most epidemiologic studies examined and found lower T cell abundance and higher serum IgE levels in association with population mean (or quantiles) concurrent blood Pb levels > 10 µg/dL.

With respect to critical lifestages of Pb exposure, animal studies provided strong evidence for immune effects induced by prenatal Pb exposures and by postnatal exposures in adult animals. There was uncertainty regarding critical lifestages of Pb exposure in humans as epidemiologic studies of children primarily were cross-sectional in design and examined concurrent blood Pb levels. Several other limitations of epidemiologic studies were noted, including small sample sizes; inconsistent adjustment for potential confounders such as age, sex, smoking, and comorbid conditions; and reliance on comparisons of immune endpoints among groups with different blood Pb levels, which provided limited information on the concentration-response function.

Collectively, the small numbers of toxicological and epidemiologic studies published since the 2006 Pb AQCD supported the previous findings of Pb-associated immune effects. Epidemiologic studies supported previous findings in children and provided new evidence for effects in nonoccupationally-exposed adults. Recent studies also expanded on the array of immunological parameters affected by Pb exposure as presented in Figure

5-42. For example, a new toxicological study indicated that Pb may modulate the function of dendritic cells. Results from new toxicological and epidemiologic studies strengthened 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 5-42). Several new epidemiologic studies examined signaling molecules such as pro-inflammatory cytokines and NO to produce findings parallel with toxicological studies. New toxicological studies expanded knowledge of the broader role of Pb-associated immune modulation in mediating Pb effects in nonlymphoid tissues (e.g., in the nervous, reproductive, and respiratory systems). Although primarily cross-sectional in design, recent epidemiologic studies improved on the design of earlier studies through greater examination of children and adults with blood Pb levels more comparable to contemporary levels in the U.S. population and greater consideration of confounding by age, sex, smoking, comorbid conditions, and SES-related variables.

5.6.2 Cell-Mediated Immunity

5.6.2.1 T Cells

A majority of the evidence indicating effects of Pb exposure on T cells was provided by older toxicological and epidemiologic studies reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b). Toxicological studies demonstrated Pb exposure-induced shifts in the partitioning of CD4+ (T helper) cell populations to favor Th2 cells (10-100 µM in vitro) and the production of Th2 cytokines and to suppress production of Th1 cytokines (wide range of Pb exposures) (Section 5.6.5.4). Previous epidemiologic findings were limited largely to associations between higher concurrent blood Pb level and lower T cell abundance in children.

Consistent with previous epidemiologic findings, in a recent study of 7 week-old Wistar rats administered 200 ppm Pb-acetate, the percentages of CD4+ and CD8+ cytotoxic T cells were decreased (with CD4-CD8- cells elevated) in the submaxillary lymph nodes, but only with i.p. Pb dosing ($p < 0.05$) and not oral exposure (200 ppm Pb-acetate both routes of administration) (Teijon et al., 2010). Limited recent toxicological investigation added mechanistic information by indicating that Pb may induce a shift to Th2 responses via T cell-dependent and -independent pathways. Previously, in cultures of human CD4+ T cells, Pb (1 µM, 30 minutes) was shown to activate transcription factor NF-κB (regulates T cell activation) (Pyatt et al., 1996) and to increase, in a concentration-dependent manner (10 and 50 µM $PbCl_2$, 24 hours), the expression of MHC class II

surface antigens (e.g., HLA-DR), which mediate the CD4+ response to exogenous antigens (Guo et al., 1996b). Recent studies examined higher Pb exposure concentrations than did previous studies. Heo et al. (2007) recently showed that Pb (25 µM) blocked production of the Th1 cytokine interferon-γ (IFN-γ) in cultures of stimulated mouse T cells not by affecting gene expression but by suppressing translation of the protein. This blockage was rescued with the addition of IL-12, which promotes Th1 activity. These results demonstrated a T cell-dependent pathway to skewing toward Th2 responses. However, Pb was able to decrease the IFN-γ to IL-4 ratio (indicating a shift to Th2) in the absence of STAT6, the preferential signaling pathway for T cells. Thus, Pb also was found to skew toward Th2 responses via a T cell-independent pathway. Similar observations were made in vivo by Kasten-Jolly et al. (2010). In this study, developmental Pb exposure of mice (100 µM Pb-acetate in drinking water of dams from GD8 to PND21, resulting in pup blood Pb levels of 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 and occurred with concomitant increases in adenylate cyclase 8 and phosphatidylinositol 3-kinase, adding to the evidence that Pb may promote Th2 activity via T cell-independent pathways.

While a few available recent epidemiologic studies found associations of blood Pb levels with lower levels of Th1 cytokines and higher levels of Th2 cytokines in humans (Section 5.6.5.4), the extant evidence for effects on T cells in humans is derived largely from older studies describing differences in the abundance of several T cell subtypes that mediate acquired immunity responses to antigens. In most studies of children, higher blood Pb levels were associated with lower T cell abundance, primarily CD3+ cells. Such associations were observed in studies that adjusted for potential confounding factors (as described below) and studies that compared mean cell abundances among groups with different blood Pb levels. Blood Pb level was less consistently associated with lower abundance of other T cell subtypes such as CD4+ and CD8+.

The weight of evidence supported lower T cell abundance in association with concurrent blood Pb level and in groups of children with levels > 10 µg/dL (Zhao et al., 2004; Sarasua et al., 2000; Lutz et al., 1999). Associations were less consistent in comparisons of children with lower blood Pb levels. In analyses of 331 children in Germany, Karmaus et al. (2005) found that children (ages 7-10 years) with concurrent blood Pb levels 2.2-2.8 µg/dL (2nd quartile) had a 9 to 11% lower abundance of several T cell subtypes ($p < 0.05$, t-test) compared with children with blood Pb levels < 2.2 µg/dL (lowest quartile). Unlike other studies in children, Karmaus et al. (2005) considered potential confounding by adjusting for sex, age, number of infections in the past 12 months, passive smoke exposure, serum lipids, and serum organochlorine levels. Other studies that examined

blood Pb levels < 10 µg/dL (population mean < 2 µg/dL or quantile 5-9 µg/dL) did not find associations with T cell abundance (Hegazy et al., 2011; Belles-Isles et al., 2002).

Another study of U.S. (multiple unspecified locations) children that considered confounding also found an association between higher concurrent blood Pb level and lower T cell abundance; however, it was limited to the youngest subjects (Sarasua et al., 2000). Among 241 children 6-35 months in age, a 1 µg/dL higher blood Pb level was associated with a 0.18% (95% CI: -0.34, -0.02) lower CD3+ cell count, a 0.10% (95% CI: -0.24, 0.04) lower CD4+ cell count, and a 0.04% (95% CI: -0.15, 0.07) lower CD8+ cell count, adjusting for city of residence, age, and sex. In older age groups (36-71 months, 6-15 years), many effect estimates were positive. Analysis of blood Pb level categories indicated that associations were driven by lower T cell abundance (3-6%) among children 6-35 months in age with blood Pb levels > 15 µg/dL. It is important to note that 76% of subjects lived near a Pb smelting operation. These subjects living near Pb sources likely had higher blood Pb levels and may have driven the observed associations. Neither Karmaus et al. (2005) nor Sarasua et al. (2000) found a monotonic decrease in T cell abundance across blood Pb level groups. Neither of these studies adjusted for SES, which has been associated with blood Pb levels and immune-related conditions such as asthma, allergy, and viral infections. However, it is difficult to assess the potential for confounding by SES as neither study reported the SES characteristics of the study population.

In the limited investigation of nonoccupationally-exposed adults, higher concurrent blood Pb levels were associated with higher T cell abundance (Boscolo et al., 2000; Sarasua et al., 2000; Boscolo et al., 1999); however, occupational studies conducted in the U.S. and Asia did not find Pb-exposed workers consistently to have lower or higher abundance of T cells (Mishra et al., 2010; Pinkerton et al., 1998; Yucesoy et al., 1997b; Undeger et al., 1996; Fischbein et al., 1993). Some studies found that compared with unexposed controls, Pb-exposed workers had a lower ratio of CD4+/CD8+ cells (Mishra et al., 2010; Fischbein et al., 1993). In particular, Fischbein et al. (1993) found this lower ratio in New York area firearms instructors with a relatively lower mean blood Pb level of 14.6 µg/dL and after adjusting for age and smoking. Changes in the CD4+/CD8+ ratio have not been examined in populations with lower blood Pb levels.

Although several epidemiologic studies have found lower T cell abundance in association with higher blood Pb levels, after adjusting for a range of potential confounding factors, it is not clear what effects these small magnitudes of change may have on the cell-to-cell interactions that mediate downstream acquired immune responses. In children, groups with higher blood Pb level had lower CD3+ cell abundance that ranged between 1 and 9%. Larger decreases (20-35%) were observed in studies of occupationally-exposed

males with higher blood Pb levels than those found in the general U.S. population (means 15 and 75 µg/dL) (Undeger et al., 1996; Fischbein et al., 1993).

In summary, a majority of the evidence indicating effects of Pb exosure on T cells was provided by previous toxicological and epidemiologic studies reviewed in the 2006 Pb AQCD. Previous toxicological studies demonstrated Pb-induced expansion of Th2 cells and increased Th2 cytokine production. A small number of new toxicological studies expanded the evidence by describing that Pb-induced Th2 skewing may occur via T cell-dependent pathways (Heo et al., 2007) and -independent pathways (Kasten-Jolly et al., 2010; Heo et al., 2007). Previous studies did not provide consistent evidence that higher blood Pb levels or Pb exposure was associated with lower T cell abundance in nonoccupationally-exposed or occupationally-exposed adults, respectively. A new study of children did not find children with higher blood Pb level to have lower T cell abundance (Hegazy et al., 2011). Epidemiologic findings were limited largely to associations between higher concurrent blood Pb level (> 10 µg/dL) and lower T cell abundance observed in previous studies of children (Karmaus et al., 2005; Zhao et al., 2004; Sarasua et al., 2000; Lutz et al., 1999).

5.6.2.2 Lymphocyte Activation

A majority of the evidence indicating that Pb exposure stimulates lymphocyte activation is provided by previous toxicological studies in which exposures to high concentrations of Pb (10-100 µM) induced an expansion of alloreactive B and T lymphocytes (U.S. EPA, 2006b). Lymphocyte activation occurs as a result of reversing the normal suppression that is mediated by a macrophage-like subpopulation. In the limited recent investigation of Pb-induced lymphocyte activation, toxicological studies characterized potential mechanisms underlying this effect. Gao et al. (2007) described a potential role for dendritic cells. Dendritic cells that matured in the presence of 25 µM $PbCl_2$ promoted enhanced alloreactive T cell proliferation compared to control dendritic cells. In addition, using the local lymph node assay (LLNA), Carey et al. (2006) found that $PbCl_2$ (injection doses 25-50 µg) was able to provide a costimulatory signal to antigens that could activate T cells in adult female mice. The exact mechanistic basis for this is not known. As discussed in Section 5.6.5.2, changes in NO production appear to be involved in Pb-induced lymphocyte activation (Farrer et al., 2008).

The available epidemiologic evidence for Pb-associated lymphocyte activation was provided by a small number of previous studies in children and nonoccupationally-exposed adults. Instead of directly measuring lymphocyte proliferation, these studies provided indirect evidence by measuring the abundance of cells that expressed HLA-DR,

an indicator of activated cells. It is important to note that HLA-DR+ cells also may indicate the presence of activated monocytes. Further, these studies did not adjust extensively for potential confounding. In the only study of children (ages 9 months-6 years, Missouri), the mean percentage of HLA-DR+ cells was approximately 2-fold higher ($p > 0.05$, Kruskal-Wallis) in the 19 children with concurrent blood Pb levels 15-19 µg/dL than in children with blood Pb levels 10-14 µg/dL ($n = 61$) or < 10 µg/dL ($n = 178$) after adjusting for age (Lutz et al., 1999). However, activated cells were not elevated in children with blood Pb levels 20-44 µg/dL. Studies of nonoccupationally-exposed adults in Italy found that concurrent blood Pb level was correlated positively with the percentage of HLA-DR expressing cells in men with and without allergies (Spearman $r = 0.51$, $p < 0.002$, $n = 17$ each, median blood Pb level both groups combined: 11 µg/dL) (Boscolo et al., 1999) but only in women without allergies (Spearman $r = 0.44$, $p < 0.05$, $n = 25$, median blood Pb level: 5.5 µg/dL) (Boscolo et al., 2000).

Comparisons of Pb-exposed workers and unexposed controls indicated similar levels of lymphocyte proliferation (≤ 1% difference) between groups (Queiroz et al., 1994b; Cohen et al., 1989) or lower lymphocyte proliferation among Pb-exposed workers (8-25%) (Mishra et al., 2003; Fischbein et al., 1993; Alomran and Shleamoon, 1988; Kimber et al., 1986) Toxicological studies have demonstrated the selective expansion of Th2 cells and suppression of Th1 cells (U.S. EPA, 2006b). Therefore, the differential activation of specific subtypes may not be discernable in epidemiologic studies that measure overall lymphocyte proliferation. Additionally, because occupational studies did not provide concentration-response information, it is difficult to infer whether findings apply to populations with lower blood Pb levels. Toxicological studies also did not provide information on Pb-induced lymphocyte activation at exposure concentrations relevant to humans without occupational Pb exposures.

5.6.2.3 Delayed-type Hypersensitivity

Although recent investigation was limited, a large body of previous toxicological studies reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) and recent reviews (Mishra, 2009; Dietert and McCabe, 2007) identified a suppressed DTH response as one of the most consistently observed and well-documented immunomodulatory effects of Pb exposure in animal models. A new study indicated that this effect may be mediated by dendritic cells. The DTH assay is commonly used to assess 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).

Previous toxicological studies demonstrated suppressed DTH responses in animals after gestational (Chen et al., 2004; Bunn et al., 2001a; Bunn et al., 2001b, c; Lee et al., 2001b; Chen et al., 1999; Miller et al., 1998; Faith et al., 1979) and postnatal (McCabe et al., 1999; Laschi-Loquerie et al., 1984; Muller et al., 1977) Pb exposures. Blood Pb level data were not available in all studies; however, DTH was suppressed in animals with a wide range of blood Pb levels (11 to > 100 µg/dL), with the associations of DTH with lower blood Pb levels occurring as a result of gestational exposure.

In some studies that examined Pb exposures at multiple stages of gestation, exposures later in gestation suppressed DTH in animals (Bunn et al., 2001c; Lee et al., 2001b). These latter findings may reflect the status of thymus and T cell development. A recent study contributed to the robust evidence 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 adult mice. Mice treated with Pb-exposed dendritic cells had a diminished OVA-specific DTH footpad response compared with mice treated with non Pb-exposed dendritic cells.

The capability 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). In some animal studies, the suppressed DTH response was accompanied by a decreased production of IFN-γ (Lee et al., 2001b; Chen et al., 1999), which is the primary cytokine that stimulates recruitment of macrophages, a key component of the DTH response. Observations of a concomitant decrease in IFN-γ further link Pb-induced inhibition of Th1 functional activities with suppression of the DTH response. Further, coherence is provided by associations observed between Pb exposure or blood Pb levels and other responses in animals related to the inhibition of Th1-driven adaptive immune responses, including decreased host resistance (Section 5.6.4.1).

5.6.2.4 Macrophages and Monocytes

As reported in the 2006 Pb AQCD, based on a large body of toxicological evidence, Pb-induced promotion of a hyperinflammatory phenotype in macrophages was considered to be a hallmark of Pb-associated immune effects (U.S. EPA, 2006b). Pb-induced hyperinflammation was indicated by the enhanced production of ROS, suppressed production of NO, enhanced production of TNF-α, excessive metabolism of arachidonic acid into immunosuppressive metabolites (e.g., PGE_2), impaired growth and

differentiation of cells, and potentially altered receptor expression [e.g., toll-like receptors]). Several of these findings are described in detail in Sections 5.6.5.2 and 5.6.5.4. Because macrophages are major resident populations in most tissues and organs and also are highly mobile in response to microbial signals and tissue alterations, their functional impairment in response to Pb exposure may serve as a link between Pb-induced immune effects and impaired host defense, tissue integrity, and organ homeostasis in numerous physiological systems (Section 5.6.4.5). A small number of available recent studies continue to support previous findings. A study in Pb-treated (40 ppm by oral gavage, once daily for 40 days) adult mice reported decreased macrophage yield and viability, and phagocytic index in the kidney (Lodi et al., 2011). In addition, several studies reinforced the effect of Pb exposure to alter a broad spectrum of functional alterations in human monocytes (the blood form of macrophages) or mouse macrophages in vitro (Khan et al., 2011; Bussolaro et al., 2008; Mishra et al., 2006a). In particular, Bussolaro et al. (2008) found reduced phagocytic activity with a relatively low concentration of Pb exposure (0.2 µM Pb nitrate, 72 hours). Khan et al. (2011) found that exposure of human monocytes to 25-100 µM Pb-acetate for 3-6 hours induced increases in the pro-inflammatory cytokine TNF-α. Mishra et al. (2006a) found that 100 ppm Pb-acetate suppressed lipopolysaccharide (LPS)-induced NO production.

Epidemiologic studies have not widely examined the effects of Pb exposure on altered macrophage functional activity in humans, and the indices of macrophage function have varied among the few available studies. Pineda-Zavaleta et al. (2004) was unique in examining the hyperinflammatory state specifically in macrophages, albeit in relation to blood Pb levels higher than those in the current U.S. population (range of blood Pb levels: 3.5-47.5 µg/dL). This study included children in Lagunera, Mexico, attending schools at varying distances from an active Pb smelter. Consistent with the large body of toxicological evidence, higher concurrent blood Pb level was associated with lower NO production and higher superoxide anion production in macrophages isolated from child sera (Section 5.6.5.2). Model covariates included sex, age, and presence of allergies.

Other studies in humans examined occupationally-exposed adults and did not find a clear association between concurrent measurements of blood Pb level and macrophage abundance. Adjusting for age, race, smoking, and workshift, Pinkerton et al. (1998) found a lower abundance of monocytes among 145 U.S. Pb smelter workers with a mean blood Pb level of 39 µg/dL (7.8%) than among 84 unexposed controls with a mean blood Pb level of <2 µg/dL (8.5%) ($p = 0.03$). Conterato et al. (In Press) examined a group of male Pb-exposed painters with blood Pb levels ranging between 1.4 and 14.0 µg/dL (mean: 5.4 µg/dL), lower than those in other occupational studies. The mean level and percentage of monocytes in the 50 painters were similar to those in the 36 controls (mean blood Pb level: 1.5 µg/dL) and in the 23 battery workers with much higher blood Pb

levels (mean: 50 µg/dL). Fischbein et al. (1993) found lower ($p < 0.001$) abundance of HLA-DR+ cells in two groups of New York metropolitan area firearms instructors with mean blood Pb levels of 14.6 µg/dL (n = 36, 8.8%) and 31.4 µg/dL (n = 15, 8.7%) than among the 36 unexposed controls (15.2%). HLA-DR+ is an indicator of activated functional state of antigen presenting cells (APCs, e.g., macrophages, dendritic cells, T cells) and is upregulated in response to cell signaling. While this study did not provide direct evidence for Pb effects on macrophages, the investigators cited previous observations that 70% of monocytes express HLA-DR antigen compared with 15% of lymphocytes to suggest that the reduced expression of HLA-DR antigen in Pb-exposed workers was due primarily to a reduction in activated monocytes.

In summary, a small body of new in vitro studies (Khan et al., 2011; Bussolaro et al., 2008; Mishra et al., 2006a) adds to the extensive base toxicological evidence indicating that Pb exposure decreases functionality of macrophages and promotes a hyperinflammatory phenotype. The sparse epidemiologic data are provided primarily by older studies and are not conclusive. Whereas an association between blood Pb level and a hyperinflammatory state of macrophages was observed in a previous study of children (Pineda-Zavaleta et al., 2004), studies of occupationally-exposed adults did not clearly indicate Pb exposure effects on macrophages. A new study did not find large differences in monocyte abundance between Pb-exposed workers and unexposed controls (Conterato et al., In Press).

5.6.2.5 Neutrophils

In the 2006 Pb AQCD, Pb exposure was not judged to have strong effects on neutrophils (U.S. EPA, 2006b). This conclusion was based on the relatively limited available toxicological evidence as compared with that for effects on other immune cells. However, the modulation of neutrophil activity may have important consequences on the dysregulation of inflammation and ability of organisms to respond to infectious agents. Studies of cultured human polymorphonuclear cells (PMNs) (Governa et al., 1987) and occupationally-exposed adults (Queiroz et al., 1994a; Queiroz et al., 1993; Valentino et al., 1991; Bergeret et al., 1990) found Pb-associated reductions in PMN functionality, as indicated by reduced chemotactic response, phagocytic activity, respiratory oxidative burst activity, or reduced ability to kill ingested antigen. Important limitations to applying epidemiologic findings broadly include male-only study populations, the relatively high blood Pb levels of workers (range of mean levels: 33.1-71 µg/dL), lack of concentration-response information for associations between blood Pb level and neutrophil function, and lack of consideration of potential confounding factors.

Instead of examining neutrophil functional activities, a few available recent studies of animals and occupationally-exposed adults examined the effects of Pb exposure on neutrophil counts, an increase in which has been interpreted by some investigators to be a compensatory response to Pb-induced impairment in neutrophil chemotactic activity and a hyperinflammatory response. In male rats with Pb spheres implanted in their brains, neutrophils were a major responding cell (Kibayashi et al., 2010). Compared with rats implanted with glass spheres, rats with Pb spheres had greater neutrophil infiltration with inflammatory-related damage that included apoptosis and indications of neurodegeneration. It is important to acknowledge the uncertain relevance of these findings to those expected from typical routes of Pb exposure in humans.

In a group of 68 ceramic, Pb recycling, or bullet manufacturing workers and 50 controls selected among food plant workers, DiLorenzo et al. (2006) provided information on the concentration-response relationship and adjusted for potential confounding variables. Among all subjects, a 1 µg/dL higher concurrent blood Pb level was associated with a 21.8 cells/µL (95% CI: 11.2, 32.4 cells/µL) higher absolute neutrophil count (ANC) adjusted for age, BMI, and smoking status. The geometric mean (range) of concurrent blood Pb levels was 20.5 µg/dL (range: 3.2 to 120 µg/dL) among workers and 3.5 µg/dL (range: 1 to 11 µg/dL) among controls. Eight workers described to have medium to high Pb exposures (exact blood Pb levels not reported), but no controls had neutrophilia (n >7,500 cells/mm^3), 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 across increasing blood Pb groups, supporting a blood Pb concentration-dependent relationship. When the three blood Pb groups were further stratified by current smoking, two-way ANOVA indicated an interaction between concurrent blood Pb level and current smoking. Higher blood Pb level was associated with higher ANC only in current smokers. Among nonsmokers, ANCs were similar across blood Pb groups. In contrast, Conterato et al. (In Press) found lower neutrophil concentrations among two groups of Pb-exposed male workers with mean concurrent blood Pb levels of 50.0 and 5.4 µg/dL than among controls with a mean blood Pb level of 1.5 µg/dL. Pb-exposed workers did not consistently have higher levels of other immune cells such as eosinophils, basophils, monocytes, or total lymphocytes either.

Additional evidence 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 serum complement C3 protein among Pb-exposed workers compared with unexposed controls, with Ewers et al. (1982) additionally observing an association between higher concurrent blood Pb level and lower C3 in a regression analysis combining Pb-exposed workers and controls. However, the broad applicability of these findings may be 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.

In summary, a majority of evidence indicating the effects of Pb exposure on neutrophils was provided by previous studies that found that compared with unexposed controls, Pb-exposed workers had lower neutrophil functionality (Queiroz et al., 1994a; Queiroz et al., 1993; Valentino et al., 1991; Bergeret et al., 1990) and lower complement, which is a mediator of phagocyte functionality (Undeger et al., 1996; Ewers et al., 1982). In the limited new epidemiologic investigation that examined neutrophil abundance, studies did not conclusively find Pb-exposed workers to have higher or lower neutrophil abundance. Overall, the epidemiologic evidence is limited by the high blood Pb levels (range of mean levels: 33.1 to 71 µg/dL) with which neutrophil functionality was observed to be decreased and the lack of consideration of potential confounding.

5.6.2.6 Dendritic Cells

Since the publication of the 2006 Pb AQCD, new results (from both an ex vivo and in vitro models) suggest 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 versus Th2 T-cell populations and cytokine profiles. But new research techniques have been developed (Gao and Lawrence, 2010) that provide 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. $PbCl_2$ (25 µM, 10 days) was found to alter 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 did 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 toward a Th2 phenotype. Strengthening the role of dendritic cells in mediating Pb immune effects were ex vivo results from the same study which showed that Pb-naïve adult BALB/c mice implanted with Pb-exposed dendritic cells were skewed toward Th2 activity as indicated by inhibited DTH (Section 5.6.2.3) and IgG2a antibody (Section 5.6.3) responses (Gao et al., 2007).

5.6.2.7 Natural Killer Cells

Based mostly on epidemiologic studies, previous and recent evidence does not clearly indicate that the innate immune natural killer (NK) cells are affected to a large extent by Pb exposure. In previous studies of nonoccupationally-exposed adults in Italy, concurrent blood Pb levels were correlated with NK cell abundance among men with and without allergy (Spearman $r = 0.49$, $p \sim 0.05$, $n = 17$ each, median blood Pb level both groups combined: 11 µg/dL) (Boscolo et al., 1999) and women without allergies (Spearman $r = 0.44$, $p < 0.05$) (Boscolo et al., 2000) In a study of children from St. Lawrence River communities in Quebec, Canada, cord blood Pb level was not significantly associated with cord blood NK cell abundance (quantitative results not reported (Belles-Isles et al., 2002). Similar means of NK cell abundance or functional activity were observed in Pb-exposed workers and unexposed controls (García-Lestón et al., 2011; Mishra et al., 2003; Pinkerton et al., 1998; Yucesoy et al., 1997b; Undeger et al., 1996; Fischbein et al., 1993; Kimber et al., 1986). The lack of evidence for a strong effect on NK cells is underscored by epidemiologic observations within individual studies that blood Pb levels are associated with T cell abundance but are not associated with NK cell abundance or their level of functional activity (Karmaus et al., 2005; Sarasua et al., 2000; Pinkerton et al., 1998). Consistent with the epidemiologic evidence, toxicological evidence is not conclusive. In a recent in vitro study comparing the toxicities of metals for different populations of immune cells, Fortier et al. (2008) found that $PbCl_2$ (7.5-20.7 µg/dL) did not affect NK cytotoxicity compared with the DMSO vehicle. However, $PbCl_2$ was not found to affect other immune parameters (e.g., monocyte phagocytic activity or lymphocyte proliferation) either. A recent study did show a decrease in NK cell activity in mice albeit with high-level Pb exposure (1,300 ppm Pb-acetate in drinking water, 10 days, blood Pb level ~100 µg/dL) (Queiroz et al., 2011).

5.6.3 Humoral Immunity

The 2006 Pb AQCD described another hallmark effect of Pb on the immune system to be an enhanced humoral immune response as characterized by increased production of IgE antibodies (U.S. EPA, 2006b). Several previous toxicological and epidemiologic studies (Table 5-28) demonstrated Pb-associated increases in IgE production, which is strongly implicated in mediating allergic responses and inflammation in allergic asthma. In studies of juvenile rodents, Pb exposures (Pb-acetate) through drinking water from gestation through lactation induced concomitant increases in IgE and IL-4 production (a Th2 cytokine) by T cells (Snyder et al., 2000; Chen et al., 1999), consistent with the hypothesis that Th2-mediated mechanisms can induce class switching of B cells to produce IgE. Evidence from both earlier toxicological and epidemiologic studies (Table 5-28) did not consistently indicate that Pb exposure was associated with changes in other classes of Igs including IgG, IgM, and IgA. Previous epidemiologic studies of children (Table 5-28) and adults [Table 5-28 with group comparisons and (Boscolo et al., 2000; Boscolo et al., 1999) with correlation analyses] did not find a consistent association between blood Pb level and the abundance of B cells, which produce IgE and other antibodies.

Although previous evidence for effects on Ig classes other than IgE was inconsistent, a small number of available new toxicological studies found Pb-induced increases in IgG and pointed to a role for T cell-mediated mechanisms in Pb-induced activation of B cells and production of Ig antibodies. Important limitations of this evidence include high Pb exposures tested and use of injection routes of exposure that may have little relevance to human routes of exposure. Fernandez-Cabezudo et al. (2007) reported evidence for a subtle shift toward a Th2 immune response following *Salmonella* infection in mice exposed to high concentrations of Pb-acetate (5,000-10,000 µM, 16 weeks, resulting in blood Pb levels of 20.5-106 µg/dL). Serum levels of *Salmonella*-specific IgG1 antibodies were increased in the Pb-exposed mice compared to controls, whereas IgG2a levels were increased in control but not Pb-exposed mice following infection. The impaired Th1 and enhanced Th2 response also was evident by the decreased secretion of IL-12 and increased production of IL-4 by spleen cells taken from Pb-exposed (blood Pb levels > 20.5 µg/dL) mice (Fernandez-Cabezudo et al., 2007).

In a highly-specialized strain of knockout adult mice lacking the ability to produce IFN-γ, i.p. injection with 50 µg $PbCl_2$ increased the IgG2a/IgG1 ratio (Gao et al., 2006). This result was surprising given evidence that IFN-γ usually directs secretion of IgG2a; however, the authors suggested that in these knockout mice, Pb may initiate a Th1 response via an IFN-γ independent pathway to enhance IgG2a production. Other animal studies that administered Pb via the i.p., found Pb-induced humoral responses to

mediated preferentially via Th2 mechanisms. 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-ovalbumin [TNP-OVA]) hapten-protein conjugate with or without co-exposure to $PbCl_2$. Seven days later, investigators examined the effects of Pb on the LLNA response to TNP-Ficoll or TNP-OVA. A bolus injection of $PbCl_2$ (25-50 µg) increased the numbers of T and B cells in the lymph node against both TNP-Ficoll and TNP-OVA. Further, in a concentration-dependent manner, Pb induced statistically significant elevations of IgM-, IgG2a-, and IgG1-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 finding 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.

In a recent microarray study in BALB/c mice, Kasten-Jolly et al. (2010) found that early-life Pb exposure (100 µM 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, several of which are new, associations between biomarkers of Pb exposure and serum IgE level were demonstrated primarily in children, although a monotonic dose-dependent increase was not consistently observed (Hegazy et al., 2011; Hon et al., 2010; Hon et al., 2009; Karmaus et al., 2005; Annesi-Maesano et al., 2003; Sun et al., 2003; Lutz et al., 1999) (Table 5-28). Karmaus et al. (2005) had the most extensive adjustment for potential confounding factors and examined differences with lower blood Pb levels. Compared with children with concurrent blood Pb level <2.2 µg/dL (quartile 1), children with blood Pb levels 2.84-3.41 µg/dL (quartile 3) and >3.4 µg/dL (quartile 4) had 28% higher serum IgE levels ($p = 0.03$, F-test). These differences were observed after the adjustment for potential confounding by age, biomarkers of various organochlorine exposures, number of infections in the previous 12 months, serum lipids, and passive smoke exposure. Similar differences in IgE count on basophils were not observed among the blood Pb quartiles; however, it is important to note that although serum IgE and basophil-bound IgE have been correlated in adults (Malveaux et al., 1978; Conroy et al., 1977), little data are available in children (Dehlink et al., 2010). A recent study in children found that serum IgE levels were not correlated

with basophil-bound IgE but correlated (Spearman r = -0.003) with other IgE receptor-expressing cells such as dendritic cells and monocytes (Spearman r = 0.43 to 0.65, $p < 0.05$) (Dehlink et al., 2010). The number of IgE-bound basophils also has been found to be highly variable across individuals, particularly children (Hausmann et al., 2011; Dehlink et al., 2010). Thus, it is not unexpected that higher blood Pb level was associated with higher serum IgE but not basophil-bound IgE counts in Karmaus et al. (2005). In this study, blood Pb level was not associated with serum levels of IgG, IgA, and IgM or B cell abundance. Lutz et al. (1999) found higher serum IgE in children after adjusting for age, albeit with concurrent blood Pb levels > 10 µg/dL.

Recent studies in children also reported associations between concurrent blood Pb level and elevated serum IgE but did not adjust for potential confounding variables (Hegazy et al., 2011; Hon et al., 2010; Hon et al., 2009). The studies by Hon and colleagues (2010; 2009) demonstrated associations in children with low blood Pb levels (range: 1.4-6.0 µg/dL) and found that blood Pb level was correlated with both serum IgE and atopic dermatitis, a condition commonly characterized by elevated IgE levels.

Table 5-28 Comparison of serum immunoglobulin levels and B cell abundance among various blood Pb groups

Study	Population/Study Details	Blood Pb Level Mean or Range (µg/dL)	IgE[a]	IgG[b]	IgM[b]	IgA[b]	B cells[c]
Children							
Karmaus et al. (2005)	331 children, ages 7-10 yr, Hesse, Germany	<2.2	46 (1.0)	1210	150	123	418[e] (1.0)
	Results were adjusted for age, sex, passive smoke exposure, number of infections in the previous 12 months, serum lipid concentration, and organochlorine exposures.	2.21 - 2.83	30 (0.65)	1214	143	121	353 (0.84)
		2.84 - 3.41	59 (1.28)	1241	153	133	389 (0.93)
		>3.41	59 (1.28)[d]	1201	148	136	393 (0.94)
Hegazy et al. (2011)	318 children, ages 6 mo-7 yr, Egypt	< 5	13.0 (1.0)				
		5-9	12.0 (0.92)				
		10 - 14	20.8 (1.60)				
		15 - 19	14.9 (1.15)				
		20 - 44	20.4 (1.57)				
		45 - 69	10.2 (0.78)[d]				
Sarasua et al. (2000)	382 children, ages 6-30 mo, Multiple U.S. locations	0.6 - 4.9		609	103	50.1	19.1 (1.0)
	Results were adjusted for age, sex, and study location.	5 - 9.9		666[d]	108	55.0	20 (1.05)
		10 - 14.9		680[d]	105	58.2	20.4 (1.07)
		≥ 15		630	124[d]	61.4[d]	22.2 (1.16)
Sarasua et al. (2000)	562 children, ages 36-71 mo, Multiple U.S. locations	0.6 - 4.9		817	120	88.6	18.4 (1.0)
	Results were adjusted for age, sex, and study location.	5 - 9.9		813	116	90.9	17.6 (0.96)
		10 - 14.9		856	125	96.3	19.2 (1.04)
		≥ 15		835	121	94.1	18.6 (1.01)
Sarasua et al. (2000)	675 children ages 5-16 yr, Multiple U.S. locations	0.6 - 4.9		1,031	128	140	16.1 (1.0)
	Results were adjusted for age, sex, and study location.	5 - 9.9		1,094[d]	131	143	15.8 (0.98)
		10 - 14.9		1,048	136	140	15.3 (0.95)
		≥ 15		1,221	106	108	20.1 (1.25)
Lutz et al. (1999)	279 children, ages 9 mo-6 yr, Springfield, MO	<10	51.8 (1.0)				13.4 (1.0)
	Results were adjusted for age.	10 - 14	74.0 (1.43)				12.6 (0.94)
		15 - 19	210.7 (4.07)				16.9 (1.26)
		20 - 44	63.7 (1.23)[d]				11.1 (0.83)
Zhao et al. (2004)	75 children, ages 3-6 yr, Zhejiang Province, China	<10					16.58 (1.0)
		≥ 10					16.82 (1.01)
Adults without Occupational Pb Exposures							
Sarasua et al. (2000)	433 children and adults, ages 16-75 yr, Multiple U.S. locations	0.6-4.9		1,099	175	252	13.9 (1.0)
		5-9.9		1,085	175	242	13.0 (0.94)
	Results were adjusted for age, sex, study location, and smoking.	10-14.9		1,231	262[d]	283	12.4 (0.89)
		≥ 15		1,169	139	193	14.8 (1.06)
Adults with Occupational Pb Exposures							
Fischbein et al. (1993)	36 unexposed controls, mean age 47 yr	NR					8.6 (1.0)
	36 firearms instructors, mean age 49 yr	14.6					10.5 (1.22)
	15 firearms instructors, mean age 48 yr	31.4					11.2 (1.30)[d]
	New York metropolitan area						
Kimber et al. (1986)	21 unexposed male controls, ages 20-60 yr	11.8		1062	1294	2235	
	39 male tetraethyl Pb plant workers, ages 25-61 yr	38.4		1018	1040	2425	
	U.K.						

Study	Population/Study Details	Blood Pb Level Mean or Range (µg/dL)	IgE[a]	IgG[b]	IgM[b]	IgA[b]	B cells[c]
Pinkerton et al. (1998)	84 unexposed controls, mean age 30 yr	<2		1,090	94.5	180	14.6 (1.0)
	145 Pb smelter workers, mean age 33 yr	39		1,110	106.2	202	13.2 (0.90)
	U.S., exact location NR						
	Results were adjusted for age, race, current smoking status, and workshift						
Heo et al. (2004)	606 Pb battery plant workers	<10	112.5 (1.0)				
	Korea	10-29	223.3 (1.99)				
		≥ 30	535.8 (4.76)[d]				
Anetor and Adeniyi (1998)	50 male controls, ages 22-58 yr	30.4		1,997	215	188	
	80 male Pb-exposed workers, ages 21-66 yr, Nigeria	56.3		1,187[d]	191	144[d]	
Ewers et al. (1982)	53 male unexposed controls, ages 21-54 yr	11.7		193[f]	161[f]	140[f]	
	72 male Pb battery/smelter workers, ages 16-58 yr, Germany	59.0		171	127	128	
Undeger et al. (1996)	25 unexposed male controls, ages 22-56 yr	16.7		1,202.1	140.4	210.3	545.5[e] (1.0)
	25 male Pb battery plant workers, ages 22-55 yr, Turkey	74.8		854.6[d]	93.3[d]	168.1	635.9 (1.17)
Alomran and Shleamoon (1988)	18 unexposed age-matched controls	NR		1713		183	545.5[e] (1.0)
	39 Pb battery workers, mean age 35 yr, Iraq	NR		1610		170	635.9 (1.17)

[a]IgE data are presented as IU/mL. (In parentheses are the ratio of IgE in the higher blood Pb group to IgE in the lowest blood Pb group.)

[b]Other Ig data are presented as mg/dL unless otherwise specified.

[c]B cell data are presented as the percentage of B cells among all lymphocytes unless otherwise specified. (In parentheses are the ratio of B cells in the higher blood Pb group to B cells in the lowest blood Pb group.)

[d]p <0.05 for group differences.

[e]Data represent the number of cells/µL serum.

[f]Data are presented as IU/mL.

Sarasua et al. (2000) found associations of higher concurrent blood Pb level with higher IgA, IgG, and IgM in U.S. children. Blood Pb level was associated with higher levels of all three Igs (0.8 [95% CI: 0.2, 1.4], 4.8 [95% CI: 1.2, 8.4], and 1.0 [95% CI: 0.1, 1.9] mg/dL higher IgA, IgG, and IgM, respectively, per 1 µg/dL higher blood Pb level, adjusted for age, sex, and location) in the youngest age group (6-35 months) but not in older age groups (36-71 months, 6-15 years, 16-75 years), suggesting elevated risk in very young children. Among infants aged 6-35 months with concurrent blood Pb levels ≥ 15 µg/dL, serum levels of all three examined Igs were elevated over levels measured in infants with blood Pb levels < 5 µg/dL. In this study, IgE was not examined.

While most epidemiologic studies examined concurrent blood Pb levels, some studies indicated that prenatal Pb exposure may impact Ig levels in newborns (Annesi-Maesano et al., 2003; Belles-Isles et al., 2002). These studies also pointed to an increased risk in infants; however, important limitations of these studies are the lack of extensive consideration of potential confounding variables. Belles-Isles (2002) examined newborns in St. Lawrence River communities in Quebec, Canada and found an association between

higher cord blood Pb level and higher cord serum IgG in a regression analysis adjusted for prenatal smoke exposure. Annesi-Maesano et al. (2003) found that infant hair Pb level but not cord or placental Pb level was associated with cord serum IgE in newborns in Paris. From these findings, the authors inferred a stronger effect of Pb exposure integrated over the entire gestational period compared to exposures closer to birth. Cotinine was not associated significantly with Pb biomarker levels or with IgE. The magnitude of association was larger in the subgroup of infants with mothers without allergies (Spearman $r = 0.21$, $p < 0.01$ in infants with mothers without allergies versus $r = 0.12$ in infants with mothers with allergies, pointing to the possible masking of a blood Pb association with IgE by the stronger association of family history of allergy.

Associations between blood Pb level and IgE also were reported in studies of adults, without (Pizent et al., 2008) and with occupational Pb exposure (Heo et al., 2004). In a study of urban adults aged 19 to 67 years and of similar SES (i.e., white-collar office workers) in Zagreb, Croatia, a statistically significant association between higher concurrent blood Pb level and higher 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 higher blood Pb level was associated with a 0.60 higher log of IgE (95% CI: 0.58, 1.18). Concurrent blood Pb levels were low in these women who were aged 19-67 years (mean: 2.16 µg/dL, range 0.56-7.35 µg/dL); however, due to the cross-sectional nature of this study, it is difficult to characterize the timing, level, frequency, and duration of Pb exposure that contributed to the observed association. Because of likely higher past Pb exposures of adults and the mobilization of Pb from bone to blood, the associations may reflect effects of higher past Pb exposures. Investigators did not report an effect estimate in men because it did not attain statistical significance. Without quantitative results, it was difficult to ascertain whether there was suggestion of association in men but insufficient power to indicate statistical significance due to the smaller number of men examined (50 men versus 166 females). Another study of 34 men in Italy also did not report quantitative results but indicated a lack of statistically significant correlation between blood Pb level and IgE in men (Boscolo et al., 1999).

A majority of the epidemiologic evidence for the effects of Pb on IgA, IgG, and IgM levels is provided by previous studies of Pb-exposed workers (Anetor and Adeniyi, 1998; Pinkerton et al., 1998; Undeger et al., 1996; Queiroz et al., 1994b; Alomran and Shleamoon, 1988; Kimber et al., 1986; Ewers et al., 1982). Consistent with the collective body of toxicological findings for these other Ig classes, epidemiologic evidence is mixed, with studies reporting higher, lower, and similar Ig levels in Pb-exposed workers compared with unexposed controls. Some studies reporting lower Ig levels in Pb-exposed

workers included workers with the highest mean blood Pb levels (> 50 µg/dL) (Anetor and Adeniyi, 1998; Undeger et al., 1996). The lack of rigorous statistical analyses in occupational studies precludes characterization of the factors that may contribute to inconsistent associations.

In summary, a majority of the evidence for Pb exposure affecting humoral immunity is provided by previous toxicological and epidemiologic studies that found Pb-associated increases in IgE. Previous toxicological studies provided evidence for the independent effects of Pb exposure increasing IgE levels. A few available recent epidemiologic studies add to the large body of extant evidence supporting associations between higher concurrent blood Pb level and higher IgE levels in children; however, they did not adjust for potential confounding variables (Hegazy et al., 2011; Hon et al., 2010; Hon et al., 2009). Collectively, epidemiologic evidence indicates higher IgE in children with concurrent blood Pb levels > 10 µg/dL. The epidemiologic evidence in children equally comprises studies that did not and did adjust for potential confounding. Studies that adjusted for potential confounding varied in the number and the specific variables included as model covariates (Table 5-28) but were consistent in finding an association between blood Pb and IgE. None of the studies in children adjusted for SES or allergen exposure. Lower SES, which may indicate poorer housing conditions, is associated with higher exposures to Pb as well as cockroach, rodent, and other allergens. Allergen exposure and lower SES are associated with higher IgE and IgE-related conditions such as allergies and asthma (Bryant-Stephens, 2009; Dowd and Aiello, 2009; Aligne et al., 2000). Studies generally did not provide detailed demographic or residential information to assess whether SES and/or allergen exposure potentially confounded the observed associations between blood Pb level and IgE. Thus, uncertainty remains as to the extent to which blood Pb-IgE associations in children are confounded by unmeasured SES and/or allergen exposure. Evidence in adults is limited and thus, inconclusive. A new study of environmentally-exposed adults found an association between concurrent blood Pb level and IgE in women (Pizent et al., 2008). Evidence integrated across toxicological and epidemiologic studies does not consistently indicate that Pb exposure affects other classes of Igs; however, a few new toxicological studies found Pb-induced increases in IgG (Fernandez-Cabezudo et al., 2007; Gao et al., 2006) or increases in the expression of genes encoding Ig antibodies (Kasten-Jolly et al., 2010).

5.6.4 Immune-based Diseases

5.6.4.1 Host Resistance

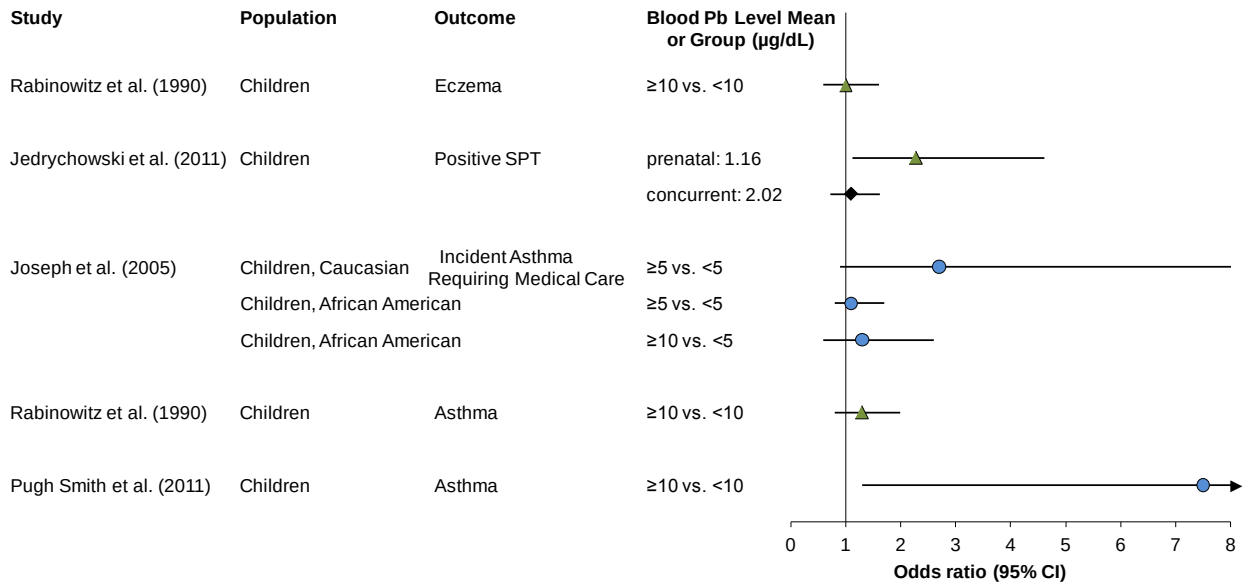
The capability of Pb to reduce host resistance of animals to bacteria has been known for almost 40 years and was supported by several animal studies described in the 2006 Pb AQCD (U.S. EPA, 2006b). A recent animal study provided supporting evidence for such an effect. Biological plausibility for these findings has been provided by observations that Pb affects mechanisms underlying diminished host resistance, e.g., suppressed Th1-driven acquired immune responses and increased inflammatory responses in target tissue resulting in further damage to host protective barriers. Host resistance to bacteria such as *Listeria* requires effective Th1-driven responses including the production of IL-12 and IFN-γ (Lara-Tejero and Pamer, 2004) and these have been found to be inhibited by Pb exposure. The lack of IFN-γ can inhibit appropriate and timely macrophage activation. Nitric oxide is produced by macrophages during cellular activation and plays a role in host defense against bacterial infection (U.S. EPA, 2006b), and NO production also has been found to be suppressed by Pb exposure. A recent animal study characterized a potential mechanism by which Pb may impact both innate immune cells and natural host defense barriers. Kasten-Jolly et al. (2010) showed that developmental exposure of mice to Pb (100 µM Pb-acetate in drinking water of dams from GD8 to PND21, resulting in pup blood Pb levels of 10-30 µg/dL) 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).

In the few available epidemiologic studies, a range of indicators of Pb exposure (i.e., cord blood Pb, concurrent blood Pb, Pb content in total deposition samples, Pb content in lichen) were associated with viral and bacterial infections in children. Collectively, study limitations, including lack of consideration for potential confounding variables (Karmaus et al., 2005; Rabinowitz et al., 1990), lack of statistical analysis (Karmaus et al., 2005), and ecological design (Carreras et al., 2009), limit the ability to draw inferences regarding the effects of Pb exposure on viral or bacterial infections in children. Similar to these studies in children, an occupational study finding higher frequency of self-reported colds or influenza among Pb battery or smelter plant workers with higher blood Pb levels than among unexposed controls is limited by a lack of statistical analysis (Ewers et al., 1982). Studies that address the aforementioned limitations are needed to characterize the relationship between Pb exposure and resistance to bacterial and viral infection in humans.

With limited investigation, the effect of Pb on host resistance to parasitic agents is unclear. The 2006 Pb ACQD described a study in which high-level Pb-exposed (≥ 10 µM) mouse macrophages had diminished ability to kill *Leishmania enrietti* parasites (Mauel et al., 1989). However, given the well-characterized effect of Pb in promoting Th2 activity, it is plausible for Pb to enhance host resistance to parasites that require robust Th2 responses (e.g., helminths) (U.S. EPA, 2006b). In a recent study, high-level Pb exposure (≥ 10 µM) enhanced host resistance to malaria (Koka et al., 2007). However, this was attributed to the capability of Pb to induce eryptosis and the rapid removal of malaria-infected erythrocytes and not to Pb-induced alterations in immune function. Nriagu et al. (2008) also reported that higher blood Pb level was associated with lower malaria prevalence among children (ages 2-9 years) from three Nigerian cities. A majority of children (75%) had blood Pb levels below 10 µg/dL. The association persisted after adjusting for age, sex, number of siblings, and other comorbidities such as headaches, depressed mood, and irritability.

5.6.4.2 Asthma and Allergy

Toxicological evidence and to a relatively limited extent, epidemiologic evidence, have supported the effects of Pb exposure on multiple immune parameters, including elevated production of Th2 cytokines such as IL-4, increased IgE antibody production (Table 5-24), and increased inflammation. These are well-recognized pathways in the development and exacerbation of allergy and allergic disease, including asthma. This mechanistic evidence is coherent with a small body of epidemiologic studies that found associations of blood Pb levels with asthma or allergy in children (Figure 5-43 and Table 5-29). Children examined in these studies encompassed a wide range of ages (i.e., less than 1 year to 12 years) and across studies, blood Pb was measured during different lifestages. This body of evidence in children included large studies with multivariate analyses and studies with prospective follow-up of subjects in which disease occurrence was ascertained after the measurement of blood Pb levels.



Note: Results are organized by endpoint and for asthma in order of increasing blood Pb level. For analyses with blood Pb level as a continuous variable, odds ratios are standardized to a 1 µg/dL increase in blood Pb level. SPT = skin prick test, BR = bronchial responsiveness. Black diamond represents associations with concurrent blood Pb levels, green triangles represent associations with prenatal (cord) blood Pb levels, and blue circles represent associations with blood Pb levels measured in childhood up to 12 months prior to outcome assessment.

Figure 5-43 Associations of blood Pb levels with asthma- and allergy-related conditions in children.

Table 5-29 Additional characteristics and quantitative results for studies presented in Figure 5-43

Study	Population/Location	Blood Pb Level Data (µg/dL)	Statistical Analysis	Outcome	Odds Ratio (95% CI)[a]
Rabinowitz et al. (1990)	1,768 children followed from birth to unspecified age Boston area, MA	Prenatal (cord) ≥ 10 vs. <10[a]	Logistic regression with no additional covariates included in model	Eczema	1.0 (0.6, 1.6)
Jedrychowski et al. (2011)	224 children followed prenatally to age 5 yr Krakow, Poland	Prenatal (cord): GM: 1.16 (95% CI: 1.12, 1.22) Concurrent: GM: 2.02 (95% CI: 1.95, 2.12)	Logistic regression adjusted for sex, parity, maternal age, maternal education, maternal atopy, maternal smoking	Positive SPT	2.3 (1.1, 4.6)[b] 1.1 (0.7, 1.6)[b]
Joseph et al. (2005)	4,634 children, ages 1-3 yr followed prospectively for 12 months Southeastern MI	Measured up to 12 mos before outcome Caucasian ≥ 5 vs. Caucasian <5[c] African American ≥ 5 vs. African American <5[c] African American ≥ 10 vs. African American <5[c]	Logistic regression adjusted for sex, birth weight, and annual income	Incident asthma requiring medical care	 2.7 (0.9, 8.1) 1.1 (0.8, 1.7) 1.3 (0.6, 2.6)
Rabinowitz et al. (1990)	1,768 children followed from birth to unspecified age Boston area, MA	Prenatal (cord blood) ≥ 10 vs. <10[a]	Logistic regression with no additional covariates included in model	Prevalent asthma	1.3 (0.8, 2.0)
Pugh Smith and Nriagu (2011)	356 children, ages 0-12 yr Saginaw, MI	Levels ascertained from statewide database, timing unreported but varied among subjects ≥ 10 vs. <10[a]	Logistic regression adjusted for age, sex, number of stories in unit, cat in home, dog in home, cockroach problem, number of persons in home, household smoking, clutter, candles/incense, type of cooking stove, main heating source, months of residency, housing tenure, type of air conditioning, peeling paint, ceiling/wall damage, age of housing, water dampness/mold/mildew	Prevalent asthma	7.5 (1.3, 42.9)

GM = geometric mean, SPT = skin prick test.

[a]Odds ratio in children with blood Pb level ≥ 10 µg/dL with children with blood Pb level <10 µg/dL serving as the reference group

[b]Odds ratio presented per 1 µg/dL increase in blood Pb level.

[c]Relative risk in each specified subgroup with children with blood Pb level <5 µg/dL serving as the reference group.

In a study of 4,634 children in southeastern Michigan, blood Pb levels were measured at ages 1 to 3 years, up to 12 months prior to asthma assessment. In analyses 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 among Caucasian children (relative risk [RR]: 2.7 [95% CI: 0.9, 8.1] compared with Caucasian children with blood Pb levels < 5 µg/dL) (Joseph et al., 2005) (Figure 5-43 and Table 5-29). In analyses restricted to African Americans, children with blood Pb levels ≥ 10 µg/dL had an elevated risk of asthma requiring medical care (RR: 1.3 [95% CI: 0.6, 2.6] compared with children with blood Pb level < 5 µg/dL) (Figure 5-43 and Table 5-29). In analyses that used Caucasian children with blood Pb level <5 µg/dL as the reference group, blood Pb level was associated with increased risk of asthma requiring medical care among African American children in all blood Pb level categories. Collectively, the results indicated a stronger association with race/ethnicity than with blood Pb level. It is important to recognize the small numbers of

children with asthma requiring medical care in the high blood Pb level categories, which likely accounted for the wide 95% CIs (5 Caucasian children with blood Pb ≥ 5 µg/dL and 9 African American children with blood Pb level ≥ 10 µg/dL).

Consistent with Joseph et al. (2005), a recent cross-sectional study conducted in Saginaw, Michigan found a higher prevalence of asthma in children (ages ≤ 12 years) with blood Pb levels ≥ 10 µg/dL (Pugh Smith and Nriagu, 2011). Of the 356 children randomly selected from a statewide surveillance database of blood Pb measurements, 78% were African American. This study had greater power than did Joseph et al. (2005) due to the larger percentage of children with blood Pb levels ≥ 10 µg/dL (18.6%). Compared with children with initial blood Pb levels < 10 µg/dL, children with initial blood Pb levels ≥ 10 µg/dL had a higher odds of having a doctor diagnosis of asthma within the past 12 months (OR: 7.5 [95% CI: 1.3, 42.9]). A strength of this study was the adjustment for a large number of potential confounding variables such as age, sex, pets in the home, housing characteristics, and household smoking. However, because children were identified from a statewide database of initial blood Pb measurements collected at unspecified ages, the timing of blood Pb varied among children. In a study that did not consider potential confounding variables, Rabinowitz et al. (1990) reported an increased risk of asthma in children (age of assessment not reported) in association with cord blood Pb levels > 10 µg/dL relative to cord blood Pb levels ≤ 10 µg/dL (Figure 5-43 and Table 5-29).

While the aforementioned studies examined blood Pb levels measured at a single point in time, a recent prospective birth cohort study compared associations of prenatal (cord and maternal) and concurrent blood Pb levels with risk of allergic sensitization at age 5 years (Jedrychowski et al., 2011). Cord and prenatal maternal blood Pb level were associated with greater risk of positive skin prick test (SPT) to dust mite, dog, or cat allergen than was child concurrent blood Pb level (Figure 5-43 and Table 5-29). For prenatal Pb biomarkers, similar effect estimates were obtained before and after adjusting for sex, parity, maternal age, maternal education, maternal atopy, and environmental tobacco smoke exposure. Cord and concurrent blood Pb levels were weakly correlated (r = 0.29), providing support for an independent association for prenatal Pb biomarkers. The independent effects of Pb also were substantiated by observations that indicators of other exposures, including blood levels of mercury, polycyclic aromatic hydrocarbon DNA adducts, and residential levels of dust mite or pet allergen were associated with lower risks of SPT than was blood Pb level. While associations were observed with relatively low cord blood Pb levels (geometric mean: 1.16 µg/dL [95% CI: 0.12, 1.22]), it is uncertain the extent to which higher past Pb exposures of the mothers may have influenced their pregnancy blood Pb levels and newborn cord blood Pb levels. Among children approximately age 10 years in Hong Kong, Hon et al. (2010; 2009) found

correlations between low concurrent blood Pb levels (population means < 2 µg/dL) and severity of atopic dermatitis (Spearman r = 0.46, p <0.001 and r = 0.33, p <0.005), another inflammatory condition commonly related to elevated IgE levels. Neither analysis considered potential confounding variables.

Among the studies in children that found associations of blood Pb level with asthma and allergy-related outcomes, several adjusted for potential confounding by SES-related variables. Joseph et al. (2005) adjusted for annual income. Pugh Smith et al. (2011), who examined a primarily low SES population of children in Michigan, adjusted for various factors associated with SES, including multiple indices of housing condition, and presence of pets and cockroaches in the home, which are indicators of allergens in the home. Jedrychowski et al. (2011) adjusted for maternal education, and found similar magnitudes of association between cord blood Pb level (and positive SPT) as those in the unadjusted analysis. Further, residential levels of dust mite or pet allergen were associated with lower risks of SPT than was blood Pb level. Thus, while SES and/or allergen exposure has been associated with both Pb exposure and asthma and allergy in children (Bryant-Stephens, 2009; Dowd and Aiello, 2009; Aligne et al., 2000), these collective findings in different populations do not demonstrate that confounding by SES and/or allergen exposure fully accounts for the associations observed between blood Pb levels and asthma and allergy. As allergic sensitization, asthma, and elevated IgE have been correlated in children, the evidence of association of blood Pb level with asthma and allergy after adjusting for SES, housing conditions, and allergen exposures, may provide support for the associations observed between higher blood Pb and higher IgE in children (Section 5.6.3).

Studies in nonoccupationally-exposed adults did not find associations of biomarkers of Pb exposure with asthma or allergy (Pizent et al., 2008; Mendy et al., In Press). The largest of these studies was a U.S. NHANES 2007-2008 analysis of adults ages 20 years and older, in which urinary Pb level was not associated with an increase in asthma (OR: 0.72 [95% CI: 0.46, 1.12] per 1 µg/g increase in creatinine-adjusted urine) (Mendy et al., In Press). The results did not provide strong evidence that urinary Pb level was associated with other respiratory conditions such as emphysema or chronic bronchitis either. In a study of 216 adults without occupational Pb exposures, Pizent et al. (2008) found that among women, the association between concurrent blood Pb level and serum IgE was statistically significant, whereas the association with positive SPT to common inhaled allergens was not. Among men, higher concurrent blood Pb level was associated with lower odds of positive SPT (OR: 0.92 [95% CI: 0.86, 0.98] but was not statistically significantly associated with IgE. These findings appeared to be discordant because an increase in IgE commonly mediates the acute inflammatory response to allergens. However, the interpretation of the findings is difficult because only statistically

significant effect estimates were reported; thus it is not known whether odds ratios were in the same direction for SPT and IgE. Bener et al. (2001b) found higher prevalence of asthma and allergy-related conditions such as rhinitis and dermatitis among Pb industrial workers than among control subjects; however, the blood Pb levels in both the Pb-exposed group and the control group (geometric means: 77.5 and 19.8 µg/dL, respectively) were higher than those in the current U.S. general adult population.

In summary, an available recent epidemiologic study added evidence for associations between blood Pb level and asthma in children (Pugh Smith and Nriagu, 2011), and a few recent studies provided new evidence for associations with allergic conditions in children (Jedrychowski et al., 2011; 2010; Hon et al., 2009). This epidemiologic evidence was strengthened by several observations that associations were robust to the adjustment of multiple potential confounding factors, in particular, SES and allergen exposures. Because of the heterogeneity in the relatively small body of evidence, it was difficult to identify whether the strength of association with asthma and allergy differed by age of children, lifestage of blood Pb measurement (prenatal, sometime in childhood prior to outcome assessment, concurrent), or level of blood Pb. The epidemiologic evidence for associations of blood Pb level with asthma and allergy is well supported by toxicological and epidemiologic evidence indicating Pb effects on increasing IgE (Section 5.6.3), Th2 cytokines (Section 5.6.5.4), and inflammation (Section 5.6.5.1). In the few recent studies that investigated nonoccupationally-exposed adults, Pb biomarker levels were not associated significantly with greater asthma or allergy (Pizent et al., 2008; Mendy et al., In Press).

5.6.4.3 Other Respiratory Effects

The respiratory effects of Pb have been examined primarily in a small number of recent epidemiologic studies of nonoccupationally-exposed adults, and as with evidence for asthma, evidence for Pb-associated respiratory effects in adults is weak. Increased bronchial responsiveness (BR) is a characteristic feature of asthma and other respiratory diseases and can result from the activation of innate immune responses and increased airway inflammation. In a study of 525 middle-aged adults in Seoul, Korea, Min et al. (2008a) found an association between concurrent blood Pb level and BR. A 1 µg/dL higher concurrent blood Pb level was associated with a higher BR index (log [% decline in forced expiratory volume in 1 second (FEV_1)/log of final methacholine concentration in mg/dL]) of 0.018 (95% CI: 0.004, 0.03), adjusting for age, sex, height, smoking, lung function, and asthma diagnosis (Min et al., 2008a). The concurrent blood Pb levels in these adults were low (mean [SD]: 2.90 [1.59] µg/dL); however, it is uncertain what timing, level, frequency, and duration of Pb exposures contributed to the observed

association. In contrast to Min et al. (2008a), Pizent et al. (2008) found that higher concurrent blood Pb level was associated with lower BR in 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 finding was not discussed in details by investigators, it was consistent with the observation that higher blood Pb level was associated with lower odds of positive SPT among men in this study.

In studies of male Pb workers, Pb-associated respiratory effects were not clearly indicated. None of the studies directly examined associations between blood Pb levels and lung function. In bus drivers in Hong Kong, China, (Jones et al., 2008; Jones et al., 2006), drivers of non-air conditioned buses had lower exposures to PM_{10}, lower blood Pb levels (mean 3.7 µg/dL versus 5.0 µg/dL in air conditioned buses) but lower indices of lung function than did drivers of air conditioned buses (Jones et al., 2006). In this study, the authors attributed the slightly higher blood Pb levels of 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 (Jones et al., 2008). Pb industrial workers in the United Arab Emirates had higher prevalence of respiratory symptoms such as cough, phlegm, shortness of breath, and wheeze than did unexposed controls (Bener et al., 2001b). Blood Pb levels in both the Pb-exposed group and the control group (geometric means: 77.5 and 19.8 µg/dL, respectively) were higher than those in the current U.S. adult general population, and the analysis did not consider potential confounding variables.

Toxicological evidence for Pb-associated respiratory effects is provided by observations that exposure to Pb-containing PM induces a range of inflammatory-related effects in the airways of animals and in cultured airway cells (Section 5.6.6). Specific effects on the lung also were demonstrated in a recent study of rats injected with Pb-acetate (25 mg/kg, 3 consecutive days, resulting in 2.1 µg/dL blood Pb) (Kaczynska et al., 2011). Pb-treated rats exhibited ultrastructural changes in lung tissue, including substantial pulmonary fibrosis containing numerous lipofibroblasts, collagens, and elastin filaments in the interstitium. Mast cells also were present in the interstitium following Pb exposure. Pulmonary inflammation was observed, evidenced by the increased recruitment of monocytes and thrombocytes inside the capillary vessels and increased macrophage accumulation in the alveolar space. Evidence of damaged surfactant lining and destruction of the laminae inside lamellar bodies of epithelial type II cells also was observed. While the observed pulmonary histological changes have been linked with functional pulmonary decrements in other studies (unrelated to Pb exposure), they were observed with injected Pb. It is not clear whether routes of Pb exposure more relevant to those in humans would result in similar effects.

5.6.4.4 Autoimmunity

Evidence for the effects of Pb on increasing the risk of autoimmunity is provided primarily by a small number of older toxicological studies reviewed in the 2006 Pb AQCD in which Pb exposure of animals (pre- and postnatal) was associated with the generation of autoantibodies (Hudson et al., 2003; Bunn et al., 2000; El-Fawal et al., 1999; Waterman et al., 1994). Whereas some evidence linked this risk of autoimmunity to a shift toward Th2 responses, other evidence pointed to a shift toward 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 (100 µM Pb-acetate in drinking water of dams from GD8 to PND21, resulting in pup blood Pb levels 10-30 µg/dL) Pb exposure of mice on changes in gene expression in the spleen. Investigators 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 $PbCl_2$-treated adult mice (25-50 µg i.p.) also indicated the potential for autoantibody generation. Evidence of Pb-associated autoimmune responses in humans is limited to an older 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). Pb also was found to modify neural proteins in rats (Waterman et al., 1994). The generation of self-peptides and modification of proteins can result in formation of neoantigens, thereby increasing the risk of autoimmune reactions.

5.6.4.5 Specialized Cells in Other Tissues

As discussed in Section 5.6.2.4, Pb exposure consistently has been found to alter the function of macrophages. Nonlymphoid tissues also contain specialized macrophages whose altered function can contribute to organ/tissue dysfunction, cell death, tissue pathology and tissue-specific autoimmune reactions. Among the specialized macrophages 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 evidence demonstrating the effects of Pb on specialized macrophages, which was provided by recent toxicological studies, is important as it demonstrates the contribution of immune dysfunction to the effects of Pb on dysfunction in nonlymphoid tissues (Figure 5-44). 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. It is important to note that in this small body of available studies, several studies treated animals by Pb injection, and it is unclear whether the effects would be observed with routes of exposure that are more relevant for humans.

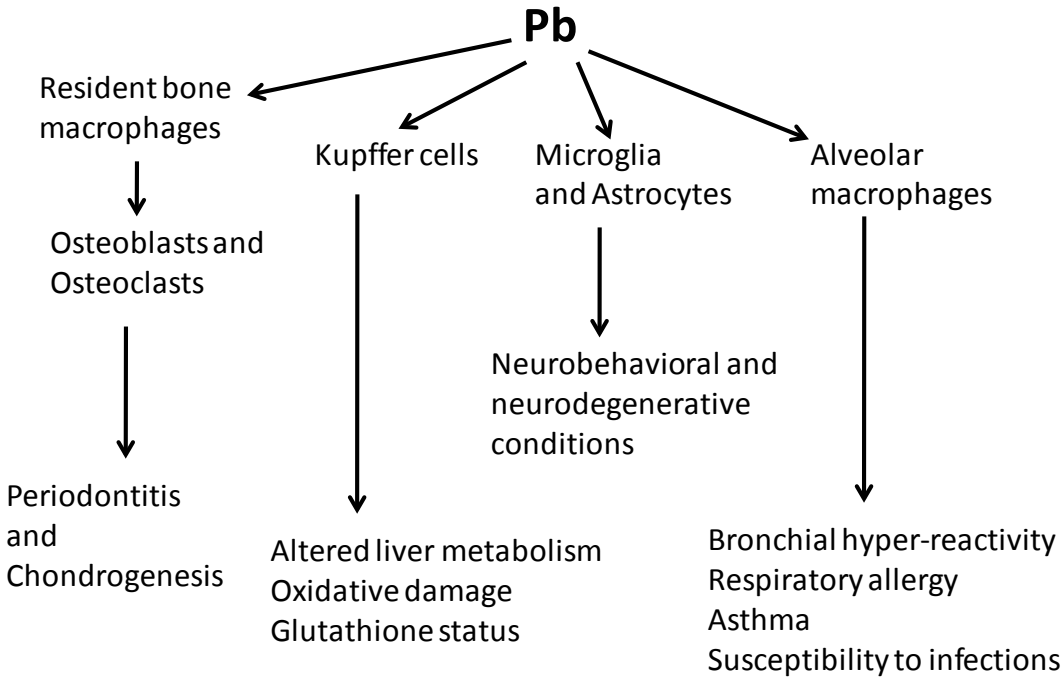


Figure 5-44 Specialized macrophages in nonlymphoid tissue may serve as a link between Pb exposure and disease in multiple organ systems.

Fan et al. (2009b) 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. Dosing of juvenile Wistar rats with Pb injections (15 mg/kg of Pb-acetate daily for 2 weeks, resulting in a mean blood Pb level of 30 µg/dL) during early postnatal maturation was observed to produce chronic glial activation, increase pro-inflammatory cytokines, and increase neurodegeneration, as indicated by an increase in GFAP, increase in IL-1β, TNF-α and IL-6 cytokines, and a decrease in synaptophysin (component of presynaptic vesicles), respectively, in brain tissue (Struzynska et al., 2007). In bone, osteoclasts regulate osteoblast function (Chang et al., 2008a), and osteoblasts have been shown to be affected by Pb exposure. Effects on these cell types can contribute to later life diseases such as arthritis [reviewed in Zoeger et al. (2006)]. Pb-induced elevation of TGF-β production was found to be involved in

chondrogenesis in bone (Zuscik et al., 2007). Kaczynska et al. (2011) reported effects on alveolar macrophages after Pb treatment (i.p. 25 mg/kg, 3 days, resulting in blood Pb levels of 2.1 µg/dL) in rats. Macrophage recruitment increased, and this macrophage infiltration limited air space available to gas exchange and contained parts of phagocytized surfactant and alveolar lining.

Resident immune cells in reproductive organs have been shown to be affected by Pb exposure. 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 indicates that high concentration Pb exposures directly promote tumor formation or induce 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 1,300 ppm of Pb-acetate in drinking water had enhanced moloney sarcoma virus-induced tumor growth compared with control animals. The findings indicated that Pb-induced immunomodulation affecting tumors likely resulted 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 immunotoxicology, particularly when early life exposures are involved (Dietert, 2011).

5.6.5 Modes of Action for Lead Immune Effects

5.6.5.1 Inflammation

The 2006 Pb AQCD indicated that 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 such as the liver, kidney, and vasculature (Section 5.2.5). In the 2006 Pb AQCD, several lines of toxicological evidence demonstrated Pb-induced inflammation: Pb-induced increases in PGE_2 and ROS (Section 5.6.5.2) and increases in pro-inflammatory cytokines (Section 5.6.5.4), both of which promote a hyperinflammatory phenotype in immune cells. As described in sections that follow, these findings are

corroborated by a small number of available recent toxicological studies. A few available recent epidemiologic studies provided new evidence of Pb-associated inflammation, as indicated by associations of blood Pb levels with pro-inflammatory cytokines and other nonspecific indicators of inflammation.

Enhanced inflammation and tissue damage occurs through the modulation of inflammatory cell function and production of pro-inflammatory cytokines and metabolites. It is important to note that while inflammation can mediate immune-related conditions such as asthma, respiratory infections, and BR, inflammation can exacerbate disease and damage in almost any organ given the distribution of immune cells as both permanent residents and infiltrating cell populations. 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). For example, toxicological studies have found that Pb-induced inflammatory damage involves the depletion of antioxidants such as glutathione and catalase (Lodi et al., 2011; Chetty et al., 2005). While several processes have been proposed to explain the mechanisms of Pb-induced oxidative damage, the exact combination of processes involved remains to be determined (Section 5.2.4).

In epidemiologic studies, Pb-associated changes in pro-inflammatory cell function (Section 5.6.2) and cytokine production (Section 5.6.5.4) have been found in the cumulative body of evidence. A few recent epidemiologic studies added to this evidence with observations of associations between blood Pb level and other nonspecific indicators of inflammation that may be related to multisystemic effects as have been demonstrated in animal and in vitro studies. Using 1999-2004 NHANES data, Songdej et al. (2010) examined the relationship between concurrent blood Pb levels and the inflammation markers, C-reactive protein (CRP), fibrinogen, and white blood cell (WBC) count in adults 40 years of age or older. Adjusting for age, sex, race/ethnicity, education, income, BMI, physical activity, smoking status, diabetes status, inflammatory disease status, and cardiovascular disease status, investigators found larger magnitudes of association between blood Pb and inflammation in men compared with women. Among women, most ORs for associations between quintiles of blood Pb level and tertiles of CRP, fibrinogen, and WBC count were less than 1.0 whereas corresponding ORs in men tended to be greater than 1.0 but were not always statistically significant. For example, compared with men with concurrent blood Pb levels less than 1.16 µg/dL, men with blood Pb levels of 1.16-<1.63 µg/dL, 1.63-<2.17, 2.17-<3.09 µg/dL, and ≥ 3.09 µg/dL had elevated odds of elevated CRP (OR [95% CI]: 2.22 [1.14, 4.32], 1.67 [0.85, 3.28], 2.12 [1.07, 4.21], and 2.85 [1.49, 5.45], respectively). For all inflammation markers, although the OR was highest in the highest quintile of blood Pb level (≥ 3.09 µg/dL), monotonic concentration-dependent increases were not observed.

Consistent with these findings, among men in Incheon, Korea without occupational Pb exposures, Kim et al. (2007) reported associations of higher concurrent blood Pb level with higher levels of WBCs and IL-6. Larger effects were estimated for men in the upper two quartiles of blood Pb levels, 2.51-10.47 µg/dL than for the full range of blood Pb levels. The findings 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 (Hage and Szalai, 2007; Fuller and Zhang, 2001). Despite the low concurrent blood Pb level of adults in these aforementioned studies, it is important to acknowledge that the relative contributions of recent versus past Pb exposures to the associations observed with concurrent blood Pb levels are not delineated.

In a genome-wide association study that included 37 children with autism and 15 children without autism (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, concurrent 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) (Tian et al., 2011). Although blood Pb levels were similar between children with and without autism and correlations were observed in both groups, they were in opposite directions (positive among children with autism and negative among children without autism). Pb has been shown to increase MHC molecule surface expression in mouse and human HLA antigen presenting cells (Guo et al., 1996a; McCabe and Lawrence, 1991); however, additional larger studies with a priori hypotheses regarding specific indices of inflammation are warranted to characterize Pb-associated changes in inflammation in children.

5.6.5.2 Increased Prostaglandin E_2 and Decreased Nitric Oxide

Consistent with the large body of evidence presented in the 2006 Pb AQCD (U.S. EPA, 2006b), a small number of available recent studies continued to indicate that Pb exposure alters the levels of signaling molecules such as PGE_2 and NO. Collectively, the weight of evidence was provided by toxicological studies. These signaling molecules are involved in mediating inflammation and host resistance (Figure 5-42). A recent in vitro study with human neuroblastoma cells found increases in PGE_2 with lower Pb concentrations (0.01-1 µM) than those previously reported (Chetty et al., 2005). A large body of evidence reviewed in the 2006 Pb AQCD demonstrated a Pb-associated decreased production of NO by macrophages [see 2006 Annex Table AX5.9.6 (U.S. EPA, 2006h)].

In adult animal models, decreases in NO were observed with short-term exposures (hours to days) to a wide range of Pb concentrations. Decreases in NO can impact not only

innate host defenses, but also, acquired immunity. In a recent study, Farrer et al. (2008) found that high-level Pb glutamate exposure (5 µM) of lymphocyte cultures led to decreased NO production, decreased inducible NO synthase function in myeloid cells, but no change in its gene expression. Additionally, Pb abrogated the myeloid cell (CD11b+)-mediated suppression of CD4+ T cell proliferation. Together, these findings indicated that Pb may indirectly enhance T cell proliferation through its effect on decreasing NO production. Combined with the observation that Pb can alter antigen processing (Farrer et al., 2005) and, hence, the quality and magnitude of the acquired immune response signal against pathogenic challenge, evidence indicated that 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 supported associations of blood Pb level with suppressed NO production (Barbosa et al., 2006a; Pineda-Zavaleta et al., 2004) and increased ROS production (Pineda-Zavaleta et al., 2004) in populations living near Pb sources. In a previous study of children in Mexico, with increasing residential proximity to the Pb smelter, mean concurrent 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 (Pineda-Zavaleta et al., 2004). NO release from macrophages (indirectly activated by phytohemagglutinin, PHA) was lower with higher blood Pb levels. After adjusting for age and sex, a 1 µg/dL higher blood Pb level was associated with a higher level of superoxide anion of 0.00389 (95% CI: 0.00031, 0.00748) µmol/mg protein and a lower level of NO of 0.00089 (95% CI: -0.0017, -0.00005) nmol/µg protein. Because PHA activates macrophages indirectly through the activation of lymphocytes and IFN-γ directly activates macrophages, these results indicated that Pb suppressed T cell-mediated macrophage activation and stimulated cytokine-induced macrophage activation. Group-level comparisons indicated that associations likely were driven by changes observed in the group of children living in closest proximity to the smelter who had blood Pb levels 10.31-47.49 µg/dL. Results also demonstrated a larger magnitude of association between blood Pb levels and superoxide anion release in males. Although not described in detail, higher blood Pb level was not associated with lower NO in girls. Barbosa et al. (2006a) also observed an association between higher blood Pb level and lower plasma NO in a group of adults in Sao Paolo, Brazil residing near a closed battery plant, particularly among adults with the TC or CC eNOS genotype ($r = 0.23$, $p = 0.048$) which is associated with reduced promoter activity and potentially reduced gene expression. Quantitative results were not reported for analysis of all adults combined or adults with the TT genotype, but p-values were greater

than 0.05. Because NO was measured in plasma, it was not possible to identify immune cells as the specific sources of NO.

Studies of occupationally-exposed adults provided less clear indication of associations of blood Pb level with NO and ROS. Despite large differences in blood Pb levels between 30 male Pb recycling plant workers (mean: 106 µg/dL) and 27 unexposed controls (mean: 4.5 µg/dL), levels of ROS released from neutrophils (indicators of respiratory burst) were similar between groups (Mishra et al., 2006a). 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) found similar plasma NO levels in controls compared with Pb-exposed workers. Also, although quantitative results were not reported, blood Pb level was reported not to be correlated with NO.

5.6.5.3 Cellular Death (Apoptosis, Necrosis)

The 2006 Pb AQCD reviewed a small number of toxicological studies in which Pb exposure had contrasting effects on the apoptosis of macrophages. Since then, a few available recent toxicological studies found that Pb exposure induces apoptosis or mediators of apoptosis in immune cells. In a study in mice, Bishayi and Sengupta (2006) found that Pb treatment of adult mice (10 mg/kg, i.p.) elevated DNA fragmentation in splenic macrophages. Using mouse resident peritoneal macrophages, Gargioni et al. (2006) found that 20 and 40 µM Pb nitrate 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 3 week-old 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. Pb also induced DNA damage (determined by the comet assay), which was accompanied by elevations in p53 and Bax expression with no change in Bcl-2 expression (creating a Bax/Bcl-2 imbalance). The authors proposed that oxidative stress was a likely route to Pb-induced apoptosis and tumorigenesis. Because of the high Pb concentrations administered and systemic administration of Pb employed, other studies are warranted to examine whether apoptosis of macrophages represent a mode of action relevant to human Pb exposures.

5.6.5.4 Cytokine Production

The 2006 Pb AQCD presented a large body of toxicological evidence that clearly demonstrated Pb-induced changes in cytokine production in vivo and in vitro (U.S. EPA, 2006b). Previous toxicological studies indicated that Pb affects immune cytokine production via action on T cells and macrophages. The combination of cytokine changes induced by Pb in multiple cell types can create a hyperinflammatory state among innate immune cells to skew acquired immunity responses away from Th1 responses and toward Th2 responses. As illustrated in Figure 5-42, downstream effects include altered IgE production, ROS production, and inflammation. In support of a Pb-induced skew toward Th2 responses, several studies in rats, mice, and chickens found that pre- and postnatal Pb exposure suppressed the production of Th1 cytokine IFN-γ and/or increased production of Th2 cytokines such as IL-4 [Table 5-7 of the 2006 Pb AQCD (U.S. EPA, 2006g)]. Recent toxicological studies continued to find such Pb-induced changes in cytokine production, and a study provided new evidence that Th2 skewing may be mediated via effects on dendritic cells.

Cheng et al. (2006) found that Pb exposure affected TNF-α production in vitro in A/J mice macrophages by affecting the mitogen-activated protein kinase (MAPK) signaling pathway. Pb-acetate (10 µM) co-administered with LPS stimulated the phosphorylation of p42/44 MAPK and TNF-α expression (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. Thus, Pb exposure may induce local tissue damage through the modulation of immune responses. These findings were consistent with those from recent studies. Gao et al. (2007) showed that treatment of mouse dendritic cells with 25 µM $PbCl_2$ produced an increased phosphorylation of the Erk/MAPK signaling molecule, and Khan et al. (2011) showed that monocytes treated with 25-50 µM Pb-acetate increased TNF-α through ERK1/2 and p38 signaling.

In an in vivo study conducted across a lifetime (developmental through adulthood) in Swiss mice (females and males) using a broad range of dietary Pb concentrations, Iavicoli et al. (2006a) found a nonlinear hierarchical cytokine response. At the lowest dietary Pb concentration (0.11 ppm Pb-acetate, resulting in blood Pb level: 1.6 µg/dL), IL-2 and IFN-γ were decreased compared to those in the controls (0.02 ppm Pb-acetate, resulting in blood Pb level: 0.8 µg/dL), indicating a suppressed Th1 response. 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 differentially modulated by low-level versus high-level Pb exposures. Other studies 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 (25 µM) promoted a slight but statistically significant increase in IL-2 production (quantitative results not reported) among lymphocytes.

In vitro studies also reported a Pb-induced shift to production of Th2 cytokines. In conjunction with other indicators of Th2 skewing described in Sections 5.6.2.3, 5.6.2.6, and 5.6.3, Gao et al. (2007) observed that 25 µM Pb elevated the production of Th2 cytokines such as IL-4, IL-5 and IL-6 in dendritic cells. In cultures of human PMNs activated with *Salmonella enteritidis* or with monoclonal antibodies of CD3, CD28, and CD40, Pb-acetate concentrations of 0.15 µg/dL and higher suppressed expression of Th1 cytokines, IFN-γ, IL-1β, and TNF-α, and increased secretion of Th2 cytokines, IL-5, IL-6, and IL-10 (Hemdan et al., 2005).

Consistent with toxicological studies, a few available epidemiologic studies also found higher concurrent blood Pb levels in children and occupationally-exposed adults to be associated with a shift toward production of Th2 cytokines relative to Th1 cytokines. The evidence in children was based on comparisons of serum cytokine levels among groups with different blood Pb levels without consideration of potential confounding factors. Among children ages 9 months to 6 years in Missouri, Lutz et al. (1999) found that children with concurrent blood Pb levels 15-19 µg/dL had higher serum levels of IL-4 ($p = 0.08$, Kruskal Wallis) and IgE (Section 5.6.3) than did children with lower blood Pb levels. However, IL-4 levels in children with blood Pb levels 20-44 µg/dL were lower than those in children with blood Pb levels < 15 µg/dL. The elevated IL-4 and IgE in children with blood Pb levels 15-19 µg/dL were consistent with the mode of action for IL-4 to activate B cells to induce B cell class switching to IgE. In another study of children in grades 5 and 6 in Taiwan, investigators did not group children by blood Pb levels but by potential for Pb exposures due to age of home and location of residence (Hsiao et al.). Concurrent blood Pb levels did not differ by residence in old versus new homes or by urban versus rural residence (means: 3.2-3.8 µg/dL) but were higher among children living near an oil refinery, in particular, among children with known respiratory allergies (mean: 8.8 µg/dL). This latter group of children also had the lowest serum levels of IFN-γ (45-fold) and highest levels of IL-4 (6-fold) (lower $p < 0.05$ for comparisons with any subgroup). There was no direct comparison of cytokine levels between blood Pb level groups in the population overall; however, cytokine levels were similar between healthy and allergy groups in the other Pb source groups that had similar blood Pb levels ($p > 0.05$ for comparisons with any subgroup). Thus, the differences in cytokine levels between healthy and allergic children living near the oil refinery may have been influenced by differences in their blood Pb levels.

Evidence of association between blood Pb levels and cytokine levels in nonoccupationally-exposed adults was equivocal. Among adult university students in Incheon, Korea, Kim et al. (2007) found associations of concurrent blood Pb level with serum levels of TNF-α and IL-6 that were larger among males in the upper two quartiles of blood Pb levels, 2.51-10.47 µg/dL. Notably, the relative contributions of recent versus past Pb exposures to these associations are not known. In models that adjusted for age, BMI, and smoking status, a 1 µg/dL higher blood Pb level was associated with a 0.75 (95% CI: 0.14, 1.36) pg/mL higher TNF-α and a 0.18 (95% CI: -0.02, 0.38) pg/mL higher IL-6. The association between levels of blood Pb and plasma TNF-α was greater among men who were GSTM1 null (1.14 [95% CI: 0.20, 2.10] pg/mL higher per 1 µg/dL higher blood Pb level) than men who were GSTM1 sufficient (0.12 [95% CI: -0.43, 0.67] pg/mL). Blood Pb also was associated with a larger increase in TNF-α among men who had the TNF-α GG genotype (0.80 [95% CI: 0.15, 1.45] pg/mL higher per 1 µg/dL higher blood Pb level) than men who had the GA or AA genotype (-0.21 [95% CI: -1.1, 0.71] pg/mL). 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, it is biologically plausible that the null variant of GSTM1, which is associated with reduced elimination of ROS, may increase the risk of Pb-associated immune effects. The results for the TNF-α polymorphism 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. Among adults in Italy, concurrent blood Pb levels were not statistically significantly correlated with either Th2 or Th1 cytokine levels in men (Boscolo et al., 1999) or women (Boscolo et al., 2000) (quantitative results not reported).

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) that did not consider potential confounding variables. The exception was a study of male foundry workers, pottery workers, and unexposed workers (Valentino et al., 2007). Multiple regression analyses were performed with age, BMI, smoking, and alcohol consumption included as covariates; however, regression coefficients describing the concentration-response functions were not reported. Pb-exposed workers had higher IL-10 and TNF-α (ANOVA, $p < 0.05$). Levels of IL-2, IL-6, and IL-10 also increased from the lowest to highest blood Pb group (ANOVA, $p > 0.05$). In contrast with most other studies, both exposed worker groups had lower IL-4 levels compared with controls (ANOVA, $p > 0.05$). In a similar analysis, DiLorenzo et al.

(2007) separated exposed workers into intermediate (9.1-29.4 µg/dL) and high (29.4-81.1 µg/dL) blood Pb level groups, with unexposed workers comprising the low exposure group (blood Pb levels 1-11 µg/dL). Mean TNF-α levels showed a monotonic increase from the low to high blood Pb level group. Results also indicated a potential interaction between blood Pb level and smoking. Among current smokers, a 12- to 16-fold difference in TNF-α levels was observed among blood Pb groups. Among nonsmokers, the differences were less than two fold. In Yucesoy et al. (1997a), levels of the Th1 cytokines, IL-1β and IFN-γ, were lower in workers than in controls.

In summary, a large body of previous toxicological evidence clearly demonstrated Pb-induced increases in Th2 cytokines and decreases in Th1 cytokines. Several recent toxicological studies added to this evidence by showing Pb-induced increases in TNF-α that were mediated by MAPK signaling pathways (Khan et al., 2011; Gao et al., 2007; Cheng et al., 2006; Iavicoli et al., 2006a; Hemdan et al., 2005), and demonstrating Pb-induced increases in cytokine production in dendritic cells (Gao et al., 2007). The collective epidemiologic evidence is sparse; however, in the few available new studies, higher concurrent blood Pb level was associated with higher levels of various pro-inflammatory cytokines in children, nonoccupationally-exposed adults, and occupationally-exposed adults. The recent epidemiologic study of children in Taiwan found an association between higher IL-4 levels in children with higher blood Pb levels (Hsiao et al.), similar to a previous study of children in Missouri (Lutz et al., 1999). Neither study of children considered potential confounding variables. In contrast to previous studies, a recent study in nonoccupationally-exposed adults found associations between higher concurrent blood Pb level and higher TNF-α and IL-6 cytokine levels in men in Korea (Kim et al., 2007). Recent studies of occupationally-exposed adults added to the evidence for higher levels of several cytokines among Pb-exposed workers compared to unexposed controls (Di Lorenzo et al., 2007; Valentino et al., 2007). Due to the limited investigation, it is difficult to draw conclusions about the effects of Pb exposure on cytokine levels in any particular group in the human population.

5.6.6 Air-Lead Studies

Although comprising a smaller body of evidence than do immune studies of Pb biomarkers, several recent studies used Pb measured in PM_{10} and $PM_{2.5}$ air samples to represent Pb exposures. Some studies analyzed the Pb component individually, whereas others analyzed Pb as part of a group of correlated components using source apportionment techniques or principal component analysis. In concordance with blood Pb studies, recent time-series epidemiologic studies that examined ambient air Pb-PM concentrations found associations with respiratory morbidity in children (Gent et al.,

2009; Hong et al., 2007b). Adjusting for season, day of week, and date, Gent et al. (2009) found that an increase in lag 0-2 average Pb-$PM_{2.5}$ was associated with elevated odds of wheeze (OR: 1.03, p = 0.13 per 5 ng/m^3 increase in Pb $PM_{2.5}$), shortness of breath (OR = 1.12, p = 0.01), and fast-acting inhaler use (OR: 1.04, p = 0.10) among children with asthma (ages 4-12 years), followed for 1 year during 2000-2003. Among children in Korea (grades 3-6), a 1 log increase in lag 1 Pb PM_{10} was associated with a -6.83 L/min decrease (p < 0.01) in morning peak expiratory flow and a −6.37 L/min decrease (p < 0.01) in daily average peak expiratory flow adjusting for age, sex, height, weight, smoking exposure, and meteorological factors (Hong et al., 2007b). Older and recent toxicological studies also found Pb-containing CAPs to induce pulmonary inflammation. Uzu et al. (2011) specifically tested particles emitted at a Pb recycling plant and found that these Pb-rich particles increased the release of the cytokine granulocyte-macrophage colony-stimulating factor from human epithelial cells. Other toxicological studies found pulmonary inflammation induced by CAPs in which Pb was one of numerous components (Wei et al., 2011; Duvall et al., 2008; Godleski et al., 2002; Saldiva et al., 2002). As with blood Pb, epidemiologic studies did not consistently find associations of ambient air Pb-PM concentrations with respiratory-related hospitalizations or mortality in older adults. Both studies adjusted for meteorological factors and for temporal trends. Among adults ages 65 years and older in 6 California counties, a 4 ng/m^3 increase in lag 3 Pb-$PM_{2.5}$ was associated with an increased relative risk of respiratory mortality all year of 1.01 (95% CI: 0.99, 1.03) and during summer months (quantitative results not reported) (Ostro et al., 2007). However, among adults ages 65 years and older in 106 U.S counties, Bell et al. (2009) found that an increase in lag 0 Pb-$PM_{2.5}$ was associated with a decrease in respiratory hospital admissions. The 95% CI was wide, indicating lack of precision in effect estimate.

Although limited available recent findings suggest a relationship between respiratory effects in children and short-term (over several days) changes in ambient air Pb-PM concentrations, it is important to note uncertainties that limit the ability to draw conclusions regarding airborne Pb exposure. Size distribution data for Pb-PM are relatively limited, so it is difficult to assess the representativeness of these concentrations to population exposure (Section 3.5.3). Moreover, data on the relationship between blood Pb and air Pb are relatively limited (see Section 4.5.1) and do not characterize relationships of varying Pb-PM size distribution with blood Pb level. In several air-Pb studies, other PM components such as elemental carbon (EC), copper (Cu), and zinc (Zn) also were associated with respiratory effects. In the absence of detailed data on correlations among all PM components, measurements on other co-occurring ambient pollutants, or results adjusted for copollutants, it is difficult to exclude confounding by ambient air exposures to other PM components or ambient pollutants. In several studies that analyzed PM component mixtures, of which Pb particles comprised one component,

it is not possible to attribute the observed associations or lack of associations specifically to Pb (Sarnat et al., 2008; Andersen et al., 2007; Veranth et al., 2006; Maciejczyk and Chen, 2005).

5.6.7 Immune Effects of Lead within Mixtures

Several toxicological studies published since the 2006 Pb AQCD examined immune effects resulting from exposures to metal mixtures. Some studies indicated that immune effects may be observed with lower levels of Pb exposure when they occur in conjunction with other metals. In a study of mice treated with Pb-acetate (10 mg/kg i.p. injection by weight, daily for 15 days), As (0.5 mg/kg i.p. injection by weight, daily for 15 days), or both, Bishayi and Sengupta (2006) reported a greater than additive effect of co-administered Pb and As on macrophages in decreasing bacterial resistance, myeloperoxidase (MPO) release, and NO production. Investigators assessed the Pb-As interaction on MPO release using the multivariate ANOVA 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.

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) found interactions between Pb and As among children in Mexico aged 6-11 years. Contamination of drinking water by both Pb and As was a concern in the study area; however, urinary As levels were higher in children who had lower blood Pb levels. In multiple regression analyses, urinary As was associated with lower NO release from macrophages (similar to blood Pb). An interaction was observed between Pb and As, which indicated that high internal doses of both metals were associated with a larger decrease in NO than was either metal alone (p for interaction = 0.037). Urinary As was associated with lower superoxide anion release (opposite direction of Pb). The interaction between Pb and As indicated that higher internal doses of both Pb and As were associated with a larger increase in superoxide anion than was blood Pb level alone (p for interaction = 0.042). Due to the high blood Pb in these children (means in three groups at varying distances from a Pb smelter: 7, 20.6, 30.4 µg/dL), it is not clear whether these relationships would apply to children with lower blood Pb levels.

Results from Institoris et al. (2006) indicated that metal co-exposures potentiated the effects of Pb. Lymph node weight decreased with exposure of 4 week-old rats to 20 mg/kg Pb-acetate by drinking water plus a second metal (Cd or Hg) but not with 20 mg/kg of Pb alone. In contrast with the aforementioned studies, Fortier et al. (2008) did not find Hg co-exposure to increase the effects of Pb. $PbCl_2$-exposed (7.5-20.7 µg/dL)

human leukocytes did not have alterations in lymphocyte proliferation, monocytic phagocytic activity, or NK cell activity. The combination of 20.7 µg/dL $PbCl_2$ plus 12.0 µg/dL methylmercuric chloride (MeHgCl) decreased lymphocyte proliferation; however, these effects were attributed to MeHgCl, which had a stronger suppressive effect independently. Other toxicological studies found immune effects of multiple metal mixtures that included Pb (e.g., decreased antibody titers, increased neutrophil counts) (Jadhav et al., 2007; Massadeh et al., 2007) but did not test each metal individually. Thus, it cannot be ascertained whether findings are due to interactions between Pb and other components within the mixture. Overall, results indicated that exposures to Pb-containing metal mixtures are associated with immune effects. Moreover, several studies found an interaction between Pb and metals such as As, Cd, and Hg, suggesting that a threshold for producing Pb-induced immune effects may be lower if additional metals are present.

5.6.8 Summary and Causal Determination

The collective body of evidence demonstrates that Pb exposure is associated with immune effects. The evidence indicates that rather than resulting in overt cytotoxicity to lymphoid tissues, Pb exposure is associated predominantly with subtle changes in a spectrum of cellular and humoral immune responses, most notably, altered function of T lymphocytes and macrophages, suppression of the DTH response and resistance to bacterial infection, increased IgE production, changes in cytokine production, and inflammation. The strength of evidence for Pb-associated immune effects is derived not only from the consistency of findings within particular endpoints but also from the coherence of findings among the spectrum of immune changes operating within the same pathway. In particular, the evidence integrated across the examined spectrum of immune outcomes clearly indicates that the prominent immune effect of Pb exposure is to shift responses from a Th1 phenotype toward a Th2 phenotype.

The weight of evidence is provided largely by an extensive body of animal studies characterizing effects on the broad range of immune endpoints as described above. Comprising a smaller body of evidence, epidemiologic studies in children are consistent with toxicological studies in reporting associations of blood Pb levels with indicators of increased Th2 activity, principally, higher levels of IgE, asthma, and allergy in children. Across outcomes, because most studies examined concurrent blood Pb levels, there is uncertainty regarding the critical timing, frequency, duration, and level of Pb exposures in children that contributed to observed associations. In toxicological studies, the shift to a Th2 phenotype is well characterized by observations that Pb exposures suppress the production of Th1 cytokines (e.g., IFN-γ) and increase production of Th2 cytokines (e.g., IL-4) (Section 5.6.5.4). In animal studies, the shift to Th2 cytokine production was

observed in juvenile animals with prenatal Pb exposures and in adult animals with long-term (> 4 weeks) Pb exposures [Table 5-7 in the 2006 Pb AQCD (U.S. EPA, 2006g)]. Blood Pb levels of animals were reported infrequently, and a wide range of Pb exposures was found to induce changes in cytokine levels. In a recent study, lifetime Pb exposure that resulted in blood Pb levels between 2-3 µg/dL suppressed Th1 cytokine production in adult mice (Iavicoli et al., 2006a). A few available epidemiologic studies in children (Hsiao et al., 2001; Lutz et al., 1999) found higher levels of IL-4, a Th2 cytokine, in groups of children with higher concurrent blood Pb levels (e.g., mean 8.8 µg/dL or range 15-19 µg/dL, respectively); however, these studies are limited by their lack of consideration of potential confounding variables and lack of information on the concentration-response function.

Coherence for a Pb-associated skewed ratio of cytokine production is provided by evidence demonstrating Pb-induced activation of T cells in animals, with recent studies describing mechanisms underlying T cell activation (Sections 5.6.2.1 and 5.6.2.2). A recent toxicological study expanded the extant evidence by showing in vivo and in vitro that Pb may promote Th2 responses by directly increasing production of Th2 cytokines in dendritic cells, the major effector in antigen response (Gao et al., 2007). Pb-induced T cell activation also is indicated by a relatively small body of evidence for prenatal and postnatal Pb exposures of animals resulting in the generation of autoantibodies in response to new antigens. These findings suggest that Pb exposure may increase the risk of developing autoimmune conditions (Section 5.6.4.4). In epidemiologic studies, evidence for the effects of Pb on T cells comprised associations of concurrent blood Pb levels > 10 µg/dL in children with lower T cell abundance, in particular CD3+ cells. However, the functional relevance of these changes is unclear.

In addition to T cell responses, a prominent effect of Pb exposure, as demonstrated in an extensive historical toxicological evidence base, was the induction of 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). Consistent with these observations, a previous epidemiologic study examined and found greater release of ROS and lower release of NO from macrophages, primarily in children with concurrent blood Pb levels 10.31-47.49 µg/dL (Pineda-Zavaleta et al., 2004). Misregulated inflammation represents one of the major modes of action for Pb-induced immune effects. Toxicological studies provide evidence for the modulation of inflammatory cell function, production of pro-inflammatory cytokines and metabolites, enhanced inflammatory chemical messengers, and pro-inflammatory signaling cascades. In addition to the associations reported with IL-4, epidemiologic evidence for Pb effects on inflammation is limited to a few recent studies in nonoccupationally-exposed adults in which concurrent

blood Pb level was associated with other indicators of inflammation such as CRP (Songdej et al., 2010) and IL-6 (Kim et al., 2007). The studies commonly adjusted for potential confounding by age, sex, BMI, and smoking status. However, because only concurrent blood Pb levels were examined, there is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposures that contributed to the observed associations.

The toxicological evidence for Pb-induced production of Th2 cytokines provides biological plausibility for the evidence linking Pb exposure with elevated IgE 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. Animal studies describe Pb-induced (resulting in blood Pb levels 10-30 µg/dL) increases in IgE (Snyder et al., 2000; Miller et al., 1998; Heo et al., 1996). Additionally, epidemiologic studies in children consistently demonstrated associations between higher concurrent blood Pb levels and higher in serum IgE (Section 5.6.3). While most studies found elevated IgE in groups of children with blood Pb levels > 10 µg/dL, Karmaus et al. (2005) found higher serum in IgE in children with blood Pb levels 2.8-3.4 µg/dL compared with children with lower blood Pb levels. A few studies considered potential confounding by factors such as age (Karmaus et al., 2005; Lutz et al., 1999), smoking exposure, serum lipids, organochlorine biomarkers, and number of previous infections (Karmaus et al., 2005) but not SES or allergen exposure. It is important to acknowledge the extensive evidence in animals for Pb-induced increases in IgE that is not subject to confounding by SES. Additional support for effects on IgE is provided by toxicological evidence for Pb-induced increases in Th2 cytokines. Epidemiologic studies did not find blood Pb level to be associated consistently with B cell abundance.

The toxicological evidence for Pb-induced suppression of Th1 cytokine production is coherent with historical observations in animals that Pb exposure decreases responses to antigens and bacterial infection (U.S. EPA, 2006b). Toxicological studies provide clear, consistent evidence that prenatal and postnatal Pb exposure of animals (resulting in blood Pb levels 11->100 µg/dL) suppresses the DTH response to antigens (Section 5.6.2.3), and a recent in vitro study indicates such effects may be mediated by dendritic cells (Gao et al., 2007). Recent toxicological studies provided additional support for Pb-induced decreased host resistance by demonstrating that Pb exposure impairs phagocytic and chemotactic activity of macrophages (Lodi et al., 2011; Bussolaro et al., 2008). These observations reflect suppressed Th1 activity given the role of Th1-dependent IFN-γ in enhancing the killing capacity of macrophages. Epidemiologic evidence for Pb-associated diminished response to bacterial infection is limited to previous observations of reduced neutrophil functionality in Pb-exposed workers with mean blood Pb levels > 30 µg/dL. While a few previous epidemiologic studies in children found higher prevalence of

respiratory infections in children with higher blood Pb levels (Karmaus et al., 2005; Rabinowitz et al., 1990), the findings are limited by the weak statistical methods of studies and the lack of consideration of potential confounding variables.

The effects of Pb exposure on macrophages also suggest a role for the immune system in mediating Pb-associated effects in multiple other physiological systems. A small body of new toxicological studies indicated Pb-induced changes in specialized macrophages in nonlymphoid tissue such as alveolar macrophages, testicular macrophages, and brain microglia (Section 5.6.4.5); however, these studies primarily used the i.p. route to administer Pb. Thus, the relevancy of observations to those expected from typical routes of human exposure is not clear.

Th2-dependent increases in IgE mediate type 1 hypersensitivity resulting in various allergic conditions and asthma. Observations of Pb-associated increases in Th2 cytokines and circulating IgE levels provide biological plausibility for the small body of available epidemiologic evidence indicating associations of blood Pb levels with asthma and allergic conditions in children (Jedrychowski et al., 2011; Pugh Smith and Nriagu, 2011; Joseph et al., 2005). Several of these studies considered a larger set of potential confounding variables than did studies of IgE in children. While the set of particular factors varied among studies, studies frequently considered SES indicators and/or residential allergen measurement. Low SES has been associated with higher blood Pb levels, poorer housing conditions, higher exposures to mouse and cockroach allergen, and with conditions such as asthma and allergy. Jedrychowski et al. (2011) found similar magnitudes of association between cord blood Pb level and cord blood IgE in models that did and did not adjust for potential confounding variables, including maternal education. Studies examining cognitive effects in children also found that associations with blood Pb levels with and without adjustment for SES-related variables (Section 5.3). Pugh Smith et al. (2011) and Jedrychowski et al. (2011) also indicated lack of confounding by allergen exposures and other indicators of housing condition by their inclusion as model covariates or analysis of their independent associations with outcomes. The robust association between blood Pb level and allergic sensitization (which indicates elevated allergen-specific IgE) observed in Jedrychowski et al. (2011), provides support for the associations observed between blood Pb and IgE, in children, in which confounding by SES and allergen was not considered. Collectively, these findings do not indicate that confounding by SES or allergen exposure alone accounts for associations observed between blood Pb levels and immune effects in children. This evidence for associations of blood Pb level with clinical conditions such as asthma, allergic sensitization, and allergic diseases in children expanded by results from recent studies also supports the public health significance for Pb-associated immune effects.

With respect to lifestages of Pb exposure, animal studies found that gestational Pb exposures, encompassing a wide range of concentrations, affected endpoints such as IgE, cytokine levels, and DTH but also found postnatal short-term (multiple days) long-term (multiple weeks) Pb exposures to affect cytokine levels in adult animals. The blood Pb levels and Pb exposure lifestage, magnitude, frequency, and duration associated with immune effects are not well characterized in humans. Several epidemiologic studies examined associations (of IgE, asthma, and allergy) with concurrent blood Pb levels of children > 10 µg/dL. A few studies found cord blood or newborn hair Pb levels to be associated with endpoints such as Ig levels (Annesi-Maesano et al., 2003; Belles-Isles et al., 2002) and allergic sensitization (Jedrychowski et al., 2011). The small body of epidemiologic studies of nonoccupationally-exposed adults examined different endpoints and found associations with concurrent blood Pb levels, which are influenced by current Pb exposures as well as cumulative Pb stores in bone.

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 that cumulatively promote a Th2 phenotype and hyperinflammatory state. The principal findings are Pb-induced increased production of Th2 cytokines, suppressed production of Th1 cytokines, increased inflammation, and elevated IgE, with the weight of evidence provided by toxicological studies. Collectively, these findings are coherent with the observed effects of Pb exposure on decreasing responses to antigens (e.g., DTH, bacterial resistance) in animals. Both toxicological and epidemiologic studies in children provide evidence for Pb-associated increases in IgE. The toxicological and epidemiologic findings for Th2 cytokines, IgE, and inflammation provide biological plausibility for associations observed for blood Pb levels with asthma and allergic conditions in children. Associations with asthma and allergy were observed after considering potential confounding by several factors, including, SES and allergen exposure. Animal studies found a range of immune effects with prenatal exposure in juvenile animals and long-term postnatal (> 4 weeks) Pb exposures in adult animals. The blood Pb levels and Pb exposure lifestage, magnitude, frequency, and duration associated with immune effects are not well characterized in children or adults. Epidemiologic studies of children and adults primarily examined concurrent blood Pb levels. Little information was provided on concentration-response functions. In epidemiologic studies, higher IgE and higher asthma prevalence were examined and found in children with blood Pb levels > 10 µg/dL. In the large body of studies of adults (mostly males) with occupational Pb exposures, the most consistent findings were decreased neutrophil functionality in workers with mean blood Pb levels 21-71 µg/dL. Recent epidemiologic studies provided new evidence in adults without occupational Pb exposures; however, each examined a different immune endpoint, for example, IgE, eNO, IL-6. These endpoints were associated with concurrent

blood Pb levels in populations of adults with mean blood Pb levels of 1.9-7 µg/dL; however, there is uncertainty regarding the contributions of current Pb exposures and cumulative Pb stores in bone. The consistency and coherence of findings across the continuum of related immune parameters that demonstrate a stimulation of Th2 responses in toxicological studies combined with the supporting epidemiologic evidence in children 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 red blood cells (RBCs) in children and occupationally exposed adults 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 Pb-induced 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 (blood Pb levels > 100 µg/dL) was found to decrease RBC survival in laboratory animals, as well as alter 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 has been shown to result 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 (Figure 5-45 presents 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 human Pb exposure, in that measurement of ALAD activity is correlated with blood Pb levels. Concentration-response changes in the ratio of activated/nonactivated ALAD activity in avian RBCs was observed to be 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 and potentially ultimately resulting in anemic conditions. The 2006 Pb AQCD indicated that perturbations in RBC energy metabolism may be related to significant decreases in levels of nucleotide pools, including nicotinamide adenine nucleotide (NAD), possibly due to decreased NAD synthase activity, and nicotinamide adenine nucleotide phosphate (NADP) accompanying significant increases in purine degradation products.

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 were Ca^{2+} or magnesium (Mg^{2+}) ATPases.

The 2006 Pb AQCD identified oxidative stress as an important potential mechanism of action by which Pb exposure induced effects on RBCs. Increased lipid peroxidation and inhibition of antioxidant enzymes (e.g., superoxide dismutase [SOD], catalase [CAT]) were observed following exposure to Pb.

5.7.2 Red Blood Cell Functions

As stated in the 2006 Pb AQCD, Pb poisoning has been 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 lower in non- or mildly anemic, iron-deficient 5- to 9-year old children in India fed an iron-fortified diet for 30 weeks compared to 14 weeks (mean [range]: 8.1 [3.1-219] µg/dL versus 12.1 [3.7-26.8] µg/dL; $p < 0.02$); blood Pb levels were not lower in children receiving the no iron diet for 30 weeks compared to 14 weeks (mean [range]: 10.2 [4.4-25.3] µg/dL versus 12.0 [3.8-25.5] µg/dL). Although a number of epidemiologic studies find decreases in RBCs and/or Hct levels associated with blood 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 Pb Uptake, Binding, and Transport into Red Blood Cells

The 2006 Pb AQCD reported that Pb uptake into human RBCs occurs via passive anion transport mechanisms. Although Pb can passively cross the membrane in both directions, little of the Pb is found to leave the cell after entry. Simons (1993b) found that in vitro uptake of ^{203}Pb (1-10 µM) occurred via an anion exchanger while the efflux occurred via a vanadate-sensitive pathway. After entry into the RBC, radioactive Pb was found to partition with Hb at a ratio estimated to be about 6000:1 bound to unbound (Simons, 1986). However, Bergdahl et al. (1997a) suggested that ALAD was the primary Pb binding protein and not Hb. The 2006 Pb AQCD also reported that the majority (approximately 98%) of Pb accumulates in RBC cytoplasm bound to protein and only about 2% is found in the membrane. This is related to the high ratio of Pb in RBCs compared to plasma Pb. Further information on Pb binding and transport in blood can be found in the kinetics section of 0 (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 Pb exposure has been consistently shown to shorten the RBC life span and alter RBC mobility, as of the 2006 Pb AQCD the mechanism of this was not well understood. While the mechanism is still not fully understood, there has been some indication for a role of free Ca^{2+}. There are also newer studies that examine the relationship between Pb and RBC survival, mobility, and membrane integrity. Occupational studies investigating severely Pb-intoxicated worker populations (mean blood Pb > 28 µg/dL) observed increased intracellular RBC free calcium levels ($[Ca^{2+}]_i$) and decreased RBC membrane Ca^{2+}-Mg^{2+}-ATPase activity in workers compared with unexposed controls (Abam et al., 2008; Quintanar-Escorza et al., 2007). $[Ca^{2+}]_i$ levels were highly correlated with blood Pb levels even among unexposed control populations with blood Pb levels < 10 µg/dL (mean 9.9 ± 2 µg/dL) (Quintanar-Escorza et al., 2007). Changes in $[Ca^{2+}]_i$ were associated with increased fragility of the RBCs and dramatic morphological alterations, including the increased presence of echinocytes (cells without normal biconcave shape) and crenocytes (speculated cells) in Pb-exposed workers.

Similar to the associations observed in Quintanar-Escorza et al. (2007), concentration-dependent effects were observed when RBCs from healthy human volunteers were incubated in vitro with Pb nitrate at physiologically-relevant concentrations. $[Ca^{2+}]_i$ increased in a concentration-dependent manner when RBCs were exposed to 0.2 or 0.4 µM Pb nitrate for 24 or 120 hours (0.4 µM Pb nitrate roughly approximates 10 µg/dL Pb, although concentrations in exposure media are not directly comparable to blood Pb levels) (Quintanar-Escorza et al., 2010). $[Ca^{2+}]_i$ levels were further increased at higher, less physiologically-relevant concentrations (i.e., 2-6 µM Pb nitrate). The increase in $[Ca^{2+}]_i$ levels was observed to be related to increased Ca^{2+} influx: RBCs exposed to 0.4 µM Pb nitrate for 24 hours incorporated twice as much Ca^{2+} as controls did, whereas exposure for 120 hours resulted in a fourfold increase. Concomitant to increased influx of Ca^{2+}, efflux of Ca^{2+} was observed due to reduced Ca^{2+}-Mg^{2+} ATPase activity; exposure to 0.4 µM Pb nitrate for 24 or 120 hours reduced activity by 33% and 83%, respectively, compared to controls. As was observed among severely Pb-intoxicated workers, changes in $[Ca^{2+}]_i$ were associated with increased fragility of the RBCs (measured as increased hemolysis) and dramatic morphological alterations, including the increased presence of echinocytes (cells without normal biconcave shape) and crenocytes (speculated cells) following exposure to 0.4 µM Pb nitrate. Similarly, Ciubar et al. (2007) found that RBC morphology was disrupted, with ≥ 50% RBCs having lost the typical discocytic morphology and displaying moderate to severe echinocytosis following exposure to Pb nitrate concentrations of 0.5 µM or higher for 24 hours at 37 °C. Exposure of RBCs to higher concentrations (concentrations not stated) 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 (resulting in mean [SD] blood Pb: 40.63 [9.21] µg/dL) for three months through drinking water compared to controls. Further, the cholesterol/phospholipid ratio was increased in the Pb-exposed 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 inter-connected and related hematological parameters. Most occupationally-exposed cohorts represent populations highly exposed to Pb, with mean blood Pb levels ranging from 26-74 µg/dL. Although effects observed within these cohorts may not be generalizable to the population as a whole, they are useful in demonstrating consistent effects on a number of hematological parameters, including Hb, MCV, MCH, MCHC, total RBCs, and packed cell volume (PCV) (Khan et al., 2008; Patil et al., 2006a; Patil et al., 2006b; Karita et al., 2005). Additionally, these studies were cross-sectional in design; thus, there is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed observations. Workers occupationally exposed to Pb may also have been co-exposed to other contaminants than can affect the hematological system although the potential for co-exposure was not assessed in most studies. A few occupational studies did investigate the effect of moderate occupational Pb exposure on hematological parameters. Ukaejiofo et al. (2009) studied the hematological effects of Pb in 81 male subjects moderately exposed to Pb at three different manufacturing companies in Nigeria for durations between six months and 20 years. 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 a mean 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) ($p < 0.05$). Pb-exposed workers had significantly reduced Hb and PCV levels and increased percentage of reticulocytes. Although the differences were statistically significant between the exposed and control subjects, the study authors state that the levels 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 controls in group II ($p < 0.01$). Conterato et al. (In Press) investigated hematological parameters in automotive painters exposed to Pb in Brazil. Exposed painters had a mean [SEM] blood Pb concentration of 5.4 [0.4] µg/dL compared to 1.5 [0.1] µg/dL in controls. The mean [SEM] duration of exposure to Pb in painters was 133.9 [14.5] months, whereas the controls were not occupationally exposed to Pb. Although hematocrit, hemoglobin concentration, and the number of RBCs were statistically significantly decreased in painters compared to controls, they were not

correlated with blood Pb levels. These parameters were correlated with blood Cd levels which were also statistically significantly elevated in painters compared to controls (mean [SEM]: 1.606 [0.074] versus 0.003 [0.001] µg/dL). MCHC and RBC distribution width (a measure of the variability of RBC widths) were correlated ($r = 0.23$ and 0.44, respectively; $p < 0.05$) with blood Pb although these measures were not statistically different in painters compared to controls. In an investigation of petrol workers in Sarajevo, Yugoslavia, examinations on hematological parameters were performed on the same population five years apart, in 2003 and 2008. Workers (mean [SD] duration of exposure: 12.1 [9.1] years) had increased blood Pb (mean: 5.96 µg/dL) in 2008 compared to 2003 (mean: 4.07 µg/dL; mean [SD] duration of exposure: 10.4 [5.5]) (Cabaravdic et al., 2010). In 2008, levels of MCH and MCHC were significantly decreased from levels in 2003, whereas RBC numbers, Hb, Hct, and MCV were elevated. Positive correlations were observed in all subjects between blood Pb and RBC count, Hb, and MCH ($r = 0.241$, 0.201, and 0.213, respectively; $p < 0.05$). Taken together, the above occupational studies provide consistent evidence that very high (blood Pb > 26 µg/dL) and moderate (blood Pb ~ 5.5-7.0 µg/dL) occupational exposure to Pb reduces the number of RBCs in circulation. Although this decrease in RBCs observed in highly exposed worker cohorts 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. In a non-occupational study, the associations between blood Pb levels, calcium, iron, and hemoglobin were investigated in 55 pregnant Brazilian women (21.9% 14-19 years old, 74.5% 20-34 years olds, and 3.6% ≥ 35 years old) (Zentner et al., 2008). The majority of women (across all age groups) had concurrent blood Pb levels below 5 µg/dL (58.2%), although the mean blood Pb level was not reported; only 5.4% of women had blood Pb levels above 10 µg/dL. The vast majority of women (78.2%) were also observed to have adequate levels of hemoglobin (≥ 11 g/dL). In a multiple linear regression model, blood Pb was observed to be negatively correlated with hemoglobin ($\beta = -0.359$ g/dL).

Studies in children were generally supportive of effects on hematological parameters (e.g., Hb, MCV, MCH) observed in occupational adult populations. In two cross-sectional studies of children measuring blood hemoglobin as the independent variable, blood Pb levels were observed to decrease with increasing blood Hb. Riddell et al. (2007) found that 21% of children 6 months to 5 years of age living in rural Philippines had concurrent blood Pb levels greater than 10 µg/dL (total population 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. Similarly, in children aged 6-36 months (n = 222) living in Montevideo, Uruguay, 32.9% of children had blood Pb greater than 10 µg/dL (population mean [SD]: 9.0 [6.0] µg/dL) (Queirolo et al., 2010). The mean [SD] Hb concentration was 10.5 [1.5] g/dL, and 44.1% of children were diagnosed as anemic (Hb < 10.5 g/dL).

Blood Pb levels were higher in anemic children compared to non-anemic (mean [SD]: 10.4 [6.8] versus 7.9 [5.1] µg/dL), and in bivariate regression models, blood Pb decreased 0.65 µg/dL for every 1 g/dL increase in Hb. In children younger than 18 months of age, blood Pb was, on average, 3.5 ± 1.1 µg/dL higher in anemic children. In children aged 5-9 years (n = 189) living in Cartegena, Columbia, a smaller percentage (4.7%) of children had blood Pb > 10 µg/dL (mean [SD]: 5.49 [0.23] µg/dL). The only hematological parameters that fell outside of their reference values were MCV and MCH, which were negatively correlated with blood Pb (r = -0.159 [p = 0.029] and -0.171 [p = 0.019], respectively) (Olivero-Verbel et al., 2007). RBC count, which was not observed to differ from reference values, was positively correlated with blood Pb level (r = 0.208, p = 0.004). Ahamed et al. (2006) studied 39 male urban adolescents in India who were separated into groups according to their blood Pb level (group 1: <10 µg/dL [mean 7.4 µg/dL], group 2: >10 µg/dL [mean 13.27 µg/dL]). Although the groups were similar in age (mean [SD]: 16.59 [0.91] versus 16.76 [0.90] years, respectively), height, weight, and body mass index, group 2 had a significantly lower PCV compared to group 1. In a related study, Ahamed et al. (2007) investigated the relationship between blood Pb, anemia, and other hematological parameters in urban children in India (n = 75). Children were split into two groups as above: group 1 had blood Pb <10 µg/dL (mean [SD]: 6.89 [2.44] µg/dL, n = 19), whereas group 2 had blood Pb >10 µg/dL (mean [SD]: 21.86 [7.58] µg/dL, n = 56). As with the earlier study, ages were similar between the two groups: mean [SD]: 4.68 [1.49] and 4.11 [1.77] years, respectively. Hb and Hct were significantly decreased in group 2, compared to group 1, and children in group 2 had an increased odds of anemia (OR: 2.87 [95% CI: 1.60, 2.87]) compared to group 1 after adjustment for age, sex, and area of residence. Similarly, in a study of 340 children (aged 1-5 years) from Karachi, Pakistan, mildly and severely anemic children (mean [SD] Hb levels: 8.9 [0.9] and 7.4 [0.5] g/dL, respectively) had higher blood Pb levels compared to non-anemic children (mean [SD] Hb: 12.1 [1.3] g/dL). Mean [SD] blood Pb levels in the mildly anemic, severely anemic, and nonanemic children were 14.9 [0.81], 21.4 [2.7], and 7.9 [1.7] µg/dL, respectively (p < 0.01) (Shah et al., 2010). Additionally, Hct, RBC count, and MCV were all decreased in anemic children versus non-anemic children. Although statistical analyses were not reported, the levels of Hb, Hct, RBC count, and MCV in anemic children all fell outside of the reported normal range for these parameters, whereas the reported values in non-anemic children did not. Blood Pb was negatively correlated with Hb level in all groups, with the magnitude of negative correlation increasing with increasing severity of anemia: r = -0.315 (non-anemic children), -0.514 (mild anemia), and -0.685 (severe anemia). In iron-deficient anemic children (n = 23) from Denizli, Turkey, mean (SD) serum Pb levels were statistically increased compared to healthy children (n = 179): 0.013 (0.004) versus 0.008 (0.001) µg/dL, respectively (Turgut et al., 2007). The iron-deficient children were

observed to have decreased Hb, MCV, and ferritin compared to controls, but increased RBC and RDW. In 140 children from southern Brazil aged 2-11 years, living within 25 km of a Pb smelter, blood Pb levels were not observed to differ between anemic and non-anemic children (mean [SD]: 10.36 [6.8] versus 9.73 [5.8] µg/dL, p = 0.98) (Rondo et al., 2006). However, blood Pb levels were significantly negatively correlated with Hb in anemic children (r = -0.41, p = 0.01); this relationship was not observed in non-anemic children (r = 0.018, p = 0.84).

Contrary to the observations in the above studies, Huo et al. (2007) found that children (less than 6 years of age) living near an area where electronic waste was recycled in China had significantly higher mean blood Pb levels than did children in the neighboring town with no waste recycling (15.3 versus 9.94 µg/dL). No difference was detected in the mean Hb levels of the children in the two towns (127.55 g/L in children from the waste recycling town versus 123.46 g/L in children from the town with no recycling). Equivocal findings in studies investigating associations of blood Pb levels with hematological effects in children may be due to the comparatively shorter time period and magnitude of exposure versus those seen in occupational studies.

A number of animal toxicology studies support associations of blood Pb levels with hematological parameters observed in epidemiologic 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 0.1–100 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. The concentration of Hb in the plasma of chronically-exposed rats, a marker of RBC hemolysis, was statistically significantly (p = 0.01) increased compared to that in control rats. The in vitro studies demonstrated a similar concentration-dependent increase in the amount of hemolysis, with a significant (fourfold) increase even at the lowest concentration tested (i.e., 0.1 µM). Pb-induced hemolysis in these experiments may be due to inhibition of RBC phosphoribosyltransferases (Section 5.7.5.1). Lee et al. (2005) observed that rats orally administered Pb (25 mg/kg) via gavage once a week for 4 weeks had an average plasma Pb level of 6.5 µg/dL (9.6-fold higher than that in controls, p < 0.05), and had significant decreases in Hct, Hb, and RBCs (p < 0.05). Male mice orally administered 50 mg/kg Pb nitrate in distilled water via gavage for 40 days had mean [SD] final blood Pb levels of 1.72 [0.02] µg/dL versus 0.09 [0.011] µg/dL in control mice. Pb-exposed mice had significantly reduced total RBC counts, total leukocyte counts, Hb, lymphocytes, and monocytes compared to controls (p < 0.001) (Sharma et al., 2010b). Rats exposed to 2 g/L Pb-acetate in drinking water for 30 days (blood Pb not reported) had significantly decreased RBCs, Hb, PCV, MCH, and MCHC compared to controls (p < 0.05) (Simsek

et al., 2009), and not a disruption of hematopoiesis. Mice exposed to 1 g/L Pb-acetate in drinking water (blood Pb not reported) for 90 days, but not those exposed for 15 or 45 days`, had significantly decreased RBC counts and Hct compared to controls ($p < 0.05$) (Marques et al., 2006). Spleen weights were observed to be increased relative to body weight in animals exposed to Pb for 45 days. Mice injected daily with 50 mg/kg Pb-acetate subcutaneously (blood Pb not reported) had significantly reduced Hb, MCV, MCH, and MCHC compared to controls injected with 5% dextrose (Wang et al.). In weanling rats (age = 25 days, n = 10) whose dams were exposed to 2.84 mg/mL Pb-acetate (approximating mean [SD] daily exposures of 342.57 [28.11] and 744.47 [29.27] mg/kg during gestation and lactation, respectively), blood Pb was significantly elevated compared to controls (mean [SE]: 698.1 [78.2] versus 5.4 [0.8] ng/g). The only hematological parameter affected by Pb exposure was Hct, which was decreased in exposed rats (mean [SE]: 27.3 [0.5] versus 33.4 [0.3] percent) (Molina et al., 2011).

Some toxicological studies found no evidence of hematological effects in animals following exposure to Pb. Male rats administered Pb-acetate in the drinking water for 4 weeks at concentrations ranging from 100–1,000 ppm had a concentration-dependent increase in blood Pb (range: 6.57–22.39 µg/dL) compared to controls (0.36 µg/dL), but there were no significant changes in any of the hematological parameters (complete blood cell count performed) measured at the end of treatment (Lee et al., 2006b). Slight, statistically nonsignificant increases in PS expression on RBC membranes were also observed. Similarly, exposure of male rats to 0.5% Pb nitrate in drinking water (blood Pb not reported) for three weeks had no affect on any measured hematological parameter (Gautam and Flora, 2010). In vitro experiments with rat and human blood did not demonstrate a significant increase in hemolysis after 4 hours of treatment with Pb-acetate at concentrations up to 10 µM.

Khaĭrullina et al. (2008) observed that the surface profiles of RBC membrane shadows incubated with 0.5-10 µM Pb-acetate for three hours were much smoother than were untreated RBC membranes when examined by atomic force microscopy. The authors postulate that the observed smoothing in Pb-treated RBC membranes may be due to clusterization of band 3 protein. Band 3 (anion exchanger 1 [AE1]), is a chloride/bicarbonate (Cl^-/HCO_3^-) exchanger and is the most abundant protein in RBC membranes. AE1 is integral in carbon dioxide (CO_2) transport and linkage of the cellular membrane to the underlying cytoskeleton (Akel et al., 2007; Su et al., 2007). The observed smoothing of the RBC membrane may due to Pb interfering with how the membrane attaches to the cytoskeletal structure of the RBC through perturbation of the normal activity of AE1.

Eryptosis

Eryptosis is the suicidal death of RBCs. It is characterized by cell shrinkage, membrane blebbing, and cell membrane phospholipid scrambling associated with PS exposure on the cell membrane that leads to cell destruction via macrophages (Föller et al., 2008; Lang et al., 2008). As previously reported in the 2006 Pb AQCD, Kempe et al. (2005) found that exposing human RBCs to Pb at concentrations ranging from 0.3–3 µM caused increased activation of K^+ channels that led to cell shrinkage and scramblase activation. The activation of scramblase increased the exposure to PS on the cell membrane, which causes an increase in the destruction of the RBCs by macrophages.

Shin et al. (2007) found that in vitro exposure of human RBCs to 1–5 µM Pb-acetate increased PS expression in a time- and concentration-dependent manner. The maximum mean [SE] increase in expression of PS was 26.8 ± 3.15% (compared to deionized water), observed 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 (mean [SE]: 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 concentration-dependent manner following exposure to Pb. To corroborate these findings in vivo, Shin et al. (2007) treated male rats i.p. to 25, 50, or 100 mg/kg Pb-acetate (blood Pb not reported). Expression of PS was observed to increase in a concentration-dependent manner at concentrations ≥ 50 mg/kg, confirming the in vitro results. No hemolysis or microvesicle formation was observed in the in vitro and in vivo experiments. In a follow-up study, the same lab observed that exposure of human RBCs to low concentrations of Pb-acetate (0.1-0.5 µM) induced PS expression. Most notably, exposure to 0.1 µM Pb for 24 hours increased PS expression on RBC membranes by approximately 20% (Jang et al., 2011). Accompanying the expression of PS were abnormal, echinocytic RBCs following incubation with Pb. Unlike the above study, incubation with low concentrations of Pb (0.1 µM) induced the generation of microvesicles, which also expressed PS on their membranes. Flippase was inhibited by 0.1 µM Pb following incubation for one hour, but scramblase activity was not changed at any Pb exposure concentration. The intracellular concentration of ATP was decreased at Pb concentrations 0.25 µM and greater, but $[Ca^{2+}]_i$ did not increase following exposure. This decrease in ATP levels, but lack of affect on $[Ca^{2+}]_i$ may explain why flippase activity, but not scramblase, was altered

following Pb incubation. At 0.5 µM, Pb-exposed RBCs with externalized PS were observed to be targeted and engulfed by differentiated macrophages. Similar in vitro effects were observed in rat erythrocytes, although higher concentrations were generally required. PS expression on rat erythrocytes was observed ex vivo in rats exposed to 10 or 50 mg/kg Pb. To corroborate these in vitro and ex vivo findings, rats were exposed to 0, 50, 250, or 1,000 ppm Pb-acetate in drinking water for 4 weeks. At 100 ppm, Hb and Hct were significantly decreased relative to control, and liver and spleen weights were increased. At doses greater than 50 ppm, iron accumulation was observed in the spleen, a clear sign of increased RBC clearance via phagocytosis.

Ciubar et al. (2007) also found that exposure to Pb nitrate (0.5–2 µM) resulted in an increase in PS exposure to RBCs and cell shrinkage, which authors stated were indicators of cell apoptosis. As reported above, Khaĭrullina et al. (2008) observed Pb-induced RBC membrane smoothing that may be due to alterations in AE1 activity. Disruptions in AE1 activity may also result in enhanced PS exposure and premature cell death. Akel et al. (2007) observed that in $AE1^{-/-}$ knockout mice, Pb-induced PS exposure was much greater than that in wild type mice. Decreased RBCs and increased reticulocytes were also observed, an indication of high cell turnover.

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 nonanemic tricycle taxi drivers (n=27) working in Kathmandu, Nepal (mean [SD] age: 5.6 [2.6] years). 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 mean level of serum erythropoietin (12.7 versus 18.8 mU/mL) compared to the nondrivers and there was an inverse relationship between the level of serum erythropoietin and blood Pb ($r = -0.68$, $p < 0.001$). Blood Pb level was not associated with any other hematological effects. 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 decreased erythropoietin is generally considered a measure of kidney toxicity, it can also indicate that Pb could possibly affect the level of RBCs through decreasing levels of serum erythropoietin.

Celik et al. (2005) observed that exposure of female rats to 140, 250, or 500 mg/kg Pb-acetate via gavage once per week for 10 weeks (blood Pb not reported) resulted in decreased numbers of polychromatic RBCs (PCE) and increased numbers of micronucleated PCEs, compared to controls ($p < 0.001$). Alghazal et al. (2008b) exposed

male and female rats to 100 mg/L Pb-acetate daily in drinking water for 125 days (blood Pb not reported) and observed increases in micronucleated PCEs in female rats ($p = 0.02$) but no significant reduction in the ratio of PCEs to normochromic RBCs (NCE). In male rats, an increase in micronucleated PCEs was observed ($p < 0.001$) along with a decrease in the PCE/NCE ratio ($p = 0.02$). While the results from Alghazal et al. (2008b) indicate that Pb is cytotoxic in male rats only, but is genotoxic in both sexes, results from Celik et al. (2005) indicate that Pb is cytotoxic in female rats as well. Mice exposed to 1 g/L Pb-acetate in drinking water for 90 days (blood Pb not reported) had statistically significant increases in micronucleated PCEs; a small, but statistically nonsignificant decrease in the PCE/NCE ratio was also observed (Marques et al., 2006). Cyto- and geno-toxicity in RBC precursor cells are strong indications of altered hematopoiesis in bone marrow.

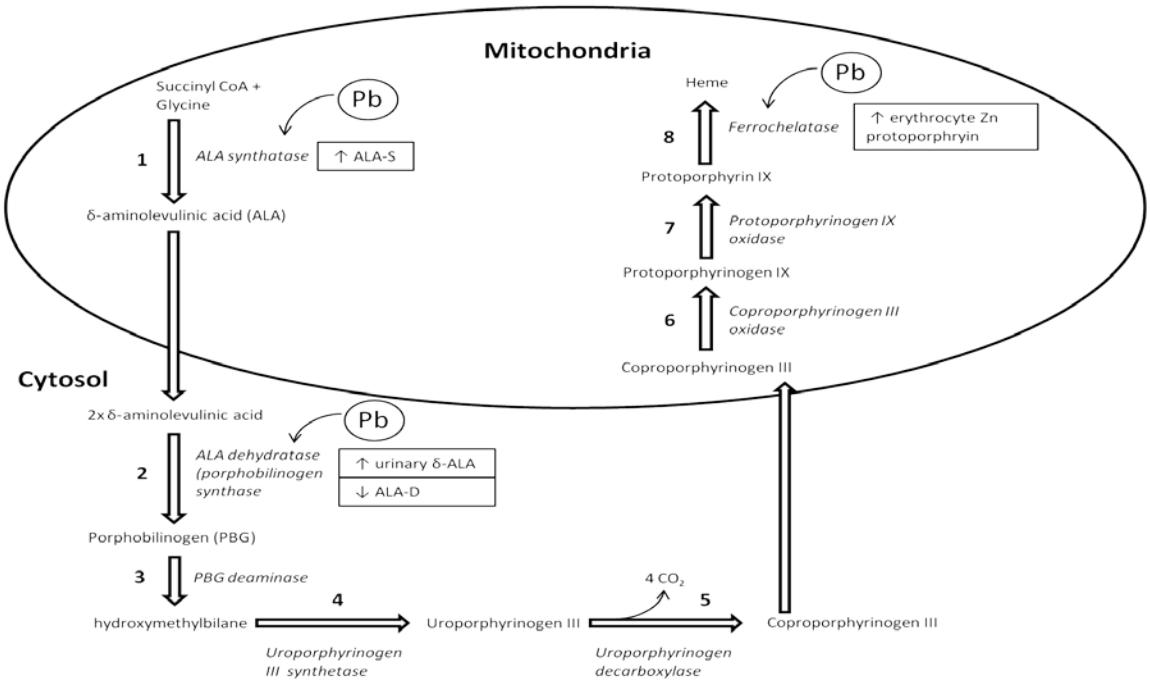
5.7.2.4 Membrane Proteins

There have been few studies examining the effects of Pb on membrane proteins since the 2006 Pb AQCD. According to the 2006 Pb AQCD, Pb has been found to affect RBC membrane polypeptides in exposed workers (Apostoli et al., 1988; Fukumoto et al., 1983). In Pb-exposed workers, 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 in association with blood Pb levels greater than 50 µg/dL. Exposure to Pb-acetate at concentrations above 0.1 µM for 60 minutes has also been found to increase the phosphorylation of proteins in human RBC membranes in vitro (Belloni-Olivi et al., 1996). Phosphorylation did not occur in cells depleted of protein kinase C (PKC), indicating a PKC-dependent mechanism.

Huel et al. (2008) found that newborn hair and cord blood Pb levels (mean [SD]: 1.22 [1.41] µg/g and 3.54 [1.72] µg/dL) 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 were cord blood Pb ($p < 0.0001$ versus $p < 0.05$). Maternal blood 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 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 5-45). The observations of decreased Hb (measured as total Hb, MCH, or MCHC) in occupationally-exposed adults (Ukaejiofo et al., 2009; Khan et al., 2008; Patil et al., 2006b; Karita et al., 2005) and Pb-exposed experimental animal models (Sharma et al., 2010b; Simsek et al., 2009; Marques et al., 2006; Lee et al., 2005) and associations with blood Pb levels in children (Riddell et al., 2007), is a direct indicator of decreased heme synthesis due to Pb exposure.



Note: Steps in the pathway potentially affected by Pb are indicated with curved arrows pointing to the affected enzyme, and effects are represented by ↑ and ↓ arrows.

Figure 5-45 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 in adults and children (see below). These studies were cross-sectional in nature. This limits their utility in assessing the magnitude, timing, frequency, and duration of Pb exposure necessary to contribute to the observed effects.

As was seen with epidemiologic studies investigating Pb-induced deficits in hematological parameters, most occupationally-exposed cohorts investigated ALAD levels in association with high blood Pb levels, i.e., > 27 µg/dL (range: 27.0-74.4 µg/dL). Although effects observed within these cohorts regarding ALAD levels may not be generalizable to the population as a whole, they are useful in demonstrating consistent and negative effects of Pb on the activity of this enzyme (Quintanar-Escorza et al., 2007; Patil et al., 2006a; Patil et al., 2006b; Ademuyiwa et al., 2005b). Occupationally-exposed adults had levels of inhibition of ALAD that were as great as 90% relative to control (Quintanar-Escorza et al., 2007). There were few studies that investigated Pb-associated decrements in ALAD levels among moderately-exposed workers. Painters in India with a mean blood Pb level of 21.92 µg/dL (mean [SD] duration of exposure: 126.08 [49.53] months) had lower ALAD levels ($p < 0.01$) compared to controls whose mean blood Pb level was 3.06 µg/dL (Mohammad et al., 2008). Stoleski et al. (2008) observed that workers in a Pb smelter in Macedonia (mean [SD]: 16.4 [8.5] µg/dL blood Pb; 18.8 [7.5] years employment) had lower ALAD activity ($p < 0.001$) and higher ALA levels ($p < 0.0005$) compared to workers with no exposure to Pb (mean [SD] blood Pb: 7.0 [5.4] µg/dL). In automotive painters exposed to Pb in Brazil (mean [SD]: 5.4 [0.4] µg/dL blood Pb; 133.9 [14.5] months duration of exposure), the ALAD reactivation index was increased over that in controls; ALAD activity did not differ between groups (Conterato et al., In Press). However, ALAD activity was negatively correlated with blood Pb ($r = -0.59$, $p < 0.05$) but not blood Cd, whereas ALAD reactivation index was positively correlated with blood levels of both metals (Pb: $r = 0.84$, $p < 0.05$; Cd: $r = 0.27$, $p < 0.05$). In a benchmark dose (BMD)-based analysis, Murata et al. (2009) calculated the BMD and 95% lower confidence limit of the BMD (BMDL) for decreased ALAD activity in RBCs of exposed Pb workers. The calculated BMD and BMDL values of 2.7 and 2.3 µg/dL, respectively, were substantially lower than the BMDs (28.7-44.2 µg/dL) and BMDLs (19.4-29.6 µg/dL) for decreased Hb, Hct, and RBC count in similarly exposed workers, indicating decreases in ALAD activity can occur at blood Pb levels that do not decrease RBC survival.

Wang et al. (2010f) found that there was also a concentration-dependent decrease in ALAD activity in both children (4-13 years old) and adults (16-77 years old) (mean blood Pb levels: 7.1 and 6.4 µg/dL, respectively) in rural southwest China. Further, Wang et al. (2010f) observed that the relationship between blood Pb and ALAD activity was nonlinear and exponential, with larger 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. (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 [mean 7.4 µg/dL], group 2: >10 µg/dL [mean 13.27 µg/dL]). Although group 1 and 2 were similar in age (mean [SD]: 16.59 [0.91] versus 16.76 [0.90] years, respectively), height, weight, and body mass index, group 2 had lower ALAD activity than did group 1 ($p < 0.001$). When all 39 adolescents were examined together, an inverse relationship was found between blood Pb and ALAD activity. Similar decreases in ALAD activity were observed in other populations of Indian children (aged 4-12 and 1-7 years) with elevated blood Pb (mean [SD]: 11.39 [1.39] and 21.86 [7.58] µg/dL) compared to children with lower blood Pb levels (mean [SD]: 3.93 [0.61] and 6.89 [2.44] µg/dL) (Ahamed et al., 2007; Ahamed et al., 2005). Decreases were also observed in children 3–6 years of age with > 10 µg/dL, compared to children < 10 µg/dL (mean blood Pb concentration for groups not reported) in northeastern China (Jin et al., 2006).

Decreased ALAD activity in response to Pb exposure is also observed in toxicological studies. 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 was associated with statistically significant decreases (approximately 50% lower than control levels) in RBC ALAD activity (Lee et al., 2005). Exposure of male Wistar rats to 0.5% Pb-acetate via drinking water for three weeks statistically significantly decreased ALAD activity 72% compared to controls (mean [SD]: 7.35 [0.35] versus 26.14 [2.19] nM/min/mL RBCs) (Gautam and Flora, 2010).

5.7.4 Other Heme Metabolism Enzymes

The 2006 Pb AQCD indicated that Pb affects RBC PBG synthase (Simons, 1995; Farant and Wigfield, 1990, 1987), PBG deaminase (Tomokuni and Ichiba, 1990), and TF endocytosis and iron transport across membranes (Qian and Morgan, 1990), all of which are directly or indirectly involved in heme synthesis. Although there are no new studies that examine the effect Pb has on the activities of other heme metabolism enzymes, a

number of studies investigated associations of blood Pb with 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 (Figure 5-45). However, Pb has been shown to inhibit this insertion of Fe^{2+} into the protoporphyrin ring and instead, Zn is inserted into the ring creating ZPP. A number of recent studies have shown that blood Pb is statistically significantly associated with increased RBC ZPP levels in adults occupationally exposed to high levels of Pb (blood Pb level > 27-54 µg/dL) (Patil et al., 2006b; Ademuyiwa et al., 2005b), workers exposed to moderate levels of Pb (blood Pb level = 21.92 µg/dL) (Mohammad et al., 2008), children aged 1-21 years (blood Pb 18-23 µg/dL) (Counter et al., 2009, 2008; Counter et al., 2007), and animals exposed to 500 ppm Pb via drinking water for 15 or 30 days (Rendón-Ramirez et al., 2007). Interestingly, Wang et al. (2010f) found that in children and adults living in a rural area of Southwest China, ZPP levels were negatively correlated with blood Pb at blood Pb levels < 10 µg/dL and were only positively correlated with blood Pb at higher blood Pb concentrations (i.e., > 10 µg/dL). The authors suggest that this may be representative of ALAD activities at low blood Pb levels, which contributes to lower ZPP levels. Scinicariello et al. (2007) performed a meta-analysis and observed that Pb-exposed individuals who carried the ALAD2 allele had slightly lower concentrations of blood ZPP levels compared to carriers of the ALAD1 allele (overall pooled standardized mean estimate: -0.09 [units not specified]; 95% CI: -0.22, 0.03, p = 0.13).

5.7.5 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 reported that Pb significantly reduces the nucleotide pool including NAD and NADP, as well as increases purine degradation products resulting in altered RBC energetics. Since the 2006 Pb AQCD, 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 a concentration range from 0.1 to 100 µM for 5–30 minutes. 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 0.1 µM concentration, but the decrease was also concentration-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 concentration-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 reported 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 is the inhibition of Ca^{2+}-Mg^{2+} ATPase. Few new studies 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 metalloproteins that use Zn to catalyze the equilibrium between carbon dioxide and bicarbonate in the cells of higher invertebrates. Although investigators found that Pb nitrate inhibited both carbonic anhydrase isozymes in a concentration-dependent manner, the concentrations used (i.e., 200-1,000 µM) were above those that would be physiologically relevant. Inhibition of isozyme I was noncompetitive, while the inhibition for isozyme II was uncompetitive. Bitto et al. (2006) examined the mechanisms of action of Pb-induced inhibition of P5N, an enzyme important in the pyrimidine salvage pathway that requires manganese for normal activity. Pb was observed to bind directly to the active site of the enzyme 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) (Nemsadze et al., 2009; Ahamed and Siddiqui, 2007). Many epidemiologic and toxicological studies have found

an association between the level of blood Pb and lipid peroxidation, antioxidant levels, or indicators of ROS production.

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. Numerous occupational studies demonstrated increased lipid peroxidation in highly-exposed worker populations (blood Pb levels ranging from 29 to 74.4 µg/dL) (Kasperczyk et al., 2009; Khan et al., 2008; Quintanar-Escorza et al., 2007; Patil et al., 2006a; Patil et al., 2006b). There was a correlation between MDA levels and blood Pb even in the unexposed workers who had lower (i.e., <12 µg/dL) blood Pb levels, although the magnitude of correlation in exposed workers was greater. (Quintanar-Escorza et al., 2007). Increases in C-reactive protein and decreases in RBC SOD, catalase, and plasma ceruloplasm were also observed in these workers, further indicating increased RBC oxidative stress due to higher Pb exposure.

Evidence of lipid peroxidation was also observed in occupational cohorts moderately exposed to Pb. These studies were cross-sectional in design; thus, there is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations. In auto repair apprentices in Turkey (mean [SD]: 16.8 [1.2] years of age, 3.8 [1.8] years duration of exposure) with blood Pb levels as low as 7.9 µg/dL (Ergurhan-Ilhan et al., 2008), increases in glutathione peroxidase (GPx) and MDA, as well as decreases in α-tocopherol and β-carotene were observed compared with controls (compared to 2.6 µg/dL in controls; mean [SD] age: 16.3 [1] years). Decreases were observed in SOD and CAT, but the results did not attain statistical significance. In painters in India (mean [SD] duration of exposure: 126.08 [49.53 months]) with a mean 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). In automotive painters in Brazil (mean [SE] blood Pb: 5.4 [0.4] µg/dL), glutathione-*S*-transferase, GPx, and SOD were positively correlated with blood Pb ($r = 0.34$, 0.38, and 0.32, respectively; $p < 0.05$) (Conterato et al., In Press). Similar effects on indices of oxidative stress were observed in in vitro studies: increased MDA and decreased SOD and catalase in RBCs exposed to 2 µM Pb (Ciubar et al., 2007), decreased glutathione reductase (GR) activity in human RBCs incubated with 5-18 µM Pb (Coban et al., 2007), and decreased GSH and increased GSSG and lipid peroxidation

in RBCs from healthy volunteers with no Pb exposure incubated with 0.4 µM Pb for 24-120 hours (Quintanar-Escorza et al., 2010).

Evidence of lipid peroxidation was also observed in children moderately exposed to Pb; as with the occupational studies, their cross-sectional design limits their utility in assessing the magnitude, timing, frequency, and duration of Pb exposure necessary to elicit the observed effects. 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 [SD] 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. The means (SDs) were: 3.93 (0.61) µg/dL group I; 7.11 (1.25) µg/dL group II; and 11.39 (1.39) µg/dL group III. Lipid peroxidation, measured as blood MDA, was statistically significantly greater in group III, compared to group II and I, whereas GSH was lower 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 [mean 7.4 µg/dL], group 2: >10 µg/dL [mean 13.27 µg/dL]). Although the groups were similar in age (mean [SD]: 16.59 [0.91] versus 16.76 [0.90] years, respectively), height, weight, and 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, investigators found a correlation between blood Pb level and blood MDA and RBC CAT levels, as well as an inverse relationship between ALAD activity and MDA and CAT levels. In a similar study, Ahamed et al. (2008) examined oxidative stress in Indian children (aged 3-12 years) with neurological disorders. There was a significantly higher mean blood Pb level in the study population compared to the control healthy 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) were found to be correlated with lipid peroxidation and oxidative stress. Children aged 3–6 years old living near a steel refinery in China with blood Pb levels ≥ 10 µg/dL also had a significant increase in plasma MDA compared to the children with blood Pb levels < 10 µg/dL. However, levels of RBC SOD, GSH, and GPx were not different from those in controls (Jin et al., 2006).

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 (Lee

et al., 2005). Other indications of Pb-induced oxidative stress included significant increases in RBC SOD and CAT levels accompanied by significant decreases in GSH and GPx. Exposure of rats to 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, with simultaneous increased activity of SOD and GPx (Kharoubi et al., 2008b). CAT activity was also slightly elevated in Pb-exposed rats, but the increase failed to reach statistical significance. Exposure of male rats to 0.5% Pb nitrate in drinking water (blood Pb not reported) for three weeks decreased GSH levels compared to that in controls (mean [SE]: 1.91 [0.02] versus 2.44 [0.09] mg/mL, respectively) (Gautam and Flora, 2010). SOD activity was significantly decreased in rats injected with 15 mg/kg Pb i.p. for seven days, but not rats treated with 5 mg/kg Pb (Berrahal et al., 2007). GPx activity and MDA concentrations were slightly elevated in the exposed groups, but differences with the control group 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 is 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. (2007), found that Pb-induced 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 2,000 ppm Pb in drinking water for 5 weeks, MDA levels were significantly increased, whereas vitamin E concentrations were significantly decreased (Caylak et al., 2008). In the case of MDA, co-exposure to Pb and a number of sulfur-containing antioxidants (e.g., L-methionine, N-acetylcysteine, and L-homocysteine) reduced concentrations to a level not statistically significantly different from that in

controls, but statistically smaller than concentrations observed with Pb alone. Exposure to L-methionine and N-acetylcysteine also reduced Pb-induced depletion of vitamin E.

5.7.7 Summary and Causal Determination

There is consistent toxicological and epidemiologic evidence indicating that exposure to Pb affects hematological endpoints, including decreased RBC survival and function, altered heme synthesis, and increased RBC oxidative stress. Pb has been shown to preferentially partition into RBCs following exposure, with RBC concentrations approximately 100-fold greater than those observed in the plasma (Jin et al., 2008; Timchalk et al., 2006).

Elaborating on the body of evidence presented in the 2006 Pb AQCD, recent epidemiologic and toxicological studies continue to demonstrate that Pb exposure is associated with decreased RBC survival and function, with the largest body of evidence consisting of populations of adults and children in which Pb is associated with effects on several inter-connected and related hematological parameters such as Hb, PCV, MCV, MCH, and MCHC. For adult populations, the largest body of evidence consists of occupationally-exposed workers in which measures of RBC survival are altered when compared with unexposed control populations (Cabaravdic et al., 2010; Ukaejiofo et al., 2009; Khan et al., 2008; Patil et al., 2006a; Patil et al., 2006b; Karita et al., 2005; Conterato et al., In Press). Although the mean blood Pb level in most occupationally exposed populations was in excess of 20 µg/dL, decreases in Hb and PCV were observed in adults with blood Pb levels of 7 µg/dL (compared to controls with blood Pb levels of 3 µg/dL), and significant correlations were observed between RBC distribution width and MCHC and blood Pb levels in adult populations with mean blood Pb levels of 5.4 µg/dL. Only one non-occupational study was found investigating the association of Pb with hematological parameters; in pregnant women, blood Pb levels were found to be negatively correlated with Hb concentrations. Studies in children measuring concurrent blood Pb levels are generally in agreement with those investigating occupationally-exposed adults regarding effects on hematological parameters (i.e., Hb, MCV, (Queirolo et al., 2010; Shah et al., 2010; Ahamed et al., 2007; Huo et al., 2007; Olivero-Verbel et al., 2007; Riddell et al., 2007; Turgut et al., 2007; Ahamed et al., 2006; Rondo et al., 2006). Any differences in the effects on specific hematological parameters observed between study findings for adults and those for children may be due to the comparatively shorter duration and lower magnitude of Pb exposure experienced by children compared to adults, although there is uncertainty regarding the timing and duration of exposure needed to induce effects in adults. In addition, Pb 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 in studies of occupationally exposed adults (Quintanar-Escorza et al., 2007) and in in vitro studies (Quintanar-Escorza et al., 2010; Ciubar et al., 2007). Heul et al. (2008) observed a reduction of RBC Ca^{2+}-Mg^{2+}-ATPase activity in children in association with a concurrent group mean cord blood Pb level of 3.54 µg/dL. 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 (Sharma et al., 2010b; Baranowska-Bosiacka et al., 2009; Simsek et al., 2009; Lee et al., 2005). Pb exposure has also been observed to increase PS expression on RBC membranes, leading to cell shrinkage, erythropoiesis, and destruction of the RBCs by macrophages (Jang et al., 2011; Ciubar et al., 2007; Shin et al., 2007). Suggestive evidence of disrupted hematopoiesis evidenced by decreased serum erythropoietin was observed in occupationally-exposed adults with a mean blood Pb level of 6.4 µg/dL (Sakata et al., 2007); toxicological studies in rats also indicate that Pb is cytotoxic to RBC progenitor cells (Alghazal et al., 2008b; Celik et al., 2005). Taken together, these studies provide consistent evidence that exposure to Pb affects 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.

Confirming effects observed in the 2006 Pb AQCD, a large body of evidence consisting of cross-sectional epidemiologic studies measuring concurrent blood Pb in adults and children have found that decreases in RBC ALAD levels and activity are strongly associated with higher blood Pb levels (Wang et al., 2010f; Mohammad et al., 2008; Ahamed et al., 2007; Quintanar-Escorza et al., 2007; Ahamed et al., 2006; Patil et al., 2006a; Patil et al., 2006b; Ademuyiwa et al., 2005b; Ahamed et al., 2005; Conterato et al., In Press). Although the body of evidence is smaller than for humans, decreases in blood ALAD activity were also seen in rats with increased blood Pb levels, compared to controls (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 children and occupationally-exposed adults (Counter et al., 2009, 2008; Mohammad et al., 2008; Counter et al., 2007; Patil et al., 2006b; Ademuyiwa et al., 2005b) and animals (Rendón-Ramirez et al., 2007). Pb has also been shown to inhibit the in vitro 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 adult populations (Ergurhan-Ilhan et al., 2008; Khan et al., 2008; Mohammad et al., 2008;

Quintanar-Escorza et al., 2007; Patil et al., 2006a; Patil et al., 2006b). Other changes in oxidative stress parameters observed included lowered activities of SOD, GR, and CAT, and increased CRP. Altered indices of RBC oxidative stress were also seen in adolescents and children in association with blood Pb levels (Ahamed et al., 2008; Ahamed et al., 2006; 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 ameliorate at least partially Pb-induced oxidative stress in RBCs (Caylak et al., 2008; Casadoa et al., 2007; 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 (Sections 7.4.1.2 and 7.4.2.2). 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 summary, new epidemiologic and toxicological studies included in the current review provide strong evidence that exposure to Pb is associated with numerous deleterious effects on the hematological system, including effects on RBC survival and function, altered heme synthesis, and increased oxidative stress, and continue to confirm previous conclusions from the 2006 Pb AQCD. The principal finding regarding RBC survival and function are consistent Pb-induced alterations in several inter-connected and related hematological parameters such as Hb, Hct, and MCV across multiple studies, with the weight of evidence provided by epidemiological studies in occupationally-exposed adult populations and children. In occupationally-exposed adults, these findings are most substantiated in populations with current blood Pb levels > 20 µg/dL, although effects on hematological parameters were observed in some occupationally-exposed populations at concurrent blood Pb levels in the range of 5-7 µg/dL. In Pb-exposed children, effects on hematological parameters were most substantiated in populations with blood Pb levels less than 15 µg/dL. The weight of evidence in adult rodents exposed long-term to Pb (i.e, ≥ 4 weeks), although less than the weight of evidence in humans, is coherent with epidemiologic studies regarding decrements in hematological parameters at blood Pb levels as low as 6.6-7.1 µg/dL. Regarding alterations in heme synthesis, the largest body of evidence again is provided by decreased ALAD activity observed in epidemiologic studies in occupationally-exposed adult populations and children. In the occupationally-exposed adult populations, the observation of decreased ALAD activity was most often observed in populations with concurrent blood Pb levels >15 µg/dL. In children, decreases in ALAD activity were observed in populations with concurrent blood Pb

levels in the range of 7-22 µg/dL. Animal toxicological studies also provide to the weight of evidence regarding altered ALAD activity, with effects seen in adult animals exposed for 3-4 weeks with blood Pb levels as low as 6.5 µg/dL. As for the effects listed above, the weight of evidence for oxidative stress (i.e., increased lipid peroxidation or alterations in antioxidant enzyme levels) primarily comes from epidemiological studies in occupationally-exposed adults and children. The majority of evidence for increased oxidative stress in Pb-exposed adults comes from occupational cohorts with concurrent blood Pb levels >15 µg/dL. In children, concurrent blood Pb levels of 7-22 µg/dL were associated with measures of oxidative stress. Due to the cross-sectional nature of the above epidemiologic studies in adults and children, and the measurement of concurrent blood Pb, the timing and duration of exposure necessary to alter RBC survival and function, heme synthesis, or the state of oxidative stress in RBCs is unclear. This uncertainty is greatest in adults as concurrent blood Pb levels reflecting recent exposures is likely to be less than blood Pb levels resulting from past exposures. The consistency of findings in epidemiologic studies investigating effects in adults and children, and the coherence of findings in the toxicological literature and coherence across the disciplines is sufficient to conclude that a causal relationship exists between Pb exposures and effects on heme synthesis and red blood cell function.

5.8 Reproductive and Developmental Effects

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 populations without occupational exposures, with environmentally-relevant levels of 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 and pregnancy or lactation. More detailed discussion of these measures and Pb transfer via umbilical cord blood Pb across the placenta, and via lactation is given in Section 4.3.5.2 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 blood Pb levels and pregnancy-induced hypertension.

Overall, the recent reproductive literature continues to support associations reported in earlier Pb AQCDs between Pb exposure and effects on various parameters of sperm (function, motility, count, integrity, histology). The toxicological and epidemiologic literature also indicates that Pb exposure is associated with delayed onset of puberty in both males and females. The new information from epidemiologic and toxicological studies is integrated with conclusions from previous Pb AQCDs below.

5.8.1 Effects on Female Reproductive Function

The epidemiologic studies on Pb and female reproductive function presented in the 2006 Pb AQCD (U.S. EPA, 2006b) provided little evidence for an association between Pb biomarkers and effects on female reproduction and fertility. However, the 1986 and 2006 Pb AQCDs (U.S. EPA, 2006b, 1986a) reported toxicological findings that Pb exposure was associated with effects on female reproductive function that can be classified as alterations in female sexual maturation, effects on fertility and menstrual cycle, endocrine disruption, and changes in morphology or histology of female reproductive organs including the placenta. Since the 2006 Pb AQCD, many epidemiologic studies have been published regarding Pb biomarker levels in women and reproductive effects. For some effects, there are inconsistent findings, but for others, such as delayed puberty, there are clear associations with blood Pb levels. 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 epidemiologic studies have examined the association between blood Pb levels and hormone levels and the estrus cycle. Epidemiologic studies (characterized in Table 5-30; all studies included in the table used measures of Pb and hormones that were either concurrent or close in time) support the toxicological findings, which are the major body of evidence on endocrine effects of Pb.

Table 5-30 Summary of recent epidemiologic studies of associations between Pb levels and hormones for females

Reference[a]	Study, Location, and Years	Outcome	Study population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Krieg et al. (2007)	U.S. 1988-1994	FSH, LH	Women aged 35-60 from the NHANES III study	Blood Pb	2.8	Linear regression slope (95% CI) for log-transformed Pb FSH: Post-menopausal 22.2 (13.5, 30.8) Pregnant 0.1 (-0.1, 0.3) Menstruating at time of exam 2.1 (-2.1, 6.3) Both ovaries removed 32.6 (10.1, 55.1) Birth control pills being used -6.3 (-10.0, -2.5) Pre-menopausal 8.3 (3.8, 12.7) LH: Post-menopausal 6.2 (3.0, 9.5) Pregnant -0.8 (-1.9, 0.4) Menstruating at time of exam -0.3 (-1.8, 1.3) Both ovaries removed 10.0 (1.1, 18.9) Birth control pills being used: -0.6 (-2.9, 1.6) Pre-menopausal 1.7 (-0.6, 4.1)
Chang et al. (2006)	Kaohsiung City, Taiwan 1999, 2000-2001	Estradiol	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)	Linear regression β(SE) for Pb 1.18 (0.60) p-value: 0.049
Pollack et al. (2011)	Buffalo, NY 2005-2007	FSH, estradiol, LH, progesterone, and cycle length	Healthy, premenopausal women aged 18-44 with menstrual cycle length of 21-35 days, BMI of 18-35 kg/m^2, not recently using birth control, not planning to become pregnant, and not breast feeding	Blood Pb	0.93 IQR: 0.68, 1.20	Mean % Estradiol 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 8.2 (-1.2, 18.6) 1.11-6.20 µg/dL: 4.7 (-4.7, 15.2) Amplitude Estradiol 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.01 (-0.06, 0.04) 1.11-6.20 µg/dL: -0.02 (-0.7, 0.03) Phase Shift Estradiol 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.09 (-0.24, 0.05) 1.11-6.20 µg/dL: 0.14 (-0.01, 0.29) Mean % FSH 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 8.0 (-0.9, 17.7) 1.11-6.20 µg/dL: 3.6 (-5.3, 13.3) Amplitude FSH 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.01 (-0.03, 0.02) 1.11-6.20 µg/dL: -0.02 (-0.04, 0.01) Phase Shift FSH

Reference[a]	Study, Location, and Years	Outcome	Study population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
						0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.06 (-0.25, 0.12) 1.11-6.20 µg/dL: -0.02 (-0.21, 0.18) Mean % LH 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 5.1 (-5.1, 16.4) 1.11-6.20 µg/dL: -0.5 (-10.5, 10.7) Amplitude LH 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.01 (-0.03, 0.02) 1.11-6.20 µg/dL: -0.02 (-0.04, 0.01) Phase Shift LH 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.16 (-0.36, 0.03) 1.11-6.20 µg/dL: -0.11 (-0.32, 0.10) Mean % Progesterone 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 7.5 (0.1, 15.4) 1.11-6.20 µg/dL: 6.8 (-0.8, 14.9) Amplitude Progesterone 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 0.07 (0.01, 0.15) 1.11-6.20 µg/dL: -0.06 (-0.13, 0.01) Phase Shift Progesterone 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 0.04 (-0.06, 0.15) 1.11-6.20 µg/dL: 0.15 (0.05, 0.26) Linear models β (95% CI) Estradiol 0.03 (-0.05, 0.11) FSH -0.01 (-0.07, 0.06) LH 0.02 (-0.06, 0.10) Progesterone 0.06 (-0.04, 0.17) OR (95% CI) for anovulation per l µg/dL 1.20 (0.62, 2.34)

Reference[a]	Study, Location, and Years	Outcome	Study population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Jackson et al. (2011)	Buffalo, NY 2005-2007	FSH, estradiol, LH, progesterone, and cycle length	Healthy, pre-menopausal women aged 18-44 with menstrual cycle length of 21-35 days, BMI of 18-35 kg/m^2, not recently using birth control, not planning to become pregnant, and not breast feeding	Blood Pb	Median: 0.87 IQR: 0.68, 1.20	Adjusted percent change (95% CI) in serum hormone level for change in blood Pb FSH: -2.5 (-11.2, 7.0) Estradiol: 4.9 (-5.0, 15.9) LH: 2.5 (-12.3, 19.9) Progesterone: 4.6 (-12.2, 24.6) Cycle length: 0.2 (-2.8, 3.3) OR (95% CI) per unit Pb <25 d vs. 25-35 d cycle length: 0.9 (0.4, 2.3) >35 d vs. 25-35 d cycle length: 0.5 (0.1, 1.9)

[a]Studies are presented in order of first appearance in the text of this section.

An epidemiologic study using the NHANES III data and including women aged 35-60 years old examined the relationship between blood Pb levels (mean 2.8 µg/dL) and serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Krieg, 2007). Deviation from normal FSH and LH levels may indicate endocrine disruption related to ovary functioning. Researchers found that higher blood Pb levels were associated with higher levels of serum FSH and LH among both postmenopausal women and women with both ovaries removed. There was also a trend of increasing serum FSH with blood Pb levels for pre-menopausal women who were not menstruating at the time of the exam or pregnant, although the association was not statistically significant for LH. A limitation of this portion of the study is that FSH and LH were measured without attention to day of a woman's menstrual cycle and LH and FSH are known to vary throughout the cycle of non-menopausal, cycling women who are not taking birth control pills. Higher blood Pb levels were associated with lower levels of serum FSH among women taking birth control pills. The inverse association was also present for LH, but it was not statistically significant. No associations between blood Pb and FSH or LH were apparent for women who were menstruating at the time of the exam or were pregnant. Further analysis indicated that the lowest level of blood Pb for which a statistically significant association between blood Pb and FSH could be observed was 1.7 µg/dL among women with their ovaries removed. For LH, the lowest level of blood Pb for which a statistically significant association between blood Pb and LH could be observed was 2.8 µg/dL among postmenopausal women. Another epidemiologic study was performed in Kaohsiung City, Taiwan among two groups of women aged 23-44 years: those who were seeking help at a fertility clinic after one year of trying to conceive, and those who had previously delivered an infant and were identified from medical records of a postpartum care unit (Chang et al., 2006). The mean (SD) blood Pb in this study was 3.12 (0.19) µg/dL. The study reported a positive association between blood Pb levels and serum estradiol

concentrations during the early follicular phase, which reflects ovary activity. Associations between hormones and blood Pb level were also investigated using the BioCycle study cohort (Jackson et al., 2011; Pollack et al., 2011). These women were premenopausal with normal cycles and not on birth control. Neither study detected an association between unit change in blood Pb and hormone levels. However, when examining tertiles of Pb, women in the highest tertile blood Pb (1.11-6.20 µg/dL) had higher mean progesterone and longer length of a phase shift compared to women in the lowest tertile (0.30-0.72 µg/dL) (Pollack et al., 2011). Other associations were observed but were not statistically significant (Pollack et al., 2011). No associations were detected for anovulation (Pollack et al., 2011) or for cycle length (Jackson et al., 2011).

The effect of Pb exposure on the female endocrine system was demonstrated in toxicological studies reviewed in the 1986 and 2006 Pb AQCD (U.S. EPA, 2006b, 1986a). However, the mechanism by which Pb affects the endocrine system has not been fully elucidated. Several recent articles continue to demonstrate that Pb alters the concentration of circulating hormones in female experimental animals. As mentioned in the previous AQCD, Pine et al. (2006) observed that maternal Pb exposure (during gestation and lactation) caused a decrease in basal LH levels in pre-pubertal female Fisher 344 rat pups as compared to control, non-Pb exposed pups. Dumitrescu et al. (2008a) observed alteration of hormone levels in female Wistar rats after ingesting Pb-acetate (50, 100, 150 ppb) in drinking water for 6 months; measurements were made during the pro-estrous stage of the estrous cycle to allow for consistent timing for comparison of cyclic hormonal variation. The authors reported decreases in FSH, estradiol, and progesterone levels with increases in LH and testosterone levels. Nampoothiri and Gupta (2008) administered Pb-acetate 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- hydroxysteroid dehydrogenase (HSD) and 17β-HSD, activity in reproductive organs, as well as a decrease in steroid hormones (progesterone and estradiol), suggesting that chronic exposure to low levels of Pb may affect reproductive function of mothers and their offspring. Similarly, Pillai et al. (2010) reported impaired ovarian steroidogenesis in Charles Foster adult female rats (PND56) from dams exposed gestationally and lactationally to Pb-acetate (subcutaneous daily injections of 0.05 µg/kg BW). Pillai observed a decrease in steroidogenic enzymes, 3β-HSD and 17β-HSD, but saw no changes in ovarian steroidogenic acute regulatory protein (StAR) or CYP11 mRNA levels indicating Pb-induced inhibition of ovarian steroidogenesis.

Kolesarova et al. (2010) conducted an in vitro study to examine the secretory activity of porcine ovarian granulose cells after Pb administration. The results of the study showed

that Pb-acetate concentrations of 0.046 mg/mL and 0.063 mg/mL statistically significantly inhibited insulin-like growth factor-1 (IGF-1) release, but concentrations of 0.25 mg/mL and 0.5 mg/mL 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 and Gupta, 2006).

No recent toxicological studies were found that examined Pb-induced effects on the estrus cycle.

Overall, toxicological studies report alterations in hormone levels related to blood Pb concentration. Similarly, epidemiologic studies reported associations between blood Pb levels and hormone levels in female adults. Although Pb-associated changes in hormone levels are observed, there are discrepancies about the direction of the hormone changes related to Pb. One explanation is that the direction of change 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, 2006b). Recent epidemiologic studies and studies in experimental animals support this finding. The epidemiologic studies are summarized in Table 5-31. Most of these studies examined biological measures of Pb collected at or during the period of possible fertilization, although Bloom et al. (2011a) measured blood Pb at baseline and followed women for at least 12 menstrual cycles (or until pregnancy).

Table 5-31 Summary of recent epidemiologic studies of associations between Pb levels and fertility for females

Reference[a]	Study, Location, and Years	Outcome	Study population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Chang et al. (2006)	Kaohsiung City, Taiwan 1999, 2000-2001	Infertility	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) Infertility ≤ 2.5 µg/dL: 1.00 (Ref) >2.5 µg/dL: 2.94 (1.18, 7.34)
Al-Saleh et al. (2008a)	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)
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
Bloom et al. (2010)	California 2007-2008	Oocyte maturity, oocyte fertilization	Women who were part of the Study of Metals and Assisted Reproductive Technologies (SMART): women referred to the Center for Reproductive Health of UCSF for infertility treatment and their first IVF procedure	Blood Pb	0.82 (0.32)	RR per 1 µg/dL Oocyte maturity (determined by Metaphase II arrest): 0.54 (0.31, 0.93) 0.25 (0.03, 2.50)* Oocyte fertilization: 0.97 (0.66, 1.43) 1.09 (0.72, 1.65)* *Controlling for Cd

Reference[a]	Study, Location, and Years	Outcome	Study population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Bloom et al. (2011b)	California 2007-2008	Embryo cell number, embryo fragmentation score	Women who were part of the Study of Metals and Assisted Reproductive Technologies (SMART) (women referred to the Center for Reproductive Health of UCSF for infertility treatment and their first IVF procedure) and who generated embryos	Blood Pb	0.83 (0.30)	OR per 1 µg/dL* Embryo cell number: 0.25 (0.07, 0.86) Embryo fragmentation score: 1.71 (0.45, 6.56) *Adjusted for Hg and Cd
Bloom et al. (2011a)	New York 1996-1997	Achieving pregnancy	Women who were aged 18-34 years, were previously part of a study five years prior about fish consumption, and were not currently pregnant	Blood Pb	No positive pregnancy test: 1.55 (0.16) Positive pregnancy test: 1.54 (0.12)	β (95% CI) -0.031 (-1.066, 1.004) per 0.6 µg/dL

[a]Studies are presented in order of first appearance in the text of this section.

Epidemiologic studies examined women having difficulty conceiving by performing studies among patients of fertility clinics or undergoing in vitro fertilization (IVF). Among women aged 23-44 years, a difference in blood Pb was reported between women who were seeking help at a fertility clinic after one year of trying to conceive and women who had previously delivered an infant and were identified from medical records of a postpartum care unit at a medical center (Chang et al., 2006). Higher odds of infertility were observed when comparing women with blood Pb levels >2.5 µg/dL to those with blood Pb levels ≤ 2.5 µg/dL. Another study examining fertility reported on women in Saudi Arabia aged 19-50 years who were undergoing IVF treatment (Al-Saleh et al., 2008a). Women were categorized as having achieved a pregnancy versus not having achieved a pregnancy and achieved fertilization versus not achieving fertilization. The majority of women had follicular Pb levels that were below the limit of detection, whereas less than 2% of women had blood Pb levels below the limit of detection. In addition, less than 2% of women had blood Pb levels that were above 10 µg/dL. Follicular Pb levels were not correlated with the blood Pb. No association was observed between blood or follicular Pb and pregnancy outcomes in either crude or adjusted models. An association was not detected between follicular Pb and fertilization, but higher blood Pb was associated with lower rates of fertilization. Finally, a study that included nine women undergoing IVF treatment in Rhode Island (Silberstein et al., 2006) found that median follicular Pb levels in women who achieved pregnancy were lower than the follicular Pb levels among nonpregnant women. 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 participants are not samples of the general population and therefore cannot be generalized to all women of childbearing age. The Study of Metals and Assisted Reproductive Technologies (SMART) enrolled women

undergoing their first round of IVF and investigated multiple steps before pregnancy as the outcomes (Bloom et al., 2011b; Bloom et al., 2010). Higher blood Pb levels were associated with lower oocyte maturity although the lack of power made interpretation of models controlling for Cd difficult. No association was observed between blood Pb and oocyte fertilization (Bloom et al., 2010). When examining markers of IVF success, inconsistent results were observed. Embryo cell number was lower in association with higher blood Pb levels but no association was observed for embryo fragmentation score (Bloom et al., 2011b). The study was limited by the small number of participants. Overall, these epidemiologic studies examine a variety of fertility-related endpoints and although some studies demonstrate an association between higher Pb levels and fertility/pregnancy, as a whole the results are inconsistent across studies.

A prospective cohort study enrolled women who previously participated in a study of fish consumption for a length of <1 to >6 menstrual cycles and investigated the relationship between blood Pb levels and having a positive pregnancy test (Bloom et al., 2011a). No association was observed between blood Pb and achieving pregnancy.

Animal toxicology studies following female fertility looked at various outcomes. Several studies observed a decrease in litter size when females were exposed to Pb before mating or during pregnancy (Dumitrescu et al., 2008b; Iavicoli et al., 2006b; Teijon et al., 2006). Pups in a study by Teijon et al. (2006) receiving 400 ppm Pb-acetate in drinking water had blood Pb of 97 µg Pb/dL blood at 1 week post-weaning and 18.2 µg Pb/dL blood at 2 week post-weaning. Dumitrescu et al. 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. (1998b), who did not observe differences in sex ratio dams and offspring were exposed only during pregnancy. Thus, Pb exposure in animal studies during or before pregnancy have shown effects on litter size and mixed effects on sex ratio.

Nandi et al. (2010) demonstrated a concentration-dependent decline in viability rate, maturation, fertilization, and cleavage rates of buffalo oocytes cultured in medium containing 1-10 µg/mL Pb-acetate. Karaca and Şimşek (2007) observed an increase in the number of mast cells in ovary tissue after Pb exposure (2,000 µg/mL in drinking water) suggesting that Pb may stimulate an inflammatory response in the ovaries which may contribute to Pb-induced female infertility.

In contrast, Nampoothiri and Gupta (2008) did not observe any statistically significant change in fertility rate or litter size in female rats subcutaneously administered Pb

(0.05 mg/kg body weight daily before mating and during pregnancy) with a resulting blood Pb of 2.49 µg/mL. Although reproductive performance was not affected in this study, the authors did report an alteration in implantation enzymes. Cathepsin-D activity decreased and alkaline phosphatase activity increased after Pb exposure.

Recent epidemiologic and toxicological studies on the effect of Pb on fertility outcomes have generated inconsistent results. However, the bulk of the evidence including the current and historical Pb literature (U.S. EPA, 2006b) indicate 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 biomarkers of pubertal development. There have also been recent epidemiologic studies examining associations between blood Pb levels and onset of puberty, which are summarized in Table 5-32 and in the text below. All of the epidemiologic studies examined concurrently measured blood Pb and puberty and are reported below. Additionally, Naicker et al. (2010) followed girls to determine their age of menarche; however, blood Pb levels were measured once at 13 years of age.

Table 5-32 Summary of recent epidemiologic studies of associations between Pb levels and puberty for females

Reference[a]	Study Location and Years	Outcome	Study Population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Tomoum et al. (2010)	Cairo, Egypt 2007	Hormones and pubertal development	Healthy children aged 10-13 yr; seeking treatment for minor health problems and living in one of two designated areas (one with high-risk for Pb contamination and one with no Pb source)	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% ≥ 10 µg/dL: Stage 2: 100% Stage 3: 0% Chi-square p-value<0.01 Pubic Hair Development <10 µg/dL: Stage 2: 36.4% Stage 3: 63.6% ≥ 10 µg/dL: Stage 2: 77.8% Stage 3: 22.2% Chi-square p-value>0.05 *Quantitative results for hormones not provided
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%
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) ≥ 5 µg/dL: 2.34 (1.45, 3.79) Delay in pubic hair development at age 13 <5 µg/dL: 1.00 (Ref) ≥ 5 µg/dL: 1.81 (1.15, 2.84) Delay in attainment of menarche at age 13 <5 µg/dL: 1.00 (Ref) ≥ 5 µg/dL: 2.01 (1.38, 2.94)

Reference[a]	Study Location and Years	Outcome	Study Population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Den Hond et al. (2011)	Flanders 2003-2004	Tanner staging, age at menarche, regular menses	Girls ages 14 and 15, in their 3rd year of secondary education and living in the same study areas for at least 5 years	Blood Pb	Median: 1.81 10th percentile: 0.88 90th percentile: 3.81	OR (95% CI) for pubic hair development with doubling of exposure 0.65 (0.45, 0.93) *Association was no longer statistically significant when PCB marker included in the model No association between Pb and breast development (results not given)
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)
Wu et al. (2003b)	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)

Reference[a]	Study Location and Years	Outcome	Study Population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
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 >10 µg/dL: NHWhites: 0.3% NHBlacks: 1.6% Mexican-Americans: 2.3%	OR (95% CI) Breast development NH Whites: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.82 (0.47, 1.42) NH Blacks: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.64 (0.42, 0.97) Mexican Americans: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.76 (0.63, 0.91) Pubic hair development NH Whites: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.75 (0.37, 1.51) NH Blacks: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.62 (0.41, 0.96) Mexican Americans: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.70 (0.54, 0.91) HR (95% CI) *included only girls 8-16 Age at menarche NH Whites: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.74 (0.55, 1.002) NH Blacks: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.78 (0.63, 0.98) Mexican Americans: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.90 (0.73, 1.11)
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) ≥ 5 µg/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) ≥ 5 µg/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

[a]Studies are presented in order of first appearance in the text of this section.

Several epidemiologic studies investigated the association between blood Pb and indicators of puberty onset. A study among girls aged 10-13 years (median: 12 years)

reported lower levels of FSH and LH levels in the group with blood Pb of at least 10 µg/dL compared to the group with blood Pb less than 10 µg/dL (Tomoum et al., 2010). In addition, there were some indications of lower Tanner stages of breast development associated with Pb levels of at least 10 µg/dL, but this relationship was not present for stages of pubic hair development. A study of girls aged 10-16.9 years of age in the Akwesasne Mohawk Nation reported a nonlinear 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 in a nonlinear model of the Pb-menarche relationship. A study conducted in South Africa reported a positive association between blood Pb levels and age at first menarche and pubertal development (Naicker et al., 2010). Another study reporting on girls with low blood Pb concentrations observed an association between blood Pb and pubic hair but not breast development (Den Hond et al., 2011). The association was no longer statistically significant when a marker for polychlorinated biphenyl exposure was included in the model. A study performed in NYC among 9 year old girls reported no association between Pb levels and pubertal development (Wolff et al., 2008), but this age group may be too young to study when investigating delayed puberty as the outcome.

Multiple studies have been performed examining blood Pb levels and puberty using NHANES III data (Gollenberg et al., 2010; Selevan et al., 2003; Wu et al., 2003b). A study that included girls aged 8-16 years and reported an association for delayed attainment of menarche and pubic hair development, but not for breast development (Wu et al., 2003b). The associations were observed even at blood Pb levels of 2.1-4.9 µg/dL compared to girls with blood Pb levels <2.1 µg/dL. Another NHANES III study included girls 8-18 years of age and reported the results stratified by race (Selevan et al., 2003). Higher blood Pb levels were associated with lower Tanner stage of breast and pubic hair development and later age at menarche among African Americans and with lower stage of breast and pubic hair development among Mexican Americans. For whites, the associations were in the same directions, but none reached statistical significance. In a study of girls ages 6-11 years old from NHANES III data, higher blood Pb levels were associated with lower inhibin B, a protein that inhibits FSH production, but no association was observed for LH. (Gollenberg et al., 2010). 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, as the authors indicated, these hormones are, “believed to be relevant for younger girls… near the onset of puberty and…serve as markers for hypothalamic-pituitary-gonadal functioning.”

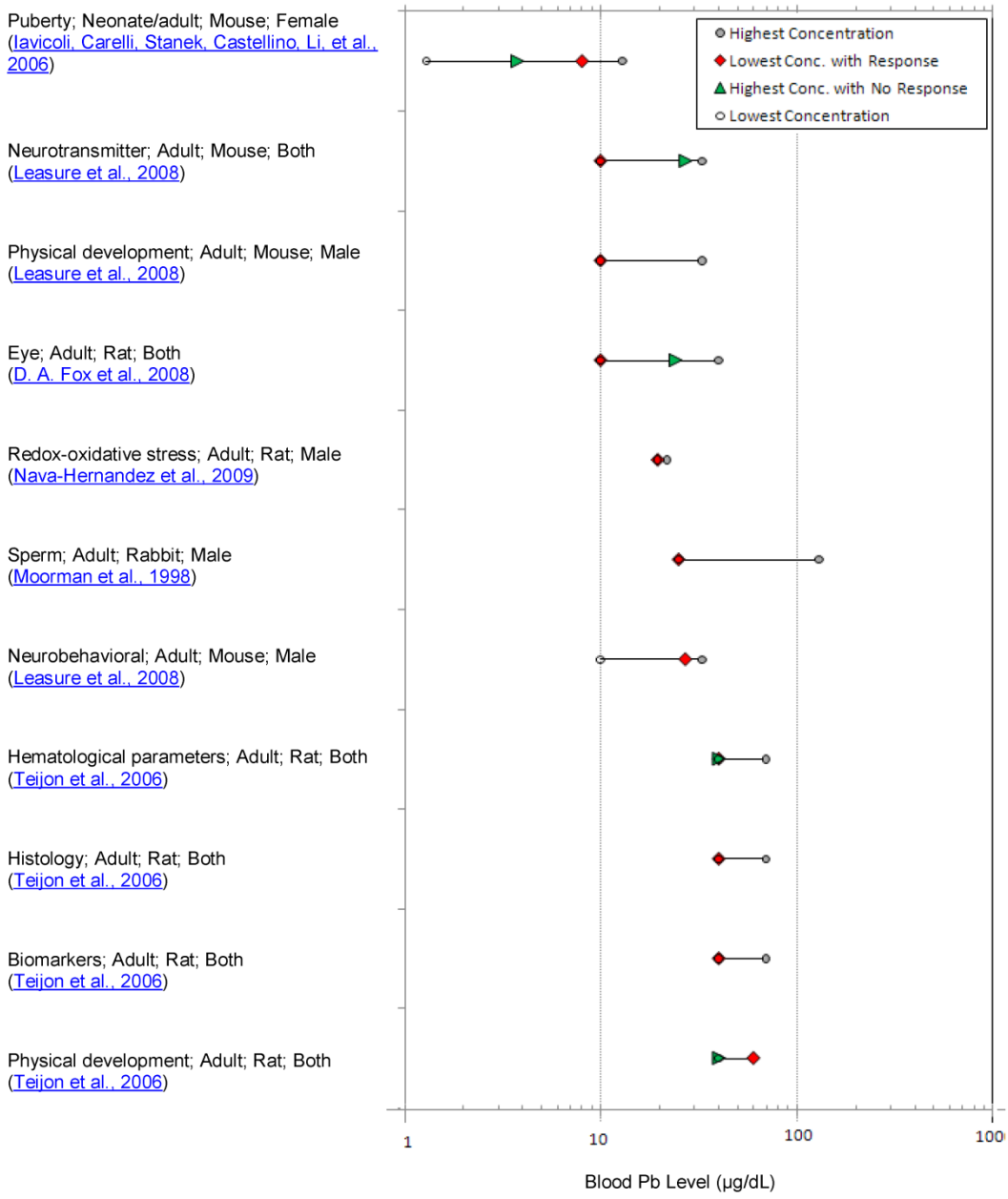


Figure 5-46 Toxicological exposure-response array for reproductive effects of Pb.

Table 5-33 Toxicological concentration-response array summary for reproductive effects of Pb presented in Figure 5-46

Reference	Blood Pb level with Effect (µg/dL)	Altered Outcome
Iavicoli I. et al. (2006b)	8 & 13	Delayed onset female puberty
Leasure et al. (2008)	10 & 42	Neurotransmitter, Dopamine homeostasis
	10, 24 & 42	Physical Development, Adult obesity (males)
	10 & 42	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	Hematology
	40 & 100	Histology-Offspring renal & hepatic
	40 & 100	Biomarker-Offspring renal function
	100	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	Hematology
	40 & 100	Histology-Offspring renal & hepatic
	40 & 100	Biomarker-Offspring renal function
	100	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 corroborate these findings and show that puberty onset is one of the more sensitive markers of effects of Pb exposure as is demonstrated in the exposure response array (Figure 5-46 and Table 5-33 Figure 5-45; including outcomes described in sections that follow). Dumitrescu et al. (2008b) exposed adult Wistar female rats to varying doses of Pb-acetate (50-150 ppb) in drinking water for 3 months before mating and during pregnancy. Vaginal opening, an indicator of sexual maturation, was statistically significantly delayed in pups from all Pb treated groups when compared to pups from non-treated dams. The age at vaginal opening in female pups from the Pb treated groups increased, in a concentration-dependent manner, from 39 days to 43-47 days. The authors also observed a correlation between body weight and age at vaginal opening meaning that as body weight decreased the age at vaginal opening increased. This effect also exhibited a concentration-dependent relationship.

In another recent study, Iavicoli et al. (2006b) reported a statistically significant delay in several indicators of sexual maturity in offspring (Swiss mice, F_1 generation) born to dams that ingested 3.5-40 ppm Pb in their daily diet; offspring had continuous dietary exposure until the termination of the experiment. 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 in this range (3.5-13 µg/dL), there was a delay in age at vaginal opening, age of first estrus, age of vaginal plug formation, and age of first parturition when compared to the group at background Pb concentration (2 µg/dL). 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 continuous through gestation, lactation and until the termination of the experiment) induced statistically significant acceleration of markers of sexual maturation in female offspring versus background Pb level animals (blood Pb of 2 µg/dL). There were statistically significant increases in time of vaginal opening (30% earlier), 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 concentration-dependent fashion with very low dose Pb having a statistically significant earlier onset of puberty than the background Pb animals (2 µg/dL). 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 (F_2 generation) across that dose range. These F_2 animals continued to be exposed to same concentrations of Pb over multiple generations through the diet. Results in the F_2 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, 2006b), it was reported that a statistically significant reduction in the circulating levels of insulin-like growth factor 1 (IGF-1), LH, and estradiol (E2) was associated with Pb-induced delayed puberty in Fisher 344 pups. Subsequently, Pine et al. (2006) evaluated whether IGF-1 replacement could reverse the effects of Pb on delayed female puberty onset. The authors reported that offspring from dams exposed to Pb during gestation and lactation (daily oral gavage of dam with 1.0 mL solution of Pb-acetate 12 mg/mL; mean maternal blood Pb level 40 µg/dL) exhibited a marked increase in LH and luteinizing hormone releasing hormone (LHRH) secretion after IGF-1 administration (200 ng^3/µL i.p. injection twice daily from PND23 until the appearance of vaginal opening which appears in control animals at ~ PND 40) resulting in restored timing of vaginal to that of control animals. 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, 2006b) and not necessarily due to a direct toxic effect on the hypothalamic-pituitary-gonadal axis (Salawu et al., 2009), and IGF-1 may play a prominent role in the process.

In sum, epidemiologic studies consistently show an association between higher concurrent blood Pb and delayed pubertal development in girls. This association is apparent even at low blood Pb levels. 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 is mediated by IGF-1.

5.8.1.4 Effects on Lactation

Experiments in laboratory animals have shown that dietary manipulation of maternal fatty acid (FA) levels in diet can worsen Pb-related behavioral effects of offspring after lactational Pb exposure (Lim et al., 2005). To determine if components of dam milk contributed to this change, dam dietary fatty acids were altered via diet. Diets deficient in n-3 fatty acids can lead to a deficiency of DHA, which is essential for proper nervous system development. Lim et al. (2005) found that dam Pb exposure (Long-Evans rats, 0.2% Pb-acetate trihydrate/BW) during lactation (PND 0-21) led to a decrement in non-essential fatty acids in the maternal organs at PND25 (mean [SD] blood Pb levels in dams: 308 [56] µg/dL). In animals with a diet deficient in n-3 FAs, there was a Pb-diet interaction on the 20-carbon n-6 PUFAs. In general, Pb exposure caused a decrement in shorter chain monounsaturated and saturated FAs in maternal organs.

Dietary supplementation with calcium can be an especially important contributor to Pb mobilization during periods of high calcium demand including pregnancy/lactation. For example, mothers with elevated blood Pb levels given calcium phosphate and ascorbic acid supplementation during lactation had a 90% decrease in placental Pb content and a 15% decrease in the concentration of Pb in breast milk (Altmann et al., 1981) versus the control group that did not receive dietary treatment. Another study (Gulson et al., 2004a) has shown that calcium supplementation during the lactation is less beneficial in modulating maternal blood Pb levels (mean blood Pb at first sampling was 2.4 µg/dL); the Gulson cohort was limited by power (n=10 women). In a cohort of women from Mexico City, daily calcium supplementation during lactation reduced maternal blood lead by 15–20% and lead in breast milk by 5–10% (Ettinger et al., 2004a). Another study by the same investigators showed that using calcium supplements daily during pregnancy also reduced blood lead levels during pregnancy (Ettinger et al., 2009) with the effect strongest in women with higher biomarkers of Pb exposure (elevated baseline bone Pb or

>5 µg/dL blood Pb) or in women with higher Pb exposure (self-reported use of lead-glazed ceramics). Thus, dietary modulation with calcium supplementation during pregnancy and lactation may decrease the amount of Pb to which the developing fetus of infant is exposed. The evidence for this seems especially strong for protection during pregnancy and more mixed for protective effects of calcium during lactation.

5.8.1.5 Summary of Effects on Female Reproductive Function

In summary, Pb exposure was found to affect female reproductive function as demonstrated by both epidemiologic and toxicological studies. At low concurrent blood Pb levels, associations are observed with delayed puberty. These associations are noted among girls with mean blood Pb levels of 10 µg/dL and lower. Some evidence is also available regarding blood Pb levels and altered hormone levels in adults, although the direction of the change varied among studies. Although studies reported inconsistent findings for the association between Pb and fertility, there is some evidence of a potential relationship. Most of the epidemiologic studies are cross-sectional, thus there is uncertainty regarding the lifestages of Pb exposure associated with the greatest risk. Toxicological studies are often dealing with prenatal or early postnatal exposures, except for puberty studies which use concurrent exposure. Although epidemiologic and toxicological studies provide information on different exposure periods, both types of studies support the conclusion that Pb affects at least some aspects of female reproductive function.

5.8.2 Effects on Male Reproductive Function

The 2006 Pb AQCD (U.S. EPA, 2006b) reported on male Pb exposure or biomarker levels and reproductive functions as measured by sperm count/motility/morphology, time to pregnancy, reproductive history, and chromosomal aberrations. Despite limitations, most of the studies found slight associations between high blood Pb levels (i.e., ≥ 45 µg/dL) and reduced male fecundity or fertility (U.S. EPA, 2006b). Evidence reviewed in the 1986 Pb AQCD (U.S. EPA, 1986a) also demonstrated that Pb exposure affects male reproductive function in humans and experimental animals. Recently published research has continued to support an association between Pb and 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 5-34. The biological samples in these studies are from concurrent measures. The biological samples measuring Pb levels and outcomes were collected concurrently in the epidemiologic studies.

Table 5-34 Summary of recent epidemiologic studies of associations between Pb levels and effects on sperm and semen

Reference[a]	Study Location and Years	Study Population	Pb Biomarker or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
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
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

Reference[a]	Study Location and Years	Study Population	Pb Biomarker or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Hsu et al. (2009b)	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)
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

Reference[a]	Study Location and Years	Study Population	Pb Biomarker or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
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
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)
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

Reference[a]	Study Location and Years	Study Population	Pb Biomarker or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Mendiola et al. (2011)	Spain 2005-2007	Men attending infertility clinics and classified as either normal sperm (controls) or oligo-astheno-teratozoospermia (cases) based on WHO semen quality criteria	Seminal plasma Pb Blood Pb	Seminal plasma: 2.90 (IQR 2.70, 3.20) Whole blood: 9.50 (IQR 7.50, 11.90) Blood plasma: 2.90 (IQR 2.70, 3.10) Cases: Seminal plasma: 3.0 (0.3) Whole blood: 9.8 (2.3) Blood plasma: 2.9 (0.2) Controls: Seminal plasma: 2.9 (0.3) Whole blood: 9.7 (2.3) Blood plasma: 2.9 (0.3)	β (95% CI) Sperm concentration Seminal plasma: -1.0 (-3.1, 2.3) Whole blood: -0.2 (-1.7, 1.6) Blood plasma: 0.08 (-4.1, 5.2) % Immotile sperm Seminal plasma: 1.5 (0.37, 1.9) Whole blood: 0.05 (-0.32, 0.43) Blood plasma: -0.49 (-1.8, 6.2) % Morphologically normal sperm Seminal plasma: -0.54 (-3.1, 2.0) Whole blood: -0.31 (-1.5, 0.89) Blood plasma: -0.08 (-3.5, 3.4) *Units not given (assume 1 µg/dL) Note: No correlation in Pb levels among bloods or seminal plasma. There was correlation between Pb and other metals (Cd and Hg) within each body fluid. Other metals were not controlled for in models

[a]Studies are presented in order of first appearance in the text of this section.

International epidemiologic studies of men occupationally exposed to Pb have reported on associations between Pb exposure or biomarker levels and sperm count and quality and semen quality. In most of these occupational studies, blood Pb levels over 40 µg/dL have been reported for individuals occupationally exposed to Pb. For example, studies performed in India (Naha and Manna, 2007; Naha and Chowdhury, 2006) reported that men in the highest exposure group (men working in battery or paint manufacturing plants for 10-15 years for 8 hours/day) had mean blood Pb levels of 77.22 µg/dL (Naha and Chowdhury, 2006) and 68.26 µg/dL (Naha and Manna, 2007). Control groups in these studies (those without occupational Pb exposure) had mean blood Pb levels below 15 µg/dL. Increases in levels of Pb in semen were also noted across exposure groups. Both studies report decreases in sperm count and in sperm velocity and motility with increasing Pb exposure. Higher Pb exposure was also associated with greater hyploidy of sperm DNA and morphologic abnormalities (Naha and Manna, 2007; Naha and Chowdhury, 2006). Decreased viability and increased lipid peroxidation were detected (Naha and Chowdhury, 2006). A study performed in Taiwan among men with high levels of blood Pb reported that men with higher 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 (Hsu et al., 2009b). 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 (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 can induce tissue damage in sperm via reactive oxygen species, was greater in the highest exposure group compared to the controls.

A study performed in Croatia recruited men who had never been occupationally exposed to metals and therefore had lower blood Pb levels than the occupational studies (Telisman et al., 2007). Increased blood Pb was associated with increased percentages of pathologic sperm, wide sperm, and round sperm. There was also a slight increase in immature sperm although it was not statistically significant. Similar results were seen when other biomarkers for Pb (erythrocyte protoporphyrin and δ-aminolevulinic acid dehydratase [ALAD]) were used instead.

A few studies examined blood or seminal plasma Pb levels and semen quality of men at infertility clinics (Mendiola et al., 2011; Slivkova et al., 2009; Meeker et al., 2008). In general, these men had lower levels of Pb biomarkers than men that were occupationally exposed. Meeker et al. (2008) detected no associations between higher blood Pb and semen concentration, morphology, or motility (although a slight positive trend was observed between higher Pb levels and motility in unadjusted models). In models that include multiple metals, blood Pb was associated with being below the WHO limit of sperm concentration levels (less than 20 million sperm/mL), although the 95% CI was wide for the 4th quartile of Pb levels and included the null. Slivkova et al. (2009) reported a negative correlation between semen Pb and pathological changes in sperm (specifically, flagellum ball), but no correlations were observed for other alterations in the sperm. Another study reported a positive association between seminal plasma Pb concentration and percentage of immotile sperm, but this analysis did not adjust for exposure to other metals reported to be correlated with Pb concentration in the seminal plasma (Mendiola et al., 2011). No association was observed for seminal plasma Pb concentration and sperm concentration or percentage of morphologically normal sperm. Additionally, neither Pb levels in whole blood or plasma were associated with sperm concentration, percentage of immotile sperm, or percentage of morphologically normal 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, resulting in blood Pb

of 16-24 µg/dL, induced statistically significant decrements in semen quality and greater testicular pathology (Moorman et al., 1998). Recent studies corroborate earlier findings that Pb alters sperm parameters such as sperm count, viability, motility, and morphology. Anjum et al. (2010) exposed 50 day old male albino Wistar/NIN rats to Pb-acetate (273 or 819 mg/L in drinking water, 0.05% or 0.15%, respectively) for 45 days. Affected endpoints included reduced epididymal sperm count, motile sperm, and viable sperm, indicating decreased sperm production and quality. Anjum did not report blood Pb values. Wistar/NIN rats (0.15% Pb-acetate in drinking water for 70 days) supplemented with the herb Centella asiatica had significant attenuation of the Pb-induced changes observed by Anjum et al. (2010). Pillai et al. (In Press) found gestational and lactational treatment with Pb-acetate in Charles Foster rats (subcutaneous injection of 0.05 mg/kg BW/day) induced effects on sperm in adults (PND65) including significant decreases in testicular sperm count, epididymal sperm count, and sperm motility. 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 exposure concentration-dependent manner (8-24 mg/kg body weight), reduced the length of certain stages of the spermatogenic cycle of rat seminiferous tubules and thus affected spermatogenesis. Oral Pb-acetate exposure (25 mg/kg bw in drinking water for 3 months, resulting in blood Pb level of 5.3 µg/dL) to adult male albino rats produced significant histological seminiferous tubule damage (epithelium, spermatocytes, acrosomes) that was attenuated with ascorbic acid treatment (Pb exposure + 100 mg/kg bw/day ascorbic acid, resulting in blood Pb level of 4.7 µg/dL) (El Shafai et al., 2011). Reshma Anjum et al. (2010) 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) which were significantly attenuated with Pb co-exposure to the herb Centella asiatica (Sainath et al., 2011). Pb induced morphological abnormalities in sperm in a concentration-dependent manner (Allouche et al., 2009; Oliveira et al., 2009; Salawu et al., 2009; Shan et al., 2009; Tapisso et al., 2009; Massanyi et al., 2007; Wang et al., 2006a). Sperm abnormalities reported after Pb exposures were amorphous sperm head, abnormal tail, and abnormal neck. Dong et al. (2009) reported decreased epididymis and body weights in mice after 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 of the aforementioned studies observed changes in every parameter. This may be due to the use of different strains or species, chemical form of the Pb compound administered,

dosage schedule, duration of exposure, and age of animals at the time of the study (Oliveira et al., 2009).

Data from recent studies 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 catalase (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 Pb-induced ROS production and prevented the Pb-induced increase in MDA formation and decrease in SOD and CAT activity. Furthermore, co-treatment of Pb with substances that are known to have antioxidant properties [i.e., tomato paste, Maca (*Lepidium meyenii*), and ascorbic acid] prevented the Pb-induced reduction in sperm count, sperm motility, and sperm viability (Salawu et al., 2009; Shan et al., 2009; Madhavi et al., 2007; Rubio et al., 2006; Wang et al., 2006a).

Recent studies also demonstrate that Pb may be directly toxic to mature spermatozoa (Tapisso et al., 2009; Hernandez-Ochoa et al., 2006) as well as primary spermatocytes (Nava-Hernandez et al., 2009; Rafique et al., 2009). Nava-Hernandez et al. (2009) exposed two groups of rodent to Pb via drinking water (L1 and L2). In their study, all Pb-treated animals had blood Pb levels statistically significantly higher than controls (L1:19.54 µg/dL and L2:21.90 µg/dL); no statistically significant difference in blood Pb levels existed between the two Pb exposure groups likely because the L2 group drank less water than did the L1 group. Piao et al. (2007) reported that Pb exposure caused DNA damage to sperm; the Pb exposed group had a blood Pb of 67 µg/l. Piao et al. (2007) also examined the effect of Zn supplementation on Pb-induced sperm aberrations and found that the proportion of abnormal sperm was statistically significantly higher in the Pb group and the Pb+Zn group than in controls. However, the proportion of abnormal sperm in Pb+Zn group was statistically significantly lower than in Pb alone group. Hernandez-Ochoa et al. (2006) reported that Pb reaches the sperm nucleus in the epididymis of mice chronically exposed (16 weeks in adult animals) to Pb (resulting in mean blood Pb of 75.6 µg/dL) by binding to nuclear sulfhydryl groups from the DNA-protamine complex, increasing sperm chromatin condensation, and thereby interfering with the sperm maturation process without altering sperm quality parameters. Tapisso et al. (2009) observed a statistically significant increase in the number of micronuclei and frequency of sister chromatid exchange with increasing treatment duration in adult male mice administered 21.5 mg/kg body weight Pb-acetate by i.p. injection. Nava-Hernandez (2009) reported a concentration-dependent increase in DNA damage in rat primary spermatocytes after a 13-week exposure period to Pb-acetate in

drinking water (resulting in mean blood Pb levels between 19.5 and 21.9 µg/dL). Rafique et al. (2009) reported degenerative changes from pyknosis to apoptosis in primary spermatocytes. Hepatic expression of spermatogenic genes was transiently down-regulated in 8 week old male Wistar-Kyoto (WKY) rats in response to Pb nitrate (100 µmol single i.v. injection) 3 hours after injection and recovered to baseline by 12 hours (Nemoto et al., 2011); this effect was not seen in the stroke-prone spontaneously hypertensive rats, which are from a WKY background, or in Sprague-Dawley rats, demonstrating strain specificity.

Pb-induced apoptosis in germ cells within the seminiferous tubules is another suggested mechanism by which Pb exerts its toxic effects on sperm production and function (Wang et al., 2006a). Dong et al. (2009) reported a exposure concentration-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. (2006a) observed a exposure concentration-dependent increase in the expression of apoptotic markers TGFβ1 and caspase-3 in spermatogenic cells, Sertoli cells, and Leydig cells. Shan et al. (2009) 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 (El Shafai et al., 2011; Shan et al., 2009; Wang et al., 2006a) suggesting ROS generation is a major contributing factor in decreased male fertility observed after chronic Pb exposure.

Similar to the results summarized in previous Pb AQCDs, recent epidemiologic and toxicological studies indicate that high levels of Pb exposure have effects on sperm and semen. In studies of men exposed to Pb in occupational settings, associations were observed between blood Pb levels as low as 20-45 µg/dL and sperm count and quality. Multiple epidemiologic studies of occupational cohorts included control populations with high blood Pb levels (close to or greater than 10 µg/dL), which makes identification of effects at lower levels difficult. Future studies are warranted to determine whether this association is observed at lower Pb levels.

5.8.2.2 Effects on Hormone Levels

The 2006 Pb AQCD (U.S. EPA, 2006b) provided evidence that Pb acts as an endocrine disruptor in males at various points along the hypothalamic-pituitary-gonadal axis. The

2006 Pb AQCD also reported inconsistencies in the effects of Pb exposure on circulating testosterone levels. Recent epidemiologic and toxicological studies are reported below. Epidemiologic studies are summarized in Table 5-35 (biological samples used for the measurement of Pb in these studies were measured concurrently with hormone levels).

Table 5-35 Summary of recent epidemiologic studies of associations between Pb levels and hormones for males

Reference[a]	Study Location and Years	Outcome	Study Population	Pb Biomarker or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
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)
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

Reference[a]	Study Location and Years	Outcome	Study Population	Pb Biomarker or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Hsieh et al. (2009a)	Taiwan 1991-NS	FSH, LH, testosterone, inhibin B	Workers at a Pb-acid battery factory with annual blood Pb measures	Current blood Pb, cumulative blood Pb, time-weighted cumulative blood Pb	Current blood Pb: <10 µg/dL: 11.6% >40 µg/dL: 17.1%	β from linear regression Inhibin B Current blood Pb: 0.40 (p-value 0.40) Cumulative blood Pb: 0.05 (p-value 0.02) Time-weighted cumulative blood Pb: 1.33 (p-value 0.007) Pearson's correlations detected no correlations between current blood Pb levels and FSH, LH, or testosterone. Cumulative blood Pb levels were correlated with FSH and LH, but not testosterone. Time-weighted cumulative blood Pb levels were correlated with LH, but not FSH or testosterone.

Reference[a]	Study Location and Years	Outcome	Study Population	Pb Biomarker or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Meeker et al. (2010)	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[a]	Study Location and Years	Outcome	Study Population	Pb Biomarker or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Mendiola et al. (2011)	Spain 2005-2007	FSH, LH, testosterone	Men attending infertility clinics and classified as either normal sperm (controls) or oligo-astheno-teratozoospermia (cases) based on WHO semen quality criteria	Seminal plasma Pb Blood Pb	Seminal plasma: 2.90 (IQR 2.70, 3.20) Whole blood: 9.50 (IQR 7.50, 11.90) Blood plasma: 2.90 (IQR 2.70, 3.10) Cases: Seminal plasma: 3.0 (0.3) Whole blood: 9.8 (2.3) Blood plasma: 2.9 (0.2) Controls: Seminal plasma: 2.9 (0.3) Whole blood: 9.7 (2.3) Blood plasma: 2.9 (0.3)	Linear regression β (95% CI) FSH Seminal plasma: 0.05 (-0.24, 0.39) Whole blood: 0.04 (-0.03, 0.04) Blood plasma: -0.20 (-0.64, 0.25) LH Seminal plasma: 0.14 (-0.13, 0.41) Whole blood: 0.05 (-0.05, 0.07) Blood plasma: -0.07 (-0.49, 0.31) Testosterone Seminal plasma: 0.11 (-0.10, 0.31) Whole blood: 0.01 (-0.05, 0.02) Blood plasma: -0.12 (-0.40, 0.14) *Units not given (assume 1 µg/dL)

[a]Studies are presented in order of first appearance in the text of this section.

Hormone levels were measured in a few recent epidemiologic studies. In a study of men non-occupationally exposed to Pb in Croatia, increased blood Pb level was associated with increasing serum testosterone and estradiol but decreasing serum prolactin (Telisman et al., 2007). In addition, the analysis of an interaction term for blood Pb and blood 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 (controls: mean blood Pb 10.25 µg/dL, low exposure: mean blood Pb 50.29 µg/dL, high exposure: mean blood Pb 68.26 µg/dL) (Naha and Manna, 2007). A study of occupationally-exposed men in Taiwan reported an association between measures of cumulative blood Pb levels and inhibin B levels, but no association was detected when using current blood Pb levels (Hsieh et al., 2009a). A correlation between cumulative blood Pb measures and LH levels was detected but correlations were not present when examining FSH or testosterone levels. No correlations were apparent between FSH, LH, or testosterone and current blood Pb levels.

Among men recruited from infertility clinics in Michigan, median blood Pb levels were much lower than those observed in the other studies of Pb and hormone levels among

men (Meeker et al., 2010). No association was detected between blood Pb and levels of FSH, LH, inhibin B, sex hormone-binding globulin (SHBG), free androgen index (FAI) or a measure of Leydig cell function (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. Similarly, another study of men recruited from infertility clinics observed no association between Pb concentrations from seminal plasma, whole blood, or blood plasma and FSH, LH, or testosterone (Mendiola et al., 2011).

In a recent toxicological study, Rubio et al. (2006) observed a decrease in testosterone levels in Pb-acetate-treated rats in a exposure concentration-related fashion (8-24 mg/kg body weight), and this decrease correlated with reduced lengths of spermatogenic cycle stages VII-VIII (spermiation) and IX-XI (onset of spermatogenesis). Anjum et al. (2010), who dosed 50 day old male rats with 273 or 819 mg/L Pb-acetate in drinking water (0.05% or 0.15%, respectively; blood Pb not reported), found significant decreases in serum testosterone and testicular 3β-HSD and 17β-HSD levels in Pb-exposed animals versus controls. Pandya et al. (2010) reported altered hepatic steroidogenic enzyme activity. Pillai et al. (In Press) found gestational and lactational exposure to Pb-acetate in Charles Foster rats (subcutaneous injection of 0.05 mg/kg BW/day, blood Pb not reported) induced significant decreases in testicular 17β-HSD and serum testosterone. Biswas and Ghosh (2006) reported a Pb-induced decrease in serum testosterone and gonadotropins (FSH, LH) with inhibition of spermatogenesis, however, there was a statistically significant increase in adrenal steroidogenic enzyme, Δ5-3β-HSD activity and serum corticosterone levels indicating disruption of the adrenocortical process. Exposure concentration-dependent decreases in serum testosterone were reported in Pb-exposed male rats (Reshma Anjum et al., 2010). 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, 2006b).

Overall, recent epidemiologic and toxicological studies report mixed findings regarding hormone aberrations in males associated with Pb exposure or Pb biomarker levels. These results are similar to those from the 2006 Pb AQCD 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 that blood and seminal plasma Pb levels were different in fertile (mean [SD] blood Pb: 23.16 [5.59] µg/dL) and infertile men (mean [SD] blood Pb: 36.82 [12.30] µg/dL)($p < 0.001$, ANOVA) (Kiziler et al., 2007). The SMART study examined the success of IVF treatment for women and their partners starting their first round of treatment (Bloom et al., 2011b; Bloom et al., 2010). A small number of the male partners participated. Their mean (SD) blood Pb level was 1.50 (0.80) µg/dL. Higher blood Pb concentration was associated with greater oocyte fertilization (OR 1.08 [95% CI: 0.97, 1.21] per 1 µg/dL increase in blood Pb when adjusted for Cd), which is not the expected direction (Bloom et al., 2010). However, higher blood Pb was associated with lower embryo cell number (a predictor of IVF success) and associated with higher embryo fragmentation score (an inverse predictor of IVF success) (OR for embryo cell number: 0.58 [95% CI: 0.37, 0.91]; OR for embryo fragmentation score: 1.47 [95% CI 1.11, 1.94] per 1 µg/dL, controlled for Cd and Hg) (Bloom et al., 2011b). 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 study populations are not a sample of the general population regarding fertility.

A couple of recent animal toxicology studies assessed paternal-mediated reproductive fitness by examining the reproductive success of Pb-exposed males with non-exposed control females. Anjum et al. (2010) found that adult male rats who were exposed to 273 or 819 mg/L Pb-acetate in drinking water (0.05%or 0.15%, respectively; blood Pb not reported) spent a significantly longer time copulating than did their control littermates. The Pb-exposed males were less successful copulators with only 73% of the 0.05% Pb-acetate exposed males, and 53% of the 0.15% exposed males generating copulatory plugs in the unexposed female mates. While the number of pregnant females did not significantly differ from controls, Pb exposed males contributed to the formation of significantly fewer implantations/dam, and significantly fewer fetuses/dam. Pb-exposed males were able to sire offspring, but produced fewer offspring per litter. In a group of males rats with co-exposure to Pb and the herb Centella asiatica, these reproductive decrements were attenuated relative to rats exposed to Pb alone (Sainath et al., 2011).

5.8.2.4 Puberty

Research has also been published examining the association between blood Pb and onset of puberty in males. These epidemiological studies are summarized in Table 5-36. The majority of studies used concurrent measures of blood Pb and puberty (Den Hond et al., 2011; Tomoum et al., 2010; Hauser et al., 2008), but Williams et al. (2010) performed a longitudinal analysis of blood Pb levels measured at ages 8-9 years and pubertal onset.

Table 5-36 Summary of recent epidemiologic studies of associations between Pb levels and puberty for males

Reference[a]	Study Location	Outcome	Study Population	Pb Biomarker	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)
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)

Reference[a]	Study Location	Outcome	Study Population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
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% ≥ 10 µg/dL: Stage 1: 33.3% Stage 2: 66.7% Stage 3: 0% Chi-square p-value<0.01 Pubic Hair Development <10 µg/dL: Stage 1: 0% Stage 2: 55.6% Stage 3: 44.4% ≥ 10 µg/dL: Stage 1: 33.3% Stage 2: 66.7% Stage 3: 0% Chi-square p-value<0.05 Penile staging <10 µg/dL: Stage 1: 11.1% Stage 2: 44.4% Stage 3: 44.4% ≥ 10 µg/dL: Stage 1: 58.3% Stage 2: 41.7% Stage 3: 0% Chi-square p-value<0.05 Mean testosterone level <10 µg/dL: 4.72 (SD 1.52) ≥ 10 µg/dL: 1.84 (SD 1.04) *Quantitative results for LH and FSH not provided
Den Hond et al. (2011)	Flanders 2003-2004	Tanner staging and gynecomastia	Boys ages 14 and 15, in their 3rd year of secondary education and living in the same study areas for at least 5 years	Blood Pb	Median: 2.50 10th percentile: 1.20 90th percentile: 5.12	OR (95% CI) for gynecomastia with doubling of exposure 1.84 (1.11, 3.05) No association between Pb and pubic hair or genital development (results not given)

[a]Studies are presented in order of first appearance in the text of this section.

Studies were performed among a cohort of Russian boys enrolled between ages 8-9 years (Williams et al., 2010; Hauser et al., 2008). The area where these studies were performed had various environmental contaminants such as dioxin, polychlorinated biphenyls, and other metals, present. Both the cross-sectional study (Hauser et al., 2008) and the

prospective study with annual follow-ups (Williams et al., 2010) demonstrated an association; higher blood Pb levels at 8-9 years of age was associated with later onset of puberty. In a study of boys in Egypt, boys with higher blood Pb had delayed pubertal 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 blood Pb group, those boys with higher blood Pb had lower testosterone, FSH, and LH levels. A study in Flanders reported no associations between blood Pb concentration and pubertal development among 14 and 15 year old boys (Den Hond et al., 2011). However, higher blood Pb levels were associated with an increased odds of gynecomastia.

No recent toxicological studies address Pb-induced male sexual maturation and development, but older studies do provide support to findings in epidemiologic cohorts. Pb exposure resulted in delayed sexual maturity as measured by prostate weight in male Sprague-Dawley pups at PND 35. These pups were exposed chronically to 0.15 or 0.45% Pb-acetate in dam or their own drinking water from GD5 until PND85 and had blood Pb ranges from low to high of 88-196 and 120-379 μg/dL, respectively (Ronis et al., 1998b). Cynomolgus monkeys exposed to Pb over a lifetime (10 years, blood Pb levels ranging from 30–60 μg/dL) had altered pituitary and Sertoli cell function along with decreases in inhibin/FSH ratio and reduced gonadotropin-releasing hormone (GnRH) stimulation of LH release in adulthood (Foster et al., 1993; Foster, 1992), all indicators that are important in proper sexual maturation. Further mechanistic understanding of the effect of Pb can be gleaned from studies in adult male Wistar rats exposed to Pb for 1 month (starting at PND56, 0.1 or 0.3% Pb-acetate in drinking water, respective blood Pb levels of 34 or 60 μg/dL) that showed significant decreases in FSH, ventral prostate weight and serum testosterone but no change in serum LH (Sokol et al., 1985). These Pb-exposed adult male rats (0.3% Pb-acetate in drinking water starting at PND56 for 30 days) demonstrated an impaired pituitary release of LH in response to challenge of the hypothalamic–pituitary–adrenal (HPA) axis with the opiate antagonist naloxone, an enhanced release of LH from the pituitary in response to direct stimulation of the pituitary with luteinizing hormone-releasing hormone (LHRH), an enhanced response to human chorionic gonadotropin (hCG) by the testes, increased pituitary LH stores, and increased GnRH mRNA levels in the hypothalamus (Klein et al., 1994; Sokol, 1987). Thus, Pb likely interferes with the male HPA axis, contributing to its reproductive toxicity.

In summary, recent epidemiologic studies have demonstrated an inverse effect of Pb on pubertal development among boys at low concurrent blood Pb levels. No recent toxicological studies were found that addressed the effect of Pb on male sexual development and maturation; however, the 2006 Pb AQCD (U.S. EPA, 2006b) supported

earlier findings that Pb exposure may result in delayed onset of male puberty and altered reproductive function later in life in experimental animals.

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 changes in the sex organs as well as germ cells. Histological changes of testes in Pb nitrate-treated animals (a single i.p. dose of 12.5, 25, or 50 mg/kg of BW and were sacrificed 48 hours later) included seminiferous tubule atrophy, Sertoli cell and Leydig cell shrinkage with pyknotic nuclei (Shan et al., 2009; Wang et al., 2006a), dilatation of blood capillaries in the interstitium, undulation of basal membrane, and occurrence of empty spaces in seminiferous epithelium (Massanyi et al., 2007). Pillai et al. (In Press) found gestational and lactational exposure to Pb-acetate in Charles Foster rats (subcutaneous injection of 0.05 mg/kg BW/day) induced significant decreases in absolute organ weight (testes and epididymis) and significant decreases in relative epididymal weight. Anjum et al. (2010), who exposed 50 day old male albino Wistar/NIN rats to Pb-acetate (273 or 819 mg/L in drinking water, 0.05% or 0.15%, respectively, blood Pb levels not reported) for 45 days, reported significant decreases in relative reproductive organ weight (epididymis, testis, vas deferens, and seminal vesicle) in Pb-exposed animals. .

5.8.2.6 Summary of Effects on Male Reproductive Function

Evidence of associations between Pb exposure and male reproductive function vary by outcome. The strongest evidence of an association is the relationship observed between Pb and negative effects on sperm and semen in both recent epidemiologic and toxicological studies and studies reviewed in previous Pb AQCDs. Many of the epidemiologic studies included occupational cohorts, which had high blood Pb levels. Recent toxicological studies also reported an association between Pb exposure and decreases in reproductive organ weight, organ histological changes in the testes and germ cells. Male rats exposed to Pb also showed subfecundity in that they produced smaller litters when mated with unexposed females. In addition, recent epidemiologic studies found blood Pb levels to be associated with delayed pubertal development in boys with low concurrent blood Pb levels (mean/median blood Pb levels <10 µg/dL). This is supported by earlier toxicological studies. Similar to the 2006 Pb AQCD (U.S. EPA, 2006b), recent epidemiologic and toxicological studies reported inconsistent results regarding hormone aberrations associated with Pb exposure. Mixed findings were also apparent among epidemiologic studies of fertility among men.

5.8.3 Effects on Ovaries, Embryo Development, Placental function, and Spontaneous Abortions

The 2006 Pb AQCD (U.S. EPA, 2006b) included studies of Pb exposure among men and women and their associations with spontaneous abortions. The 2006 Pb AQCD concluded that overall there was little evidence to support an association between Pb exposure among women and spontaneous abortion (U.S. EPA, 2006b). Most of the studies examined in the 2006 Pb AQCD assigned exposure based on living near a smelter or working in occupations that often result in Pb exposure and the results of these studies were inconsistent. Little evidence was available in the 2006 Pb AQCD to suggest an association with paternal Pb levels (U.S. EPA, 2006b), and no recent studies have been performed to examine paternal Pb levels and spontaneous abortion. Since the 2006 Pb 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 5-37 Summary of recent epidemiologic studies of associations between Pb levels and spontaneous abortions

Reference[a]	Study Location	Outcome	Study population	Pb Biomarker	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
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
Vigeh et al. (2010)	Tehran, Iran 2006-2008	Pregnancy ended before 20 weeks of gestation	Women who were non-smokers, non-obese, had no chronic health conditions, had their last menstrual period less than 12 weeks prior, and were pregnant with a singleton infant	Maternal blood Pb during weeks 8-12 of pregnancy	3.8 (2.0) Spontaneous abortion: 3.51 (1.42) Non-spontaneous abortion: 3.83 (1.99)	T-test for difference in mean values: 0.41 OR: 0.331 (95% CI: 0.011, 10.096) for an increase in log-transformed blood Pb (units not given, assume 1 µg/dL)
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)
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)

[a]Studies are presented in order of first appearance in the text of this section.

Table 5-37, above, provides a summary of the recent epidemiologic studies examining the association between Pb biomarker 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 plasma Pb level was inversely correlated with folate and vitamin B12, but this correlation was not observed among those who delivered at term; no models examining plasma Pb levels adjusted for nutrient status. Another study

examining spontaneous abortions occurring early in the pregnancy was conducted in Iran (Vigeh et al., 2010). Mean blood Pb concentrations were similar in women who did and did not have spontaneous abortions. Higher blood Pb levels were not associated with greater odds of spontaneous abortions before 20 weeks of pregnancy. 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 did women in the lowest tertile. The authors state that the plasma/whole blood ratio represents the bioavailability of Pb, which is 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 (Gundacker et al., 2010). It is important to note that the number of women included in the study was small (only 8 women reported previously having a miscarriage)

In toxicological studies, isolated embryo cultures are often used to understand the mechanisms responsible for aberrant embryo development as it may contribute to teratogenesis, fetal loss or negative postnatal pup outcomes. Nandi et al. (2010) demonstrated an exposure concentration-dependent decline in embryo development of fertilized buffalo oocytes cultured 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, 2006b). Wang et al. (2009e) observed that elevated maternal blood Pb (0.6-1.74 µM, ~12.4-36.0 µg/dL) compared to control (0.04 µM, ~0.83 µg/dL) 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 concentration-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-level Pb exposure condition which correlated with low blood Pb 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. (2010) that had an exposure concentration-dependent decreases in serum testosterone, decreased male reproductive organ weight and decreased sperm were mated to untreated females. These untreated dams had male related exposure concentration-dependent decreased implantation rate and higher pre- and post-implantation loss, indicating paternally mediated fetal loss. The magnitude of these effects in dams was dependent on the concentration of Pb exposure in their male mating partners.

As observed in sperm cells, Pb stimulates changes in antioxidant enzyme activity in rat ovaries indicating that oxidative stress may be a contributing factor in Pb-induced ovarian. 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, 2006b). In support of historical studies, recent studies demonstrate Pb-induced histological changes in ovarian cells of pigs (Kolesarova et al., 2010) and rats (Nampoothiri et al., 2007; Nampoothiri and Gupta, 2006). Kolesarova et al. (2010) observed a reduction of the monolayer of granulosa cells after Pb addition (0.5 mg/mL). 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, 2006b).

Overall, the recent studies support the conclusions of the 2006 Pb AQCD that there is insufficient evidence among epidemiologic studies to suggest an association between Pb and spontaneous abortions. It is important to note that studies of spontaneous abortions are difficult to conduct. The majority of spontaneous abortions 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 exposure to spontaneous abortions. These laboratory data show that Pb exposure impaired placental function, induced oxidative stress and histological changes in the ovaries, and affected embryo development. The toxicological and epidemiologic data provide mixed evidence on the role of Pb in spontaneous abortions.

5.8.4 Infant Mortality and Embryogenesis

The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that Pb exposure can increase fetal mortality and produce sublethal effects (disrupt growth and development) in offspring of Pb exposed dams at concentrations that do not result in clinical toxicity to the dams by disrupting implantation and pregnancy, particularly at the blastocyst stage of development. In rodent studies gestational exposure to Pb (blood Pb 32 to >70 μg/dL) resulted in smaller litters and fewer implantation sites and in non-human primates pre- and perinatal mortality was reported in squirrel monkeys exposed to Pb (mean dam blood Pb of 54 μg/dL) in the last two-thirds of gestation (U.S. EPA, 2006b). There is substantial evidence to show that there is no apparent maternal-fetal barrier to Pb and it can easily cross the placenta and accumulate in fetal tissue during gestation (Pillai et al., 2009; Wang et al., 2009e; Uzbekov et al., 2007). No recent studies have reported on the relationship between Pb levels and infant mortality.

5.8.5 Birth Defects

The 2006 Pb AQCD (U.S. EPA, 2006b) reported the possibility of small associations between high Pb exposure and birth defects, but many of the epidemiologic studies used occupational histories instead of actual measures of blood Pb levels. Among the studies included in the 2006 Pb AQCD, a couple studies reported possible associations between parental exposure to Pb and neural tube defects (Irgens et al., 1998; Bound et al., 1997). Recent studies also examined indicators of Pb exposure and neural tube defects (Table 5-38). No other recent epidemiologic studies of Pb exposure and birth defects were identified in the literature. No recent toxicological studies were found that investigated Pb-induced changes in morphology, teratology effects, or skeletal malformations of developing fetuses as a result of maternal Pb exposure; however, in the 2006 Pb AQCD toxicological studies demonstrated associations between exposure to high doses of Pb and increased incidences of teratogenic effect in experimental animals.

Table 5-38 Summary of recent epidemiologic studies of associations between Pb levels and neural tube defects

Reference[a]	Study Location	Study Population	Pb Biomarkers and Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
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
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)
Huang et al. (2011b)	China 2002-2004	Live and still births of women living in the study area (villages in the Lvliang region of Shanxi province)	2 soil samples from each village	56.14 µg/g (11.43 µg/g)	N/A

[a]Studies are presented in order of first appearance in the text of this section.

Among the recent epidemiologic studies (described in Table 5-38), a study of women in Turkey detected no difference between the blood Pb of mothers or the umbilical cord blood Pb of the newborns for healthy infants compared with infants with neural tube defects (cases of spina bifida occulta were excluded, but other forms of spina bifida were included) (Zeyrek et al., 2009). Brender et al. (2006) performed a study of Mexican-American women living in Texas. Measurements were taken 5-6 weeks postpartum, which is a limitation of this study because the blood Pb levels may be different from those during the developmental period of gestation. The OR comparing women with at least 6 µg/dL blood Pb to those with less than 6 µg/dL blood Pb was 1.5 (95% CI: 0.6, 4.3). This increased after adjusting for breast feeding, although this variable was not a confounder because it cannot be associated with neural tube defects. For these women, neither occupational exposure to Pb nor proximity of residence to a facility with Pb air emissions at the time of conception was associated with increased odds of neural tube defects. A study with an ecologic design was performed in China and did not use individual-level biomarkers to determine Pb levels (Huang et al., 2011b). A positive association between Pb levels in soil samples and neural tube defects was reported Exposure to multiple other trace elements also demonstrated a positive association but no control for co-exposures was included in the models for Pb.

Previous studies included in the 2006 Pb AQCD observed associations between Pb and neural tube defects but were limited due to the lack of biologically measured Pb [Pb was measured in drinking water (Bound et al., 1997) and estimated from occupational reports (Irgens et al., 1998)]. A recent ecologic study reported an association between Pb in the soil and neural tube defects but was also limited by its lack of biological samples, as well as a lack of individual-level data and the prevalence of several other metals (Huang et al.,

2011b). Other recent epidemiologic studies of maternal blood Pb levels and neural tube defects observed no associations (Zeyrek et al., 2009; Brender et al., 2006).

5.8.6 Preterm Birth

Epidemiologic studies on preterm birth included in the 2006 Pb AQCD (U.S. EPA, 2006b) reported inconsistent findings regarding the relationship between Pb and gestational age. Recent studies have examined this potential association and again mixed results were reported (Table 5-39). Of these studies, the ones that categorized births as preterm or term all defined preterm birth as less than 37 weeks of gestation. One limitation to note for these studies is that if Pb affects spontaneous abortion and length of gestation via a similar pathway, then the studies that only collect data at delivery and not at earlier stages of pregnancy would be biased toward the null.

Table 5-39 Summary of recent epidemiologic studies of associations between Pb levels and preterm birth

Reference[a]	Study Location	Outcome	Study Population	Pb Biomarkers or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect estimates
Berkowitz et al. (2006)	Idaho 1970-1981	Preterm birth (<37wk)	Singleton births 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)
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
Wells et al. (2011a)	Baltimore, MD 2004-2005	Gestational age	Singleton births from the Baltimore Tracking Health Related to Environmental Exposures (THREE) study	Umbilical cord Pb	0.84 (95%: CI 0.72, 0.96) ≥ 5 µg/dL: 0.7%	Ratio for Pb concentration per 10 days of gestation: 0.99 (0.93, 1.06)
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) ≥ 40 µg/dL: 4.7 (1.1, 19.9) <10 µg/dL: 1.00 (Ref) ≥ 10 µg/dL: 3.2 (1.2, 7.4)
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)

Reference[a]	Study Location	Outcome	Study Population	Pb Biomarkers or Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect estimates
Cantonwine et al. (2010a)	Mexico City 1997-1999	Preterm birth (<37wk), Gestational age	Births to mothers with at least 1 blood Pb measurement during pregnancy and no chronic diseases requiring medication	Maternal blood Pb during pregnancy	Blood Pb Visit at <20wks pregnant 7.2 (5.2) Visit at 20-28 weeks pregnant 6.3 (4.3) Visit at >28 weeks pregnant 6.8 (4.5) Plasma Pb Visit at <20wks pregnant 0.17 (0.16) Visit at 20-28 weeks pregnant 0.13 (0.10) Visit at >28 weeks pregnant 0.16 (0.26)	Linear regression β (95% CI) Blood Pb Visit at <20wks -2.76 (-5.21, -0.31) Visit at 20-28 weeks -1.77 (-3.39, -0.15) Visit at >28 weeks -0.47 (-1.78, 0.84) Average -1.49 (-3.63, 0.64) Plasma Pb Visit at <20wks -2.38 (-4.97, 0.21) Visit at 20-28 weeks -1.34 (-2.98, 0.29) Visit at >28 weeks -1.28 (-2.63, 0.06) Average -0.28 (-2.81, 2.25) Plasma-to-blood Pb ratio Visit at <20wks -3.23 (-6.01, -0.44) Visit at 20-28 weeks -1.41 (-3.10, 0.29) Visit at >28 weeks -1.30 (-2.67, 0.07) Average -1.27 (-3.89, 1.35) Cord blood Pb -0.68 (-2.37, 1.00)
Zhu et al. (2010)	New York 2003-2005	Preterm birth (<37 completed wk)	Singleton births to mothers aged 15-49 with blood Pb measures before or on the date of delivery and blood Pb measuring <10 µg/dL	Maternal blood Pb	2.1	Odd Ratios: ≤ 1.0 µg/dL: 1.00 (Ref) 1.1-2.0 µg/dL: 1.03 (0.93, 1.13) 2.1-3.0 µg/dL: 1.01 (0.92, 1.10) 3.1-9.9 µg/dL: 1.04 (0.89, 1.22)
Chen et al. (2006a)	Taiwan 1993-1997	Preterm birth (<37 wk)	Infants born to at least one parent who was part of the Program to Reduce Exposure by Surveillance System – Blood Lead Levels cohort that monitored workers occupationally exposed to Pb	Maternal blood Pb during pregnancy (or if that wasn't available, the 1 year prior to fertilization) and/or paternal blood Pb during spermatogenesis (the 64 days before fertilization, or if that wasn't available, the 1 year prior to spermatogenesis)	Maternal blood Pb 10.1 (10.4) Paternal blood Pb 12.9 (13.8)	Risk Ratios Maternal blood Pb <10 µg/dL: 1.00 10- 19 µg/dL: 1.97 (0.92, 3.86) ≥ 20 µg/dL: 1.86 (0.68, 4.28) Paternal blood Pb <10 µg/dL: 1.00 10- 19 µg/dL: 1.17 (0.53, 2.32) ≥ 20 µg/dL: 0.55 (0.19, 1.28)
Orun et al. (2011)	Turkey NS	Preterm birth (<37 wk)	Births to mothers not occupationally exposed to toxic metals and living in a suburban but non-industrial area	Breast milk 2 months post-partum	Median: 20.6 µg/L >WHO limit (5 µg/L): 87%	Median Pb (IQR) >37 wk:20.6 (11.2, 29.2) µg/L ≤ 37 wk: 20.4 (14.4, 27.9) µg/L p-value for Mann-Whitney U test: ≥ 0.05

[a]Studies are presented in order of first appearance in the text of this section.

A study of preterm birth included women living in two different residential areas over three different time periods (Berkowitz et al., 2006). One residential area had consistently lower exposures but the other had a period of high Pb emissions due to damage at a local

factory. Preterm birth rates were examined during three time periods: before, during, and after the time of higher Pb 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 exposure time periods, but the number of preterm infants born during the period of higher exposure was small.

In another study, measurements of umbilical cord blood were taken after birth at a hospital in Nagpur, India (Patel and Prabhu, 2009). A sample of women had their blood Pb measured and among this sample, maternal blood Pb was correlated with the umbilical cord Pb levels. Mean gestational age differed between infants with >5 µg/dL cord blood Pb and infants with ≤ 5 µg/dL cord blood Pb. In a linear regression model, gestational age was found to decrease with increasing umbilical cord Pb levels. A study of women in Tennessee consisted primarily of African American women living in an urban setting (Jones et al., 2010). The mean level of umbilical cord blood Pb was slightly higher among infants born preterm but the difference was not statistically significant. Using umbilical cord blood Pb measures, a study reported no association between cord blood Pb levels and gestational age. The concentrations of cord blood Pb among study participants were overall low (99.3% had umbilical cord blood Pb ≤ 5 µg/dL) (Wells et al., 2011a).

In a study taking place in California, women with information on blood Pb levels during pregnancy based on their participation in a surveillance program (reason for participation in the surveillance program was unknown but the authors speculate it was likely because of potential Pb exposure) 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 higher 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 further refined into additional categories, the odds of preterm birth were elevated among women with maximum blood Pb measurement ≥ 20 µg/dL compared with women with maximum blood Pb levels ≤ 5 µg/dL. A study in Iran also reported higher maternal blood Pb for preterm births than for term births (Vigeh et al., 2011). The women in this study had lower blood Pb levels than did those observed in the Jelliffe-Pawlowski et al. study (Jelliffe-Pawlowski et al., 2006). Higher maternal blood Pb level was associated with higher odds of preterm birth. Another study examining blood Pb and gestational age among women with lower blood Pb levels reported an inverse association between maternal blood Pb concentration and gestational age, especially for blood Pb levels early in pregnancy (Cantonwine et al., 2010a). However, a study conducted in New York among women with lower blood Pb levels (inclusion criteria mandated that blood Pb concentration be less than 10 µg/dL), no association was observed between blood Pb levels and preterm birth (Zhu et al., 2010). Similarly, a study

of maternal and paternal blood Pb concentrations reported no association between maternal or paternal blood Pb levels and preterm birth (Chen et al., 2006a).

A study of breast milk in the second month postpartum reported no difference in breast milk Pb levels for those infants born preterm or term; however, a limitation of this study is that Pb levels were not measured until two months after the birth (Örün et al., 2011).

In sum, as in the 2006 Pb AQCD, recent epidemiologic studies report inconsistent findings for a relationship between indicators of Pb exposure and preterm birth. No patterns were apparent within type of exposure measurement or Pb level.

5.8.7 Low Birth Weight/Fetal Growth

The 2006 Pb AQCD reported inconsistent epidemiologic study results for 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, 2006b). Since then, multiple epidemiologic studies on the relationship between Pb exposure and birth weight and fetal growth have been published using various measures of exposure, such as air levels, umbilical cord blood, and maternal blood and bone. These studies are summarized in Table 5-40 below. Additionally, there have been a few recent toxicological studies evaluating the effect of Pb exposure during gestation on birth weight.

Table 5-40 Summary of recent epidemiologic studies of associations between Pb levels and low birth weight and fetal growth

Reference[a]	Study Location	Outcome	Study population	Pb Biomarkers and Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
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)
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) ≥ 40 µg/dL: -- <10 µg/dL: 1.00 (Ref) ≥ 10 µg/dL: 3.6 (0.3, 40.0) Small for gestational age ≤ 5 µg/dL: 1.00 (Ref) 6-9 µg/dL: -- 10-19 µg/dL: 2.3 (0.6, 9.2) 20-39 µg/dL: 2.1 (0.7, 6.7) ≥ 40 µg/dL: -- <10 µg/dL: 1.00 (Ref) ≥ 10 µg/dL: 4.2 (1.3, 13.9)
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

Reference[a]	Study Location	Outcome	Study population	Pb Biomarkers and Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
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)
Zhu et al. (2010)	New York 2003-2005	Birth weight, small for gestational age (birth weight for gestational age <10th percentile based on national birth weight by gestational week from weeks 25-42	Singleton births to mothers aged 15-49 with blood Pb measures before or on the date of delivery and blood Pb measuring <10 µg/dL	Maternal blood Pb	2.1	Difference in birthweight in grams: 0 µg/dL: Ref 1 µg/dL: -27.4 (-37.8, -17.1) 2 µg/dL: -38.8 (-53.4, -24.1) 3 µg/dL: -47.5 (-65.4, -29.6) 4 µg/dL: -54.8 (-75.5, -34.2) 5 µg/dL: -61.3 (-84.4, -38.2) 6 µg/dL: -67.2 (-92.5, -41.8) 7 µg/dL: -72.5 (-99.9, -45.2) 8 µg/dL: -77.6 (-106.8, -48.3) 9 µg/dL: -82.3 (-113.3, -51.2) 10 µg/dL: -86.7 (-119.4, -54.0) After exclusion of blood Pb <1 µg/dL, a 1 µg/dL increase in blood Pb was associated with a 7.0 g decrease in birthweight Odd Ratios for small for gestational age: ≤ 1.0 µg/dL: 1.00 (Ref) 1.1-2.0 µg/dL: 1.07 (0.98, 1.17) 2.1-3.0 µg/dL: 1.06 (0.98, 1.16) 3.1-9.9 µg/dL: 1.07 (0.93, 1.23)

Reference[a]	Study Location	Outcome	Study population	Pb Biomarkers and Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Chen et al. (2006a)	Taiwan 1993-1997	Low birth weight (<2,500 g), small for gestational age (birth weight ≤ 10th percentile of sex- and gestational wk weights for singletons in 1993-1996)	Infants born to at least one parent who was part of the Program to Reduce Exposure by Surveillance System – Blood Lead Levels cohort that monitored workers occupationally exposed to Pb	Maternal blood Pb during pregnancy (or if that wasn't available, the 1 year prior to fertilization) and/or paternal blood Pb during spermatogenesis (the 64 days before fertilization, or if that wasn't available, the 1 year prior to spermatogenesis)	Maternal blood Pb 10.1 (10.4) Paternal blood Pb 12.9 (13.8)	Risk Ratios Low birth weight Maternal blood Pb <10 µg/dL: 1.00 (Ref) 10- 19 µg/dL: 2.22 (1.06, 4.26) ≥ 20 µg/dL: 1.83 (0.67, 4.20) Paternal blood Pb <10 µg/dL: 1.00 (Ref) 10- 19 µg/dL: 0.83 (0.34, 1.75) ≥ 20µg/dL: 0.42 (0.12, 1.06) SGA Maternal blood Pb <10 µg/dL: 1.00 (Ref) 10- 19 µg/dL: 1.62 (0.91, 2.75) ≥ 20µg/dL: 2.15 (1.15, 3.83) Paternal blood Pb <10 µg/dL: 1.00 (Ref) 10- 19 µg/dL: 0.94 (0.49, 1.66) ≥ 20 µg/dL: 0.94 (0.51, 1.62)
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
Afeiche et al. (2011)	Mexico City 1994-2005	Birth weight	Term, singleton births, at least 2,500 grams enrolled in one of three birth cohorts recruited for other longitudinal studies	Maternal patella and tibia Pb measured at 1 month postpartum	Patella Pb 10.4 (11.8) µg/g Tibia Pb 8.7 (9.7) µg/g	β (95% CI) for 1 SD increase in maternal patella Pb Girls -45.7 (-131.7, 40.2) Boys 72.3 (-9.8, 154.4) No association for birth weight and tibia Pb among girls. A positive association was observed for tibia Pb and birth weight among boys. (results not given)

Reference[a]	Study Location	Outcome	Study population	Pb Biomarkers and Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Cantonwine et al. (2010b)	Mexico City 1994-1995	Birthweight	Infants who were part of a clinical trial to assess maternal calcium supplementation on bone lead mobilization during lactation	Umbilical cord blood Pb Maternal tibia and patella Pb one month after delivery	Umbilical cord blood Pb varied by genotype from 6.3 to 6.9 Umbilical cord blood Pb ≥ 10 µg/dL: 12.6%	Regression models β (95% CI) Umbilical cord blood Pb -31.1 (-105.4, 43.3) Maternal tibia Pb Overall -4.4 (-7.9, -0.9) <1-4.1 µg/g: Ref 4.1-9.2 µg/g: 17.2 (-75.6, 110.1) 9.2-15.4 µg/g: -19.1 (-112.1, 73.9) 15.4-43.2 µg/g: -95.4 (-189.9, -0.8)
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
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) (i.e., in this study, Pb is assessed as the outcome)
Atabek et al. (2007)	Turkey NS	Birth weight, birth length, head circumference, mid-arm circumference	Term, singleton infants born to healthy mothers living in urban areas and assumed to have high Pb concentrations	Umbilical cord blood Pb	14.4 (8.9) Umbilical cord blood Pb ≥ 10 µg/dL: 53.7% Umbilical cord blood Pb ≥ 25 µg/dL: 9.2%	Regression models β (p-value) Birth weight -0.81 (0.01) Birth length 0.41 (0.05) Mid-arm circumference 0.30 (0.05)
Al-Saleh et al. (2008b)	Saudi Arabia 2004	Head circumference	Infants with a gestational age of at least 34 weeks born to healthy mothers aged 17-46 years and non-occupationally exposed to Pb	Umbilical cord blood Pb	2.210 (1.691) Umbilical cord blood Pb >10 µg/dL: 1.23%	Regression models for those above the 75th percentile of cord blood Pb levels β (SE) per unit of log-transformed Pb -0.158 (0.718), p-value: 0.036
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) ≥ 10 µg/dL: 0.82 (0.57, 1.17)
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

Reference[a]	Study Location	Outcome	Study population	Pb Biomarkers and Exposure Measurement	Mean Pb (SD) in µg/dL	Adjusted Effect Estimates
Wells et al. (2011a)	Baltimore, MD 2004-2005	Birth weight	Singleton births from the Baltimore Tracking Health Related to Environmental Exposures (THREE) study	Umbilical cord Pb	0.84 (95%: CI 0.72, 0.96) ≥ 5 µg/dL: 0.7%	Ratio for Pb concentration per 100g birth weight: 1.01 (0.99, 1.02)
Örün et al. (2011)	Turkey NS	Birth weight and head circumference	Births to mothers not occupationally exposed to toxic metals and living in a suburban but non-industrial area	Breast milk 2 months post-partum	Median: 20.6 µg/L >WHO limit (5 µg/L): 87% Median (IQR) <2500g: 20.4 (8.5, 27.1) µg/L ≥ 2500g: 20.6 (11.8, 29.5) µg/L	Correlations for breast milk Pb and z-scores of head circumference Girls 0.087 Boys 0.029 Correlations for breast milk Pb and z-scores of birth weight Girls 0.097 Boys 0.045 *All p-values for correlations>0.05
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
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)

[a]Studies are presented in order of first appearance in the text of this section.

Women residing in two different towns in Yugoslavia (one with a Pb smelter and one without a Pb smelter) were recruited during their first prenatal visit (Lamb et al., 2008) (study based on previous work by Factor-Litvak et al. (1991). The mid-pregnancy blood Pb levels were greater in women from the town with a Pb smelter. No association was reported between maternal blood Pb and height or BMI at birth for the infants of these women despite the differences in maternal blood Pb between the two towns. Multiple studies were conducted that examined the association between maternal blood Pb and birth weight/fetal growth. In California, blood Pb measurements of women during pregnancy were matched with the corresponding birth certificates (Jelliffe-Pawlowski et

al., 2006). The adjusted OR for low birth weight that compared women with blood Pb levels ≥ 10 µg/dL to women with levels <10 µg/dL was elevated. However, it was difficult to draw conclusions about the relationship between blood Pb and birth weight due to small numbers (n = 9 for low birth weight) and the subsequently wide 95% CI. An association was detected for high blood Pb and having an infant who was small of his/her gestational age (SGA). 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). A study in Vienna, Austria reported an inverse association between maternal blood Pb levels and birth weight but no associations for birth length or head circumference (Gundacker et al., 2010). Similarly, increased maternal blood Pb was associated with decreased birth weight, with the strongest associations observed at the lowest blood Pb level (Zhu et al., 2010). No association was observed between maternal blood Pb and SGA. A study in Taiwan examined both maternal and paternal blood Pb levels among those occupationally exposed to Pb and their associations with birth weight and SGA (Chen et al., 2006a). Paternal blood Pb levels were not associated with increased risk of low birth weight or SGA. Higher maternal blood Pb concentration was associated with higher risk of low birth weight and SGA, although not all of the associations were statistically significant. There were small numbers of infants with low birth weight or SGA, especially at the highest blood Pb levels (≥ 20 µg/dL).

A study examining the association between Pb biomarker levels and birth weight used tibia bone measurements from mothers living in Mexico City (Kordas et al., 2009). Tibia Pb levels were inversely associated with birth weight but not with birth length. This association between Pb and birth weight was not modified by maternal folate consumption or maternal or infant MTHFR genotype, although the association between tibia Pb levels and birth weight was greater in magnitude among women with certain genotypes (statistical tests not reported). Another study in Mexico City reported no association between maternal tibia Pb levels and birth weight among girls but reported a positive association for boys (Afeiche et al., 2011). No associations were observed with maternal patella Pb concentration, although among boys, the relationship was positive but not statistically significant. One of the cohorts used by Afeiche et al. (2011) was also evaluated in another study (Cantonwine et al., 2010b). An inverse association was observed between tibia Pb and birth weight, especially at higher levels. This association was stronger among those mothers with variants of the hemochromatosis iron gene (HFE).

Multiple studies examined the relationship between Pb level and birth weight using Pb measured from the placenta or umbilical cord. Researchers in Chile collected the placentas from term births and compared the Pb levels for those born with normal birth

weights to those with low birth weights (Llanos and Ronco, 2009). Pb levels were greater in the placentas of infants with low birth weights. In addition, the authors note that 3 low birth weight infants had extremely high Pb levels in the placentas (>1.5 µg/g) and were excluded from these analyses. A study in Brazil examined Pb levels in umbilical cord blood from term births of women residing within 5 km of a Pb smelter (Zentner et al., 2006). The cord blood Pb level was found to be inversely correlated with length and weight of the infants. A study with high Pb concentrations in umbilical cord blood reported an inverse association between Pb levels and birth weight (Atabek et al., 2007). However, no correlation was detected in an analysis restricted to umbilical cord Pb less than 10 µg/dL. No association with other measures of growth, such as birth length and mid-arm circumference, were detected. In Saudi Arabia, a study was conducted among non-occupationally exposed women (Al-Saleh et al., 2008b). Umbilical cord blood Pb concentrations were low and an association was observed between umbilical cord Pb and head circumference. Another study recruited women in Pakistan (Janjua et al., 2009). Umbilical cord blood Pb levels were not associated with low birth weight. The study by Iranpour et al. (2007) discussed above investigated the association with umbilical cord blood Pb levels in addition to their examination of maternal whole blood Pb. They again report no difference in levels between term infants of normal and low birth weight. A study comparing geometric mean umbilical cord blood Pb levels reported no difference in the levels for normal and low birth weight infants born to women living primarily in urban areas of Memphis, TN (Jones et al., 2010). A study performed in Baltimore, MD also reported no association between umbilical cord blood Pb concentration and birth weight (Wells et al., 2011a). This study had low blood Pb levels, with only 0.7% of participants having umbilical cord blood Pb measuring > 5 µg/dL. A study previously mentioned that observed an inverse association between maternal tibia Pb and birth weight in Mexico City reported no association between umbilical cord blood Pb concentration and birth weight (Cantonwine et al., 2010b). Finally, a study in Vienna measured Pb in the placenta (Gundacker et al., 2010). A positive correlation was observed between placenta Pb and birth length and weight; however, in the same study, maternal blood Pb was inversely related to birth weight.

A study performed in Turkey examined the relationship between Pb levels in breast milk two months postpartum and size at birth (Örün et al., 2011). No association was observed between breast milk Pb concentration and birth weight or head circumference.

A few studies examined air exposures and reported inverse associations between air Pb concentrations and birth weight. 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. Another study of air Pb levels was conducted in Idaho and included two areas over three time periods. One study area was affected by damage to 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 infants born to women living in the high exposure area during the period of high exposure compared to those living in the lower exposure area. The difference in birth weight of term births remained, but was reduced, between the two areas during the time period after the exposure ended. During the period of higher exposure, the odds of low birth weight among term births was increased among those living in the higher exposed area compared to those in the lower exposed area, but the odds were not different between the two study areas during the time periods before or after the high level of exposure. An increase in SGA infants (defined as infants with weights less than or equal to the lowest 5th percentile of birth weight for their sex and age) was also associated with living in the higher exposed area during the time period of higher exposure. The odds of SGA infants decreased during the time period after the exposure but the odds were still elevated compared to those residing in the lower exposed area.

Evidence from previous toxicological studies has shown an association between gestational Pb exposure and reduced birth weight and impaired postnatal growth (U.S. EPA, 2006b). More recent studies have reported conflicting results. Wang et al. (2009e) demonstrated a statistically significant decrease in fetal body weight and body length of Wistar rats after maternal exposure to 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, litter weight was significantly decreased (400 ppm Pb only) versus controls due to significant decrements in female pup birth weight; male birth weight was unaffected. 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 (exposure during gestation and lactation resulting in mean blood Pb level of 22.8 µg/dL), but no changes in body length were reported. In contrast, Leasure et al. (2008) reported a statistically significant inverse relationship between Pb exposure and body weight for male mice exposed to low (27 ppm)-and high (55 ppm)-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 Effects on Postnatal Stature and Body Weight

Findings from previous toxicological studies of rodents and primates have demonstrated Pb induced impairment of postnatal growth (U.S. EPA, 2006b). Several recent epidemiologic studies examining the association of various biomarkers of Pb exposure with stature and body weight have been conducted and the evidence reported is mixed.

Table 5-41 Summary of recent epidemiologic studies of associations between Pb levels and postnatal growth

Reference[a]	Study Location and Years	Study Population	Pb Biomarker	Mean Pb (SD)	Effect Estimates
Afieche et al. (2011)	Mexico City, Mexico Children born between 1994 and 2005	n=522 boys n=477 girls	Maternal bone Pb	Patella: 10.4 (11.8) µg/g	Change in weight at 5 years of age (g) per 1 SD increase in maternal bone Pb Girls: 130.9 (95% CI: -227.4 to -34.4) Boys: 13.0 (95%CI -73.7, 99.9) Adjusted for cohort, maternal age, calf circumference, height, education, number of pregnancies, breast feeding for 6 months, calcium treatment, child's gestational age at birth, height.
Sanna et al. (2011)	Sardinia, Italy Data collected in 1998, 2002 and 2007	n=825 children 11-14 yrs old	Pb in hair	1998: 5.84 (6.56) µg/g 2002: 1.49 (1.72) µg/g 2007: 0.78 (0.93) µg/g	Height 1998: β log Pb= -0.121 (p=0.0021) 2002: β log Pb= -0.115 (p=0.0349) 2007: β log Pb= -0.011 (p=0.8665) Sitting Height 1998: β log Pb=-0.117 (p=0.0017) 2002: β log Pb=-0.036 (p=0.5149) 2007: β log Pb=0.028 (p=0.6633) ELL 1998: β log Pb=-0.103 (p=0.0209) 2002: β log Pb=-0.164 (p=0.0057) 2007: β log Pb=-0.008 (p=0.9058) Adjusted for age and sex
Ignasiak et al. (2006)	South-western Poland 1995 (Industrial area with copper smelters and refineries)	school children 7-15 years n=463 boys n= 436 girls	Concurrent blood Pb	7.7 (3.5) µg/dL	Estimated decrement per 10 µg/dL increase in blood Pb Weight: Boys: 2.8 kg Girls: 3.5 kg Height: Boys: 3.2 cm Girls: 4.0 cm Trunk length: Boys: 1.2 cm Girls: 1.1 cm Leg length: Boys: 2.1 cm Girls: 2.9 cm Arm length: Boys: 1.8 cm Girls: 1.9 cm Adjusted for age, age2, and education level of mother

Reference[a]	Study Location and Years	Study Population	Pb Biomarker	Mean Pb (SD)	Effect Estimates
Hauser et al. (2008)	Chapaevsk, Russia May 2003 – May 2005	n=489 boys 8-9 yrs old	Concurrent blood Pb	3 (2-5) µg/dL Median (25-75 percentile)	Regression coefficient (95% CI) Height (cm): -1.439 (-2,25, -0.63) Weight (kg): -0.761 (-1.54, 0.02) BMI: -0.107 (-0.44, 0.23) Adjusted for birth weight, gestational age, and age at exam
Little et al. (2009)	Dallas, Texas 1980-1989 and 2002	n=191 (1980s) n=169 (2002) 2-12 yrs old	Concurrent blood Pb	1980s: 23.6 (1.3 SE) µg/dL 2002: 1.6 (0.2 SE) µg/dL	Blood Pb effect per 10µg/dL Pb increase – Mean(95%CI) Height (cm): -2.1 (-1.9, -2.3) Weight (kg): -1.9 (-1.7, -2.1) BMI (kg/m2): -0.5 (-0.4, -0.7) Adjusted for age, age2, sex and cohort effect
Min et al. (2008b)	Seoul, South Korea Date(s) not specified	n=62 boys n= 46 girls 5-13 yrs	Concurrent blood Pb	2.4 (0.7) µg/dL	Linear model estimate (SE; p) Height: -1.449 (0.639; p=0.026) Total arm length: -1.804 (0.702; p=0.012) Body weight: -0.646 (0.718; p=0.370) BMI: -0.006 (0.272; p=0.982) Adjusted for age, sex, and father's education
Schell et al. (2009)	Albany, New York 1986-1992, 1992-1998	n=244	Maternal blood Pb during second trimester, third trimester, and delivery; Infant blood Pb at delivery, 6 months, and 12 months	Maternal blood Pb during second trimester 2.8 (2.6) µg/dL, maternal blood Pb during third trimester: 2.6 (2.2) µg/dL, maternal blood Pb at delivery: 2.8 (2.4) µg/dL Infant blood Pb at delivery: 2.3 (2.7) µg/dL, infant blood Pb at 6 months: 3.2 (3.3) µg/dL, and infant blood Pb at 12 months: 6.3 (4.8) µg/dL	Linear model for maternal second trimester Pb: -0.242 (p-value 0.01) for 6-month head circumference. When examining second trimester maternal Pb ≥ 3 µg/dL, associations were observed for 6 mo weight for age, 6 mo weight for length, 6 and 12 mo head circumference, and 12 mo upper arm circumference for age Adjusted for infant sex, infant birth weight, infant nutrition, maternal age, marital status, employment, race, height, parity, second trimester smoking, and education.

Reference[a]	Study Location and Years	Study Population	Pb Biomarker	Mean Pb (SD)	Effect Estimates
Lamb et al. (2008)	Kosovo, Yugoslavia 1985-1986	n=309 mother child pairs	Maternal blood Pb	Pristina: 5.60 (1.99) µg/dL Mitrovica: 20.56 (7.38) µg/dL	Regression coefficients relating maternal blood Pb to height (95% CI): Pristina Birth: 0.35 (-0.64, 1.34) 1 yr: -0.61 (-2.24, 1.03) 4 yr: 0.79 (-1.71, 3.29) 6.5 yr: 0.15 (-2.43, 2.74) 10 yr: -0.09 (-3.69, 3.52) Mitrovica Birth: 0.43 (-0.83, 1.69) 1 yr: -0.30 (-2.55, 1.96) 4 yr: -0.72 (-3.26, 1.82) 6.5 yr: -1.87 (-4.38, 0.64) 10 yr: -2.87 (-6.21, 0.47) To BMI (95% CI): Pristina Birth: -0.14 (-0.69, 0.42) 1 yr: 0.61 (-0.28, 1.50) 4 yr: 0.17 (-0.67, 1.00) 6.5 yr: 0.61 (-0.09, 1.30) 10 yr: -0.49 (-1.45, 0.46) Mitrovica Birth: -0.18 (-0.69, 0.33) 1 yr: 0.23 (-0.84, 1.30) 4 yr: 0.16 (-0.66, 0.98) 6.5 yr: -0.12 (-0.90, 0.66) 10 yr: 1.31 (-0.95, 3.57) Adjusted for sex, ethnicity, parity, maternal height or maternal BMI, maternal education, gestational age at delivery, gestational age at blood sample, and HOMES score
Zalina et al. (2008)	Kuala Lumpur, Malaysia	n=269 children 6.5-8.5 yrs old n=169 urban n=100 industrial	Concurrent blood Pb	Industrial: 3.75 µg/dL Urban: 3.56 µg/dL	Correlation with blood lead: Height for age: Urban: -0.095 (p=0.219) Industrial: -0.037 (p=0.716) Weight for age: Urban: 0.019 (p=0.806) Industrial: -0.063 (p=0.535) Weight for height: Urban: 0.136 (p=0.079) Industrial: -0.069 (p=0.493) Left arm circumference: Urban: 0.041 (p=0.595) Industrial: -0.055 (p=0.587)

Reference[a]	Study Location and Years	Study Population	Pb Biomarker	Mean Pb (SD)	Effect Estimates
Tomoum et al. (2010)	Cairo, Egypt Jan-Jun 2007	n=45 boys and girls 10-13 yrs old	Concurrent blood Pb	9.46 (3.08) µg/dL	Percentage of the median (SD): Pb<10 µg/dL Weight: Boys: 127.56 (16.26)% Girls: 114.8 (10.8)% Height: Boys: 98.06 (3.19)% Girls: 96.75 (2.91)% Pb≥ 10 µg/dL Weight: Boys: 122.0 (16.71)% Girls: 123.11 (12.52)% Height: Boys: 99.5 (5.04)% Girls: 100.33 (4.53)% Adjusted for age and sex
Olivero-Verbel et al. (2007)	Cartegena, Columbia Jun-Aug 2004	n=189 children 5-9 yrs old	Concurrent blood Pb	5.49 (0.23) µg/dL	Spearman correlation coefficient (p-value) between blood Pb and body size: -0.224 (0.002) *no significance in partial correlation between blood Pb and size when controlled for age: -0.096 (0.189)
Liu et al. (2011a)	Guiyu, China Chendian, China Jan-Feb 2008	n=303 3-7 yrs old	Concurrent blood Pb	Guiyu: 13.2 (4.0-48.5) µg/dL Chendian: 8.2 (0-21.3) µg/dL Median (range)	Mean chest circumference in girls was lower among those with higher blood Pb levels (>10µg/dL) Mean chest and head circumference in children > 6 years old greater among those with higher blood Pb levels (>10 µg/dL) No multivariate adjustment, stratification by age/sex
Mahram et al. (2007)	Zanjan province,, Iran Date(s) not specified	n=42 boys n= 39 girls 7-11 yrs	Concurrent blood Pb	Area with lead smelters: 37.0 (24.7) µg/dL Area without lead smelters: 15.6 (13.4) µg/dL	Comparison of control and study groups Height, standardized for age: p-value 0.52 Weight, standardized for age: p-value 0.8

*Estimated Lower Limb Length

[a]Studies are presented in order of first appearance in the text of this section.

Results from recent epidemiologic studies of postnatal growth are summarized in Table 5-41. Afeiche et al. (2011) conducted a longitudinal study of children in Mexico City, born between 1994 and 2005. Maternal bone Pb during pregnancy was associated with a significant decrease in BW at age 5 years in girls but not in boys. The findings were robust to additional adjustment for child's blood Pb level. In a study of children in Sardinia Italy, Sanna et al. (2011) measured Pb in hair at three points in time (1997, 2002, and 2007) and reported cross-sectional results from regression analyses for each of these time periods. Pb in hair decreased over time and significant associations of Pb in hair

with height were observed only in earlier time periods when hair Pb levels were relatively high.

Ignasiak et al. (2006) studied school children aged 7-15 years living close to copper smelters and refineries in Poland to assess the impact of Pb exposure on their growth status. There was a statistically significant linear relationship between concurrent blood Pb and reduced weight, height, trunk, leg and arm lengths. This decrease in height was more influenced by decreases in leg length than trunk length. These results also indicated that there was attenuation in osteoblast activity associated with higher blood Pb levels, consistent with animal toxicological studies (Long et al., 1990). Hauser et al. (2008) investigated the relationship between blood Pb and height in boys living in Chapaevsk, Russia. In a multivariate adjusted regression analysis, height significantly decreased with increasing blood Pb. Statistically nonsignificant decreases in weight and BMI were also observed. The association of blood Pb with height, weight and BMI was examined among two cohorts of children living near Pb smelters in Texas (Little et al., 2009). The first cohort included children 2-12 years old in 1980 and the second cohort included children of the same age in 2002 when blood Pb levels were substantially lower. Decreases in height, weight and BMI with increasing blood Pb levels were observed among children in both cohorts and increases in height and weight were observed comparing children from the 2002 cohort to those from the 1980 cohort. In a study with Korean children, Min et al. (2008b) observed that height and total arm length decreased significantly with increasing blood Pb in multivariate adjusted regression models. A statistically nonsignificant decrease in body weight was observed with increasing blood Pb while no effect on BMI reported. A study in New York reported an association between maternal blood Pb during the second trimester of pregnancy and various measures of growth, especially among those mothers with blood Pb levels of at least 3 µg/dL (Schell et al., 2009). These associations did not persist for those with maternal blood Pb levels less than 3 µg/dL. Among infants, 6 month blood Pb levels were not associated with 12 month measures of growth. In comparisons of changes in blood Pb levels over time, high maternal blood Pb combined with low 12 month blood Pb among infants (indicating a decrease in blood Pb over time) resulted in the greatest growth, even compared to those with both low maternal and infant blood Pb measures.

Contrary to the results summarized above, several studies do not observe associations between blood Pb levels and impaired growth. In a prospective study of 309 mother-child pairs from Yugoslavia, the relationship between maternal blood Pb and attained height in children was investigated in those living in an highly exposed town with a smelter and battery plant and those living in a relatively lower exposed town (Lamb et al., 2008). In multivariate adjusted regression models, neither attained height (at birth, 1, 4, 6.6, or 10 years age) nor rate of height change per month (at birth-1 year, 1-4 years, 4-6.5 years,

6.5-10 years age) was associated with maternal pregnancy blood Pb levels in either the industrial or less exposed town. Weight was also not associated with maternal blood Pb in this study. In a study with a similar design, Zailina et al. (2008) studied the relationship of blood Pb and height in 7 year-old Malaysian school children comparing those attending two schools in an urban setting to those attending a school near an industrial area. After adjustment for age no statistically significant associations between concurrent blood Pb and physical development were observed. Tomoum et al. (2010) investigated the association between blood Pb and height in pubertal children in Cairo, Egypt. Neither boys nor girls with concurrent blood Pb levels > 10 µg/dL differed significantly in height or weight when compared to those with blood Pb < 10 µg/dL. In a simple correlation analysis of children aged 5-9 years in Colombia, Olivero-Verbel et al. (2007) reported that concurrent blood Pb levels were negatively associated with body size ($r = -0.224$, $p < 0.002$). However, when a partial correlation analysis was performed controlling for age, the association between blood Pb and body size was no longer statistically significant. In a study of school children in China chest and head circumference were found to differ between high (>10µg/dL) and low concurrent blood Pb level groups; however, the direction of the difference was not consistent (Liu et al., 2011a). Among girls, comparing those with high and low blood Pb levels, a reduction in head circumference was observed. Among children greater than 6 years of age, those with higher blood Pb levels were reported to have greater head and chest circumferences. In a study of children aged 7-11 years and living in an area of Iran with or without Pb smelters, age-standardized weight and height did not vary by study area (Mahram et al., 2007).

Recent toxicological studies report significant changes in postnatal or adult body weight after Pb exposure during different developmental windows. Masso-Gonzalez and Antonio-Garcia (2009) found Pb-induced decreased body weights at weaning (PND21) in rat pups from dams exposed to Pb during pregnancy and lactation. Blood Pb level in the control group was 1.43 µg/dL, in the Pb group it was 22.8 µg/dL. Dong et al. (2009) reported decreased body weight in adult Kunming mice after exposure to 0.6% Pb-acetate in drinking water for 8 weeks. In contrast, Leasure et al. (2008) reported a statistically significant inverse relationship between Pb exposure and body weight for male mice exposed to lower (27 ppm), moderate (55 ppm) and higher levels (109 ppm) levels of Pb during gestation and lactation (2 weeks before mating, through gestation and to PND10) with those exposed to the lowest dose having the highest adult body weight among the overweight Pb-exposed animals. Male mice exposed to the lower and higher Pb concentrations during gestation were 26% and 13% heavier than were controls at 1 year of age, respectively. In this study, dams were administered 27 ppm (low), 55 ppm (moderate), and 109 ppm (high) Pb in drinking water beginning which resulted in respective blood Pb levels from 10 µg/dL or less in the low-exposure offspring to 42 µg/dL in the high-exposure offspring at PND10. Leasure et al. (2008) also exposed a

separate group of mice to Pb only during the postnatal period (PND0-PND21, lactation only exposure) and mice exposed to the same aforementioned low or high dose of Pb did not exhibit a difference in body weight when compared to control offspring. Wang et al. (2009e) observed a statistically significant decrease in fetal body weight and body length of Wistar rats at GD20 after maternal exposure to 0.025% Pb-acetate during gestation days 1-10, 11-20, or 1-20. Also, associations were reported between elevated maternal blood Pb levels (0.6, 1.3, or 1.74 µM, respectively or ~12.4, 26.9, or 36.0 µg/dL, respectively) compared to control (0.04 µM or ~ 0.83 µg/dL) and decreased pup body length, and placental weight in Wistar rats at GD20. The greatest decrease in fetal body weight and length was observed in the group exposed to Pb during gestation days 1-20 followed by the group exposed to Pb during gestation days 11-20. Teijón et al. (2006) observed reductions in birthweight of litters administered 200 ppm or 400 ppm Pb-acetate in drinking water, but found that this effect did not persist in the postnatal growth of the rats.

Notably, previous toxicological studies observed reductions in postnatal weight as well as birth weight after exposure to Pb, albeit often at higher concentrations of Pb exposure. Ronis et al. (2001; 1998a; 1998b; 1996) have published a series of papers exposing rats to Pb over different developmental windows, showing associations between Pb exposure and deficits in growth. Sprague-Dawley rats with lifetime Pb exposure to 0.6% Pb-acetate in drinking water (gestational-termination of experiment Pb exposure, maximum blood Pb of 316 µg/dL in males and 264 µg/dL in females) had sex-independent pre-pubertal growth suppression, male-specific suppression of pubertal growth and loss of growth effects postnatally but still maintained an overall decreased body size out to PND60 due to earlier deficits. In a follow up study using the same exposure duration with a dose of 0.45% Pb-acetate (resulting in blood Pb of 263 µg/dL at PND85) yielded the same results (Ronis et al., 1996) with mechanistic insight showing decrements in insulin-like growth factor 1 (IGF1) accompanying the decreases in growth rates.

The body of toxicological literature on postnatal growth with Pb exposure indicates that Pb exposure can induce decrements in both height/body length and BW that may be persistent and differ by sex. However, findings from epidemiologic studies of postnatal growth are not consistent. Animal toxicology studies give insight to mechanistic changes that may contribute to this Pb-induced decrement and to the windows of exposure that may contribute greatest to these decrements.

5.8.9 Toxicological Studies of Developmental Effects

5.8.9.1 Developmental Effects on Blood and Liver

The 1986 and 2006 Pb AQCDs reported studies that suggest Pb may alter hematopoietic and hepatic function during development. Some recent studies provide evidence that support these findings; however recent results are not consistent among the studies.

Massó et al. (2007) reported a decrease in liver weights of pups born to dams that consumed 300 mg/L Pb in drinking water during gestation and lactation. They also reported an increase in the number of erythrocytes; however the erythrocyte size was diminished by 62%. Pb produced microcytic anemia as evidenced by decreased hemoglobin content and hematocrit values without changes in mean corpuscular hemoglobin (MCH) concentration. Alkaline phosphatase (ALP) activity, CAT activity, or thiobarbituric acid reactive substances (TBARS) production did not change in pups at 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 (2009) reported increases in TBARS and CAT activity in the liver after Pb exposure. Intoxication with Pb also resulted in decreased liver protein concentrations and 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 by a study by Uzbekov et al. (2007). The authors reported an initial increase in SOD activity in livers of pups exposed to 0.3 mg/L and 3.0 mg/L Pb nitrate during gestation for 1 month (mean daily consumption 27 µg/kg). In contrast, long-term

exposure (5 months) to the same concentrations of Pb nitrate concentration during gestation resulted in decreased hepatic SOD activity.

Effects on hepatic Phase I and Phase II enzymes after early developmental exposure of offspring to Pb during gestation and lactation was evaluated by Pillai et al. (2009). In the study, pregnant Charles Foster rats were administered 0.05 mg/kg body weight Pb subcutaneously throughout gestation until PND21. Pups were evaluated on PND56. Results of the study show that Phase I xenobiotic-metabolizing enzymes (NADPH- and NADH cytochrome c reductase) and Phase II xenobiotic- and steroid-metabolizing enzymes (δ-glutamyl transpeptidase, UDPGT, glutathione-s-transferase, and 17β-hydroxysteroid oxidoreductase) were reduced in both male and female pups by PND56. Only inhibition in glutathione-s-transferase and 17β-hydroxysteroid oxidoreductase activities demonstrated a sex-specific pattern (glutathione-s-transferase inhibition in males; 17β-hydroxysteroid oxidoreductase inhibition greater in females). Observed Pb-induced histological changes included massive fatty degeneration in hepatocytes, large vacuoles in cytoplasm, appearance of pyknotic nuclei, and infiltration of lymphocytes in the liver. 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.

5.8.9.2 Developmental Effects on Skin

The 2006 Pb AQCD (U.S. EPA, 2006b) reported a study that demonstrated Pb-induced abnormalities in skin development. No current studies were identified that addressed Pb-induced skin alterations.

5.8.9.3 Developmental Effects on the Retina

The 2006 Pb AQCD concluded that Pb exposure during early postnatal development (resulting in blood Pb levels ~20 µg/dL) impaired retinal development in female Long-Evans hooded rats. A more recent study (Fox et al., 2008) exposed female Long-Evans hooded rats to 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 measured in these pups on postnatal days 0-10 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 (ERGs) (Figure 5-46, Table 5-33) 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 (Figure 5-46, and Table 5-33). High-level Pb exposure (109 ppm) statistically significantly decreased the number of rods in central and peripheral retina. Pb-exposure induced concentration-dependent decreases in adult rat retinal dopamine synthesis and utilization/release.

5.8.9.4 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.

5.8.10 Summary and Causal Determination

Many epidemiologic and toxicological studies of the effects of Pb on reproductive outcomes have been conducted since the 2006 Pb 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 concentration-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 Pb AQCD examined 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 Pb AQCD on male fertility showed Pb exposure or biomarkers of Pb-exposure were associated with decrements in 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, recent epidemiologic studies of reproductive factors among males and females investigated whether Pb biomarker 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.

Delayed puberty has been linked to decreased peak bone mass and increased risk of osteoporotic fractures (Gilsanz et al., 2011; Naves et al., 2005). Multiple epidemiologic studies of Pb and puberty have shown associations between concurrent blood Pb levels and delayed pubertal development for girls and boys (evidence is stronger among girls). These associations are consistently observed in multiple epidemiologic studies in populations with blood Pb levels <10µg/dL. Confounders considered in the epidemiologic studies that performed regression analyses varied. Most studies controlled for age and BMI. Other variables, such as measures of diet and SES and race/ethnicity, were included in some of the studies. Many of the studies demonstrating positive associations adjusted for many of the potential confounders. No patterns were detected in the associations between Pb and puberty based on inclusion of specific confounders.

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 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 2006 Pb AQCD included a few studies that reported potential associations between Pb and neural tube defects, but the recent epidemiologic studies found no association. Some 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 (maternal blood Pb or umbilical cord and placenta Pb were the biomarkers most commonly used in studies of low birth weight). Effects of Pb exposure during early development on toxicological studies included reduction in litter size, implantation, birth weight and postnatal growth. Findings from epidemiologic studies of postnatal growth are inconsistent.

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 eyes 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 (Sections 7.3.5.2, 7.2.4.2, and 7.3.5.3).

In conclusion, the recent toxicological and epidemiologic literature provides strong evidence that Pb exposure is associated with effects on reproduction and development. The weight of the evidence supports the association of Pb exposure with delayed onset of puberty in both males and females and detrimental effects on sperm and semen quality in occupationally-exposed males and in laboratory animals. In cross-sectional epidemiologic studies of girls (ages 6-18 years) with mean and/or median concurrent blood Pb levels less than 5 µg/dL consistent associations with delayed pubertal development (measured by age at menarche, pubic hair development, and breast development) were observed. Toxicological studies indicate that prenatal and lactational exposures to Pb can cause a delay in the onset of female puberty at blood Pb levels as low as 8 µg/dL (Iavicoli et al., 2006b; Iavicoli et al., 2004). Recent studies show that pubertal onset is one of the more sensitive markers of Pb exposure with effects observed after maternal exposures leading to blood Pb levels in the pup of 3.5-13 µg/dL (Iavicoli and Carelli, 2007). In boys (ages 8-15 years), fewer studies were conducted but associations were observed in most. Male animal toxicology studies have reported delayed sexual

maturity as measured with prostate weight, among other outcomes, seeing significant decrements at blood Pb levels of 34 µg/dL (Sokol et al., 1985). Additionally, Pb exposure has been shown to have detrimental effects on sperm. These were observed in epidemiologic studies at population mean blood Pb levels of 30 µg/dL and greater among men occupationally exposed (mean blood Pb levels in study controls around 10 µg/dL) and in animal toxicological studies with rabbits exposed to subcutaneous Pb 3 times per week for 15 weeks with blood Pb levels of 20 µg/dL (Moorman et al., 1998). 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. The collective body of evidence integrated across epidemiologic and toxicological studies with a focus on the strong relationship observed with detrimental effects on sperm and delayed pubertal onset is sufficient to conclude that there is a causal relationship between Pb exposures and reproductive and developmental effects.

5.9 Effects on Other Organ Systems

5.9.1 Effects on the Hepatic System

Hepatotoxic effects of Pb indicated in various animal models and human populations 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 Pb. Induction of oxidative stress by Pb exposure 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 the large experimental animal database indicated hepatotoxic effects, including liver hyperplasia, at very high dose Pb exposures. Other effects noted in the liver following exposure to Pb included altered cholesterol synthesis, DNA synthesis and glucose-6-phosphotase dehydrogenase (G6DP) activity . The 2006 Pb AQCD reported that cytochrome (CYP) P450 levels decreased following single doses of 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*lpha* (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. The 2006 Pb AQCD further suggested that alterations in liver heme metabolism may involve changes in 5-aminolevulinic acid dehydrogenase (ALAD) activity, porphyrin metabolism, transferrin (TF) gene expression and changes in iron metabolism.

With regard to human studies, the 2006 Pb AQCD stated that nonspecific liver injury generally observed as increases in liver enzymes in the serum was reported in adults with occupational Pb exposure, although associations specifically with Pb exposures have not been well established. In addition, similar to effects noted in animal studies, cytochrome P450 activity was also suppressed in children and adults (drawn from the general population) following exposure to Pb. The 2006 Pb AQCD reported that hepatic effects occurred only at high Pb exposure levels (blood Pb levels > 30 µg/dL).

5.9.1.2 New Epidemiologic Studies

A few occupational epidemiologic studies examined liver biochemical parameters effects on antioxidant status and oxidative stress resulting from exposure to Pb. However all of these occupationally-exposed cohorts represented populations highly exposed to Pb, with blood Pb levels ranging from 29 to 53 µg/dL. Although the hepatotoxicity observed within these cohorts may not be generalizable to the general population as a whole, they are useful in demonstrating consistent effects on a number of liver outcomes, including altered liver function (i.e., changes in the level of liver function enzymes), oxidative stress, and antioxidant status (Can et al., 2008; Khan et al., 2008; Patil et al., 2007). Additionally, these studies were cross-sectional in design; thus, there is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations.

In one of the occupational cohorts (spray painters in western Maharashtra, India, exposed to Pb for > 6 hours/day for 2 to 20 years) examined by Patil et al. (2007), mean (SD) blood Pb levels in workers were 22.32 (8.87) µg/dL. The blood Pb levels in workers were statistically significantly higher compared to those in the concurrent control group (mean [SD]: 12.52 [4.08] µg/dL), who had no history of Pb exposure. Liver function enzymes, including serum glutamic oxaloacetic transaminase (SGOT)/AST, and serum glutamic pyruvic transaminase (SGPT)/ALT levels were statistically significantly increased in spray painters compared to those in controls, whereas total serum protein levels were decreased. In another occupational epidemiolgic study, Conterato et al. (In Press) investigated liver function parameters in automotive painters exposed to Pb in Brazil. Exposed painters had a mean (SD) blood Pb concentration of 5.4 (0.4) µg/dL compared to 1.5 (0.1) µg/dL in controls. The mean (SD) duration of exposure to Pb in painters was 133.9 (14.5) months, whereas the controls were not occupationally exposed to Pb. In exposed workers, the levels of aspartate aminotransferase (AST), but not γ-glutamyltranseferase, were increased approximately 2-fold compared to levels in controls ($p < 0.05$). The activity of AST was positively correlated with blood Pb levels ($r = 0.26$, $p < 0.05$). The authors suggested that confounding exposures to toxic constituents of the paints regularly used by painters, and not Pb, may be the etiological cause of decrements in AST function as these effects were not also seen in battery workers with much higher blood Pb levels (49.8 µg/dL). Co-exposure to other environmental contaminants may also explain the effects seen in occupationally-exposed spray-painters in Patil et al. (2007).

5.9.1.3 New Toxicological Studies

Hepatic Metabolism

As stated in the 2006 Pb AQCD, acute exposures of rodents to Pb nitrate and other Pb compounds cause a decrease in Phase I enzymes accompanied by a simultaneous increase in Phase II enzymes. The conclusions presented in the 2006 Pb AQCD were also reviewed by Mudipalli (2007).

Changes in biochemical parameters, suggestive of liver damage, in undernourished male Wistar rats (fed low-protein diet without mineral supplements) 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 increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), and gamma glutamyl transpeptidase (GGT) levels (DS et al., 2009). In Pb-treated animals, the blood Pb levels steadily increased throughout the initial portion of the study period, reaching a maximum of approximately 30 µg/dL after 2 months. After this time, blood Pb levels rapidly

increased to approximately 110 µg/dL (greatly in excess of blood levels in the general human population) by six months time and remained at this level until the termination of exposure at 10 months. The study authors reported that similar biochemical changes were not observed in animals treated with Pb-acetate maintained on protein-adequate, mineral rich diet and concluded that nutritional management is important in managing Pb-related poisoning. Mice gavaged with 50 mg/kg Pb nitrate for 40 days demonstrated similarly increased activities of AST, ALT, ALP, and acid phosphatase (ACP) compared to controls (Sharma et al., 2010a). Upadhyay et al. (2009) reported that treatment of Sprague-Dawley rats with 35 mg/kg Pb via i.p. injection for 3 days (blood Pb not reported) significantly increased the activities of ALT, AST, serum ALP, and acid phosphatase over those in controls, whereas alkaline phosphatase activity was decreased in Pb-treated animals. Concomitant treatment with zinc and varying levels of ascorbic acid were observed to ameliorate the toxic effects of Pb. The serum activities of glutamic pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) were similarly significantly increased over those in controls in mice subcutaneously injected with 50 mg/kg Pb-acetate daily for 15 days (blood Pb not reported) (Wang et al.). 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 (mean [SD] blood Pb: 86 [6] µg/dL, greatly in excess of blood levels in general human populations) compared to control cows (mean [SD] 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.

Pillai et al. (2009) investigated effects on hepatic phase I and II enzymes in male and female rats born to dams that were treated with 50 µg/kg Pb-acetate via subcutaneous injection daily throughout gestation and continuing until PND21. Thus, the offspring of treated dams were exposed to Pb via placental and lactational transfer. The female and male pups were then allowed to reach sexual maturity (PND55-56) 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 (blood Pb not reported), compared to controls. In rats treated with 25 µg/kg Pb and Cd, the effect on phase I enzymes was increased. Pb treatment additionally decreased the activities of phase II enzymes uridine diphosphate-glucoronyl transferase and GST in males and females, but no effect was observed on GGT or 17β-hydroxysteroid oxidoreductase. Additionally, no effect was observed in Pb-treated rats on 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 treatment. 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 (mean [SE]: 1.19 µg Pb/g tissue [0.30] versus 0.76 [0.06], respectively), but were similar between the 2 dosing regimens at 3 months postweaning (mean [SE]: 0.054 [0.06] versus 0.55 [0.07], respectively). 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 not in a concentration-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, treatment of mice with low doses of Pb and LPS together caused a statistically significant increase in TNF-α induction as well as enhanced liver injury, suggesting that Pb potentiated LPS-induced inflammation. In 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 increasing LPS- induced TNF-α levels via the protein kinase C (PKC)/MAPK pathway. Similarly, Pb chloride potentiated bovine serum albumin (BSA)-induced inflammation in the livers of mice subcutaneously injected with Pb (Sá et al., In Press).

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 mean (SD) blood Pb levels of 40.63 (9.21), 61.44 (4.63), and 39.00 (7.90) µg/dL, respectively. Animals exposed to 200 ppm Pb had mean (SD) liver Pb concentrations of 10.04 (1.14) µg/g, compared to 3.24 (1.19) and 2.41 (0.31) in animals exposed to 300 or 400 ppm Pb, respectively. 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 Pb; a decrease in phospholipid levels was observed at 400 ppm Pb. The authors also reported positive correlations between tissue cholesterol and

phospholipids and Pb accumulation in liver across all doses. In contrast, the association between tissue triglyceride levels and Pb accumulation was negative. In related studies, Khotimchenko and Kolenchenko (2007) reported that adult male albino rats treated with 100 mg/kg Pb-acetate for as little as 14 days (blood Pb not reported) exhibited disorders in lipid metabolism that were supported by increased levels of total cholesterol and triglyceride levels in the liver tissue, whereas Sharma et al. (2010a) reported increased liver cholesterol in mice gavaged with 50 mg/kg Pb nitrate for 40 days. 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 by Pb may cause subtle effects at the cellular level that may lead to hepatotoxicity. Kojima and Degawa (2006) examined the sex-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 (blood Pb not reported). 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 Pb-exposed 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 Pb-exposed 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 in response to Pb 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 (blood Pb not reported) 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 well-nourished 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 treated

with 0.025 mg/kg Pb via i.p. injection (blood Pb not reported) 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 fractions (Pandya et al., 2010). Statistically nonsignificant decreases were also observed in GSH levels and GPx and GR activities in Pb-treated animals. In mice gavaged with 50 mg/kg Pb nitrate for 40 days, lipid peroxidation was increased and SOD, CAT, and GSH were decreased compared to controls (Sharma et al., 2010a). Additionally, exposure to Pb nitrate resulted in histopathological changes in the structure of the liver: hepatocytes were damaged and were marked by cytoplasmic vacuolization and pycnotic nuclei. Yu et al. (2008) reported similar concentration-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 Pb-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 demonstrated 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 (blood Pb not reported) 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 role of low molecular weight thiols on peroxidative mechanisms, 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. Metallthionein 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 treated with 35 mg/kg Pb via i.p. injection (blood Pb not reported), whereas hepatic GSH was significantly decreased (Upadhyay et al., 2009). In vitro exposure of human embryonic hepatocytes (WRL-68) to 5 µM Pb-acetate for 30 days resulted in increase production of reactive oxygen species (ROS) throughout the incubation period (Hernández-Franco et al., 2011). Concurrent with this increase in ROS generation, the activities of SOD and the levels of membrane

lipid peroxidative damage also increased throughout the first 24 days of exposure, but returned to normal levels by the end of the incubation period.

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. Blood Pb levels were higher at parturition (mean [SD]: 31.5 [0.80] µg/dL) than at weaning (mean [SD]: 22.8 [0.50] µg/dL). Pups exhibited liver damage that was accompanied by an 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. Control rats had a mean (SD) blood Pb level of 16.1 (0.63) µg/dL, whereas rats exposed to 0.3 and 3.0 mg/L Pb had mean blood Pb levels that were 26.5% (20.4 µg/dL) and 51.8% (24.4 µg/dL) higher, respectively. In the fetuses from dams exposed for 1 month prior to pregnancy, a concentration-dependent increase in liver SOD activity was observed, whereas SOD activity was decreased in the 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. 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 (blood Pb not reported) (Pillai et al., 2009).

The studies presented above all support 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.

Hepatic Apoptosis

Fan et al. (2009b) reported that a single i.v. injection (tail vein) of 200 µmol/kg Pb nitrate resulted in an increase in the expression of ferritin light-chain (FLT) in rats (mean [SD]: 3.5 [1.0]-fold increase) over that in controls. Immunohistochemical analysis revealed that hepatocytes around the central vein were heavily stained by anti-FTL antibodies, as were nonparenchymal cells identified as Kupffer cells. The authors hypothesized that the expression of FTL in Kupffer cells may be the result of phagocytosis of apoptotic cells: in Pb-treated rats, apoptotic hepatocytes represented a mean (SD) 2.5 (1.4)% of total cells

whereas only a mean (SD) 0.31 (0.31)% of hepatocytes were apoptotic in control animals. FTL expression in Kupffer cells was not increased in rats treated with clofibrate, which induced hepatocellular proliferation, but not apoptosis.

5.9.2 Effects on the Gastrointestinal System

Gastrointestinal effects of Pb exposure resulting in blood Pb levels ranging from 30 up to 80 µg/dL in humans primarily include abdominal pain, constipation, and internal paralysis. In animals, degeneration of the intestinal epithelial mucosa and a decrease in duodenal motility has been reported following Pb exposure.

5.9.2.1 Summary of Key Findings on the Effects on the Gastrointestinal System from the 2006 Lead AQCD

The 2006 Pb AQCD stated 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. In suckling rat pups, 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 in rats, calcium, and vitamin D indicated competition in absorption between Pb and calcium. Dietary supplement with vitamin D led 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 stated that studies in rat intestine have shown that the largest amount of Pb absorption occurs in the duodenum with the mechanisms of absorption involving active transport and diffusion via the intestinal epithelial cells. Absorption has been reported to occur, through both saturable and nonsaturable pathways based on results from various animal studies. The 2006 Pb AQCD reported evidence that symptoms associated with gastrointestinal colic (abdominal pain, constipation, intestinal paralysis) were prevalent in occupationally exposed adults with blood Pb levels $\geq$ 50 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. Kuruvilla et al. (2006) reported gastrointestinal effects including stomach pain and gastritis along with other Pb-related clinical manifestations in painters (mean [SD] blood Pb: 8.04 [5.04] µg/dL) occupationally exposed to Pb in India. Case reports involving children and individuals occupationally exposed to Pb with very high blood Pb levels (> 15 µg/dL) were consistent with these observations of GI pain and gastritis (Fonte et al., 2007).

5.9.2.3 New Toxicological Studies

A few new studies pertaining to gastrointestinal effects of Pb exposure were identified. Santos et al. (2006b) examined the impact of Pb exposure on nonadrenergic noncholinergic (NANC) relaxations in rat gastric fundus. Male Wistar rats treated with 0.008% Pb-acetate (80 ppm) via drinking water for 15, 30, and 120 days (blood Pb not reported) exhibited a 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 for 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), resulted in a concentration-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 (blood Pb not reported). 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 that in the controls. Additionally, there was a significant increase in gastric lipid peroxidation at 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 found to be impacted by Pb exposure include changes in the thyroid, such as changes in the thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4).

5.9.3.1 Summary of Key Findings of the Effects on the Endocrine System from the 2006 Lead AQCD

The 2006 Pb AQCD reported that endocrine processes impacted by occupational Pb exposure include thyroid hormone levels, changes in male sex hormone levels, as well as changes in the production of 1,25-(OH)2D3 levels. However, these effects were reported to be observed only with blood Pb levels exceeding 30–40 µg/dL. In addition, alterations in calcitropic hormones were affected in children with blood Pb levels ranging from 10-120 µg/dL. A summary of key findings pertaining to reproductive hormones in males and females in the current document is presented in the section on reproductive and developmental effects (Sections 5.8.1 and 5.8.2).

5.9.3.2 New Epidemiologic Studies

Recent epidemiologic studies have reported associations between indicators of exposure to Pb and thyroid hormone levels. In workers highly exposed to Pb (blood Pb = 71.1 µg/dL) thyroid stimulating hormone (TSH) and free T4, but not free T3, were increased over levels in controls (mean blood Pb level of 0.2 µg/dL), although these results are most likely not generalizable to the general public due to the high blood Pb levels of exposed workers (Pekcici et al., 2010). Abdelouahab et al. (2008) performed a cross-sectional study in a Canadian population characterized by high consumption of freshwater fish. The median concurrent blood Pb level was 3.1 µg/dL for men and 1.7 µg/dL for women. It is important to note that the median blood Pb level for women was 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 who consumed fish contaminated with Pb and other environmental pollutants. No associations 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. The authors also concluded that environmental contaminants not investigated (e.g., As) may be influencing TSH levels. Dundar et al. (2006) examined associations of blood Pb with thyroid function in 42 male adolescent auto repair workers exposed long term to Pb. A control group comprising 55 healthy subjects was also used for comparison purposes. Mean blood Pb levels were reported to be higher in the auto repair workers compared to the control subjects (mean [SD]: 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 Pb 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 in 1,679 adolescents residing in nine study areas with varying exposures to multiple industrial pollutants including Pb. The median concurrent 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 adjustment for potential confounding, significant interregional differences were observed FT3 hormone levels. When individual neighborhoods were analyzed within the larger study areas, altered levels of FT3 levels were also observed. Though varying levels of FT3 levels were observed, the study authors reported that these changes were 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 have contributed to the effects noted. In a prospective study of 309 mother-child pairs from Yugoslavia, the relationship between maternal TSH and T4 and blood Pb was investigated in those living in a highly exposed town with a smelter and battery plant (n = 156 mother-child pairs) and those living in a relatively unexposed town (n = 153 mother-child pairs) (Lamb et al., 2008). The mid-pregnancy blood Pb levels were highly elevated in the industrial town compared to the unexposed town (mean [SD]: 20.56 [7.38] versus 5.60 [1.99] µg/dL). Mid-pregnancy maternal free T4 levels were observed to be inversely related with maternal blood Pb levels, but this association was not observed in the unexposed town. In 24 newborns delivered in Tokyo, Japan, neither TSH nor free T4

(sampled 4-6 days postpartum) was associated with cord blood Pb sampled at delivery (mean: 0.67 µg/dL) (Iijima et al., 2007).

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 a mean (SD) age of 2.62 (1.2) years, respectively. For prenatal blood Pb, the children were divided into the following quartiles: ≤ 1, 1.1-1.4, 1.5-1.9, and 2.0-6.3 µg/dL. For postnatal blood Pb, the quartiles were 1.5-2.8, 2.9-4.1, 4.2-5.4, and 5.5-13.1 µg/dL. The study authors reported that blood Pb was not associated with initial salivary cortisol levels. However following an acute stressor, which comprised submerging the dominant arm for a minute in a gallon of one part ice to one part water, 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 had increased salivary cortisol responses compared to children in the 1st quartile. When blood Pb was treated as a continuous variable, regression analysis showed that both prenatal and postnatal blood Pb levels were 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 adrenocortical responses of children 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 Pb levels in 142 young, U.S. urban African-American and Hispanic children in winter and summer to determine the seasonal increase in blood Pb and its association with vitamin D (1,25-(OH)2D3), age and race. There was a winter/summer (W/S) increase in blood Pb levels in children aged between 1 and 3 years (mean [SE]: 4.94 [0.45] µg/dL in winter, 6.54 [0.82] µg/dL in summer), with a smaller W/S increase observed in children aged between 4 and 8 years (mean [SE]: 3.68 [0.31] µg/dL in winter, 4.16 [0.36] µg/dL in summer). 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. In children aged 4-8 years, the concentrations of 1,25-(OH)2D3 were greater in the summer compared to the winter (mean [SE]: 33.8 [1.1] µg/L in summer versus 25.3 [1.2] µg/L in winter). No difference in seasonal 1,25-(OH)2D3 was observed in children 1-3 years old. Additionally, winter and summer concentrations of 1,25-(OH)2D3 were highly correlated ($r = 0.635$, $p < 0.0001$). 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

. 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 (mean [SD] blood Pb: 86 [6] µg/dL) and near closed Pb and operational zinc smelters (mean [SD] blood Pb: 51 [9] µg/dL) when compared to cows in unpolluted areas (mean [SD] blood Pb: 7 [1] µg/dL). Regression analyses 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 treatment on adrenal and male gonadal functions in Wistar rats treated with 8.0 mg/kg Pb-acetate via i.p. injection for 21 days (blood Pb not reported). Pb treatment 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-treated animals, whereas adrenal weights were increased. Spermatogenesis was decreased and the percent of spermatid degeneration was increased in animals treated with Pb. Lastly, serum concentrations of testosterone, FSH, and LH, were decreased in Pb-treated animals. Supplementation with testosterone during the last 14 days of Pb treatment was observed to ameliorate these effects.

5.9.4 Effects on Bone and Teeth

Primary effects on bone as a result of Pb exposure have included an increase in osteoporosis, increased frequencies of falls and fractures, changes in bone cell function as a result of replacement of bone calcium with Pb and depression in early bone growth. Similar to bone, calcium in the teeth can be easily substituted by Pb following Pb exposure. Exposure of animals to high levels of Pb (30 mg/kg body weight) 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 in animals and following exposure to Pb. Pb easily substituted for calcium in bone and was taken up by the bone causing changes in bone cell function. Exposure of animals to Pb during gestation and immediate postnatal period was reported to significantly depress early bone growth with the effects showing a concentration-dependent trends,. In mature animals, long-term exposure (up to one year) to Pb, along with poor nutrition (low calcium) affected bone growth as well bone density. Systemic effects of Pb exposure included disruption in bone mineralization during growth, alteration in bone cell differentiation and function due to alterations in plasma levels of growth hormones and calcitropic hormones such as 1,2-[OH]2D3 and impact on calcium binding proteins and increases in calcium and phosphorus concentrations in the bloodstream. Bone cell cultures exposed to Pb had altered vitamin D-stimulated production of osteocalcin accompanied by inhibited secretion of bone-related proteins such as osteonectin and collagen. In addition, Pb exposure caused suppression in bone cell proliferation most likely due to interference from factors such as growth hormone (GH), epidermal growth factor (EGF), transforming growth factor-beta 1 (TGF-β1), and parathyroid hormone-related protein (PTHrP).

As in bone, Pb 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 does during growth, most of the Pb in the teeth remains in a state of permanent storage. High dose exposure of Pb to animals (30 mg/kg body weight) 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 2006 Pb 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 appears to be capable of causing effects in bones of humans and animals following exposure. The association between blood Pb levels and osteoporosis was examined in several epidemiologic studies. Most studies were cross-sectional in design; thus, there is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations. Campbell and Auigner (2007) examined subjects ≥ 50 years of age using NHANES III for association between concurrent blood Pb level and osteoporosis. The study authors used the bone mineral density in the hip as the primary outcome in groups comprising 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 bone mineral density in the non-Hispanic white males who had the lowest blood Pb levels (actual concentration not reported) was statistically significantly higher than that in the males with higher blood Pb levels. Similar associations, although not statistically significant, were reported among white females. Likely 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 sex/race group. Since the NHANES study comprised 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. (2008b) examined the association between concurrent blood Pb levels and osteoporosis in 155 males and 37 females in China occupationally-exposed to Pb (mean blood Pb: 20.22 and 15.5 µg/dL, respectively). Bone mineral density 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 bone mineral density in groups that had high urinary Pb levels (≥ 5 µg/g creatinine) compared to groups with low urinary Pb in both sexes. In contrast, a significant difference was observed between blood Pb and bone mineral density only 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 blood Pb level and osteoporosis in older women. The study authors conducted a prospective study using 533 women aged 65-87 years with a mean (SD) blood Pb of 5.3 (2.3) µg/dL to determine the association between blood Pb and recurring fractures. Analysis of bone mineral density was conducted in 1986-1990 and 1993-1994, while

blood Pb was measured from 1990-1991. The bone mineral density 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 bone mineral density. 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 non-spine 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 osteoporosis-related fractures.

To examine the association between biomarkers of joint tissue metabolism and blood Pb levels, Nelson et al. (In Press) performed a cross-sectional analysis of 329 male and 342 female participants in the Johnson County Osteoarthritis Project Metals Exposure Sub-study. In women (mean age = 62 years), the median concurrent blood Pb level was 1.9 µg/dL (range: 0.5-25.4 µg/dL). Blood Pb levels were higher in African-American compared to Caucasian women. Unadjusted correlation analyses demonstrated significant positive correlations between blood Pb and uNTX-I (a marker of bone resorption/turnover), uCTX-II (a marker associated with the progression of radiographic knee and hip osteoarthritis), and COMP (a cartilage biomarker related to osteoarthritis); however, significant associations only remained for uNTX-I and uCTX-II after adjusting for age, BMI, race, and smoking status. In men (median age = 65), the median blood Pb level was 2.2 µg/dL (range: 0.5-25.1 µg/dL). As with women, African-American men had higher blood Pb levels than did Caucasian men. In unadjusted correlation analyses, blood Pb levels in men were positively associated with uCTX-II, COMP, and C2C:CPII ratio (an indication of the balance between cartilage collagen degradation and synthesis), and negatively associated with CPII (a marker of collagen synthesis). After controlling for age, BMI, race, and smoking, only the positive association between blood Pb and COMP remained borderline statistically significant in men. The authors concluded that blood Pb is associated with bone turnover and mineralized cartilage turnover in women and non-mineralized cartilage turnover in men; however, as this study was cross-sectional in nature, it is impossible to conclude whether increased cartilage turnover is a product of increased blood Pb, or whether cartilage turnover itself results in increased Pb. Similarly, Machida et al. (2009) investigated bone matrix turnover rates in Japanese women related to menopause status and blood Pb. Perimenopausal women (n = 319) were observed to have significantly higher geometric mean blood Pb (2.0 µg/dL), than did premenopausal women (n = 261, blood Pb = 1.6 µg/dL), younger postmenopausal women (n = 397, blood Pb = 1.8 µg/dL), or older postmenopausal women (n = 248, blood Pb = 1.7 µg/dL).

In all subjects and perimenopausal women, markers of bone matrix turnover (osteocalcin [OC], bone-specific alkaline phosphatase [BALP], and N-telopeptide cross-linked collagen type I [NTx]) were significantly positively associated with blood Pb in unadjusted correlation analyses. In multivariate regression models controlling for bone-mineral density, NTx, and age, OC was additionally statistically positively associated with blood Pb in all subjects and perimenopausal women. Bone mineral density and NTx were also positively associated with blood Pb in these models for all subjects. As with Nelson et al. (In Press), the cross-sectional nature of this study precludes a determination whether higher blood Pb is cause of increased bone matrix turnover biomarkers, or a consequence of increased bone turn over.

To understand the importance 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 1,000 or 3,000 µM Pb, a concentration-dependent impact on the CRACI was observed, as was a concentration-dependent increase in the influx of Pb into human OLC. These results suggest that Pb interferes with CRACI 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 is enhanced after CRACI is induced. Since studies have found associations between higher blood Pb level and reduced skeletal growth in children, 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 to assess 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 NF-κB 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 NF-κB.

Effects of Pb exposure on teeth were examined in a few epidemiologic studies. Since individuals may be impacted by the 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 NAS. Tooth loss in men was categorized as 0, 1-8 or ≥ 9. Individuals with ≥ 9 teeth missing had significantly higher tibia and patella Pb concentrations (measured within 3 years of dental assessment) compared to those with

no tooth loss; no significant difference in blood Pb levels (measured within 3 years of dental assessment [(Hu et al., 1996a)]) was observed between the categories of teeth loss. Following adjustment for age, education, smoking status, pack-years of smoking, and diabetes, men with the highest tibia Pb concentrations (>23 µg/g) had higher odds of tooth loss (OR: 3.03 [95% CI: 1.60, 5.75]) compared to men with tibia Pb ≤ 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. Tooth loss was 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 U.S, Saraiva et al. (2007) analyzed data for 2,500 men and 2,399 women aged between 30 and 56 years from NHANES III. The analysis took into account various covariates including age, NHANESIII phase, cotinine levels, poverty ration, race/ethnicity, education, bone mineral density, diabetes, calcium intake, dental visits, and menopause in women. After adjusting for these covariates and comparing individuals with a concurrent 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 association between periodontitis and blood 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 (mean [SD]: 7.38 [4.41] versus 2.27 [1.49] µg/dL, respectively). No clinical periodontal or oxidative stress parameters were significantly different 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 were observed. These results demonstrate that there is significant association between clinical periodontal parameters and oxidative stress/damage indices in Pb-exposed apprentices. In a 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 indicated to be associated with 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 2006 Pb 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 Toxicological and Epidemiologic Studies

A small number of human studies pertaining to ocular effects of Pb were 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 (mean [SD]: 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). Blood 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. Erie et al. (2009) investigated the association between age-related macular degeneration and Pb and Cd in retinal tissue of human eye donors. The authors observed that Pb, but not Cd, was significantly elevated in the neural retina tissue of donors with age-related macular degeneration (n = 36 donors, 72 eyes; median [IQR]: 12.0 [8-18] ng/g Pb) versus normal control donors (n = 25 donors, 50 eyes; median [IQR]: 8.0 [0-11] ng/g Pb). Neither heavy metal was significantly elevated in the retinal pigment epithelium-choroid complex in donors with age-related macular degeneration and normal controls.

New animal studies pertaining to the ocular effects of Pb have investigated endpoints such as retinal progenitor cell proliferation and neurogenesis,(Section 5.3.4.3). An in vitro study was found that investigated whether exposure of cultured lenses from rats (4-6 weeks age) to 1 µM Pb nitrate increased opacity with or without secondary oxidative challenge (Neal et al., 2010b). Lenses incubated for 3 days in the presence of 1 µM Pb were transparent, with no difference in amino acid incorporation compared to control lenses. The authors concluded this indicated that short-term Pb exposure does not induce osmotic swelling or lens shrinkage. However, exposure to lenses to Pb for 5 days dramatically decreased the percentage of transparent lenses (30%) compared to controls (80%). In Pb-exposed lenses, 30% displayed "definite cataracts" compared to only 2.5% in control lenses. By culture day 8, all exposed lenses were described either as clearly opaque or definite cataracts, while only 7% of control lenses displayed these characteristics, indicating that prolonged exposure of lenses to Pb induced an accelerated formation of opacity/cataract compared to unexposed lenses. Pb-exposed lenses cleared the media of hydrogen peroxide more rapidly than did control lenses, potentially due to increased CAT activity. Exposure to hydrogen peroxide resulted in total opacity in Pb-exposed lenses at culture day 7, compared to less than 20% in control cells. Exposure to Pb additionally altered epithelial nutrient transport and lens histology relative to that in controls.

5.9.6 Effects on the Respiratory System

The collective body of toxicological and epidemiologic studies demonstrates Pb-associated 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 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 5.6.6). Collectively, panel and time-series epidemiologic 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, and asthma hospitalizations in children but not adults

(Section 5.6.4.3). Toxicological studies have found pulmonary inflammation induced by CAPs in which Pb was one of numerous components (Wei et al., 2011; Duvall et al., 2008; Godleski et al., 2002; Saldiva et al., 2002). Despite this evidence for respiratory effects related to air-Pb concentrations, it is important to note the limitations of air-Pb studies, including the limited data on the size distribution of Pb-PM (Section 3.5.3), the uncertain relationships of Pb-PM_{10} and Pb-$PM_{2.5}$ with blood Pb levels, and the lack of adjustment for other correlated PM chemical components.

5.9.7 Summary

There is evidence from epidemiologic and toxicological studies that substantial exposure to Pb can result 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 occupationally-exposed humans (blood Pb > 22 µg/dL) (Can et al., 2008; Khan et al., 2008; Patil et al., 2007) mature animals exposed to high levels of Pb during adulthood (Sharma et al., 2010a; Wang et al.; DS et al., 2009; Cheng et al., 2006), and animals exposed during gestation and lactation (Pillai et al., 2009; Teijon et al., 2006). In humans with mean blood Pb levels 5.4 µg/dL, altered AST levels were observed, but increases may be related to exposure to contaminants other than Pb (Conterato et al., In Press). Increased hepatic cholesterogenesis, altered triglyceride and phospholipid levels, and disorders in lipid metabolism accompanied by increased levels of total cholesterol and triglycerides have been reported in the animal literature (Ademuyiwa et al., 2009; Khotimchenko and 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 (Pandya et al., 2010; Sharma et al., 2010a; Khan et al., 2008; Yu et al., 2008; Adegbesan and Adenuga, 2007; Jurczuk et al., 2007; Khotimchenko and Kolenchenko, 2007; Jurczuk et al., 2006). Indices of increased oxidative stress were additionally observed in the livers of fetuses exposed to Pb throughout gestation (Massó et al., 2007).

Relatively few human studies have been conducted on the gastrointestinal toxicity of Pb since the completion of the 2006 Pb AQCD. A case study reporting on GI symptoms in a child reported that elevated blood Pb was associated with nonlocalized abdominal pain, vomiting, nausea, constipation, lack of appetite, fatigue, and headaches (Cabb et al., 2008). Symptoms were reported to diminish following cessation of exposure. 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 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; Santos et al., 2006b). The observation of oxidative stress was accompanied gastric mucosal damage.

The endocrine processes most impacted by exposure to Pb include changes in thyroid function. FT4, but not FT3, was decreased in adolescent male auto repair workers (Dundar et al., 2006) and mid-pregnancy T4 levels were negatively associated in women living in a highly contaminated town in Yugoslavia, whereas this association was not observed in a non-contaminated town (Lamb et al., 2008). Toxicological evidence for similar effects was found in adult cows reared in an environment contaminated with Pb. A positive correlation was reported between blood Pb and plasma T3 and T4 levels (Swarup et al., 2007). In children challenged with an acute stressor, increasing prenatal maternal and age 2-year blood Pb levels were associated with significant increases in salivary cortisol responses at age 9 years, even with blood Pb levels less than 10 µg/dL (Gump et al., 2008). A summary of key findings pertaining to reproductive hormones in males and females in the current document is presented in the section on reproductive and developmental effects (Sections 5.8.1 and 5.8.2).

Numerous epidemiologic studies investigated the association between Pb biomarkers and osteoporosis in adults. Higher blood Pb was observed to be associated with decreased bone mineral density in non-Hispanic white males (Campbell and Auinger, 2007), whereas urinary Pb, but not blood Pb, was associated with decreased bone mineral density in Chinese individuals occupationally exposed to Pb (Sun et al., 2008b). In elderly women, blood Pb levels were positively associated with risk of falls and osteoporosis-related fractures (Khalil et al., 2008). Linear skeletal growth was reduced in children living near copper smelters and refiners (concurrent mean blood Pb level = 7.7 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 resulting in altered skeletal development (Jang et al., 2008; Zuscik et al., 2007). Blood Pb levels have also been shown to be related to biomarkers of joint tissue metabolism in elderly populations, but the cross-sectional nature of these analyses prevents conclusions being drawn on whether Pb increases bone and joint turnover biomarkers, or whether increased turnover releases Pb into the bloodstream (Machida et al., 2009; Nelson et al., In Press). Epidemiologic studies investigating Pb exposure and tooth loss indicate 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) due to Pb exposure (Section 5.3.4.3). and studies in humans report associations between heavy smoking, increased blood Pb levels, and cataracts (Mosad et al., 2010) and retinal Pb concentrations and age-related macular degeneration (Erie et al., 2009). Investigation of the respiratory effects of Pb exposure has been limited; however, cross-sectional studies have indicated an association of higher blood Pb levels with increased prevalence asthma in children (Section 5.6.4.2).

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 supportive of those effects noted in the 2006 Pb AQCD. However, recent evidence of these effects is relatively limited, 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, 2006b) "provide[d] only very limited evidence suggestive of Pb exposure associations with carcinogenic or genotoxic effects in humans" and the studies were summarized as follows:

> "The epidemiologic data …suggest a relationship between Pb exposure and cancers of the lung and the stomach… Studies of genotoxicity consistently link Pb-exposed populations with DNA damage and micronuclei formation, although less consistently with chromosomal aberrations."

The International Agency for Research on Cancer (IARC) 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, 2006a; Rousseau et al., 2005). Additionally, the National Toxicology Program has listed Pb and Pb compounds as "reasonably anticipated to be human carcinogens" (NTP, 2004). The typical cancer bioassays used by IARC or NTP as evidence of Pb-induced carcinogenicity used rodents that were continuously exposed to Pb-acetate in chow or drinking water for 18 months to two years in duration. These two year cancer bioassays and the doses administered are typical of cancer bioassays used with other chemicals.

In the following sections, recent epidemiologic and toxicological studies published since the 2006 Pb AQCD regarding Pb and cancer mortality and incidence are examined. In

addition, recent studies of Pb and DNA and cellular damage, as well as epigenetics studies, are summarized. When the information is available, the form of the Pb compound under study (e.g., inorganic, organic) is indicated. In epidemiologic studies, various biological 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 or exposure dose. 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 5-42 provides an overview of the study characteristics and results for the epidemiologic studies that reported effect estimates. This section also evaluates toxicological evidence on the potential carcinogenicity of Pb.

Table 5-42 Summary of recent epidemiologic studies[a] of cancer incidence and mortality

Reference[a]	Study Location	Cancer Outcome	Study Population	Measure of Pb Exposure	Mean Pb (SD)	Adjusted Effect Estimates
Cancer Mortality:						
Menke et al. (2006)	Multiple U.S. locations	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 at baseline	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)	Multiple U.S. locations	Overall cancer mortality	NHANES III cohort At least 40 years of age	Blood Pb at baseline	Blood Pb<5 µg/dL: 67.7% Blood Pb 5-9 µg/dL: 26.0% Blood Pb>=10 µg/dL: 6.3%	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	NAS Included men only Mean follow-up period for this study: 8.9 yr	Blood Pb at baseline, Patella Pb at baseline	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. (2009a)	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 at baseline	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)
Lung Cancer:						
Lundstrom et al. (2006)	Sweden	Lung cancer (incidence and mortality)	Male Pb smelter workers first employed for ≥ 3 months between 1928 and 1979 Followed up for mortality from 1955 - 1987	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[a]	Study Location	Cancer Outcome	Study Population	Measure of Pb Exposure	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%	Organic Pb exposure compared to no exposure: Lung 1.3 (95% CI: 0.5, 3.1) Inorganic Pb exposure compared to no exposure: Lung 1.1 (95% CI: 0.7, 1.7) Pb in gasoline emissions exposure compared to no exposure: Lung 0.8 (95% CI: 0.6, 1.1) Note: results are for comparisons using population-based controls; results for controls with other types of cancers were similar
Brain Cancer:						
van Wijngaarden and Dosemeci (2006)	Multiple U.S locations	Brain cancer mortality	National Longitudinal Mortality Study – included individuals with occupational information -included follow-up from 1970-1989	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

Reference[a]	Study Location	Cancer Outcome	Study Population	Measure of Pb Exposure	Mean Pb (SD)	Adjusted Effect Estimates
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
Breast Cancer:						
Pan et al. (2011)	Canada	Breast cancer incidence	National Enhanced Cancer Surveillance System (NECSS) – population-based sample of cancer cases and controls with information collected from 1994-1997	Self-reported previous addresses and their proximity to Pb smelters (determined using Environmental Quality Database [EQDB])	NA	Residing >3.2 km from Pb smelter or no nearby smelter: 1.00 Residing 0.8-3.2 km from Pb smelter: 0.41 (95% CI: 0.11, 1.51) Residing <0.8 km from Pb smelter: 0.61 (95% CI: 0.11, 3.42)
Multiple and Other Cancers:						
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)
Mendey et al. (In Press)	Multiple U.S. locations	Incidence of cancer or "malignancy of any kind"	2007-2008 NHANES cohort – at least 20 years of age	Concurrently measured creatinine-adjusted urinary Pb	Geometric mean for creatinine-adjusted urinary Pb marker: 0.59 µg/g (95% CI: 0.57, 0.61)	Greater than log-transformed mean creatinine-adjusted urinary Pb level compared to less than log-transformed mean creatinine-adjusted urinary Pb level: 0.76 (95% CI: 0.44, 1.33)

Reference[a]	Study Location	Cancer Outcome	Study Population	Measure of Pb Exposure	Mean Pb (SD)	Adjusted Effect Estimates
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) 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) 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) 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[a]	Study Location	Cancer Outcome	Study Population	Measure of Pb Exposure	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

[a]Studies listed in order of appearance in the text.

5.10.1.1 Overall Cancer Mortality

Several recent epidemiologic studies examined the association between Pb levels and cancer mortality, including multiple analyses of the NHANES III population. In one NHANES III analysis, the cohort was followed for 12 years and individuals with blood Pb levels greater than 10 µg/dL were excluded from the study (mean baseline blood Pb level was 2.58 µg/dL). No association was observed between blood Pb and cancer mortality (HR of highest tertile [≥ 3.63 µg/dL]compared to lowest tertile [<1.93 µg/dL]: 1.10 [95% CI: 0.82, 1.47]) (Menke et al., 2006). Another analysis of the NHANES III population, which was restricted to individuals 40 years and older at the time of blood Pb collection and included individuals with all blood Pb levels (including those greater than 10 µg/dL), reported associations between blood Pb and cancer mortality (Schober et al., 2006). In this study, median follow-up time was 8.6 years. The RRs were 1.69 (95% CI: 1.14, 2.52) for individuals with blood Pb levels of at least 10 µg/dL and 1.44 (95% CI: 1.12, 1.86) for blood Pb levels of 5-9 µg/dL compared to individuals with blood Pb levels less than 5 µg/dL. When stratified by age, point estimates comparing blood Pb levels of 5-9 versus less than 5 µg/dL were similar across all age groups but only statistically significant among 75-84 year olds. The odds of mortality associated with blood Pb levels ≥ 10 µg/dL in the groups aged 40-74 years and 85 years and older were elevated. A study of men from the greater Boston area enrolled in the NAS found no association between

blood or bone Pb and cancer mortality in adjusted analyses. The mean (SD) blood Pb level for this population was 5.6 (3.4) µg/dL and blood Pb was poorly correlated with measured bone Pb (Weisskopf et al., 2009). As part of the Study of Osteoporotic Fractures , white women aged 65-87 were included in a sub-study of blood Pb level and cancer mortality and were followed for approximately 12 years (Khalil et al., 2009a). The mean (SD) blood Pb levels were 5.3 (2.3) µg/dL and no association was detected between blood Pb and cancer mortality in the study population.

Overall, epidemiologic studies of blood Pb levels and cancer mortality reported inconsistent results. One epidemiologic study using NHANES III data demonstrated an association between blood Pb and increased cancer mortality; however, other studies reported weak or no associations.

5.10.1.2 Lung Cancer

Most of the evidence regarding lung cancer incidence is provided by studies of occupationally-exposed adults. In a study of smelter workers, no association was observed between several metrics of Pb exposure (peak blood Pb values, number of years Pb samples were obtained, and cumulative blood Pb index) and lung cancer incidence and mortality combined (Lundstrom et al., 2006). The median follow-up in the study was about 30 years and the median peak blood Pb values during employment were 49.7 µg/dL for lung cancer cases and 55.9 µg/dL for controls. In a study of tin smelters workers, no association was observed between Pb exposure and lung cancer mortality in unweighted analyses, but when the analyses were weighted by age and time since exposure, positive associations were apparent (Jones et al., 2007). In this study, Pb exposure was calculated 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. It is important to note that the smelter workers 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.

Studies of Pb and lung cancer that compared the lung tissue of individuals with lung cancer to those without lung cancer were also conducted. 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). Findings are mixed among the studies. De Palma et al. (De Palma et al., 2008)

reported higher Pb concentrations in the cancerous and non-cancerous lung tissue of individuals with non-small cell lung cancer compared to control groups (although the authors report these results may be confounded by smoking). Kuo et al. (Kuo et al., 2006) found no statistical difference in Pb levels for lung tissue of individuals with lung cancer compared to controls .

Some studies in the 2006 Pb AQCD reported associations between Pb exposure and lung cancer; however, more recent epidemiologic studies of lung cancer reported no associations. Overall, these recent epidemiologic studies included only men, limiting the generalizability. The studies by Jones et al. (2007) and Rousseau et al. (2007) also have the disadvantage of not obtaining actual measures of Pb levels. In addition, studies limited to occupational exposures may be confounded by other workplace exposures.

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. Interpretation of these results is limited due to the lack of biological Pb measures and the potential confounding by other occupational 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 and 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 (OR 2.4 [95% CI 0.7, 8.8] comparing individuals ever exposed to Pb with those not exposed to Pb; OR 12.8 [95% CI 1.4, 120.8] comparing individuals with cumulative Pb exposure $\geq$ 100 $\mu g/m^3y$ to those not exposed to Pb) (Rajaraman et al., 2006). This association was not present among individuals with the *ALAD1* genotypes (OR 0.5 [95% CI 0.3, 1.0] comparing individuals ever exposed to Pb with those not exposed to Pb; OR 0.7 [95% CI 0.2, 1.8] comparing individuals with cumulative Pb exposure $\geq$ 100 $\mu g/m^3y$ to those not exposed to Pb). Another study of the association between Pb exposure (measured using self-reported occupational exposure history) and brain tumors reported none or slight overall associations with types of brain tumors; however, positive associations were observed among individuals with certain genetic single nucleotide polymorphisms (SNPs) (Bhatti et al., 2009). After control for multiple comparisons, individuals with *GPX1* variants (rs1050450) had positive associations between cumulative Pb exposure and glioblastoma

multiforme and meningioma. Individuals without *RAC2* variants (rs2239774) showed a positive association between Pb and glioblastoma multiforme. Also, individuals without *XDH* variants (rs7574920) displayed a positive association between Pb and meningioma.

Overall, associations between Pb exposure and brain cancer incidence and mortality were found to vary according to several genetic variants. These studies were limited in their methods because they do not have individual level biological measures and the potential for confounding by other workplace exposures exist. Future research will be important in confirming these associations and the modification by various genetic variants.

5.10.1.4 Breast Cancer

A population-based case-control study in Canada examined the proximity to a Pb smelter based on residential addresses (Pan et al., 2011). No association was reported between proximity of a Pb smelter and breast cancer incidence, but the study was limited by the small number of women who resided near a Pb smelter (n=13 lived ≤ 3.2 km from Pb smelter).

A few studies examined Pb levels and breast tumors among individuals with and without breast tumor and/or cancer present. A study of newly diagnosed breast cancer patients and controls examined Pb levels in blood and hair samples and reported higher levels of both for cancer cases, although the difference in the Pb content in hair samples was not statistically significant (Alatise and Schrauzer, 2010). Siddiqui et al. (2006) observed higher blood Pb levels in women with benign and malignant tumors compared to controls. Additionally, although blood Pb levels were higher among those with malignant breast tumors compared to those with benign tumors, both had similar levels of Pb detected in breast tissues. Another study of Pb levels present in breast tissue also reported no statistical difference in Pb levels (Pasha 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 higher 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 all cancers combined, multiple different 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 and 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). The Pb concentrations were not correlated with incidence of neoplasms. A recent study using the 2007-2008 NHANES cohort reported no association between higher creatinine-adjusted urine Pb levels and having ever had cancer or a malignancy (Mendy et al., In Press). The timing of cancer diagnosis in relation to the urine sample collection was not identified.

A study performed among men evaluated multiple cancer outcomes and determined exposures to organic Pb, inorganic Pb, and Pb from gasoline emissions via interviews regarding job histories and then subsequent exposure approximations by chemists and hygienists (Rousseau et al., 2007). Adults exposed to organic Pb exposure had greater odds of stomach cancer compared to adults never exposed to organic Pb. A positive association was also observed for rectal cancer when population-based controls were used but was null when the control population was limited to individuals with other types of cancers. No association was detected for cancers of the esophagus, colon, pancreas, prostate, bladder, kidney, melanoma, or non-Hodgkin's lymphoma. None of the cancers were associated with 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 as the comparison group; however the association was not present when population controls were utilized as the control group). Another case-control study using participant interviews and a job exposure matrix, including only men, reported no association between Pb exposure and esophageal squamous cell carcinomas, but an association was present between high Pb exposure and adenocarcinoma of the esophagus (Santibanez et al., 2008). However, neither of these studies was able to quantify Pb levels using biological measurements.

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, higher Pb levels were observed in the blood and bladder tissue of individuals with bladder cancer (Golabek et al., 2009), the kidney tissue of individuals with renal cell carcinoma (with highest levels among those with the highest stage tumors) (Calvo et al., 2009), the tissue (but not serum) of individuals with laryngeal cancer (Olszewski et al., 2006), the blood of individuals with gastric cancer (Khorasani et al., 2008), the plasma and hair of individuals with gastrointestinal cancer (Pasha et al., 2010), the blood and hair of individuals with non-specified types of cancer (Pasha et al., 2008c; Pasha et al., 2007), and the hair of individuals with benign tumors (Pasha et al., 2008a). No statistical difference in Pb levels was reported for colon tissue of individuals with colorectal polyps (Alimonti et al., 2008) or urine of individuals with bladder cancer (Lin et al., 2009) compared to control groups. A study examining Pb levels in kidney tissue reported the highest levels of Pb in normal kidney tissue samples that were adjacent to neoplastic tumors. The Pb levels reported in the kidney tissue of neoplastic tumors were elevated compared to those detected in corpses without neoplastic tumors of the kidney (Cerulli et al., 2006). All of these comparison studies are limited by the inability to examine temporality as Pb biomarkers were measured after the cancer diagnosis; the level of Pb may be due to changes that result from having cancer, not changes that result in cancer. Many of these studies attempted to control for this by including only cases 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.

In sum, epidemiologic studies reported no associations between various measures of Pb exposure and overall cancer incidence. Studies examining specific cancers reported varying associations. Associations were null for Pb and most cancer sites examined; however a positive association was observed between Pb exposure and adenocarcinoma of the esophagus as well as exposure to organic Pb and stomach cancer. Associations between organic Pb exposure and rectal cancer and exposure to Pb in gasoline and stomach cancer were inconsistent. These conclusions are limited by the small number of studies and a lack of biological measurements of Pb.

5.10.1.6 Toxicological Models of Carcinogenicity

Carcinogenicity in Animal Models

Previous AQCDs have established that Pb has been shown to act as a carcinogen in animal toxicology models, albeit at relatively high concentrations. The 2006 Pb AQCD pointed out that because Pb is a "well-established animal carcinogen…., focus has been more on the mechanism of neoplasia and possible immunomodulatory effects of Pb in the promotion of cancer." This focus continues to date. The kidneys are the most common target of Pb-dependent carcinogenicity (Kasprzak et al., 1985; Koller et al., 1985; Azar et al., 1973; Van Esch and Kroes, 1969) but the testes, brain, adrenals, prostate, pituitary, and mammary gland have also been affected (IARC, 2006a). The typical cancer bioassays used by IARC or NTP as evidence of Pb-induced carcinogenicity were designed using rodents, typically males but sometimes animals of both sexes, that were continuously exposed to Pb-acetate in chow (i.e., 0.1 or 1% Pb-acetate) or drinking water (i.e., 26 or 2,600 ppm Pb-acetate) for 18 months to two years in duration (Kasprzak et al., 1985; Koller et al., 1985; Azar et al., 1973; Van Esch and Kroes, 1969). These two year cancer bioassays and the doses employed are typical of cancer bioassays employed by other chemicals. Recognition of the importance of windows of exposure in Pb-induced cancer bioassays is a focus of more recent studies. In one study, early life gestational and lactational exposure of laboratory rodents to inorganic Pb induced carcinogenicity in adult offspring (Waalkes et al., 1995). Another recent study considered Pb-dependent carcinogenesis in laboratory animals with early life Pb exposure. Tokar et al. (2010) considered tumorigenesis in homozygous metallothionein I/II knockout mice and their corresponding wild type controls (groups of ten mice each) that were exposed by drinking water to 2,000 or 4,000 ppm Pb-acetate and compared to untreated controls. Study animals were exposed in utero, through birth and lactation, and then postnatally to drinking water until 8 weeks old. The Pb-exposed metallothionein I/II knockout mice had increased testicular teratomas and renal and urinary bladder preneoplasia. Pb exposed wild-type mice were not statistically significantly different than controls. The data suggest that metallothionein can protect against Pb-induced tumorigenesis. Concerns with the study are that the doses are at levels of Pb to which humans would not likely be exposed and there is no metallothionein null condition in humans, though there is variability in the expression of metallothionein. The data do not address whether this variability would have any impact on Pb-dependent carcinogenesis in humans. Thus, the animal toxicology data demonstrate that Pb is a well-established animal carcinogen in studies employing high dose Pb exposure over a continuous extended duration of exposure (i.e., 2 years), which is typical of cancer bioassays. Newer studies are showing

early life maternal Pb exposure can contribute to carcinogenicity in offspring and have shown that metallothionein is protective against cancer in this pathway.

Neoplastic Transformation Studies, Human Cell Cultures

Carcinogenesis can be measured in cell culture systems through neoplastic transformation models that monitor change by following morphological transformation of cells, i.e., formation of a focus (or foci) of cell growth.. Xie et al. (2007) treated BEP2D cells (human papilloma virus- immortalized human bronchial cells) with 0, 1, 5, or 10 µg/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. Mre11 was required to prevent $PbCrO_4$-induced neoplastic transformation.

Immune Modulation of Tumorigenesis by Pb

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), Pb-induced immunotoxicity can contribute to increased risk of cancer, primarily due to the intersection of suppressed Th1 responses and misregulated inflammation. First, Pb-induced misregulation of inflammation involving innate immune cells has been shown to result in chronic insult to tissues. These insults, excessive lipid and DNA oxidation production by overproduction of ROS and weakened anti-oxidant defenses, can increase the likelihood of mutagenesis, cellular instability, and tumor cell formation. For example, Xu et al. (2008) found toxicological evidence that supports the association with Pb exposure and DNA damage and concluded that it is a possible route to increased Pb-induced tumorigenesis. The second component of increased risk of cancer involves Pb-induced suppression of Th1-dependent anti-tumor immunity as acquired immunity shifts statistically significantly toward Th2 responses. With cytotoxic T lymphocytes and other cell-mediated defenses dramatically lessened, the capacity to resist cancer may be compromised.

5.10.2 Cancer Biomarkers

A study of men aged 21-40 years without occupational history of metals exposure examined prostate specific antigen (PSA), a biomarker for prostate cancer. This study reported a positive association between Pb levels and PSA levels (measured in the same blood samples) in regression models adjusted for confounders, including other metals

(Cd, Zn, Se, and Cu) (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 are 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). SCEs are exchanges of homologous DNA material between chromatids on a chromosome and is a test for mutagenicity or DNA damage. A study of male policemen reported mean blood Pb levels for the study population of 43.5 µg/dL (Wiwanitkit et al., 2008). When dichotomized 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 in which the SCEs per cell were higher than the 95th percentile of the population. Finally, a study of children aged 5-14 years old (mean [SD] blood Pb levels of 7.69 [4.29] µg/dL) reported no correlation between blood Pb levels and SCE (Mielzyńska et al., 2006). However, the study did report a positive association between blood Pb and micronuclei (MN) levels.

Other studies of DNA damage have reported mixed results. A study of children ages 6-11 years old and environmentally-exposed to Pb (children attending a school far from a Pb smelter: median blood Pb levels 4.6 µg/dL; children attending a school near a Pb smelter: median blood Pb levels 28.6 µg/dL for) reported no association between blood Pb and baseline DNA 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, MN frequency, or oxidative DNA damage (median blood Pb level of the study population: 3.92 µg/dL) (De Coster et al., 2008). A study conducted among workers exposed to Pb (mean blood Pb level: 30.3 µg/dL) and unexposed controls (mean blood Pb level: 3.2 µg/dL) reported greater cytogenetic damage (measured by MN frequency), chromosomal aberrations, and DNA damage in the Pb-exposed group

(although this was not statistically significant in linear regression models controlling for age) (Grover et al., 2010). A study of painters in India, where Pb concentrations in paint are high, reported a mean (SD) blood Pb level of 21.56 (6.43) µg/dL among painters who reported painting houses for 8-9 hours/day for 5-10 years (Khan et al., 2010b); the mean (SD) blood Pb level was 2.84 (0.96) µg/dL for healthy workers who had not been occupationally exposed to Pb. Cytogenetic damage was higher among the painters compared to the healthy controls. Another study compared the blood Pb of metal workers and office workers and reported higher blood Pb levels (both current and 2 year average) among the metal workers (blood Pb level ≥ 20 µg/dL) compared to the office workers (blood Pb level < 10 µg/dL for) (Olewińska et al., 2010). Overall, the workers had increased DNA strand breaks versus the office workers (this held true at various blood Pb levels). Finally, a study of Pb battery workers with symptoms of Pb toxicity and a group of controls were examined (Shaik and Jamil, 2009). Higher chromosomal aberrations, MN frequency, and DNA damage were reported for the battery workers as compared to the controls.

5.10.3.2 Toxicological Evidence for DNA and Cellular Damage

Sister Chromatid Exchanges

Tapisso et al. (2009), considered sister chromatid exchanges (SCE) in Algerian mice (groups of six mice each) that were exposed by i.p. injection to 5 or 10 doses of 0.46 mg/kg Pb-acetate. 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.

SCE was also followed in cultured human cells. Ustundag and Duydu (2007), considered the ability of N-acetylcysteine and melatonin to reduce Pb nitrate-induced SCE in a single human donor. Cells were treated with 0, 1, 5, 10, or 50 µM Pb nitrate. SCE statistically significantly increased at every Pb concentration in a concentration dependent manner. Both 1 and 2 mM N-acetylcysteine and melatonin were able to statistically significantly reduce SCE levels in Pb-exposed animals. In another study, Turkez et al. (In Press) 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. For both studies, exposure times were not provided and the full interpretation of these data is limited by the limited number of donors and the absence of an exposure time for the SCE assay.

Micronuclei Formation

The 2006 Pb AQCD stated "studies of genotoxicity consistently find associations of Pb exposure with DNA damage and MN formation" and the current document continues to report these associations. Alghazal et al. (2008b), considered the ability of Pb-acetate 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 in Pb-exposed animals than in unexposed controls. Tapisso et al., (2009), considered Pb alone, Pb plus zinc and Pb plus cadmium-induced MN in rodents. Algerian mice were exposed by i.p. injection to 5 or 10 doses of 0.46 mg/kg Pb-acetate and compared to untreated controls. The MN in bone marrow were elevated after Pb exposure and increased with time. Co-exposure with cadmium or zinc did not further increase MN levels.

MN formation has also been followed in cultured human cells. Ustundag and Duydu (2007) considered the ability of N-acetylcysteine and melatonin to reduce Pb nitrate-induced MN in a single human donor. Cells were treated with 0, 1, 5, 10, or 50 µM Pb nitrate. MN formation statistically significantly increased at the two highest Pb concentrations in a concentration dependent manner. Both 1 and 2 mM N-acetylcysteine and melatonin were not able to statistically significantly reduce MN levels. In another study, Turkez et al. (In Press) considered the ability of boron compounds to prevent Pb chloride-induced MN in human lymphocytes. Cells were obtained from 4 non-smoking donors. Both 3 and 5 ppm Pb chloride induced a statistically significant increase in MN levels over controls. Boron induced a statistically significant attenuation of these Pb-induced levels. For both studies, exposure times were not provided, and the full interpretation of these data is limited by the limited number of donors and the absence of an exposure time for the MN assay. Gastaldo et al. (2007) evaluated the ability of Pb to induce MN. Human endothelial HMEC cell line was treated with 1–1,000 µM Pb nitrate for 24 hours. MN increased in a statistically significant, concentration-dependent manner.

Hypoxanthine-guanine phosphoribosyltransferase Mutations

The potential mutagenicity of Pb in human or animal cells was evaluated by monitoring mutations at the hypoxanthine-guanine phosphoribosytransferase (HPRT) locus. Li et al. (2008a) evaluated Pb-acetate-induced HPRT in the non-small-cell lung carcinoma tumor cell line, CL3 and in normal human diploid fibroblasts (specific tissue source not reported). All cells were exposed to 0, 100, 300 or 500 µM Pb-acetate for 24 hours in serum-free medium ± a 1-hour pretreatment with a MKK1/2 inhibitor or a PKC-alpha inhibitor. Pb alone did not induce HPRT mutations. Inhibiting the ERK pathway via either inhibitor statistically significantly increased Pb-induced mutagenesis. Wang et al.

(2008c), investigated Pb-acetate -induced HPRT mutations in CL3 cells. All cells were exposed to 0, 100, 300 or 500 µM Pb-acetate for 24 hours in serum-free medium ± a 1-hour pretreatment with a PKC-alpha inhibitor or siRNA for PKC-alpha. Pb alone did not induce HPRT mutations. Inhibiting PKC-alpha via either inhibitor statistically significantly increased Pb-induced mutagenesis. McNeill et al. (2007) considered Pb-acetate induced HPRT mutations in Chinese hamster ovary AA8 cells and AA8 cells overexpressing human Ape1. Cells were treated with 5 µM Pb-acetate for 6 hours. No increases in HPRT mutations were observed after Pb exposure in either cell line but with specific pathway perturbations (PKC-alpha or ERK) Pb was able to induce HPRT mutations.

Chromosomal Aberrations

Chromosomal aberrations, an indicator of cancer risk, were followed in Pb-exposed rodents (El-Ashmawy et al., 2006). Dietary exposure to Pb-acetate administered as a single dose of 0.5% w/w to male Swiss albino mice caused statistically significant increased levels of chromosomal aberrations in the Pb treatment alone group, particularly with respect to fragments, deletions, ring chromosomes, gaps, and end-to-end associations. In addition, the authors found turmeric and myrrh powders were protective. Concerns with the study include the use of only a single dose of Pb-acetate along with the high levels of unusual aberrations such as ring chromosomes and end-to-end associations. Typically, these aberrations are rare after metal exposure, but were the most commonly observed aberration in this study raising questions about the quality of the metaphase preparations. An additional concern was that only 50 metaphases per dose were analyzed instead of the more common 100 metaphases per dose. The authors did not explain why their spectrum of aberrations was so different, why they only used one dose, or analyzed fewer metaphases per dose.

Multiple studies considered the ability of Pb to induce chromosomal aberrations in cultured human cells. The ability of Pb nitrate to induce chromosomal aberrations was examined in primary human peripheral blood lymphocytes obtained from healthy, nonsmoking donors (Pasha Shaik et al., 2006). Cells were treated with 0, 1.2 or 2 mM Pb-nitrate for 2 hours. No increase in chromosomal aberrations was reported. Some aneuploidy was observed. Concerns with the study are that only a 2 hour exposure was used, which may not be long enough for DNA damage to be expressed as a chromosomal aberration. It also appears from the data presentation that only three subjects were used; one for a control, one for the low dose and one for the high dose. Experiments were not repeated, thus given the small number of subjects, this study may not have had sufficient power to detect any effects. Holmes et al. (2006a), treated WHTBF-6 cells (hTERT-immortalized human lung cells) with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24-120

hours or with 0, 0.1, 0.5, 1, 5 or 10 µg/cm^2 Pb oxide for 24 or 120 hours. Pb chromate induced statistically significant, concentration-dependent increases in centrosome abnormalities and aneuploidy. Wise et al. (2006b) treated BEP2D cells with 0, 0.5, 1, 5, or 10 µg/cm^2 Pb chromate for 24 hours. Pb chromate induced statistically significant concentration-dependent increases in chromosomal aberrations. Holmes et al. (2006b), treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24-72 hours. Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations. The effects of the chromate anion cannot be ruled out as causative in inducing these chromosomal aberrations. Wise et al. (2006a), treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24-120 hours. Pb chromate induced statistically significant, concentration-dependent increases in spindle assembly checkpoint disruption, effects of mitosis and aneuploidy. By contrast, chromate-free Pb oxide did not induce centrosome amplification. The effects were likely attributable to the chromate anion. Xie et al. (2007) treated BEP2D cells with 0, 1, 5, or 10 µg/cm^2 Pb chromate for 24 hours. Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations and aneuploidy. Wise et al. (2010) treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24 hours in a study comparing 4 chromate compounds. Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations

Multiple investigators considered the ability of Pb chromate to induce chromosome aberrations in rodent cell cultures. Grlickova Duzevik et al. (2006) treated Chinese hamster ovary (CHO) cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24 h. Specific CHO lines used included AA8 (wildtype) EM9 (XRCC1-deficient), and H9T3 (EM9 complemented with human XRCC1 gene). Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations that were statistically significantly increased by 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 Pb chromate (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 Pb chromate for 24 h. Specific CHO lines used included AA8 (wildtype), KO40 (*Fancg*-deficient), and 40BP6 (*Fancg*-complemented). Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations that were increased by *Fancg*-deficiency. The *Fancg* gene plays an important role in cellular resistance to DNA interstrand crosslinks, protecting against genetic instability. Camrye et al., (2007) treated CHO cells with 0, 0.1, 0.5, 1, 5, or 10 µg/cm^2 Pb chromate for 24 hours. Specific CHO lines used included CHO-K1 (parental), xrs-6 (Ku80 deficient), and 2E (xrs-6 complemented with Ku80 gene). Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations that were not affected by Ku80 deficiency. Ku80 is a gene a gene involved in nonhomologous end-joining repair

and its absence can contribute to genetic instability. Stackpole et al. (2007) treated CHO and Chinese hamster lung (CHL) cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24 hours. Specific CHO lines used included AA8 (wildtype), irs1SF (XRCC3-deficient), and 1SFwt8 (XRCC3 complemented). XRCC3 is DNA repair enzyme involved in homologous recombination. CHL lines used included V79 (wildtype), irs3 (Rad51C deficient) and irs3#6 (Rad51C complemented). Rad51C is a gene which encodes strand-transfer proteins that are thought to be involved in recombinational repair of damaged DNA and in meiotic recombination. Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations that were statistically significantly increased by both XRCC3 and Rad51C deficiency.

Multiple studies considered the ability of Pb chromate to induce chromosome aberrations in marine mammal cell cultures. Li Chen et al. (2009) treated primary North Atlantic right whale lung and skin fibroblasts with 0, 0.5, 1.0, 2.0, and 4.0 µg/cm^2 Pb chromate for 24 hours. Wise et al. (2009) treated primary Steller sea lion lung fibroblasts with 0, 0.1, 0.5, 1 and 5 µg/cm^2 Pb chromate for 24 hours. Wise et al. (2011) treated primary sperm whale skin fibroblasts with 0, 0.5, 1, 3, 5, and 10 µg/cm^2 Pb chromate for 24 hours. In all three studies, Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations.

In summary, exposure of various cell models and an in vivo model to Pb (acetate, chromate, or nitrate) induced significant increases in chromosomal aberration that often responded in a concentration dependent manner. The use of various cell lines deficient in specific DNA repair enzymes helped to elucidate which pathways may be most sensitive to Pb-dependent chromosomal aberration.

COMET Assay

Multiple studies considered the ability of Pb to induce DNA single strand breaks in laboratory animals using the comet assays. The COMET assay measures DNA damage assessed by single cell electrophoresis of a lysed cell and measurement of the fragmented DNA or tail length. Xu et al. (2008) considered the ability of Pb-acetate to induce DNA damage measured by the comet assay in lymphocytes of male ICR mice. Animals (5 per group) were given Pb-acetate by gavage at doses of 0, 10, 50, or 100 mg/kg body weight every other day for 4 weeks. Pb exposure statistically significantly increased both tail length and tail moment in a dose-dependent manner. Nava-Hernandez et al. (2009) considered the ability of Pb-acetate to induce DNA damage in primary spermatocyte DNA of male Wistar rats. Animals (3 per group) were treated for 13 weeks with 0, 250 or 500 mg/L Pb in their drinking water. There was statistically significantly less DNA damage in the controls compared to the two treatment groups. Narayana and Al-Bader

(2011) considered the ability of Pb nitrate to induce DNA damage 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. Drosophila melanogaster larvae (72 hours old) exposed to Pb nitrate (2,000, 4,000, and 8,000 µM in culture media for 24 hours) yielded haemocytes that tested positive in the comet assay; Pb chloride (8,000 µM) did not cause DNA damage with the comet assay (Carmona et al.).

Other studies used the COMET assay in cultured human cells. Pasha Shaik et al. (2006) treated primary human peripheral blood lymphocytes obtained from healthy, nonsmoking donors with 0, 2.1, 2.4, 2.7, 3.0, 3.3 Pb nitrate for 2 hours and found dose-dependent increases in Comet tail length. 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 $\mu g/cm^2$ Pb chromate for 24 hours. Mre11 is a component of the MRN complex and plays a role in telomere maintenance and double-strand break repair. Pb chromate induced a concentration-dependent increase in DNA double strand breaks measured by the comet assay. In another study, Pb nitrate exposure (30 µg/mL) induced statistically significant increased DNA damage in human liver HepG2 cells that was statistically significantly attenuated with co-exposure with the antioxidant NAC (500 µM) (Yedjou et al., 2010).

Other studies used the comet assay to examine Pb-induced DNA single strand breaks in rodent cell cultures. Xu et al. (2006), treated PC12 cells with 0, 0.1, 1 or 10 µM Pb-acetate. Both tail length and tail moment statistically significantly increased in a concentration-dependent manner. Kermani et al. (2008) exposed mouse bone marrow-mesenchymal stem cells to 60 µM Pb-acetate for 48 hours. There was an increase in several comet assay measurements including tail length.

Other Assays

Other studies considered the ability of Pb to induce DNA double strand breaks by measuring gamma-H2A.X foci formation in cultured human cells. Xie et al. (2008) treated BJhTERT cells (hTERT-immortalized human skin fibroblasts) and ATLD-2 cells (hTERT-immortalized human skin fibroblasts deficient in Mre11) with 0, 0.1, 0.5, and 1 $\mu g/cm^2$ Pb chromate for 24 hours. Pb chromate induced a concentration-dependent increase in DNA double strand breaks measured by gamma-H2A.X foci formation.

Gastaldo et al. (2007) evaluated the ability of Pb to induce DNA double strand breaks with both gamma-H2A.X foci formation and pulse-field gel electrophoresis in cultured human cells. The human endothelial HMEC cell line was treated with 1 to 1,000 µM Pb nitrate for 24 hours. DNA double strand breaks increased in a concentration-dependent manner. Wise et al. (2010) treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24 hours in a study comparing four chromate compounds. Pb chromate induced statistically significant, concentration-dependent increases in DNA double strand breaks measured by gamma-H2A.X foci formation, at a similar level to the other three compounds. Two studies demonstrated the ability of Pb to destabilize DNA by forming DNA-histone cross links, which can lead to histone aggregation. Extracts of rat liver co-incubated with Pb nitrate (<300 µM) were shown to react with chromatin components and induce chromatin aggregation via histone-DNA cross links (Rabbani-Chadegani et al., 2011; Rabbani-Chadegani et al., 2009).

Genotoxicity testing of Drosophila melanogaster larvae (72 hours old) using the Wing Spot test showed that neither Pb chloride nor Pb nitrate (at concentrations of 2,000, 4,000 and 8,000 µM in culture media with exposure until pupation) was able to induce significant increases in the frequency of wing spots (Carmona et al.). Further, wing spot assays employing Pb co-exposure with gamma radiation showed no effect of Pb on gamma radiation induced spotting frequency. The wing spot test can detect mitotic recombination and multiple mutational events such as point mutations, deletions, and certain types of chromosome aberrations (Graf and Würgler, 1986).

Multiple studies considered Pb and DNA repair. Most were conducted in cultured cells, and one was done in an animal model. El-Ghor et al. (In Press) followed microsatellite instability (MSI) in Pb-acetate trihydrate exposed adult male rats; MSI reflects impaired DNA mismatch repair, and contributes to an increased risk of cancer. DNA from leukocytes of male albino rats exposed to Pb-acetate (acute: single oral dose of 467 mg/kg BW or sub-chronic: 47 mg/kg BW six d/wk for 4 wk) showed increased MSI at three microsatellite loci (D6mit3, D9mit2, and D15Mgh1). This study is limited by its small sample size (n=2 to 3 rodents per treatment group). Li et al., (2008a), evaluated Pb-acetate-induced effects on nucleotide excision repair efficiency in CL3 cells. All cells were exposed to 0, 100, 300 or 500 µM Pb-acetate for 24 hours in serum-free medium. Pb increased nucleotide excision repair efficiency. Gastaldo et al. (2007) evaluated the ability of Pb to affect DNA repair in cultured human cells. The human endothelial HMEC cell line was treated with 100 µM Pb nitrate for 24 hours. Pb inhibited non-homologous end joining (NHEJ) repair, over activated MRE11-dependent repair and increased Rad51-related repair. Xie et al. (2008) treated BJhTERT cells (hTERT-immortalized human skin fibroblasts) and ATLD-2 cells (hTERT-immortalized human skin fibroblasts deficient in Mre11) with 0, 0.1, 0.5, and 1 µg/cm^2 Pb chromate for 24 or 120 hours. Mre11 was

required to prevent Pb chromate-induced DNA double strand breaks. McNeill et al, (2007) considered Pb-acetate effects on Ape1. Chinese hamster ovary cells (AA8) were treated with 0, 0.5, 5, 50, or 500 µM Pb-acetate and then whole cell extracts were used to determine AP site incision activity. The data show that Pb reduced AP endonuclease function. Finally, studies considered Pb-induced cellular proliferation in laboratory animals. An earlier study in rats showed Pb nitrate-induced increased proliferation of liver cells after a partial hepatectomy, effects that were more prominent in males than females (sexual dimorphism) (Tessitore et al., 1995). The newer studies showed similar trends in males. Fortoul et al. (2005) exposed adult male CD1 mice (24 animals per group) to 0.01 M Pb-acetate, 0.006 M cadmium 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-acetate for 48 hours. As measured by the MTT assay, Pb decreased cell proliferation at all concentrations tested.

5.10.4 Effects of Lead within Mixtures

Several studies considered the impact of mixtures with Pb. All considered genotoxicity. Mendez-Gomez et al., (2008), evaluated 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 urinary arsenic or blood 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 (i.p.) to 5 or 10 doses of 0.46 mg/kg Pb-acetate and compared to untreated controls. The MN in bone marrow were elevated after Pb treatment alone and increased with time. Co-exposure with cadmium or zinc did not further increase MN levels but did increase SCE levels. Glahn et al., (2008) performed a gene array study in primary normal human bronchial epithelial cells from four donors treated with 550 µg/L Pb chloride, 15 µg/L cadmium sulfate, 25 µg/L cobalt chloride or all three combined for 72 hours. There was a clear interaction of all three metals impacting RNA expression.

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 5.10.3.2 and 5.10.6).

A new study details Pb and selenium interactions in virus-dependent carcinogenesis in laboratory animals. Schrauzer (2008) considered the impact of selenium on

carcinogenesis by studying 4 groups of weanling virgin female C3H/St mice infected with murine mammary tumor virus (groups of 20-30 mice), which induces mammary tumor formation. One set of two groups were fed a diet containing 0.15 ppm selenium and then were exposed via drinking water to acetic acid (control group) or 0.5 ppm Pb-acetate (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-acetate. The study was primarily focused on the general effects of a low selenium diet. The data suggest that selenium is anticarcinogenic as in the groups without Pb exposure, the animals exposed to the higher selenium levels had fewer mammary tumors and these tumors had a delayed onset of appearance. Pb exposure with low selenium caused the same delayed onset as did 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.

5.10.5 Modes of Action

The carcinogenic mechanism of action of Pb is poorly understood. It is unclear whether the mechanism of action of Pb is best understood within the framework of multistage carcinogenesis, genomic instability or epigenetic modification. For example, multistage carcinogenesis involves a series of cellular and molecular changes that result from the progressive accumulation of mutations that induce alterations in cancer-related genes. Pb does not appear to follow this paradigm and the literature suggests it is weakly mutagenic. Pb does appear to have some ability to induce 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. These processes have been rarely studied for Pb, thus there are few data that suggest Pb may interfere with some of these processes. Furthermore, the bulk of the literature in this area involves Pb chromate and it is unclear if the effects are due to Pb or chromate. Epigenetic modifications can lead to cancer by altering cellular functions without altering the genetic material. The most commonly studied epigenetic change is methylation alterations. A small number of studies show that 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. However, Pb-dependent carcinogenicity is it not likely to occur by a multistage paradigm. More work is needed to determine the mechanism.

Exposure to mixtures can also contribute to understanding of modes of action. 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 protected 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 and point to the relevance of mixtures in assessing toxicity.

5.10.5.1 Epigenetics

Air pollution exposure is being linked increasingly with epigenetic changes (Pavanello et al., 2010; Baccarelli and Bollati, 2009; Tarantini et al., 2009; Bollati et al., 2007). Epigenetic changes involve changes in DNA expression without actual changes in the DNA sequence and these changes may be heritable. Epigenetic changes are mediated by histone modification, DNA methylation, miRNA changes, or pathways that affect these three mediators. Differential epigenetic modification has the possibility to contribute to disease. Epigenetic studies have been conducted to examine the associations between Pb biomarker levels and global DNA methylation markers [Alu and long interspersed nuclear element-1 (LINE-1)] in humans (Wright et al., 2010; Pilsner et al., 2009). Wright et al. (2010) utilized a sample of participants from the NAS with mean (SD) Pb levels of 20.5(14.8)g/g for tibia measures, 27.4 (19.7)g/g for patella measures, and 4.1 (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, both of which are indicators of

global methylation. When examining the relationship between patella Pb and LINE-1 more closely, a non-linear trend was observed with a smaller magnitude of effect estimated for higher patella Pb (≥ 40 µg/g). No associations were observed for tibia or blood Pb and either LINE-1 or Alu. Another study included maternal-infant pairs from the Early Life Exposures in Mexico to Environmental Toxicants study and measured LINE-1 and Alu methylation in umbilical cord blood samples (Pilsner et al., 2009). In unadjusted models, maternal pregnancy tibia Pb levels [mean (SD) 10.5 (8.4) µg/g] were inversely associated with Alu methylation; maternal patella Pb levels [mean (SD) 12.9 (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 four donors after in vitro treatment of the cells with 550 µg/L Pb chloride, 15 µg/L cadmium sulfate, 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 epigenetic 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. A recent publication using HepG2 cells in tissue culture showed that cells exposed to a high dose of Pb (100 µM Pb-acetate) experienced ALAD gene promoter hypermethylation and decreased ALAD transcription. This was in agreement with findings in battery plant workers who showed ALAD hypermethylation (versus non-occupationally exposed controls) and an association of this hypermethylation with elevated risk of Pb poisoning (Li et al., 2011).

5.10.6 Summary and Causal Determination

In summary, the toxicological literature on the genotoxic, mutagenic, and carcinogenic potential of Pb provide 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. Multiple toxicological studies showed neoplastic transformation in cultured cells, but all focused on Pb chromate and the positive response to the chromate ion and not the Pb likely contributed to these findings. Multiple epidemiologic studies have been performed examining the association between cancer incidence and mortality and Pb exposures, estimated with biological measures and exposure databases. Mixed results have been reported for cancer mortality studies; a large NHANES epidemiologic study demonstrated a positive association between blood Pb and cancer mortality, but the other studies reported null results. Although the 2006 Pb AQCD reported some studies that found an association between Pb exposure indicators and lung cancer, current studies mostly included occupationally-exposed adults and observed no associations. Most studies of Pb and brain cancer were null among the overall study population, but positive associations were observed among individuals with certain genetic variants. However the studies of Pb and brain cancer were all performed among occupational cohorts using interviews instead of biological measurements to represent Pb exposure. A limited amount of research has been performed on other types of cancer. The 2006 Pb AQCD reported evidence that suggested an association between Pb exposure and stomach cancer, but recent studies of stomach cancer 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, high Pb levels were associated with SCEs 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 in animal and in vitro models, inducing SCE, MN and DNA strand breaks, but continues to not produce chromosomal aberrations except for Pb chromate; 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.

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. Metallothionein has been shown to be protective against the effect of Pb on carcinogenicity. Pb is clastogenic and mutagenic in some but not all models. Clastogenicity and mutagenicity may be possible mechanisms contributing to cancer but are not absolutely associated with the induction of cancer. Because Pb has a higher atomic weight than does zinc, Pb replaces zinc at many zinc binding sites 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 negatively alter protein structure and DNA repair.

Epigenetic changes associated with Pb exposure, particularly with respect to methylation and effects on DNA repair, 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. A small number of epidemiologic studies examining Pb and global epigenetic changes including LINE-1 and Alu demonstrated an inverse association between patella Pb and LINE-1 methylation, an emerging area of research. Toxicological studies show that Pb can activate or interfere with a number of signaling and repair pathways, though it is unclear whether these are due to epigenetic responses or genotoxicity. Thus, an underlying mechanism is still uncertain, but likely involves either genomic instability, epigenetic modifications, or both.

In conclusion, the toxicological literature provides the strongest evidence for Pb exposure and cancer with supporting evidence provided by the epidemiologic literature. This is substantiated by the findings of other agencies including IARC, which has classified inorganic Pb compounds as a probable human carcinogen and the National Toxicology Program, which has listed Pb and Pb compounds as "reasonably anticipated to be human carcinogens." Strong evidence from toxicological studies demonstrates an association between Pb and cancer, genotoxicity/clastogenicity or epigenetic modification. Carcinogenicity in historical animal toxicology studies with Pb exposure has been reported in the kidneys, testes, brain, adrenals, prostate, pituitary, and mammary gland, albeit at high doses of Pb. Epidemiologic studies of cancer incidence and mortality reported inconsistent results; one strong epidemiologic study demonstrated an association between blood Pb and increased cancer mortality, but the other studies reported weak or no associations. In the 2006 Pb AQCD, Pb exposure was found to be associated with stomach cancer, but there was only one recent study on stomach cancer and Pb exposure, which reported mixed findings. Similarly, some studies in the 2006 Pb AQCD reported associations between Pb exposure and lung cancer. More recent epidemiologic studies of lung cancer focused on occupational exposures and reported no associations. The majority of epidemiologic studies of brain cancer had null results overall, but positive associations between Pb exposure and brain cancer were observed among individuals with certain genotypes. In toxicological studies, chromosomal aberrations after Pb exposure are most often reported with Pb chromate exposure, which is likely due to toxicity of the chromate moiety. Mechanistic understanding of Pb and its effect on cancer and genotoxicity is expanding through toxicological work focusing on antioxidants and other proteins that sequester Pb or reduce its bioavailability. The collective body of evidence integrated across toxicological and epidemiologic studies is sufficient to conclude that there is a likely causal relationship between Pb exposure and cancer.

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CHAPTER 6 POTENTIALLY AT-RISK POPULATIONS

The NAAQS are intended to provide an adequate margin of safety for both the population as a whole and those groups with unique factors that make them potentially at increased risk for health effects in response to ambient air pollutants (Preface to this ISA). 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. To facilitate the identification of populations at greater risk for Pb-related health effects, studies have evaluated various factors that may contribute to susceptibility and/or vulnerability to Pb. The definitions of susceptibility and vulnerability vary across studies, but in most instances "susceptibility" refers to biological or intrinsic factors (e.g., age, sex) while "vulnerability" refers to nonbiological or extrinsic factors (e.g., socioeconomic status [SES]) (U.S. EPA, 2010a, 2009). Additionally, in some cases, the terms "at-risk" and "sensitive" populations have been used to encompass these concepts more generally. In this ISA, "at-risk" groups are defined as those with characteristics that increase the risk of Pb-related health effects in a population. These characteristics include various factors, such as genetic background, race and ethnicity, sex, age, diet, pre-existing disease, SES, and characteristics that may modify exposure or the response to Pb.

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 at greater risk for Pb-related health effects. Toxicological studies, using animal disease models, also provide support and biological plausibility for factors that may lead to increased risk for Pb-related health effects. These epidemiologic and toxicological studies provide the scientific basis for an overall weight of the evidence evaluation for the increased risk of specific populations to Pb-related health effects.

The first section of this chapter summarizes physiological factors that possibly influence Pb levels in the body. The second section of this chapter summarizes information on factors potentially related to differential Pb exposure. The studies presented in this section supplement the material provided in Chapters 3 and 4 by examining how factors such as age, sex, race and ethnicity, SES, proximity to Pb sources, and residential factors may affect Pb exposure. The third section of this chapter discusses the epidemiologic and toxicological studies evaluated in Chapter 5 that provide information on potential factors related to increased risk of Pb-induced health effects. Highlighted studies include only those where the population was stratified into subgroups (e.g., males versus females or

smokers versus nonsmokers) for comparative analysis. In the case of many biomarker studies and the epidemiologic studies considered, this approach allowed for a comparison between populations exposed to similar Pb concentrations and within the same study design. Numerous studies that focused on only one potentially at-risk population were described in previous chapters (Chapter 5) but are not discussed in detail in this chapter because they lacked stratified analysis with adequate comparison groups. For example, pregnancy is a lifestage with potentially increased risk for mothers and fetuses, but because there are no comparison groups for stratified analyses, these studies were presented in Chapter 5 but are not included here. Included toxicological studies may have categorized the study populations by age, sex, diet/nutrition status, genetics, etc. or are those that examined animal models of disease.

Additionally, it is understood that some of the stratified variables/factors discussed in this third section may not be effect measure modifiers but instead may be mediators of Pb-related health effects. Mediators are factors that fall on the causal pathway between Pb and health outcomes, whereas effect measure modifiers are factors that result in changes in the measured associations 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 “at-risk” factors. Some of the factors discussed in this third section could be mediators and/or modifiers. These are noted in Table 6-4.

6.1 Physiological Factors that Influence the Internal Distribution of Lead

Blood and bone Pb measures are influenced to varying degrees by biokinetic processes (absorption, distribution, metabolism, etc.), which are discussed in detail in 0. These processes can be affected by multiple factors, such as age, genetics, diet, and co-exposure with other metals and non-metals.

Age influences the biokinetic response to Pb within the body. Infants may be an at-risk population because Pb easily crosses the placental barrier and accumulates in fetal tissue during gestation (Pillai et al., 2009; Wang et al., 2009e; Uzbekov et al., 2007). This transfer of Pb from mother to fetus is partly due to the remobilization of the mother’s bone stores (O'Flaherty, 1998; Franklin et al., 1997). This also results in increased maternal blood Pb levels (Lamadrid-Figueroa et al., 2006; Gulson et al., 2004a; Hertz-Picciotto et al., 2000; Gulson et al., 1997; Lagerkvist et al., 1996; Schuhmacher et al., 1996; Rothenberg et al., 1994a). Bone growth rate is high during childhood. The majority of a child’s Pb body burden is not permanently incorporated in the bone, but some Pb does remain in the bone until older age (McNeill et al., 2000; O'Flaherty, 1995; Leggett,

1993). Older adults are more likely to have age-related degeneration of bones and organ systems and a possible redistribution of Pb stored in the bones into the blood stream (Popovic et al., 2005; Garrido Latorre et al., 2003; Gulson et al., 2002).

Various genes can also affect Pb biomarker concentrations. Genetic variants of the vitamin D receptor (VDR) in humans have been associated with varied bone and plasma Pb levels (Rezende et al., 2008; Theppeang et al., 2004; Schwartz et al., 2000a). Multiple studies have also examined the association between the aminolevulinate dehydratase (ALAD) polymorphism and blood Pb levels and found that the ALAD-2 polymorphism may be biologically related to varying Pb levels, although some studies report no difference for ALAD alleles (Miyaki et al., 2009; Shaik and Jamil, 2009; Sobin et al., 2009; Chen et al., 2008c; Rabstein et al., 2008; Scinicariello et al., 2007; Zhao et al., 2007; Montenegro et al., 2006; Wananukul et al., 2006).

It is well established that diets sufficient in minerals such as calcium, iron, and zinc offer some protection from Pb exposure by preventing or competing with Pb for absorption in the GI tract. A study in China reported that children who regularly consumed breakfast had lower blood Pb levels than those children that did not eat breakfast (Liu et al., 2011b). 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). A toxicological study reported negative effects of Pb on osmotic fragility, TBARS production, catalase activity, and other oxidative parameters, but most of these effects were reduced to the levels observed in the control group when the rats were given supplementation of zinc and vitamins (Massó-González and Antonio-García, 2009). Toxicological studies by Jamieson et al. (2008; 2006) also reported that a zinc-deficient diet increases bone and renal Pb content and impairs skeletal growth and mineralization. A zinc-supplemented diet attenuated bone and renal Pb content. Toxicological studies have shown that dietary deficiency of calcium induces increased Pb absorption and retention (Fullmer, 1992; Mykkanen and Wasserman, 1981; Six and Goyer, 1970). Increased calcium intake reduces accumulation of Pb in bone and mobilization of Pb during pregnancy and lactation (Bogden et al., 1995). Additionally, studies have reported that iron deficiencies may result in higher Pb absorption or altered biokinetics (Schell et al., 2004; Marcus and Schwartz, 1987; Mahaffey and Annest, 1986).

Finally, co-exposures with other metals and non-metals have also been studied to assess how they affected the uptake and absorption of Pb. Recent toxicological studies examined the addition of arsenic (As) to Pb and cadmium (Cd) mixtures, and reported increased bioavailability of Pb (Wang and Fowler, 2008). A toxicological study by Sawan et al. (2010) reported co-exposure with fluoride increased Pb deposition in calcified tissues.

In summary, age, genetics, diet, and other exposures affect the biokinetics of Pb, which in turn affects the internal distribution of Pb. These factors were discussed in greater detail in 0 where more information on overall biokinetics and physiological factors affecting Pb distribution was provided.

6.2 Population Characteristics Potentially Related to Differential Lead Exposure

Elevated or differential Pb exposure and related biomarker levels (such as blood Pb), have been shown to be statistically related to several population characteristics, including age, sex, race and ethnicity, SES, proximity to Pb sources, and residential factors (U.S. EPA, 2006b). In most cases, exposure, absorption, and biokinetics of Pb are all influenced to varying degrees by such characteristics. Additionally, the relative importance of population characteristics on exposure, absorption, and biokinetics varies on an individual basis and is difficult to quantify. This section presents recent studies demonstrating a relationship between each population characteristic and exposure status. The studies presented in this section build upon the current body of literature suggesting that population characteristics differentially influence Pb exposure; the new literature does not alter our previous understanding of the differential influence of population characteristics on Pb exposure. Differential response to given Pb exposures is discussed in Section 6.3.

6.2.1 Age

6.2.1.1 Early Childhood

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 that typically result in increased ingestion compared with adults (U.S. EPA, 2006b). Children can also 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 sex. Among children, highest blood Pb levels occurred in the 1-5 year age group

(children under age 1 were not included), and within this subgroup (not shown on the table), 1 year old children had the highest blood Pb levels (99th percentile: 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) or from contemporaneous Pb exposures if the mothers were located near sources. Jones et al. (2009a) analyzed the NHANES datasets for the years 1999-2004 to study trends in blood Pb among two different age groups of children over time (see Table 6-2). They observed greater percentages of children aged 1-2 years having blood Pb levels of 2.5 to <5 µg/dL, 5 to <7.5 µg/dL, and ≥ 10 µg/dL, compared with 3-5 year-old children, but no age difference was noted for the 7.5 to <10 µg/dL bracket. These distribution differences may be attributable to differences in exposure, age-dependent variability in biokinetics or diet (e.g., milk versus solid diets). Yapici et al. (2006) studied the relationship between blood Pb level and age among a cohort of children 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 by age and sex, 2007-2008 NHANES

Age	Sex	N	Avg.	Std. Dev.	5%	25%	50%	75%	95%	99%
1-5 yr	**Total**	**811**	**2.03**	**2.01**	**0.69**	**1.08**	**1.54**	**2.34**	**4.50**	**10.60**
	Male	440	2.01	2.14	0.71	1.10	1.50	2.40	4.21	8.56
	Female	371	2.05	1.85	0.66	1.02	1.60	2.28	4.65	10.70
6-11 yr	**Total**	**1,002**	**1.27**	**0.87**	**0.49**	**0.75**	**1.06**	**1.50**	**2.84**	**4.80**
	Male	500	1.29	0.92	0.48	0.74	1.08	1.50	2.90	4.76
	Female	502	1.25	0.83	0.50	0.78	1.03	1.49	2.80	4.80
12-19 yr	**Total**	**1,089**	**0.99**	**0.73**	**0.40**	**0.59**	**0.81**	**1.13**	**2.11**	**4.00**
	Male	586	1.13	0.82	0.44	0.69	0.94	1.30	2.41	4.11
	Female	503	0.83	0.57	0.37	0.52	0.69	0.91	1.70	3.36
20-64 yr	**Total**	**4,278**	**1.76**	**1.57**	**0.53**	**0.91**	**1.40**	**2.10**	**4.20**	**7.42**
	Male	2,079	2.13	1.93	0.68	1.12	1.65	2.49	5.22	9.07
	Female	2,199	1.41	1.01	0.47	0.78	1.13	1.73	3.13	5.10
65+ yr	**Total**	**1,086**	**2.31**	**1.64**	**0.80**	**1.30**	**1.90**	**2.70**	**5.22**	**8.54**
	Male	542	2.63	1.66	0.99	1.52	2.19	3.20	5.86	9.03
	Female	544	1.98	1.56	0.75	1.17	1.62	2.40	4.14	6.95

Age	Sex	N	Avg.	Std. Dev.	5%	25%	50%	75%	95%	99%
Overall	**Total**	**8,266**	**1.70**	**1.53**	**0.51**	**0.86**	**1.30**	**2.04**	**4.10**	**7.20**
	Male	4,147	1.94	1.77	0.58	0.99	1.5	2.3	4.72	8.69
	Female	4,119	1.45	1.19	0.46	0.76	1.14	1.78	3.35	5.77

Source: (NCHS, 2010).

Table 6-2 Percentage of children within six categories/brackets of blood Pb levels, 1999-2004 NHANES

Pb Units: µg/dL (95% CI)	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	2,532	1.9 (1.8-2.0)	14.0 (11.6-16.6)	55.0 (52.1-57.9)	23.6 (21.1-26.1)	4.5 (3.3-5.9)	1.5 (1.0-2.1)	1.4 (1.0-2.0)
Sex								
Female	1,211	1.9 (1.7-2.0)	14.1 (10.8-17.7)	54.5 (51.1-57.8)	23.9 (20.3-27.8)	4.5 (3.3-5.8)	1.4 (0.8-2.3)	1.7 (0.9-2.6)
Male	1,321	1.9 (1.7-2.0)	14.0 (11.4-16.7)	55.5 (51.4-59.5)	23.2 20.3-26.3)	4.6 (3.0-6.5)	1.5 (0.9-2.3)	1.3 (0.7-2.6)
Age								
1-2 yr	1,231	2.1 (2.0-2.2)	10.6 (7.7-13.9)	51.0 (46.7-55.3)	27.9 (24.9-31.0)	6.7 (5.0-8.6)	1.4 (0.8-2.2)	2.4 (1.4-3.5)
3-5 yr	1,301	1.7 (1.6-1.9)	16.2 (12.9-19.9)	57.6 (53.8-61.4)	20.7 (17.9-23.7)	3.1 (1.9-4.6)	1.5 (0.8-2.3)	0.9 (0.4-1.5)
Race/Ethnicityc								
Non-Hispanic Black	755	2.8 (2.5-3.0)	4.0 (2.5-5.7)	42.5 (37.8-47.2)	36.2 (33.1-39.3)	9.4 (6.9-12.2)	4.6 (3.0-6.5)	3.4 (1.8-5.5)
Mexican American	812	1.9 (1.7-2.0)	10.9 (8.6-13.4)	61.0 (56.9-65.1)	22.1 (18.0-26.5)	3.4 (2.2-5.0)	1.3 (0.6-2.2)	1.2 (0.4-2.6)
Non-Hispanic White	731	1.7 (1.6-1.8)	17.6 (14.0-21.5)	57.1 (52.4-61.7)	19.7 (16.1-23.5)	3.6 (1.9-5.8)	0.8 (0.3-1.6)	1.2 (0.6-2.0)
Poverty-Income Ratio (PIR)c								
≤ 1.3	1,302	2.4 (2.2-2.5)	6.7 (4.6-9.2)	49.3 (44.9-53.7)	32.5 (28.6-36.4)	6.9 (2.2-8.8)	2.8 (1.7-4.1)	1.8 (1.1-2.7)
>1.3	1,070	1.5 (1.4-1.6)	19.9 (16.3-23.8)	60.4 (56.9-63.8)	16.0 (12.9-19.3)	2.3 (1.2-3.7)	0.6 (0.1-1.4)	0.8 (0.3-1.6)

Source: Reprinted with permission of the American Acedemy of Pediatrics; Jones et al. (2009a)

Fetal and child Pb biomarkers have been demonstrated to relate to maternal Pb biomarkers; several older studies in the literature were presented in the 2006 Pb AQCD (U.S. EPA, 2006b). Kordas et al. (2010) observed that maternal hair Pb concentration was a statistically significant predictor of child hair Pb concentration ($\beta = 0.37 \pm 0.07$, $p < 0.01$). Miranda et al. (2010) observed that pregnant women (ages 30-34 years and 35-39 years) had statistically significant higher odds of having greater blood Pb levels than younger pregnant women in the (25- to 29-year-olds) reference age category. These results could be related to a historical component to Pb exposure among mothers. These findings were also consistent with observations that Pb storage in bones increased with age before subsequent release with bone loss occurring during pregnancy, as described in Section 4.2 and summarized in Section 6.1. Elevated blood Pb levels among mothers

present a potential exposure route to their children in utero or through breast milk. Additionally, maternal pica presents a potential Pb source to a developing fetus (Hamilton et al., 2001).

6.2.1.2 Adulthood

Blood Pb levels tend to be higher in older adults compared with the general adult population (U.S. EPA, 2006b). 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 blood Pb ($p < 0.001$). Average blood Pb levels among 14- to 24-year-old subjects was 2.60 ± 0.16 µg/dL compared with 4.29 ± 0.56 µg/dL among subjects aged 55 years or older. Higher average and median levels among older adults could potentially be due to a shared experience of higher historical Pb exposures stored in bone in conjunction with remobilization of stored Pb during bone loss (Section 4.2).

Theppeang et al. (2008b) studied Pb concentrations in the blood, tibia, and patella of subjects age 50-70 as part of the Baltimore Memory Study. They found a statistically significant relationship between age and tibia Pb ($\beta = 0.37$, $p < 0.01$ in a model including age, race/ethnicity, Yale energy index, and 2 diet variables; $\beta = 0.57$, $p < 0.01$ in a model including age, sex, and an interaction term for sex and age, which was also statistically significant at $p = 0.03$). Theppeang et al. (2008b) also noted that patella Pb concentrations 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 level.

6.2.2 Sex

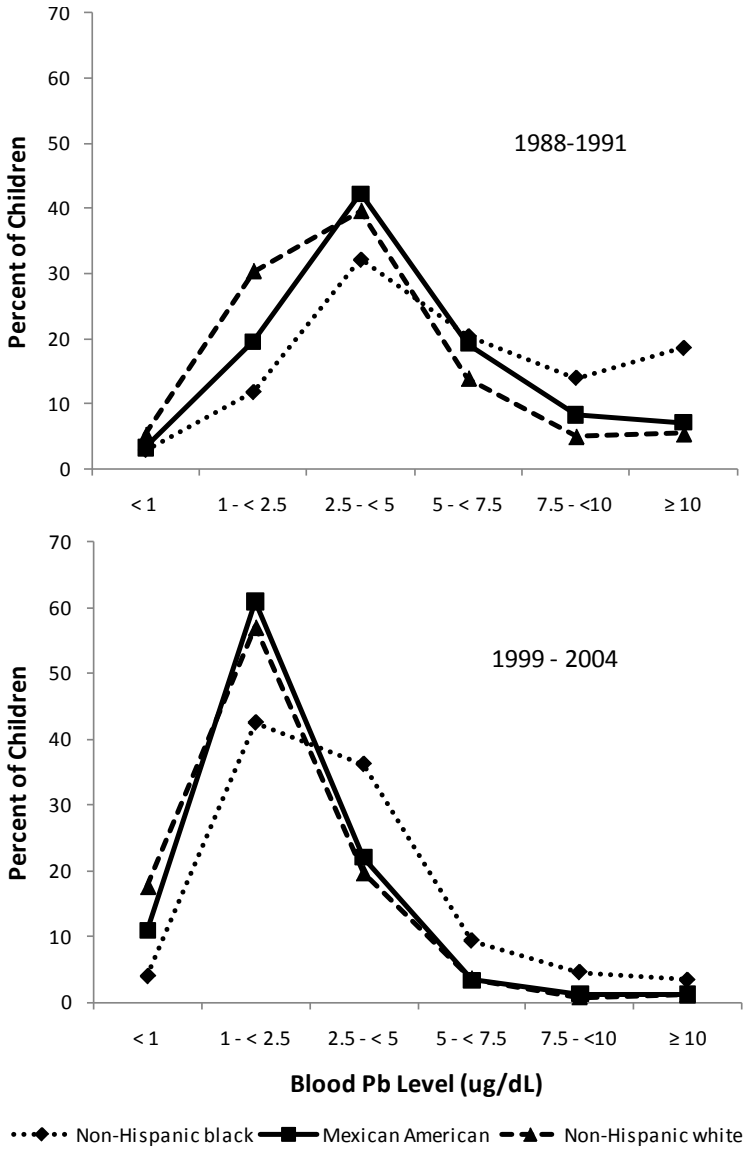
Several studies have suggested that sex influences levels of Pb biomarkers because differences in behavior between sexes may cause a differential increase in exposure. The 2007-2008 NHANES showed that overall, males have significantly higher blood Pb levels (average: 1.94 µg/dL) than females (average: 1.45 µg/dL) ($p < 0.0001$). Among adults aged 20-64 years, average blood Pb levels were 51% higher for males compared with females ($p < 0.0001$). Among adults 65 years or older, average blood Pb levels were

33% higher for males compared with females ($p < 0.0001$). In their study of Pb burden among Baltimore adults aged 50-70 years, Theppeang et al. (2008b) observed that average blood Pb levels were statistically significantly higher ($p < 0.01$) among men (4.4 µg/dL) than women (3.1 µg/dL). For average tibia Pb levels, Theppeang et al. (2008b) noted no difference ($p = 0.12$) between men (18.0 µg/g) and women (19.4 µg/g).

Among U.S. children, the 2007-2008 NHANES data showed that blood Pb levels were 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 than females 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 sex-based differences in blood Pb levels are not substantial until adolescence.

6.2.3 Race and Ethnicity

Higher blood Pb and bone Pb levels among African Americans has been well documented (U.S. EPA, 2006b). Recent studies are consistent with those previous findings. For instance, Levin et al. (2008) and Jones et al. (2009a) both analyzed NHANES survey data to examine trends in childhood blood Pb levels. Data from the Jones et al. (2009a) study, using NHANES data (NCHS, 2010) from 1988-1991 and 1999-2004 are shown in Figure 6-1. The authors found that differences among children from different racial/ethnic groups with regard to the percentage with blood Pb levels ≥ 2.5 µg/dL over the period 1999-2004 have decreased since the period of 1988-1991 . The non-Hispanic black group still had higher percentages with blood Pb levels ≥ 2.5 µg/dL compared with non-Hispanic whites and Mexican Americans, with large observable differences for blood Pb levels between 2.5 and <10 µg/dL. It is notable that the distributions of blood Pb levels among Mexican American and non-Hispanic white children were nearly identical in the 1999-2004 dataset. Theppeang et al. (2008b) also explored the effect of race and ethnicity on several Pb biomarkers in a study of older adults living in Baltimore, MD. They observed a statistically significant difference between African American (AA) and Caucasian (C) subjects with respect to tibia Pb (AA: 21.8 µg/g, C: 16.7 µg/g, $p < 0.01$) but not patella Pb (AA: 7.1 µg/g, C: 7.1 µg/g, $p = 0.46$) or blood Pb levels (AA: 3.6 µg/dL, C: 3.6 µg/dL, $p = 0.69$). Greater tibia (but lower patella) Pb levels may indicate greater historical exposure among African Americans compared to Caucasians in the Baltimore population studied by Theppeang et al. (2008b).

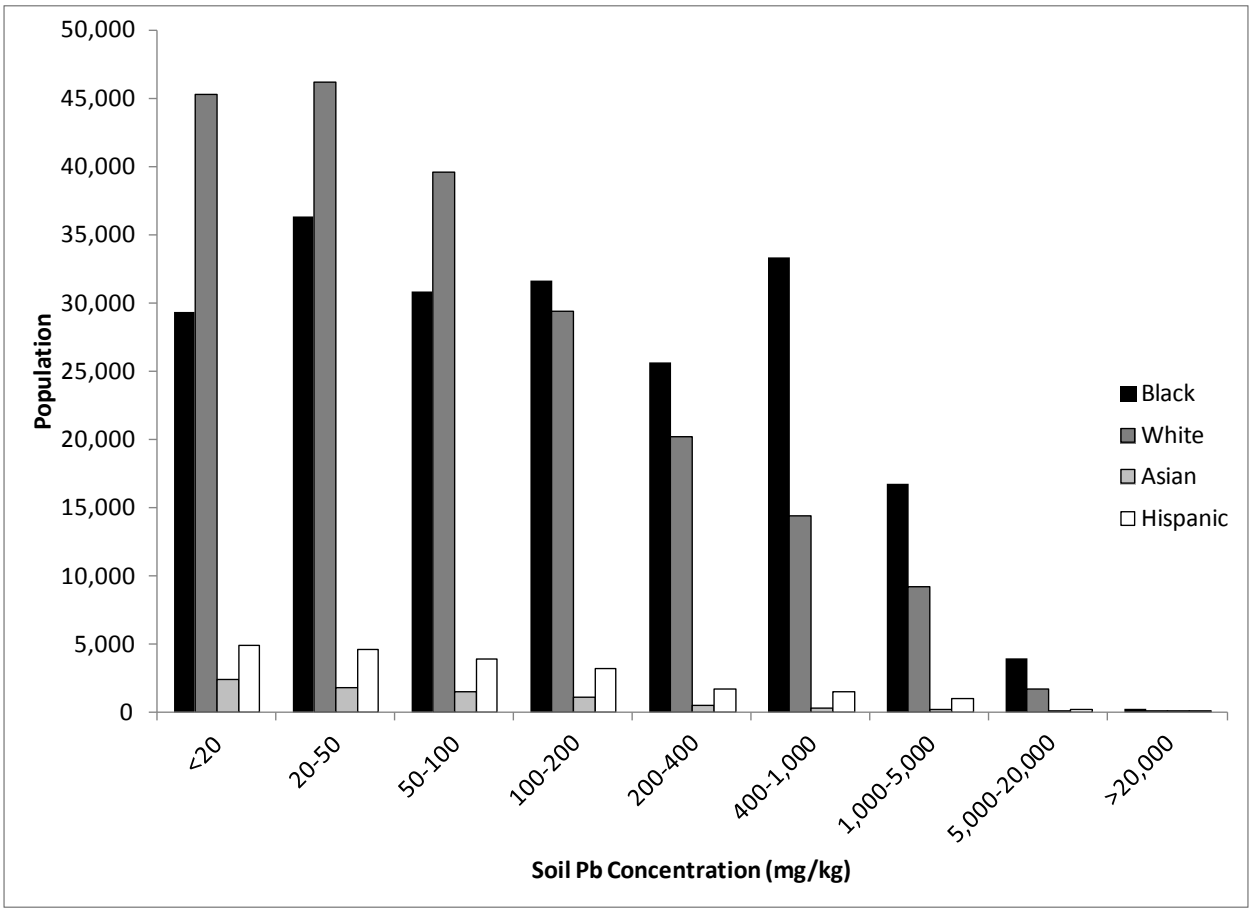


Data used with permission of the American Academy of Pediatrics, Jones et al. (2009a)
Note: from the NHANES survey, 1988-1991 (top) and 1999-2004 (bottom).

Figure 6-1 Percent distribution of blood Pb levels by race/ethnicity among U.S. children (1-5 years).

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 36% black, 55% white, 3.0% Asian, and 6.0% Hispanic, based on the 2000 Census, with the percentage based on the total number living in Census blocks with the same soil Pb levels. In contrast, they found that for Census blocks in which soil Pb levels were between 1,000 and 5,000 mg/kg, the population was 62% black, 34% white, 1% Asian,

and 4% Hispanic (Figure 6-2). As described in Section 6.2.4, the differences observed by Campanella and Mielke (2008) may also be attributable to SES factors, or SES may be a confounding factor in the relationship between Pb soil levels and race/ethnicity of nearby residents.



Source: Data used with permission of Springer Science; Campanella and Mielke (2008).
Note: By Census 2000 race/ethnicity demographic groups.

Figure 6-2 Soil Pb concentration exposure among the population of three parishes within greater metropolitan New Orleans.

6.2.4 Socioeconomic Status (SES)

Socioeconomic factors have sometimes been associated with Pb exposure biomarkers, although these relationships have not always been consistent (U.S. EPA, 2006b). Nriagu et al. (2006) performed a multiple regression analysis of blood Pb and saliva Pb levels on various socioeconomic, demographic, and exposure variables among an adult population

in Detroit, Michigan. Blood and saliva Pb were both used as indicators of Pb in unbound plasma that is available to organs. Nriagu et al. (2006) found that education ($p < 0.001$), income ($p < 0.001$), and employment status ($p = 0.04$) were all statistically significant predictors of blood Pb levels, with blood Pb decreasing with some scatter as education and income level increased. Statistically significant relationships were also reported by Nriagu et al. (2006) for saliva Pb level with respect to education ($p < 0.001$), income ($p < 0.001$), and employment ($p = 0.06$). However, the highest educational attainment and income categories had higher saliva Pb levels compared with other groups; Nriagu et al. (2006) attributed these inconsistencies to small sample sizes among the high educational attainment and income categories.

On a national level, the gap between income levels with respect to blood Pb has been decreasing. For example, Levin et al. (2008) cited 1991-1994 NHANES data [analyzed in Pirkle et al. (1994)] that the percentage of children aged 1-5 years with blood Pb levels ≥ 10 µg/dL was 4.5% for the lowest income group compared with 0.7% for the highest income group. Levin et al. (2008) also analyzed data from the 1999-2002 NHANES and found no statistically significant difference between the percent of children with blood Pb levels above 10 µg/dL for Medicaid-enrolled children (1.7%) compared with non-enrolled children (1.3%). However, Medicaid-enrolled children did have higher median blood Pb levels (2.6 µg/dL) compared to children not enrolled in Medicaid (1.7 µg/dL). 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 ≥ 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 (Levin et al., 2008). Likewise, Jones et al. (2009a) analyzed blood Pb levels with respect to poverty-income ratio (PIR), which is the ratio of family income to the poverty threshold appropriate for a given family size. They found statistically significant differences in median blood Pb for PIR ≤ 1.3 compared with PIR >1.3. The percentage of 1- to 5-year-old children having blood Pb ≥ 10 µg/dL was higher for PIR ≤ 1.3 (1.8 versus 0.8); however, this difference was not statistically significant. Additionally, 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 a Census block-averaged median household income of $40,000 per year, while soil Pb between 5,000 and 20,000 mg/kg was associated with a Census block-averaged median household income of $24,000 per year.

6.2.5 Proximity to Lead Sources

Airborne and soil Pb concentrations are higher in some industrialized and urbanized areas, as described in Sections 3.2, 3.3, 3.5 and 4.1, as a result of historical and contemporaneous Pb sources. The highest air Pb concentrations measured using the Pb-TSP monitoring network have been measured at monitors located near sources emitting Pb. Elevated soil Pb concentrations have also been measured in urbanized areas compared with less urbanized or rural locations (Mielke et al., 2010a; Laidlaw and Filippelli, 2008; Weiss et al., 2006). Several studies describe mechanisms by which larger particles present in soil can become resuspended to the air by perturbation from traffic- and wind-generated turbulence (Harris and Davidson, 2008; Lough et al., 2005; Zereini et al., 2005; Nicholson, 1988; Gillette et al., 1974). However, there are no recent monitoring studies of this process, and the current routine monitoring network is not designed for the assessment of the specific contribution of resuspension to ambient air concentrations. Air Pb concentrations exhibit high spatial variability even at low concentrations (~0.01 $\mu g/m^3$) (Martuzevicius et al., 2004). Proximity to an industrial source likely contributes to higher Pb exposures, as described in the 2006 Pb AQCD (U.S. EPA, 2006b) for several studies of Superfund and other industrial sites. This is consistent with the observation of higher air concentrations at source oriented Pb monitoring sites compared with non-source oriented sites in the 2007-2009 data presented in Section 3.5.

Jones et al. (2010) found that neonates born near a Pb-contaminated hazardous waste site had significantly higher umbilical cord blood Pb levels (median: 2.2 µg/dL [95% CI: 1.5, 3.3 µg/dL]) compared with a reference group of neonates not living near a potentially contaminated site (median: 1.1 µg/dL [95% CI: 0.8, 1.3 µg/dL]), suggesting that Pb-contaminated hazardous waste sites contribute to neonatal Pb levels. The population studied in Jones et al. (2010) was 88% African American; 75% had a high school degree or equivalent, while 20% had a college degree and 5% attended but did not graduate from high school. However, 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 maternal groups (which did and did not report nearby hazardous waste sites) confounded these results.

Studies have suggested that soil Pb exposure is related to land use type and historical exposures of the soil, as described in Section 3.6.1. For instance, Wu et al., (2010) observed that bioavailable Pb concentrations in Los Angeles soils were significantly associated with traffic-related variables and parcel age (i.e., length of time since the parcel was first developed), with parcel age being a highly significant predictor of bioavailable soil Pb in most models ($p < 0.0001$). Zahran et al. (2011) observed that soil Pb levels dropped following Hurricanes Katrina and Rita, from 329 mg/kg to 203 mg/kg

when comparing samples from 2000 with those from 2006. The reduction in soil Pb was thought to occur because sediments with lower Pb concentrations were distributed by the storms and coated existing soils having higher Pb concentrations. At the same time, blood Pb levels obtained from children ages 0-6 years at the same time periods declined by 1.55 µg/dL for each 1% reduction in soil Pb ($p \leq 0.05$). Following this observation, that lower Pb concentration material can be used to contain older soils with higher concentrations of Pb, Mielke et al. (2011b) initiated an experiment where a playground was covered with geotextile material topped with 15 cm of river alluvium. Median soil Pb concentrations decreased from 558 mg/kg to 4.1 mg/kg after the mitigation.

6.2.6 Residential Factors

A recent study of the association between blood Pb and housing factors by Dixon et al. (2009), which drew inference from the NHANES national survey for 1999-2004, is consistent with previous studies presented in the 2006 Pb AQCD that drew associations between blood Pb and house dust (U.S. EPA, 2006b; Lanphear et al., 1998; Laxen et al., 1987). Dixon et al. (2009) used NHANES data from 1999-2004 to perform a linear regression of blood Pb among children 12-60 months old on several factors including year of construction, floor surface condition, floor dust Pb level, windowsill dust Pb level, and renovation in homes built before 1978. They found that blood Pb (log transformed) was significantly associated with homes built after 1950 ($p = 0.014$), windowsill Pb level ($p = 0.002$), dust Pb level ($p < 0.001$), and renovation in pre-1978 homes ($p = 0.045$). Detailed results of this regression are shown in Table 6-3. As part of the same study, Gaitens et al. (2009) performed a regression analysis of floor dust Pb (PbD) and windowsill dusts Pb on several factors. Floor dust Pb (log transformed) was significantly associated with the following housing-related factors: floor surface condition ($p < 0.001$), windowsill dust Pb (log transformed) ($p < 0.001$), year of construction ($p < 0.001$), and renovation in a pre-1950 home ($p < 0.001$). Windowsill dust Pb (log transformed) was significantly associated with the following housing-related factors: year of construction ($p < 0.001$), window surface condition (0.001), and deteriorated indoor paint ($p = 0.028$).

Table 6-3 Regression of log-transformed blood Pb level of children 12-60 months old on various factors related to housing condition, from 1999-2004 NHANES dataset

Variables	Overall *p*-value	Levels	Estimate (SE)	*p*-Value
Intercept	0.172		–0.517 (0.373)	0.172
Age (in years)	< 0.001	Age	2.620 (0.628)	< 0.001
		Age^2	–1.353 (0.354)	< 0.001
		Age^3	0.273 (0.083)	0.002
		Age^4	–0.019 (0.007)	0.008
Year of construction	0.014	Intercept for missing	–0.121 (0.052)	0.024
		1990–present	–0.198 (0.058)	0.001
		1978–1989	–0.196 (0.060)	0.002
		1960–1977	–0.174 (0.056)	0.003
		1950–1959	–0.207 (0.065)	0.003
		1940–1949	–0.012 (0.072)	0.870
		Before 1940	0.000	—
PIR	< 0.001	Intercept for missing	0.053 (0.065)	0.420
		Slope	–0.053 (0.012)	< 0.001
Race/ethnicity	< 0.001	Non-Hispanic white	0.000	—
		Non-Hispanic black	0.247 (0.035)	< 0.001
		Hispanic	–0.035 (0.030)	0.251
		Other	0.128 (0.070)	0.073
Country of birth	0.002	Missing	–0.077 (0.219)	0.728
		U.S.[b]	0.000	—
		Mexico	0.353 (0.097)	< 0.001
		Elsewhere	0.154 (0.121)	0.209
Floor surface/condition × log floor PbD	< 0.001	Intercept for missing	0.178 (0.094)	0.065
		Not smooth and cleanable	0.386 (0.089)	< 0.001
		Smooth and cleanable or carpeted	0.205 (0.032)	< 0.001
Floor surface/condition × $(\text{log floor PbD})^2$		Not smooth and cleanable	0.023 (0.015)	0.124
		Smooth and cleanable or carpeted	0.027 (0.008)	0.001
Floor surface/condition × $(\text{log floor PbD})^3$		Uncarpeted not smooth and cleanable	–0.020 (0.014)	0.159
		Smooth and cleanable or carpeted	–0.009 (0.004)	0.012
Log windowsill PbD	0.002	Intercept for missing	0.053 (0.040)	0.186
		Slope	0.041 (0.011)	< 0.001
Home-apartment type	< 0.001	Intercept for missing	–0.064 (0.097)	0.511
		Mobile home or trailer	0.127 (0.067)	0.066
		One family house detached	–0.025 (0.046)	0.596
		One family house attached	0.000	—
		Apartment (1–9 units)	0.069 (0.060)	0.256
		Apartment (≥ 10 units)	–0.133 (0.056)	0.022
Anyone smoke inside the home	0.015	Missing	0.138 (0.140)	0.331
		Yes	0.100 (0.040)	0.015
		No	0.000	—
Log cotinine concentration (ng/dL) in blood	0.004	Intercept for missing	–0.150 (0.063)	0.023
		Slope	0.039 (0.012)	0.002
Window cabinet or wall renovation in a pre-1978 home	0.045	Missing	–0.008 (0.061)	0.896
		Yes	0.097 (0.047)	0.045
		No	0.000	—

n = 2,155; R^2 = 40%.

Source: (Dixon et al., 2009)

Renovation activities on older homes have been shown to produce excess Pb dust concentrations. Gaitens et al. (2009) performed a regression analysis on dust Pb concentrations from 1994-2004 NHANES on demographic and housing variables and found that renovation of windows, cabinets, or walls in a pre-1950 home was

significantly associated with floor dust Pb concentration ($p < 0.001$). Paint scraping within the last twelve months was nearly significantly associated with windowsill dust Pb concentration ($p = 0.053$). Dixon et al. (2009) performed a regression analysis on log-transformed blood Pb levels from NHANES (1999-2004) on several demographic and housing variables and found that renovation of windows, cabinets, or walls in pre-1978 homes was significantly associated with blood Pb concentration ($p = 0.045$). Mielke et al. (2001) tested dust produced roughly six months after a home (having paint containing ~130,000 mg Pb/kg) was power sanded. Dust concentrations in various locations around the house ranged from 390-27,600 mg/kg after the first sampling. Following several rounds of cleaning, Pb concentrations dropped. Eight months later, most interior Pb concentrations were < 3 mg/kg, but five sites ranged from 17-1,340 mg/kg. Two children had blood Pb tested as part of this study. A 33 month-old child exhibited no change in blood Pb before or after renovation (blood Pb = 4 µg/dL), while a twelve-month old child had an increase from 4 µg/dL to 12 µg/dL. Note that blood was acquired in different manners from the children because the younger child had smaller veins; the older child had a venous sample while the younger child had a finger-stick. It is unclear if either of these methods is more prone to contamination than the other. In an occupational study of men performing home renovations in the U.K., window renovation and wood-stripping workers specializing in renovation of old houses had significantly higher median blood Pb levels compared with all workers in similar occupations (wood strippers: 37 µg/dL; window renovators: 32 µg/dL; all workers: 13.7 µg/dL; $p < 0.001$) (Mason et al., 2005).

6.3 Factors Potentially Related to Increased Risk of Lead Induced Health Effects

This section evaluates factors examined in recent studies as effect measure modifiers that potentially increase the risk of various Pb-related health effects. Table 6-4 provides an overview of the factors examined and populations identified as “at-risk” of Pb-related health effects based on the recent evidence integrated across disciplines. Each characteristic is described in greater detail in the following subsections.

Table 6-4 Summary of evidence for factors that potentially increase the risk of lead-related health effects

Factor Evaluated	Potentially Related to Increased Risk
Age (Section 6.3.1)	Children
Sex (Section 6.3.2)	Males,[a] Females[a]
Genetics (Section 6.3.3)	ALAD[a], VDR[a]*, DRD4[a]*, GSTM1[a], TNF-α[a], eNOS[a], APOE[a], HFE[a]
Pre-existing Disease (Section 6.3.4)	Autism[a], Atopy[a,b], Hypertension[b]
Smoking (Section 6.3.5)	Smokers[a]
Race/Ethnicity (Section 6.3.6)	Non-Hispanic Blacks[a], Hispanics[a]
Socioeconomic Status (SES) (Section 6.3.7)	Low SES[a]
Nutrition (Section 6.3.10)	Iron deficiency[a]
Stress (Section 6.3.11)	High stress[a]
Cognitive Reserve (Section 6.3.12)	Low cognitive reserve[a,b]
Other Metals (Section 6.3.13)	High/co-exposure to Cd[a], As[a], Mn[a]

[a]Evidence for this factor was limited.
[b]Possible mediator

6.3.1 Age

Below is information from epidemiologic and toxicological studies regarding studies of increased risk for Pb-related health effects among children and older adults. Other age groups, such as adolescents, have not been evaluated here, if they were not part of stratified studies of lifestage.

6.3.1.1 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 CensusScope, 2010a). It is recognized that Pb can cross the placenta and affect the developing nervous system of the fetus (Sections 4.2.2.4 and 5.3.9) and there is strong evidence of increased risk to the neurocognitive effects of Pb exposure during several lifestages throughout gestation, childhood, and into adolescence (for more detail, Section 5.3.2.1). However, most recent studies among children do not have adequate comparison groups between children of various age groups or between children and adults, and were therefore only presented in Chapter 5.

A study including multiple U.S. locations examined associations of blood Pb levels with various immune parameters among individuals living near Pb industrial sites and matched controls (Sarasua et al., 2000). For several of these endpoints, the association in the youngest group (ages 6-35 months) and the oldest group (ages 16-75 years) were in opposite directions. For example, among children ages 6-35 months, the associations between blood Pb levels and Immunoglobulin A (IgA), Immunoglobulin M (IgM), and B-cell abundance were positive, whereas the associations among 16-75 year olds were negative. The opposite associations were also present for T cell abundance. Ig antibodies, which are produced by activated B cells, are important mediators of the humoral immune response to antigens. T cells are important mediators of cell-mediated immune responses that involve activation of other immune cells and cytokines. These findings by Sarasua et al. (2000) indicate that very young children may be at increased risk for Pb-associated activation of humoral immune responses and perturbations in cell-to-cell interactions that underlie allergic, asthma, and inflammatory responses (for more information, see Sections 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 noted 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.

Overall evidence indicates early childhood as a lifestage of increased risk 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. Toxicological studies have reported that younger animals, whose nervous systems are developing (i.e., laying down and pruning neuronal circuits) and whose junctional barrier systems in the brain (i.e., the blood brain barrier) and GI system (i.e., gut closure) are immature, are more at risk from the effects Pb exposure (Fullmer et al., 1985).

6.3.1.2 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 CensusScope, 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 CensusScope, 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). Positive hazard ratios were observed in both age groups but the hazard ratios were greater in those less than 60 years old. 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 ≥ 10 µg/dL to blood Pb levels <5 µg/dL and all-cause mortality for all age groups (40-74, 75-84, and 85+ year olds), although the association for 75-84 year olds did not reach statistical significance. The association was also present when comparing blood Pb levels of 5-9 µg/dL to blood Pb levels <5 µg/dL among 40-74 year olds and 75-84 year olds, but not among those 85 years and older. None of the associations between blood Pb and cardiovascular disease-related mortality reached statistical significance but the point estimates for cardiovascular disease-related mortality comparing blood Pb levels ≥ 10 µg/dL to blood Pb levels <5 µg/dL were elevated among all age groups. Finally, the association between blood Pb levels ≥ 10 µg/dL and cancer mortality was positive among those 40-74 years old and 85 years and older but the association was null for those 75-84 years old. Among 75-84 year olds the association was positive comparing blood Pb levels of 5-9 µg/dL to <5 µg/dL. The other age groups had similar point estimates but the associations were not statistically significant.

A study using the Normative Aging Study cohort reported an interaction between Pb and age (Wright et al., 2003b). The inverse association between age and cognitive function was greater among those with high blood or patella Pb levels. Effect estimates were in the same direction for tibia Pb but the interaction was not statistically significant.

Finally, a study of current and former Pb workers reported that an interaction term of Pb and age (dichotomous cutpoint at 67th percentile but exact age not given) examined in models of Pb (measured from blood and patella) and blood pressure was not statistically significant (Weaver et al., 2008). Thus, no modification by age was observed in this study of Pb and blood pressure.

Toxicological studies have demonstrated Pb-related health effects among older populations. The kidneys of older animals appear to be more at-risk for Pb-related health effects from the same dose of Pb (i.e., continuous 50 mg/L Pb-acetate drinking water) than younger animals (Berrahal et al., 2011). Increased risk related to older age is also observed for effects on the brain. Recent studies have demonstrated the importance of Pb exposure during early development in promoting the emergence of Alzheimer's like pathologies in aged animals. Development of pathologies of old age in brains of aged animals that were exposed to Pb earlier in life has been documented in multiple species (mice and monkeys, for more details see Section 5.3.6). These pathologies include the development of neurofibrillary tangles and increased amyloid precursor protein and its product beta-amyloid (Basha et al., 2005; Zawia and Basha, 2005). Some of these findings were seen in animals that no longer had elevated blood Pb levels.

In summary, results for age-related modification of the association between Pb and mortality had mixed results. Limited evidence was available for the associations between Pb and cognitive function or other health effects among older adults. Toxicological studies have shown increases in Pb-related health effects by age that may be relevant in humans. Future studies will be instrumental in understanding older age as a factor that potentially affects the risk of Pb-related outcomes.

6.3.2 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% was female. The distribution of sex varied by age with a greater prevalence of females ≥ 65 years old compared to males (SSDAN CensusScope, 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, 2006b).

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. (1987b) and Ris et al. (2004) observed interactions between blood Pb (prenatal and postnatal) and sex ; associations of prenatal and postnatal blood Pb and subsequent decrements in memory, attention, and visuoconstruction were observed only among male adolescents. More recently, Wright et al. (2008) examined early life blood Pb levels and criminal arrests in adulthood. The attributable risks were greater among males than females. Additionally, the association between childhood blood Pb levels and adult gray matter volume loss was greater among males than females (Cecil et al., 2008). In an expanded analysis of the developmental trajectory of childhood blood Pb levels on adult gray

matter, researchers found that associations between yearly mean blood Pb levels and volume of gray matter loss were more pronounced in the frontal lobes of males than females (Brubaker et al., 2010). Multiple studies were also conducted in Port Pirie, Australia that examined blood Pb levels at various ages throughout childhood and adolescence (Tong et al., 2000; Baghurst et al., 1992; McMichael et al., 1992). These studies observed Pb effects on cognition deficits were stronger in girls throughout childhood and into early adolescence. A study in Poland also investigated the association between umbilical cord blood Pb levels and cognitive deficits and reported a positive association for boys at 36 months but not for girls (Jedrychowski et al., 2009a). No association was detected for boys or girls at 24 months.

An epidemiologic study examined the association between concurrent blood Pb levels and kidney function among 12-20 year olds using the NHANES III study cohort (Fadrowski et al., 2010). The results were stratified by sex and no effect measure modification was apparent.

Similarly, a study of current and former Pb workers examined an interaction term between sex and Pb for the study of blood Pb and blood pressure (Weaver et al., 2008). No modification by sex was present.

Epidemiologic studies have also been performed to assess differences between males and females for Pb-related effects on various biomarkers. A study comprised mostly of females reported positive associations between blood Pb and total immunoglobulin E (IgE) for women not taking hormone replacement therapy or oral contraceptives (Pizent et al., 2008). No association was reported in males, but other associations, such as bronchial reactivity and reactive skin prick tests were observed in the opposite of the expected direction, which questions the validity of the results among the male study participants. Analysis of an NHANES dataset detected no association between blood Pb levels and inflammatory markers (Songdej et al., 2010). Although there was no clear pattern, a few of the associations were positive between blood Pb and C-reactive protein for males but not females. A study of children living at varying distances from a Pb smelter in Mexico reported that blood Pb was associated with increased release of superoxide anion from macrophages, which was greater among males than females (Pineda-Zavaleta et al., 2004).

Epidemiologic investigations of cancer have also examined the associations by sex. A study of the association between occupational exposure to Pb and brain tumors reported no sex-specific associations for gliomas, but a positive association for cumulative Pb exposure and meningiomas for males but not females (Rajaraman et al., 2006). An ecologic analysis of Pb pollution levels and cancer incidence among children reported

weak correlations overall and the weak correlations were more apparent among males, whereas no correlation was observed among females (Absalon and Slesak, 2010).

A study of all-cause and cardiovascular mortality using the NHANES III cohort reported no modification of the association between blood Pb and all-cause or cardiovascular mortality by sex (Menke et al., 2006). This did not differ among women when classified as pre- or post-menopausal.

Toxicological studies have also reported sex differences in Pb-related effects to various organ systems. Donald et al. (1986) reported a different time course of enhanced social investigatory behavior between male and female mice exposed to Pb. In a subsequent publication, Donald et al. (1987) showed that non-social behavior in mice decreased in females and increased in males exposed to Pb. Males also had a shorter latency to aggression with Pb treatment versus controls. Pb affected mood disorders differently for males and females. Behavioral testing in rats showed males experienced emotional changes and females depression-like changes with Pb exposure (de Souza Lisboa et al., 2005). In another study, gestational exposure to Pb impaired memory retrieval in male rats at all 3 doses of Pb exposure; memory retrieval was only impaired in low-dose female rats (Yang et al., 2003). Sex-specific differences in mice were also observed for gross motor skills; at the lowest Pb dose, balance and coordination were most affected among males (Leasure et al., 2008).

Pb and stress are co-occurring factors that act in a sex-divergent manner to affect behavior, neurochemistry, and corticosterone levels. Pb and stress act synergistically to affect fixed interval operant behavior and corticosterone in female rat offspring. Virgolini et al. (2008a) found that effects on the offspring's central nervous system by developmental Pb exposure (maternal exposure and transferred to the offspring through lactation) were enhanced by combined maternal and offspring stress and females were most at risk. Behavioral related outcomes after gestational and lactational Pb exposure (with and without stress) exhibited sex-differences in exposed offspring (Virgolini et al., 2008b). Pb-induced changes in brain neurochemistry, with or without concomitant stress exposure, are complex with differences varying by brain region, neurotransmitter type, and sex of the animal.

The brain is known to have a sexually dimorphic area in the hypothalamus, termed the sexually dimorphic nucleus (SDN). Lesions in this area affect sex-specific phenotypes including behavior. Across species the SDN has a greater cell number and larger size in males versus females. This sexually dichotomous area is especially vulnerable to perturbation during fetal life and the early postnatal period. This may be one area of the brain that could explain some of the sexually dichotomous effects that are seen with Pb exposure. One study supporting this line of thought showed that high-dose in utero Pb

exposure (pup blood Pb level 64 µg/dL at birth) induced reductions in SDN volume in 35% of Pb-exposed male rats (McGivern et al., 1991). Interestingly, another chemical that is known to cause a hypothalamic lesion in this area, monosodium glutamate, is associated with adult onset obesity (Olney, 1969); adult onset obesity is seen in the Pb literature.

Obesity in adult offspring exposed to low-dose Pb in utero was reported for male but not female mice (Leasure et al., 2008). Obesity was also found in male rat offspring exposed in utero to high doses of Pb that persisted to 5 weeks of age/end of the study, but among female rats, body weight remained elevated over controls only to 3 weeks of age (Yang et al., 2003). Additionally, low-dose Pb exposure induced retinal decrements in exposed male mice offspring (Leasure et al., 2008).

A toxicological study of Pb and antioxidant enzymes in heart and kidney tissue reported that male and female rats had differing enzymatic responses, although the amount of Pb in the heart tissue or the disposition of Pb also varied between males and females (Sobekova et al., 2009; Alghazal et al., 2008a). The authors reported these results could be due to greater deposition of Pb in female rats or greater clearance of Pb by males (Sobekova et al., 2009).

Multiple associations between Pb and various health endpoints have been examined for effect measure modification by sex. 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 an at-risk population, whereas studies from Australia pointed to females as an at-risk population. A difference in sex is supported by toxicological studies. Further research is needed to confirm the presence or absence of sex-specific associations between Pb and various health outcomes and in which sex the associations are greater.

6.3.3 Genetics

The 2006 Pb AQCD stated that, "genetic polymorphisms in certain genes have been implicated as influencing the absorption, retention, and toxicokinetics of Pb in humans" (U.S. EPA, 2006b). The majority of discussion there focused on the aminolevulinate dehydratase (ALAD) and vitamin D receptor (VDR) polymorphisms. These two genes, as well as additional genes examined in recent studies, are discussed below.

6.3.3.1 Aminolevulinate Dehydratase

The aminolevulinate dehydratase (ALAD) gene encodes for an enzyme that catalyzes the second step in the production of heme and is also the principal Pb-binding protein (U.S. EPA, 2006b). Studies have examined whether ALAD variants altered associations between Pb and various health effects.

Associations between Pb and brain tumors observed in an epidemiologic study varied by ALAD genotype status (Rajaraman et al., 2006). Positive associations between Pb exposure (determined via interview about occupational exposures) and meningioma were reported among ALAD2 individuals, but this association was not found among individuals who had the ALAD1 allele. No associations were observed between Pb and glioma regardless of ALAD genotype.

Studies investigating the association between Pb levels and cognitive function have also examined modification by ALAD polymorphisms. The evidence is provided by an NHANES analysis (Krieg et al., 2009) as well as multiple analyses from the NAS cohort examining different tests of cognitive function (Rajan et al., 2008; Weuve et al., 2006). In the study using a cohort from NHANES III, for several indices of cognitive function, associations with concurrent blood Pb levels were more pronounced in groups with CC and CG ALAD genotypes (i.e., ALAD2 carriers) (Krieg et al., 2009). In the NAS cohort of men, Weuve et al. (2006) found that concurrent blood Pb level but not bone Pb level was associated with a larger decrease in a test of general cognitive function among ALAD2 carriers. Another NAS study examined functioning of specific cognitive domains (e.g., vocabulary, memory, visuospatial skills) and found variable evidence for effect modification by ALAD genotype across tests (Rajan et al., 2008). For example, among ALAD2 carriers, concurrent blood Pb level was associated with a more pronounced decrease in vocabulary score but less pronounced decrease in a memory index and no difference in the associations with other cognitive tests. For tibia and patella Pb levels, ALAD genotype was found to modify associations with different tests, for example, executive function and perceptual speed. It is not clear why the direction of effect modification would vary among different cognitive domains. The limited number of populations examined, and the different cognitive tests performed in each study, make it difficult to conclusively summarize findings for effect modification by ALAD variants. However, in the limited available body of evidence, blood and bone Pb levels were generally associated with lower cognitive function in ALAD2 carriers.

A study of current and former workers exposed to Pb examined the association between blood Pb and blood pressure and reported no modification by ALAD genotype (Weaver et al., 2008). However, another study of blood Pb and blood pressure reported

interactions between blood Pb and ALAD, but this varied by race/ethnicity (non-Hispanic white, non-Hispanic black, and Mexican American) (Scinicariello et al., 2010).

Individuals with ALAD2 variants had greater associations between Pb and kidney effects; among those with the variant, higher Pb was associated with higher glomerular filtration measures (Weaver et al., 2006; Weaver et al., 2005b; Weaver et al., 2003b). A study of workers at a battery plant storage facility in China reported workers with the ALAD2 allele demonstrated greater associations between blood Pb levels and renal injury (Gao et al., 2010a). Another study of renal function among Pb workers in Asia also reported greater associations between blood Pb concentrations and renal function by ALAD, especially at high blood Pb levels (Chia et al., 2006).

6.3.3.2 Vitamin D Receptor

The vitamin D receptor (VDR) is a regulator of calcium absorption and metabolism. A recent study of the NHANES III population examined the association between blood Pb levels and various neurocognitive tests with assessment of effect measure modification by SNPs and haplotypes of VDR (Krieg et al., 2010). The results were varied, even among specific SNPs and haplotypes, with some variants being associated with greater modification of the relationship between Pb and one type of neurocognitive test compared to the modification of the relationship between Pb and other neurocognitive tests. In an epidemiologic study of blood Pb levels and blood pressure among a group of current and former Pb-exposed workers, no modification was reported by VDR (Weaver et al., 2008).

6.3.3.3 Methylenetetrahydrofolate reductase

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which in turn, is involved in homocysteine remethylation to the amino acid methionine. A study in Mexico of the association between Pb and Bayley's Mental Development Index (MDI) score at 24 months reported no effect measure modification by MTHFR 677T allele (Pilsner et al., 2010). Another study in Mexico examined the association between maternal Pb and birth weight (Kordas et al., 2009). No modification of the Pb-birth weight association by MTHFR was observed.

6.3.3.4 Apolipoprotein E

Apolipoprotein E (APOE) is a transport protein for cholesterol and lipoproteins. The gene appears to regulate synapse formation (connections between neurons), which 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 tibia Pb levels to be associated with greater cognitive decrements in some, but not all, neurobehavioral 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 blood Pb and Bayley's MDI than children with this allele, although the interaction term was not statistically significant (Wright et al., 2003a).

6.3.3.5 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 (Wang et al., 2007a). No association between tibia Pb and cognitive function was present for men with HFE wildtype, but a decline in function was associated with tibia Pb levels among men with any HFE allele variant. A study of bone Pb levels and HFE reported no difference in effect estimates for bone Pb and pulse pressure between different HFE variants and HFE wild-type (Zhang et al., 2010a). An interaction was observed between an HFE variant in mothers and maternal tibia Pb in a study of maternal Pb and birth weight (Cantonwine et al., 2010b). The inverse association between maternal tibia Pb levels and birth weight was stronger for those infants whose mothers had the HFE variant. The interaction was not present between the HFE variants and maternal blood Pb or cord blood Pb concentrations.

6.3.3.6 Other Genetic Polymorphisms

Some other genetic polymorphisms were also examined as to whether they modify Pb-related health effects, but only limited data were available for these polymorphisms. These include dopamine receptor D4 (DRD4), dopamine receptor D2 (DRD2), dopamine transporter (DAT1), glutathione S-transferase Mu 1 (GSTM1), tumor necrosis factor-*alpha* (TNF-α), endothelial nitric oxide synthase (eNOS), and various SNPS.

A prospective birth cohort reported that increasing blood Pb levels were associated with poorer rule learning and reversal, spatial span, and planning in their study population (Froehlich et al., 2007). These inverse associations were exacerbated among those lacking DRD4-7. A study of prenatal and postnatal Pb levels in Mexico City reported no modification of the associations between Pb levels and neurocognitive development by DRD2 or DAT1 (Kordas et al., 2011).

A study of university students in South Korea reported blood Pb levels to be associated with biomarkers of inflammation among individuals with GSTM1 null genotype and not among individuals with GSTM1 present (Kim et al., 2007). This study of blood Pb levels and inflammation also examined individuals with TNF-α GG, GA, or AA alleles. An association was present for those with TNF-α GG but not for those with TNF-α GA or AA.

A study of blood Pb and plasma NO_X reported no overall association but did report an inverse correlation among subjects with the eNOS TC+CC genotype (Barbosa et al., 2006a). No correlation was observed for subjects with the eNOS TT genotype; however the number of subjects in this group was small, especially for those with high blood Pb levels.

One study examined how the association between occupational Pb exposure and brain tumors varied among multiple single nucleotide polymorphisms (SNPs) (Bhatti et al., 2009). No effect measure modification of the association between Pb and glioma was observed for any of the SNPs. GPX1 (the gene encoding for glutathione peroxidase 1) modified the association for glioblastoma multiforme and meningioma. The association between Pb and glioblastoma multiforme was also modified by a RAC2 (the gene encoding for Rac2) variant, and the association between Pb and meningioma was also modified by XDH (the gene encoding for xanthine dehydrogenase) variant.

Overall, studies of ALAD observed increased Pb-related health effects associated with certain gene variants. Other genes, such as VDR, APOE, HFE, DRD4, GSTM1, TNF-α, and eNOS, may also affect the risk of Pb-related health effects but conclusions are limited due to the small number of studies.

6.3.4 Pre-existing Diseases/Conditions

Studies have also been performed to examine whether certain morbidities increase an individual's risk of Pb-related effects on health. Recent studies have explored relationships for autism, atopy, diabetes, and hypertension.

6.3.4.1 Autism

Rates of individuals with autism have increased in recent years. A study reported a prevalence rate in 2006 of 9.0 per 1,000 individuals (95% CI: 8.6, 9.3) determined from a monitoring network (Autism and Developmental Disabilities Monitoring Network) with 11 sites across the U.S. (CDC, 2009).

A cross-sectional study of children with and without autism examined the association between blood Pb levels and various immune function and inflammation genes (Tian et al., 2011). Blood Pb levels of children with and without autism were associated with expression of the genes under study; however, the associations observed were in opposite directions (for children with autism, increased blood Pb levels were associated with increased expression, whereas for children without autism, increased blood Pb levels were associated with decreased expression).

6.3.4.2 Atopy

Atopy, a type of allergic hypersensitivity, was evaluated as a factor affecting risk in a study of Pb and IgE (Annesi-Maesano et al., 2003). The study examined hair Pb levels 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 mothers with atopy, the correlation was positive, although smaller, and was not statistically significant.

6.3.4.3 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 modifying factor for Pb and various health outcomes.

Differences in the association between bone and blood Pb levels and renal function for individuals with and without diabetes at baseline was examined using the Normative Aging Study cohort (Tsaih et al., 2004). Tibia and blood Pb levels were positively associated with measures of poor renal function among individuals with diabetes but not among individuals without diabetes. However, this association was no longer statistically significant after the exclusion of individuals who were hypertensive or who used diuretic medications. Another study with this cohort reported no associations between bone Pb and heart rate variability, which did not differ among those with and without diabetes (Park et al., 2006).

The NHANES III data were used to evaluate whether the association between blood Pb and both all-cause and cardiovascular mortality varied among individuals with and without diabetes (Menke et al., 2006). The 95% CIs among those with diabetes were large and no difference was apparent among those with and without diabetes.

Overall, recent epidemiologic studies found that associations between Pb concentrations and health outcomes did not differ for individuals with and without diabetes. However, results from the 2006 Pb AQCD found that individuals with diabetes are at "increased risk of Pb-associated declines in renal function" (U.S. EPA, 2006b). Future research examining associations between Pb and renal function, as well as other health outcomes, among individuals with and without diabetes will inform further on the potential for increased risk among individuals with diabetes.

6.3.4.4 Hypertension

Hypertension affects approximately 24% of adults in the U.S. and the prevalence of hypertension increases with age (61% of individuals ≥ 75 years old have hypertension) (Pleis et al., 2009).

The Normative Aging Study mentioned above evaluating modification of the association between Pb levels and renal function by diabetes also examined modification by hypertensive status (Tsaih et al., 2004). The association between tibia Pb and renal function, measured by change in serum creatinine, was present among individuals with hypertension but not among individuals that were normotensive. Models of the follow-up serum creatinine levels demonstrated an association with blood Pb for individuals with hypertension but not individuals without hypertension (this association was not present when using tibia or patella Pb). Another study using this population examined modification of the association between bone Pb and heart rate variability, measured by low frequency power, high frequency power, and their ratio (Park et al., 2006). Although a statistically significant association between bone Pb and heart rate variability was not observed among individuals with or without hypertension, the estimates were different, with greater odds for individuals with hypertension (bone Pb levels were positively related to low frequency power and the ratio of low frequency to high frequency power and were inversely related to high frequency power).

A study using the NHANES III cohort reported a positive association between blood Pb levels and both all-cause and cardiovascular mortality for individuals with and without hypertension but the associations did not differ based on hypertensive status (Menke et al., 2006).

The 2006 Pb AQCD reported that individuals with hypertension had increased risk of Pb-related effects on renal function (U.S. EPA, 2006b). This is supported by recent epidemiologic studies. As described above, studies of Pb-related effects on renal function and heart rate variability have observed some differences among individuals with hypertension, but the difference between adults with and without hypertension was not observed for Pb-related mortality.

6.3.5 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 blood Pb levels and all-cause and cardiovascular mortality reported no modification of this association by smoking status, measured as current, former, or never smokers (Menke et al., 2006). The Normative Aging Study also examined the association between blood and bone Pb levels and renal function and also reported no interaction with smoking status (Tsaih et al., 2004).

A study of Pb-exposed workers and controls reported similar levels of absolute neutrophil counts (ANC) across Pb exposure categories among 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-α and granulocyte colony-stimulating factor (G-CSF) among both smokers and nonsmokers, but this association was greater among smokers (Di Lorenzo et al., 2007). A recent study of fertile and infertile men examined blood and seminal plasma Pb levels for smokers and non-smokers (Kiziler et al., 2007). The blood and seminal plasma Pb levels were higher for smokers of both fertile and infertile groups. Additionally, the Pb levels were lowest among non-smoking fertile men and highest among smoking infertile men.

Prenatal smoking exposure was examined in a study of children's concurrent blood Pb levels and prevalence of attention-deficit/hyperactivity disorder (ADHD) among children aged 8-15 years. An interaction was observed between children's current blood Pb levels and prenatal tobacco smoke exposure; those children with high Pb levels and prenatal tobacco smoke exposure had the highest odds of ADHD (Froehlich et al., 2009).

Overall, the studies have mixed findings on whether smoking modifies the relationship between Pb levels 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 changes in risk by this factor.

6.3.6 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 CensusScope, 2010b). Studies of multiple Pb-related health outcomes examined effect measure modification by race/ethnicity.

A study of adults from the NHANES III cohort examined the association between blood Pb levels and all-cause and cardiovascular mortality (Menke et al., 2006). Stratified analyses were conducted for non-Hispanic whites, non-Hispanic blacks, and Mexican Americans and no interaction for race/ethnicity was reported. Other studies have also used NHANES cohorts to study blood Pb levels and hypertension (Scinicariello et al., 2010; Muntner et al., 2005). While no association was observed between blood Pb and hypertension for non-Hispanic whites or Hispanics, a positive association was reported for non-Hispanic blacks in a study using the NHANES III cohort (Scinicariello et al., 2010). In another study, although none of the associations between blood Pb levels and hypertension were statistically significant, increased odds were observed among non-Hispanic blacks and Mexican Americans but not for non-Hispanic whites (Muntner et al., 2005).

A study of girls aged 8-18 years from the NHANES III cohort reported an inverse association between blood Pb levels and pubertal development among blacks and Mexican Americans (Selevan et al., 2003). For non-Hispanic whites, the associations were in the same direction but did not reach statistical significance. Of note, less than 3% of non-Hispanic whites had blood Pb levels over 5 µg/dL, whereas 11.6% and 12.8% of blacks and Mexican Americans, respectively, had blood Pb levels greater than 5 µg/dL.

A study linking educational testing data for 4th grade students in North Carolina reported declines in reading and mathematics scores with increasing levels of blood Pb (Miranda et al., 2007b). Although not quantitatively reported, a figure in the study depicted the association stratified by race, and the slopes appeared to be similar for white and black children.

Blood Pb and asthma incidence was examined for white and black children living in Michigan (Joseph et al., 2005). When utilizing separate referent groups for the two races, the only association is an increase among whites (although not statistically significant), but when restricting to the highest blood Pb levels, the association was no longer apparent. Whites with low blood Pb levels were used as the referent group for both races in additional analysis. Although the estimates were elevated for black children compared

to white children (including at the lowest blood Pb levels), the confidence intervals for the associations overlapped indicating a lack of a difference by race.

The results of these recent epidemiologic studies suggest that there may be race/ethnicity-related increased risk for some outcomes, although the overall understanding of potential effect measure modification by race/ethnicity is limited by the small number of studies. Additionally, these results may be confounded by other factors, such as socioeconomic status.

6.3.7 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 CensusScope, 2010c). Ris et al. (2004) examined modification of the associations between early-life Pb levels and Learning/IQ among adolescents in the Cincinnati Lead Study. In models examining the association between Pb and Learning/IQ, the prenatal and 78-month blood Pb levels were associated with larger decrements in Learning/IQ in the lower two quintiles of socioeconomic status (SES) (measured based on family SES levels).

6.3.8 Body Mass Index

In the U.S. self-reported rates of obesity were 26.7% in 2009, up from 19.8% in 2000 (Sherry et al., 2010). The NHANES III cohort was utilized in a study of blood Pb levels and all-cause and cardiovascular mortality, which included assessment of the associations by obesity (Menke et al., 2006). Positive associations were observed among individuals within both categories of body mass index (BMI; normal [<25 kg/m^2] and overweight/obese [≥ 25 kg/m^2], determined using measured values of height and weight) but there was no difference in the association between the two categories. Using the Normative Aging Study data, an investigation of bone Pb levels and heart rate variability was performed and reported slight changes in the association based on the presence of metabolic syndrome; however, none of the changes resulted in associations that were statistically significant (Park et al., 2006). Overall, no modification by BMI or obesity was observed among recent epidemiologic studies.

6.3.9 Alcohol Consumption

There are a limited number of studies examining alcohol as a factor affecting Pb-related risk. A study using the Normative Aging Study cohort investigated whether the association between blood and bone Pb levels and renal function would be modified by an individual's alcohol consumption (Tsaih et al., 2004). No interaction with alcohol consumption was observed. However, a toxicological study reported that ethanol potentiated the effect of Pb exposure by decreasing renal total protein sulfhydryls (endogenous antioxidants) in rats. Pb and ethanol also decreased other endogenous renal antioxidants (glutathione and non-protein sulfhydryls) (Jurczuk et al., 2006).

6.3.10 Nutritional Factors

Different components of diet may affect the association between Pb concentrations and health outcomes. Recent epidemiologic and toxicological studies of specific mineral intakes/dietary components are detailed below.

6.3.10.1 Calcium

Using the Normative Aging Study cohort, researchers examined the association between Pb levels and hypertension, modified by calcium intake (Elmarsafawy et al., 2006). The associations between Pb levels (measured and modeled separately for blood, patella, and tibia) and hypertension did not differ based on dichotomized calcium intake (800 mg/day).

6.3.10.2 Iron

The 2006 Pb AQCD included studies that indicated individuals with iron-deficiency and malnourishment had greater inverse associations between Pb and cognition (U.S. EPA, 2006b). A recent epidemiologic study of pubertal development among girls observed inverse associations between blood Pb and inhibin B, but this association was modified by iron deficiency; girls with iron deficiency had a stronger inverse association between Pb and inhibin B than those who were iron sufficient (Gollenberg et al., 2010). Toxicological studies also reported 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 GD6-GD14, had decreased litter size, more pups with reduced fetal weight and reduced crown-rump length, increased litter resorption, and a

higher damblood Pb level in the highest exposure groups (Singh et al., 1993; Saxena et al., 1991). Thus, in this model, iron deficiency makes rat dams more at risk for Pb-dependent embryo and fetotoxicity (Singh et al., 1993).

6.3.10.3 Folate

A study by Kordas et al. (2009) examined Pb levels and birth size among term births in Mexico City. The authors reported no interaction between maternal tibia Pb and folate levels.

6.3.10.4 Protein

No recent epidemiologic studies have evaluated protein intake as a factor affecting Pb-related health effects. However, a toxicological study demonstrated that differences in maternal protein intake levels could affect the extent of Pb-induced immunotoxicity among offspring (Chen et al., 2004).

6.3.11 Stress

A study of bone (tibia and patella) Pb levels and hypertension reported modification of the association by perceived stress levels (Peters et al., 2007). Among individuals with greater perceived stress levels, stronger associations between blood Pb levels and hypertension were present. Among the same study population, higher perceived stress was also reported to affect the association between blood Pb levels and cognitive function; the higher stress group showed a greater inverse association between Pb and cognitive function than those in the low stress group (Peters et al., 2008). In another study, the inverse association between tibia Pb levels and some measures of cognitive function were similarly strengthened by neighborhood psychosocial hazards (Glass et al., 2009).

Toxicological studies have demonstrated that early life exposure to Pb and maternal stress can result in toxicity related to multiple systems (Rossi-George et al., 2009; Cory-Slechta et al., 2008; Virgolini et al., 2008a; Virgolini et al., 2008b), including dysfunctional corticosterone responses (Rossi-George et al., 2009; Virgolini et al., 2008b). Additionally, toxicological studies have demonstrated that stressors to the immune system can also affect associations with Pb exposure. Chickens with low Pb exposure in ovo, with additional viral stressors, had increased immune cell mobilization

and trafficking dysfunction (Lee et al., 2002). Similarly, mice with neonatal Pb exposure, and an additional immune challenge, had a sickness behavior phenotype, likely driven by IL-6 production (Dyatlov and Lawrence, 2002).

Similar to studies of stress in animals, maternal self-esteem has also been shown to modify associations between blood Pb levels and health effects in children. Surkan et al. (2008) studied the association between children's blood Pb levels and Bayley's MDI and Psychomotor Development Index (PDI) among mother-child pairs. High maternal self-esteem was independently associated with higher MDI score and also appeared to attenuate the negative effects of the child's increased blood Pb levels on MDI and PDI scores. Greater decreases in MDI and PDI were associated with increased blood Pb levels among children whose mothers were in the lower quartiles of self-esteem. The investigators indicated that high maternal self-esteem may serve as a buffer against stress by improving mother-child interactions and care giving practices, and maternal self esteem may also 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 various nervous system health effects by stress-level. Increased risk of Pb-related health effects by stress is further supported by toxicological studies.

6.3.12 Cognitive Reserve

Cognitive reserve has been defined as "the maintenance of cognitive performance in spite of ongoing underlying brain pathology" (Bleecker et al., 2007b). A study of Pb smelter workers reported that an inverse association between lifetime weighted blood Pb levels and cognitive function was present among workers with low cognitive reserve (measured using a reading achievement test) but no association was present in workers with high cognitive reserve (Bleecker et al., 2007b). Inverse associations between lifetime-weighted blood Pb levels and motor functions existed among all workers regardless of cognitive reserve. No other recent epidemiologic studies were performed examining cognitive reserve as a factor affecting risk of Pb-related health outcomes.

6.3.13 Other Metal Exposure

The 2006 Pb AQCD reported that the majority of studies that examined other toxicants did so as confounders and not as effect measure modifiers (U.S. EPA, 2006b). Recent epidemiologic studies have begun to explore the possible interaction between Pb

exposure and co-exposures with other metals. These studies, as well as toxicological studies of these metals, are described below.

6.3.13.1 Cadmium

In a study of girls in the NHANES III cohort, inverse associations were observed between blood Pb and inhibin B concentrations (Gollenberg et al., 2010). These inverse associations were stronger among girls with high cadmium (Cd) and high Pb compared to those with high Pb and low Cd. Additionally, higher blood Pb and Cd levels together were positively associated with albuminuria and reduced estimated glomerular filtration rate, compared to those with the lowest levels of Pb and Cd (Navas-Acien et al., 2009).

Toxicological studies reported that in rats, the addition of Cd to Pb exposure reduced the histological signs of renal toxicity from each element alone; however, urinary excretion of porphyrins were increased, indicating that although measured tissue burdens of Pb were reduced, the biologically available fraction of Pb was actually increased (Wang and Fowler, 2008). In other studies, Cd synergistically exacerbated Pb-dependent renal mitochondrial dysfunction (Wang et al., 2009c).

Overall, epidemiologic and toxicological studies have reported increased risk of Pb-related health effects among those with high Cd levels as well; however, the number of studies examining both metals is small.

6.3.13.2 Arsenic

In a study of immune function among children living at varying distances from a Pb smelter in Mexico, higher levels of both Pb and arsenic (As) were associated with greater decreases in NO and greater increases in superoxide anion (Pineda-Zavaleta et al., 2004).

6.3.13.3 Manganese

Among children in South Korea taking part in a study of IQ, an interaction was reported between Pb and manganese (Mn) blood levels (Kim et al., 2009b). Compared to children with low blood Mn levels, those with high blood Mn levels had greater reductions in full scale IQ and verbal IQ associated with increased blood Pb levels. No effect measure modification by Mn was observed for the association between blood Pb levels and performance IQ. A study performed among children in Mexico City observed greater decreases in neurodevelopment with increases in blood levels of Pb and Mn at 12

months, compared to decreases in neurodevelopment observed for increased Pb levels with low levels of Mn (Claus Henn et al.). No interaction was observed between the two metals and neurodevelopment at 24 months.

6.4 Summary

Among children, the youngest age groups were observed to be most at risk of elevated blood Pb levels, with levels decreasing with increasing age of the children. Recent epidemiologic studies of infants/children detected increased risk of Pb-related health effects, and this was supported by toxicological studies. However, this is based on a limited number of epidemiologic studies, and more studies are needed for comparing various age groups and examining adolescents.

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. Studies of older adults had inconsistent findings for effect measure modification of Pb-related mortality but no difference was observed for other health effects. However, toxicological studies support the possibility of age-related differences in Pb-related health effects.

Some studies suggest that males at some ages have higher blood Pb levels than comparably aged females; this was supported by stratifying the total sample of NHANES subjects. Sex-based differences appeared to be prominent among the adolescent and adult age groups but were not observed among the youngest age groups (1-5 years and 6-11 years). Studies of effect measure modification of Pb and various health endpoints by sex were inconsistent, although it appears that there are some differences in associations for males and females. This is also observed in toxicological studies.

Regarding race and ethnicity, recent data suggest that the difference in blood Pb levels between black and white subjects is decreasing over time, but black subjects still tend to have higher Pb body burden and Pb exposures than white subjects. Similarly, the gap between SES 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/ethnicity as a factor affecting risk indicate that some modification of associations between Pb levels and health effects may be present. Compared to whites, non-white populations were observed to be more at risk of Pb-related health effects; 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, individuals with lower SES appear to represent an at-risk 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 that ALAD variants may increase the risk of Pb-related health effects. Other genes examined that may also affect risk of Pb-related health effects were VDR, DRD4, GSTM1, TNF-α, eNOS, APOE, and HFE, although the number of studies examining effect measure modification by these genes was small.

Evidence for other factors (pre-existing diseases/conditions, smoking, BMI and/or obesity, alcohol consumption, nutritional factors, stress, cognitive reserve, and co-exposure with other metals) was limited regarding their effect on Pb-related health outcomes. Pre-existing diseases/conditions have the potential to affect the risk of Pb-related health effects. Recent epidemiologic studies did not support modification of associations between Pb and health endpoints by the prevalence of diabetes; however, past studies have found individuals with diabetes to be an at-risk population with regard to renal function. Hypertension was observed to be a factor affecting risk in both past and recent epidemiologic studies. Studies of Pb levels and both renal effects and heart rate variability demonstrated greater odds of the associations among hypertensive individuals compared to those that are normotensive. Epidemiologic studies also examined autism and atopy as potential factors affecting Pb-related health effects; differences were observed but few studies were available to examine these factors. Recent epidemiologic studies examining smoking as a factor potentially affecting risk reported mixed findings. It is possible that smoking modifies the effects of only some Pb-related health outcomes. BMI, alcohol consumption, and nutritional factors were also examined in recent epidemiologic and toxicological studies. Modification of associations between Pb and various health effects (mortality and heart rate variability) was not observed for BMI/obesity. Also, no modification was observed in an epidemiologic study of renal function examining alcohol consumption as a modifier, but a toxicological study supported the potential of alcohol to affect risk. Among nutritional factors, those with iron deficiencies were observed to be an at-risk population for Pb-related health effects in both epidemiologic and toxicological studies. Other nutritional factors, such as calcium, zinc, and protein intake, 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 effect modification. Folate was also examined in an epidemiologic study of birth size but no interaction was reported between Pb and folate. Stress was evaluated as a factor that potentially increases the risk of Pb-related health outcomes 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. An epidemiologic study evaluated cognitive reserve as a modifier of the associations between Pb and cognitive and motor functions. Cognitive reserve was an effect measure modifier for the association between Pb and cognitive function but not motor function. Finally, interactions between Pb and co-exposure with other metals were evaluated in recent epidemiologic and toxicological studies of health effects. High levels of other metals, such as Cd, As, and Mn, were observed to result in greater effects for the associations between Pb and various health endpoints.

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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 important general ecology concepts. 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. A similar discussion of the effects of Pb on freshwater and saltwater ecosystems is presented in Section 7.3, including water-only exposures and sediment related effects. Both the terrestrial and aquatic 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 effects of Pb in both terrestrial and aquatic systems are presented in Section 7.4. Although terrestrial and aquatic ecosystems are considered separately in this chapter, the deposition of Pb to land and water and the subsequent flux of Pb through watersheds are interconnected by fate and transport processes discussed in Section 3.3. Areas not addressed here include literature related to ingestion of Pb shot or pellets and studies that examine human health-related endpoints which are described in other chapters of this document.

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 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

For this assessment, an ecosystem is defined as the interactive system formed from all living organisms (biota) and their abiotic (chemical and physical) environment within a given area (IPCC, 2007).The boundaries of what could be called an ecosystem are somewhat arbitrary, depending on the focus of interest or study. Thus, the extent of an ecosystem may range from very small spatial scales to, ultimately, the entire Earth (IPCC, 2007). Ecosystems cover a hierarchy of spatial scales and can comprise the entire globe, biomes at the continental scale, or small, well-circumscribed systems such as a small pond (U.S. EPA, 2008e). A pond may be a small but complex system with multiple trophic levels ranging from phytoplankton to several feeding guilds of fish plus fish-eating birds or mammals. A large lake, on the other hand, may be a very simple ecosystem, such as the Great Salt Lake in Utah that covers approximately 1,700 square miles but contains only bacteria, algae, diatoms, and two invertebrate species. All ecosystems, regardless of size or complexity, share the commonality of multiple interactions between biota and abiotic factors, and a reduction in entropy through energy flow from photosynthetic organisms to top predators. This includes both structural (e.g., soil type and food web trophic levels) and functional (e.g., energy flow, decomposition, nitrification) attributes. Changes are often considered undesirable if important structural or functional components of ecosystems are altered following pollutant exposure (U.S. EPA, 1998).

Ecosystems are most often defined by their structure, and are based on the number and type of species present. Structure may refer to a variety of measurements including the species richness, abundance, community composition and biodiversity as well as landscape attributes. Individual organisms of the same species are similar in appearance and genetics, and can interbreed and produce fertile offspring. Interbreeding groups of individual organisms within the same species 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 organisms 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 and Latham, 1999); and (3) alterations in trophic structure.

Alternatively, ecosystems may be defined on a functional basis. "Function" refers to the suite of processes and interactions among the ecosystem components and their environment that involve nutrient and energy flow as well as other attributes including water dynamics and the flux of trace gases such as rates of photosynthesis, decomposition, nitrification, or carbon cycling. Pollutants may affect abiotic conditions (e.g., soil chemistry), which indirectly influences biotic structure and function (Bartell, 2007). Feedback loops or networks influence the stability of the system, and can be mathematically described through simplistic or complex process, or energy flow, models (Bartell, 2007). For example, the Comprehensive Aquatic Systems Model (CASM) is a bioenergetics-based multi compartment model that describes the daily production of biomass (carbon) by populations of aquatic plants and animals over an annual cycle (DeAngelis et al., 1989). CASM, originally designed to examine theoretical relationships between food web structure, nutrient cycling, and ecosystem stability, has since been adapted for risk assessments and has been applied to numerous lakes with a variety of pollutants (Bartell, 2007). Likewise, other theoretical ecosystem models are being modified for use in assessing ecological risks from pollutant exposures (Bartell, 2007).

Some ecosystems, and some aspects of particular ecosystems, are less vulnerable to long-term consequences of pollutant exposure. Other ecosystems may be profoundly altered if a single attribute is affected. Thus, spatial and temporal definitions of ecosystem structure and function become an essential factor in defining impacted ecosystem services and critical loads of particular pollutants, either as single pollutants or in combination with other stressors. Both ecosystem services (Section 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 (UNEP, 2003). Ecosystem services are defined as the varied and numerous ways that ecosystems are important to human welfare and how they provide many goods and services that are of vital importance for the functioning of the biosphere. This concept has gained recent interest and support because it recognizes that ecosystems are valuable to humans, and are important in ways that are not generally appreciated (Daily, 1997). Ecosystem services also provide a context for assessing the collective effects of human actions on a broad range of the goods and services upon which humans rely.

In general, both ecosystem structure and function play essential roles in providing goods and services. Ecosystem processes provide diverse benefits including absorption and breakdown of pollutants, cycling of nutrients, binding of soil, degradation of organic waste, maintenance of a balance of gases in the air, regulation of radiation balance and climate, and fixation of solar energy (WRI, 2000; Daily, 1997; Westman, 1977). These ecological benefits, in turn, provide economic benefits and values to society (Costanza et al., 1997; Pimentel et al., 1997). Goods such as food crops, timber, livestock, fish and clean drinking water have market value. The values of ecosystem services such as flood control, wildlife habitat, cycling of nutrients and removal of air pollutants are more difficult to measure (Goulder and Kennedy, 1997).

Particular concern has developed within the past decade regarding the consequences of decreasing biological diversity (Tilman, 2000; Ayensu et al., 1999; Wall, 1999; Chapin et al., 1998; Hooper and Vitousek, 1997). Human activities that decrease biodiversity also alter the complexity and stability of ecosystems and change ecological processes. In response, ecosystem structure, composition and function can be affected (Daily and Ehrlich, 1999; Wall, 1999; Chapin et al., 1998; Levlin, 1998; Peterson et al., 1998; Tilman, 1996; Tilman and Downing, 1994; Pimm, 1984). Biodiversity is an important consideration at all levels of biological organization, including species, communities, populations, and ecosystems. Human-induced changes in biotic diversity and alterations in the structure and functioning of ecosystems are two of the most dramatic ecological trends of the past century (U.S. EPA, 2004; Vitousek et al., 1997).

Hassan (2005) identified four broad categories of ecosystem services:

- Supporting services are necessary for the production of all other ecosystem services. Some examples include biomass production, production of atmospheric O_2, soil formation and retention, nutrient cycling, water cycling and provisioning of habitat. Biodiversity is a supporting service in that it is

increasingly recognized to sustain many of the goods and services that humans enjoy from ecosystems. These supporting services provide a basis for an additional three higher-level categories of services.

- Provisioning services such as products (Gitay et al., 2001) i.e., food (including game meat, roots, seeds, nuts, and other fruit, spices, fodder), water, fiber (including wood, textiles) and medicinal and cosmetic products.
- Regulating services that are of paramount importance for human society such as (1) carbon sequestration, (2) climate and water regulation, (3) protection from natural hazards such as floods, avalanches, or rock-fall (4) water and air purification, and (5) disease and pest regulation.
- Cultural services that satisfy human spiritual and aesthetic appreciation of ecosystems and their components.

7.1.3 Critical Loads as an Organizing Principle for Ecological Effects of Atmospheric Deposition

A critical load is defined as, "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge" (Nilsson and Grennfelt, 1988). Critical loads are a powerful organizing principle for information that links atmospheric deposition with ecological impairment. They allow for heterogeneity in ecosystem sensitivity and exposure which often results in critical load values that vary by ecosystem (e.g., aquatic-water; aquatic-sediment; terrestrial), and differ by endpoint of concern. It is important to consider that critical loads are often calculated assuming steady state conditions (i.e., how much input is required to balance the rate of output), and there may be time required to reach the critical load (i.e., the lag time between onset of exposure and induction of measurable effects). The following types of information are required to calculate a critical load, each of which is discussed in more detail in the subsequent sections of this chapter:

- Ecosystem at risk;
- Receptors of concern (plants, animals, etc.);
- Endpoints of concern (organism, population or community responses, changes in ecosystem services or functions);
- Dose (concentration) - response relationships and threshold levels of effects;

- Bioavailability and bioaccumulation rates;
- Naturally occurring (background) Pb (or other metal) concentrations; and
- Biogeochemical modifiers of exposure.

There is no single "definitive" critical load for a pollutant, partly because critical load estimates reflect the current state-of-knowledge and policy priorities, and also because of local or regional differences among ecosystems (U.S. EPA, 2008e). Changes in scientific understanding may include, for example, expanded information about dose-response relationships, better understanding of bioavailability factors, and improved quantitative models for effects predictions. Changes in policy may include new mandates for resource protection, inclusion of perceived new threats that may exacerbate the effects of the pollutant of concern (e.g., climate change), and a better understanding of the value of ecosystem services.

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 modifying factors that affect Pb bioavailability in soil, sediments, and water (e.g., pH, organic matter)(Sections 7.2.2. and 7.3.2). 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.

$$t_{1/2} = \frac{0.69 \times d \times 10{,}000}{y \times TF + \frac{R}{\rho K d}}$$

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)

P 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:

$$t_{1/2} = \frac{0.69 \times d \times 10{,}000}{\frac{R}{\rho K d}}$$

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, Fairbrother 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:

$$\boldsymbol{\Lambda} = \frac{\boldsymbol{R}}{\boldsymbol{C}}$$

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 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD. The focus of this ISA is on the effects of Pb on terrestrial organisms including plants, invertebrates and vertebrates, with particular focus on current ambient level. The most extensive survey of background soil Pb concentration in the contiguous U.S. was conducted between 1961 and 1976, and comprised 1,319 non-urban, undisturbed sampling locations, where 250 cm^3 of soil was collected at a depth of 20 cm (Shacklette and Boerngen, 1984). The lower detection limit was 10 mg Pb/kg, and 14% of the 1,319 samples were below it. The mean Pb concentration was 19.3 mg Pb/kg, the median 15 mg Pb/kg, and the 95th percentile 50 mg/kg. Sixteen locations had Pb concentrations between 100 and 700 mg Pb/kg. These results were in agreement with 3 previous surveys. When creating the Ecological Soil Screening Level (Eco-SSL) guidance document, the U.S. EPA (2007d, 2003b) augmented these data with observations from an additional 13 studies conducted between 1982 and 1997, most of them limited to one state. The resulting data were summarized using state means for each of the fifty states. Those state means ranged between 5 and 38.6 mg Pb/kg, with an overall national mean of 18.9 mg Pb/kg.

No new data on background concentrations of Pb in U.S. soils have been published since 2005. Data on levels of Pb in U.S. soils are reviewed in Section 3.6.1 and summarized in Table 2-1. The literature on terrestrial ecosystem effects of Pb, published since the 2006 Pb AQCD, is considered with brief summaries from the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD where relevant. Section 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 is reviewed in Section 7.2.2 Section 7.2.3 considers the bioavailability and uptake of Pb by plants, invertebrates, and vertebrates in terrestrial systems. Biological effects of Pb on terrestrial ecosystem components including plants and lichen, invertebrates, and vertebrates (Section 7.2.4) are followed by data on exposure and response of terrestrial species (Section 7.2.5). Effects of Pb at the ecosystem level of biological organization are discussed in Section 7.2.6 Section 7.2 concludes with a discussion of critical loads in terrestrial systems (Section 7.2.7), soil screening levels (Section 7.2.8), characterization of sensitivity and vulnerability of

ecosystem components (Section 7.2.9), and effects on ecosystem services (Section 7.2.10).

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, CEC and Salinity

Models of metal bioavailability calibrated from 500+ soil toxicity tests on plants, invertebrates, and microbial communities indicated that soil pH and CEC are the most important factors governing metal solubility and toxicity (Smolders et al., 2009). The variability of derived EC_{50} values was most closely associated with CEC. Smolders et al. (2007) determined that 12 to 18 months of artificial aging of soils amended with metal decreased the soluble metal fraction by approximately one order of magnitude. Miretzky et al. (2007) also showed that the concentration of mobile Pb was increased in acidic soils, and discovered that Pb adsorption to sandy loam clay was a function of weak electrostatic bonds with charged soil surfaces and was influenced by Fe and Mn oxide. Relatedly, lower soil pH in forest environments relative to adjacent agricultural land resulted in higher solubility, and the mobility of smelter-produced metals was found to be greater in forest than in agricultural lands (Douay et al., 2009). Further, decreasing the soil pH via simulated acid rain events increased naturally occurring Pb bioavailability in field tests (Hu et al., 2009b).

Salinity can also alter Pb mobility and bioavailability in soils. Application of $CaCl_2$, MgCl, or NaCl salts to field-collected soils containing 31 to 2,764 mg Pb/kg increased the proportion of mobile metal. As the strength of the salt application was increased from 0.006 to 0.3 M, the proportion of released Pb increased from less than 0.5% to over 2% for $CaCl_2$ and from less than 0.5% to over 1% for MgCl (Acosta et al.). However, the majority of salinity-induced effects occurred in soils containing less than 500 mg Pb/kg, and the proportion of released Pb decreased with increasing total soil Pb concentrations. In addition, the authors noted that Pb release from soils under increasing salinity was reduced at higher carbonate concentrations, indicating that the effect of soil salinity on Pb release is dependent on still other soil factors. A sequential extraction procedure was employed by Ettler et al. (2005) to determine the 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. However, the validity of associating sequentially extracted fractions with discrete geochemical components has not been definitively established, and as a consequence, the association between fractionation and bioavailability remains uncertain.

7.2.2.2 Organic Matter

Organic matter decreases bioavailability of Pb, but as it is turned over and broken down, pedogenic minerals become more important in Pb sequestration (Schroth et al., 2008). Shaheen and Tsadilas (2009) noted that soils with higher clay content, organic matter, total calcium carbonate equivalent, and total free sesquioxides also exhibited higher total Pb concentration, indicating that less Pb had been taken up by resident plant species. Huang et al. (2008) examined the re-mobilization potential of Pb in forest soils, and determined that mobilization of total Pb was strongly associated with dissolved organic matter (DOM). Groenenberg et al. (2010) used a non-ideal competitive adsorption Donnan model to explain the variability of organic matter binding affinity and uncertainties associated with metal speciation. They found that natural variations in fulvic acid binding properties were the most important variable in predicting Pb speciation. Guo et al. (2006b) determined that the -COOH and -OH groups associated with soil OM were important factors in Pb sequestration in soil, and Pb sorption was increased as pH was

raised from 2 to 8. Because organic content increased the Pb sequestration efficiency of soils, OM content had an inhibitory effect on Pb uptake by woodlouse species *Oniscus asellus* and *Porcellio scaber* (Gál et al., 2008). Vermeulen et al. (2009) demonstrated that invertebrate bioaccumulation of Pb from contaminated soils was dependent on pH and OM, but that other unidentified habitat-dependent factors also contributed. The relationship of bioaccumulation and soil concentration was modified by pH and OM, and also by habitat type. Kobler et al. (2010) showed that the migration of atmospherically deposited Pb in soil matrices was strongly influenced by soil type, indicating that certain soil types may retain Pb for longer periods of time than others. In soils characterized by well-drained substrate and limestone bedrock, Pb concentration decreased over time, likely as a result of water drainage and percolation. The authors contrasted this observation with reports of prolonged residence time in humic soils, particularly at the lower depths of the humus layer. They theorized that the most significant Pb migration route was transportation of particulate-bound Pb along with precipitation-related flow through large soil pores.

A number of recent laboratory studies have further defined the relationship of soil biogeochemical characteristics and Pb uptake by plants. 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 between 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). Ma et al. (2010) also reported that long-term agricultural cultivation can decrease the rate of Pb desorption in soil through a gradual OM-enrichment. Phosphorous soil amendments equivalent to 35 mg P/kg soil were observed to reduce the quantity of DPTA-extractable Pb from an average of 19 and 24 mg Pb/kg in unamended soils to 12 to 15 mg Pb/kg in P-amended soils. As a result, maize and soybean seedlings accumulated significantly less Pb: average concentrations in soybean shoot and root ranged from 4.4 to 5.2 mg Pb/kg with P addition (versus 9.21 mg Pb/kg without), while maize shoot concentrations average between 4.8 to 5.3 mg Pb/kg in P-amended soils (as compared with 10.16 mg Pb/kg in controls) (Xie et al., 2011).

7.2.2.3 Aging

Smolders et al. (2007) defined aging as the process responsible for decreasing the bioavailability of metals in soils independently of their persistence. Smolders et al. (2009) reviewed the effects of aging of Pb in soils on the toxicity of Pb to plants and soil invertebrates, with aging achieved in most studies primarily by leaching amended soil, but also through natural binding and complexation. In nearly half of the Pb soil studies reviewed, responses that were observed with freshly amended soil could no longer be detected following soil leaching, indicating that aged soils likely contain less bioavailable Pb. The authors concluded that competitive binding between soil ligands and biotic ligands on plant roots or invertebrate guts can be used to model the relationship of observed availability and toxicity of metals in soils. Because this concept is the basis of the Biotic Ligand Model (BLM) (Section 7.2.3), 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 Pb/L, whereas reproduction was always significantly affected at Pb concentrations of 0.7 mg Pb/L and higher, independent of the soil treatment. Leaching soils prior to use in bioassays had only a slight effect on Pb toxicity to resident springtails, suggesting that

among the processes that constitute aging of Pb in field soils, leaching is not particularly important with respect to bioavailability.

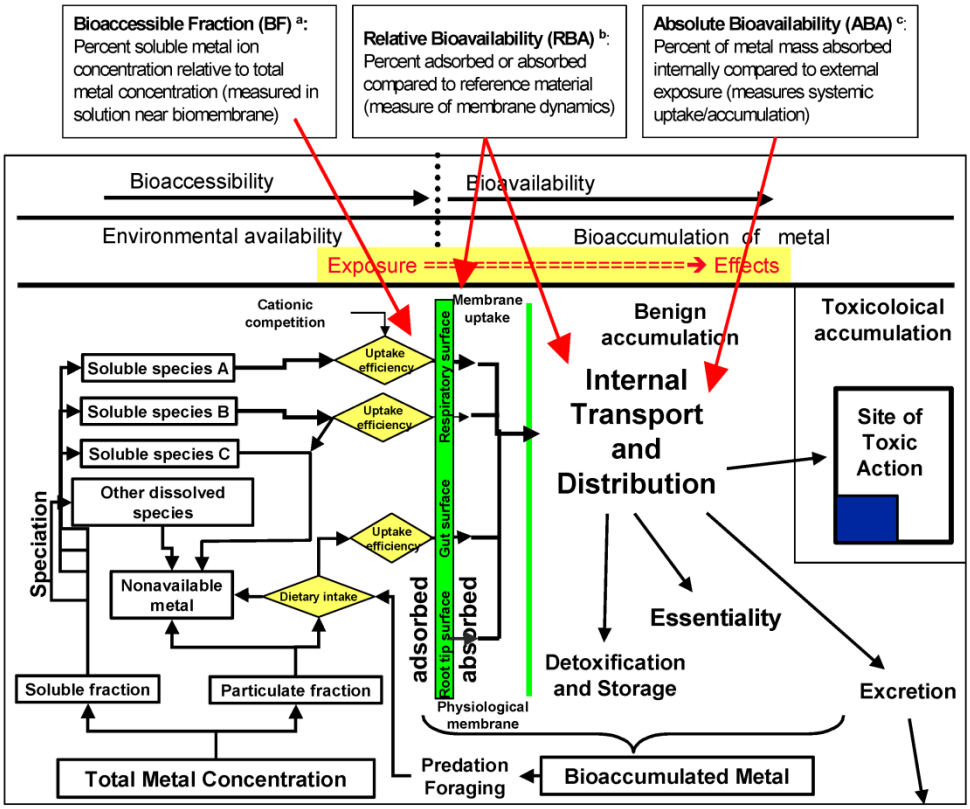
Red-backed salamanders (*Plethodon cinereus*) exposed to Pb-amended soils (553 mg Pb/kg, 1,700 mg Pb/kg, 4,700 mg Pb/kg, and 9,167 mg Pb/kg) exhibited lowered appetite and decreased white blood cell counts at the two highest concentrations, as compared to controls (Bazar et al., 2010). However, salamanders tolerated field-collected, aged soils containing Pb concentration of up to 16,967 mg Pb/kg with no significant deleterious effects.

In summary, studies published during the past 5 years continue to substantiate the important role that soil geochemistry plays in sequestration or release of Pb. Soil pH and CEC have long been known to be the primary controlling factors of the amount of bioavailable Pb in soils, and a recent review of more than 500 studies corroborates these findings (Smolders et al., 2009). Fe and Mn oxides are now known to also play an important role in Pb sequestration in soils. Pb binds to OM, although relatively weakly, and as the OM is broken down the Pb may be released into soil solution. Leaching of metal through soil pores may be the primary route for loss of bioavailable soil Pb; OM may reduce leaching and thus appear to be associated with Pb sequestration. Aging of Pb in soils through incorporation of the metal into the particulate solid phase of the soil results in long term binding of the metal and reduced bioavailability of Pb to plants and soil organisms.

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)" (U.S. EPA, 2006c). In 2007, EPA took cases of bioactive adsorption into consideration and revised the definition of bioavailability as "the extent to which bioaccessible metals absorb onto, or into, and across biological membranes of organisms, expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time and under defined conditions" (Fairbrother et al., 2007). The bioavailability of metals varies widely depending on the physical, chemical, and biological conditions under which an organism is exposed (U.S. EPA, 2007c). Characteristics of the toxicant itself that affect bioavailability are: (1) chemical form or species, (2) particle size, (3) lability, and (4) source. The bioavailability of a metal is also dependent upon the bioaccessible fraction of metal. As stated in the Framework for Metals Risk Assessment (U.S. EPA, 2007c), the bioaccessible fraction of a metal is the portion (fraction or

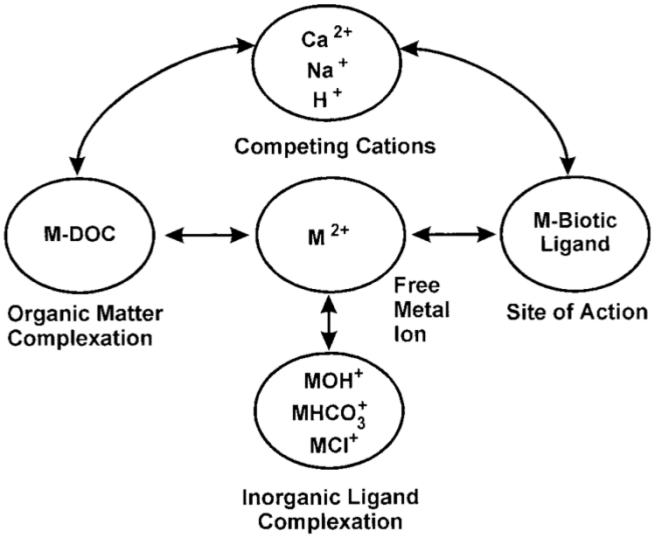
percentage) of environmentally available metal that actually interacts at the organism's contact surface and is potentially available for absorption or adsorption by the organism. The Framework states that "the bioaccessibility, bioavailability, and bioaccumulation properties of inorganic metals in soil, sediments, and aquatic systems are interrelated and abiotic (e.g., organic carbon) and biotic (e.g., uptake and metabolism). Modifying factors determine the amount of an inorganic metal that interacts at biological surfaces (e.g., at the gill, gut, or root tip epithelium) and that binds to and is absorbed across these membranes. A major challenge is to consistently and accurately measure quantitative differences in bioavailability between multiple forms of organic metals in the environment." A conceptual diagram presented in McGeer et al. (2004) and the Framework for Metals Risk Assessment summarizes metals bioavailability and bioaccumulation in aquatic, sediment and soil media (Figure 7-1).



[a]BF is most often measured using in vitro methods (e.g., artificial stomach), but it should be validated by in vivo methods.
[b]RBA is most often estimated as the relative absorption factor, compared to a reference metal salt (usually calculated on the basis of dose and often used for human risk, but it can be based on concentrations).
[c]ABA is more difficult to measure and used less in human risk; it is often used in ecological risk when estimating bioaccumulation or trophic transfer.
Source: ERG (2004) and U.S. EPA (2007c).

Figure 7-1 Conceptual diagram for evaluating bioavailability processes and bioaccessibility for metals in soil, sediment, or aquatic systems.

The BLM attempts to integrate the principal physical and chemical variables that influence Pb bioavailability. The model considers the reactions of Pb with biological surfaces and membranes (the site of action) to predict the bioavailability and uptake of the metal (Figure 7-2), and integrates the binding affinities of various natural ligands and the biological uptake rates of organisms to predict both the bioaccessible and bioavailable fraction of Pb in the environment, and to determine the site-specific toxicity of the bioavailable fraction. In principle, the BLM can be used for determining toxicity in water, sediment, and soil media, however, the parameter values that influence BLM are, in general, characterized to a greater extent in aquatic systems than in terrestrial systems (Section 7.3.4).



Source: Reprinted with permission of John Wiley and Sons ; from Di Toro et al. (2001)

Figure 7-2 Schematic diagram of the biotic ligand model.

New information on sources of Pb in terrestrial ecosystems, and their influence on subsequent bioavailability, was reviewed in Chapter 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. 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. The 2006 Pb AQCD also reported bioaccumulation factors (BAF) and bioconcentration factors (BCF) for some terrestrial and aquatic biota. BAF is defined as the field measurement of metal concentration in tissues, including dietary exposures, divided by metal concentration in environmental media (Smolders et al., 2007). BCF is defined as the same measurement carried out in artificial media in the laboratory that does not include dietary exposure (Smolders et al., 2007). The EPA Framework for Metals Risk Assessment states that the latest scientific data on bioaccumulation do not currently support the use of BCFs and BAFs when applied as generic threshold criteria for the hazard potential of metals (U.S. EPA, 2007c).

7.2.3.1 Terrestrial Plants

At the time of the 1977 Pb AQCD, it was understood that Pb uptake in plants was influenced by plant species and by the available Pb pool in the soils (U.S. EPA, 1977). The role of humic substances in binding Pb was better characterized by the 1986 Pb

AQCD where it was stated that most plants cannot survive in soil containing 10,000 µg/g (mg Pb/kg) dry weight if the pH is below 4.5 and the organic content is below 5% (U.S. EPA, 1986b). Pb can be absorbed across the leaf surface into internal plant tissues although the vast majority of uptake is via roots (U.S. EPA, 1986b). 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. Data on ambient Pb levels associated with vegetation are summarized in Section 3.6.6.

Leaf and Root Uptake

Although Pb is not an essential metal, it is taken up from soils through the symplastic route, the same active ion transport mechanism used by plants to take up water and nutrients and move them across root cell membranes (U.S. EPA, 2006c). As with all nutrients, only the proportion of a metal present in soil pore water is directly available for uptake by plants. In addition, soil-to-plant transfer factors in soils enriched with Pb have been found to better correlate with bioavailable Pb soil concentration, defined as DTPA-extractable Pb, than with total Pb concentration (U.S. EPA, 2006c).

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. Angelova et al. (2010) examined Pb uptake by rapeseed plants (*Brassica napus*) grown in heavy metal contaminated soils 0.5 km and 15 km from the Non-Ferrous Metal Works, in Bulgaria. Average surface soil Pb concentration decreased with distance from the plant (200.3 and 24.6 mg Pb/kg, respectively), as did average DTPA-extractable Pb (69.7 and 4.9 mg Pb/kg, respectively). Pb content in stems and leaves in rapeseed grown at 0.5 km from the plant averaged 1.73 and 8.69 mg Pb/kg ; average stem and leaf Pb concentrations in rapeseed grown at the more distant location were reported as 0.72 and 1.42 mg Pb/kg, respectively (Angelova et al., 2010).

Pb plant BAFs for plants grown in 70 actively cropped fields in California averaged 0.052 for vegetable crops and 0.084 for grains; the highest reported Pb BAF (0.577) was found in onions. Authors compared the BAFs based on total Pb and Pb in solution and determined that both were accurate predictors of plant uptake (Chen et al., 2009b). Likewise, Zhang et al. (2011) compiled Pb uptake data for several crop species in China, and reported an average BAF for grains (rice) of 0.009 (0.0009-0.03) and 0.41(0.0007-0.17) for leafy vegetables, such as spinach, Chinese cabbage and celery (Zhang et al., 2011). Chrastny et al. (2010) characterized the Pb contamination of an agricultural soil in the vicinity of a shooting range. Pb was predominantly in the form of PbO and $PbCO_3$, and Pb was taken up by plants through both atmospheric deposition onto the plant and by root uptake.

The Pb content of ripe date palm (*Phoenix dactylifera*) fruit collected in Riyadh, Saudi Arabia was determined to be indicative of areas of heavy industrialization and urbanization; Pb concentrations in fruit flesh ranged from 0.34 to 8.87 µg Pb/g dry weight, with the highest Pb date concentrations detected near freeways and industrial

areas (Aldjain et al., 2011). Likewise, Pb concentrations in rosemary (*Rosmarinus officinalis*) flowers, stems, and leaves were significantly higher in the urban areas of Al-Mafraq and Irbid, Jordan than in the smaller town of Ma'an, Jordan (53.6 to 86.5 mg Pb/kg versus 16.2 to 16.7 mg Pb/kg). Authors noted a significant difference between Pb concentrations in washed and unwashed rosemary samples, indicating that aerial deposition and surface dust is likely a significant source of plant-associated lead (El-Rjoob et al., 2008).

Bilberry (*Vaccinium myrtillus*), accumulated the highest amount of Pb out of four total herbaceous species growing in Slovakian spruce ecosystems with variable soil lead concentrations, giving BAFs of 0.09 to 0.44, depending on location (Kuklova et al., 2010). Because of their long life spans, trees can provide essential information regarding the sources of bioavailable Pb. A Scots pine forest in northern Sweden was found to incorporate atmospherically derived Pb pollution directly from ambient air, accumulating this Pb in bark, needles, and shoots (Klaminder et al., 2005). Nearly 50% of total tree uptake was estimated to be from direct adsorption from the atmosphere, as determined using isotopic ratios and a binary mixing model. Further, Aznar et al. (2009b) found that the Pb content of black spruce (*Picea mariana*) needles collected along a metal contamination gradient emanating from a Canadian smelter in Murdochville, Quebec, showed a significant decrease in Pb concentration with increasing distance from the smelter. Interestingly, older needles were determined to accumulate larger quantities of Pb than younger ones. Foliar damage and growth reduction were also observed in the trees (Aznar et al., 2009b). They were significantly correlated with Pb concentration in the litter layer. In addition, there was no correlation between diminished tree growth and Pb concentration in the deeper mineral soil layers, strongly suggesting that only current atmospheric Pb was affecting trees (Aznar et al., 2009a). Similarly, Kuang et al. (2007) noted that the Pb concentration in the inner bark of *Pinus massoniana* trees growing adjacent to a Pb-Zn smelter in the Guangdong province of China was much higher (1.87 mg Pb/kg dry weight) than in reference-area trees. Because concentration in the inner bark was strongly correlated with concentration in the outer bark, they concluded that the origin of the Pb was atmospheric.

Dendrochronology (tree ring analysis) has become an increasingly important tool for measuring the response of trees to Pb exposure (Watmough, 1999). Tree ring studies reviewed in the 1977 Pb AQCD showed that trees could be used as indicators of increasing environmental Pb concentrations with time. Additional studies in the 1986 Pb AQCD indicated that Pb could be translocated from roots to the upper portions of the plant and that the amounts translocated are in proportion to concentrations of Pb in soil (U.S. EPA, 1986b). The advent of laser ablation inductively coupled plasma mass spectrometry has made measurement of Pb concentration in individual tree rings possible

(Witte et al., 2004; Watmough, 1999). This allows for close analysis of the timing of Pb uptake relative to smelter activity and/or changes in soil chemistry. For example, Aznar et al. (2008b) measured Pb concentration in black spruce tree rings to determine the extent and timing of atmospheric deposition near the Murdochville smelter. Variability in tree-ring Pb content seemed to indicate that trees accumulated and sequestered atmospheric Pb in close correlation with the rates of smelter emission, but that sequestration lagged about 15 years behind exposure. However, the ability to determine time of uptake from the location in growth rings is weakened in species that transfer Pb readily from outer bark to inner bark. Cutter and Guyette (1993) identified species with minimal radial translocation from among a large number of tree species, and recommended the following temperate zone North American species as suitable for metal dendrochronology studies: white oak (*Quercus alba*), post oak (*Q. stellata*), eastern red cedar (*Juniperus virginiana*), old-growth Douglas fir (*Pseudotsuga menziesii*), and big sagebrush (*Artemisia tridentata*). In addition, species such as bristlecone pine (*Pinus aristata*), old-growth redwood (*Sequoia sempervirens*), and giant sequoia (*S. gigantea*) were deemed suitable for local purposes. Patrick and Farmer (2006) determined that European sycamore (*Acer pseudoplatanus*) are not suitable for this type of dendrochronological analysis because of the formation of multiple annual rings.

Pb in sapwood and heartwood is more likely a result of soil uptake than of direct atmospheric exposure (Guyette et al., 1991). Differentiation of geogenic soil Pb in tree tissue from Pb that originated in the atmosphere requires measurement of stable Pb isotope ratios (Patrick, 2006). Tree bark samples collected from several areas of the Czech Republic were subjected to stable Pb isotope analysis to determine the source and uptake of atmospheric Pb (Conkova and Kubiznakova, 2008). Results indicated that beech bark is a more efficient accumulator of atmospheric Pb than spruce bark. A decrease in the $^{206}Pb/^{207}Pb$ ratio was measured in bark and attributed to increased usage of leaded gasoline between 1955 and 1990; an increased $^{206}Pb/^{207}Pb$ ratio was ascribed to coal combustion (Conkova and Kubiznakova, 2008). Similarly, Savard et al. (2006) compared isotope ratios of $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ in tree rings from spruce trees sampled at a control site near Hudson Bay, with those sampled near the Horne smelter active since 1928, in Rouyn-Noranda, Canada. The concentration of total Pb showed a major increase in 1944 and a corresponding decrease of the $^{206}Pb/^{207}Pb$ ratios, suggesting that the smelter was responsible for the increased Pb uptake (Savard et al., 2006). The authors suggested that the apparent delay of 14 years may have been attributable to the residence time of metals in airborne particles the buffering effect of the soils and, to a lesser extent, mobility of heavy metals in tree stems. Furthermore, through the use of the two different isotope ratios, Savard et al. (2006) were able to differentiate three types of Pb in tree rings: natural (derived from the mineral soil horizons), industrial (from coal

burning urban pollution), and mining (typical of the volcanogenic massive sulfide ore deposits treated at the Horne smelter).

Devall et al. (2006) measured Pb uptake by bald-cypress trees (*Taxodium distichum*) growing in a swamp near a petroleum refinery and along a bank containing Pb-contaminated dredge spoils. They measured Pb in tree cores and showed greater uptake of Pb by trees in the swamp than by trees growing on the dredge spoil bank, attributing the difference to exposure source (refinery versus dredge spoils) and differences in soil chemistry between the swamp and the dredge spoil bank (Devall et al., 2006). Similarly, Gebologlu et al. (2005) found no correlation between proximity to roadway and accumulated Pb in tomato and bean plants at sites adjacent to two state roads in Turkey (average Pb concentration 5.4 and 6.0 mg Pb/kg), indicating that uptake may be influenced by multiple factors, including wind direction, geography, and soil chemistry. Average Pb levels in leaves were 0.6 and 0.5 mg Pb/kg for tomato and bean plants, respectively, while fruit concentration averaged 0.4 mg Pb/kg for both species. Conversely, if foliar contamination is due primarily to dust deposition, distance from a source such as a road may be easily correlated with Pb concentration on the plants. For example, Ai-Khlaifat and Al-Khashman (2007) collected unwashed date palm (*Phoenix dactylifera*) leaves at 3-meter trunk height from trees in Jordan to assess the extent of Pb contamination from the city of Aqaba. Whereas relatively low levels of Pb were detected in leaves collected at background sites (41 mg Pb/kg), leaves collected adjacent to highway sites exhibited the highest levels of Pb (177 mg Pb/kg). The authors determined that Pb levels in date palm leaves correlated with industrial and human activities (e.g., traffic density) (Ai-Khlaifat and Al-Khashman, 2007). Likewise, Pb concentrations were significantly enriched in tree bark samples and road dust collected in highly urbanized areas of Buenos Aires, Argentina (approximate average enrichment factors of 30 and 15 versus reference samples) (Fujiwara et al., 2011). However, decreases in tissue Pb concentration with increasing distance from 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).

Fungal species, as represented by mushrooms, accumulate Pb from soils to varying degrees. Based on the uptake of naturally occurring ^{210}Pb, Guillen et al. (2009) established that soil-associated Pb was bioavailable for uptake by mushrooms, and that the highest ^{210}Pb accumulation was observed in *Fomes fomentarius* mushrooms, followed by *Lycoperdon perlatum*, *Boletus aereus*, and *Macrolepiota procera*, indicating some species differences. Benbrahim et al. (2006) also showed species differences in uptake of Pb by wild edible mushrooms, although they found no significant correlations between Pb content of mushrooms and soil Pb concentration. Pb concentrations in mushroom carpophores ranged from 0.4 to 2.7 mg Pb/kg from sites with soil concentrations ranging from 3.6 and 7.6 mg Pb/kg dry soil. Likewise, Semreen and Aboul-Enein (2011), reported the heavy metal uptake of wild edible mushrooms collected in various mountainous regions of Jordan. Pb BCFs ranged between 0.05 *(Russula delica*) and 0.33 (*Bovista plumbea)* for six mushroom species. Pb BAFs for edible mushrooms collected from quartzite acidic soils in central Spain (containing 19.2 mg Pb/kg) ranged from 0.07 (*Macrolepiota procera*) to 0.45 (*Lepista nuda*) (Campos and Tejera, 2011).

Translocation and Sequestration of Lead in Plants

In the 1977 Pb AQCD it was recognized that most Pb taken up from soil remains in the roots and that distribution to other portions of the plant is variable among species (U.S. EPA, 1977). The 2006 Pb AQCD 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 Pb/kg) by Probst et al. (2009) revealed dark ultrastructural abnormalities that were demonstrated to be metal-rich particles located in or on root cell walls. It is unclear whether the presence of these structures had any effect on overall plant health.

Clark et al. (2006) investigated Pb bioavailability in garden soils in Roxbury and Dorchester, MA. The sources of Pb were considered to be Pb from paints and from leaded gasoline additives, with 40 to 80% coming from paint. The average Pb concentration in foliar tissue of bean plants was 14 ± 5 mg Pb/kg while the concentration in the bean pod was only 20.6 mg Pb/kg. For mustard plants, there was a linear relationship (R^2=0.85) between Pb concentration in plant tissues and Pb concentration in the soil (both for plants grown in situ and those grown under greenhouse conditions).

Murray et al. (2009) investigated the uptake and accumulation of Pb in several vegetable species (carrot [*Daucus carota*], radish [*Raphanus sativus*], lettuce [*Lactuca sativa*], soybean [*Glycine max*], and wheat [*Triticum aestivum*]) from metal-contaminated soils, containing 10 to 40 mg Pb/kg and demonstrated that most Pb remained in the roots. No Pb was measured in the above-ground edible soybean and wheat tissues, while carrots, the most efficient accumulator of Pb, contained a maximum Pb tissue concentration of 12 mg Pb/kg dry mass. Similarly, (Cho et al., 2009) showed that green onion (*Allium fistulosum*) plants also take up little Pb when planted in soil spiked with Pb nitrate. No plant tissues contained a Pb concentration greater than 24 mg Pb/kg when grown for 14 weeks in soils of up to 3,560 mg Pb/kg, and the majority of bioavailable Pb was determined to be contained within the roots. Chinese spinach (*Amaranthus dubius*) also translocates very little Pb to stem and leaf tissue, and uptake from Pb-containing soils (28 to 52 mg Pb/kg) is minimal (Mellem et al., 2009). Wang et al. (2011c) determined tissue-specific BCFs for wheat grown in soils containing 93 to 1,548 mg Pb/kg. Although the average calculated root BCF was 0.3, very little Pb was translocated to shoots (average BCF=0.02), shells (0.006), and kernels (0.0007) (Wang et al., 2011c). Sonmez et al. (2008) reported that Pb accumulated by three weed species (*Avena sterilis*, *Isatis tinctoria*, *Xanthium strumarium*) grown in Pb-spiked soils was largely concentrated in the root tissues, and little was translocated to the shoots (Sonmez et al., 2008).

The Pb BCFs for alfalfa (*Medicago sativa*) and crimson clover (*Trifolium incarnatum*) grown in mixtures of heavy metals (Pb concentrations of 10 to 500 µg Pb/kg) were reportedly low. For alfalfa, BCFs ranged from 0.02 to 0.12, while for crimson clover, these values were between 0.04 and 0.06 (Comino et al., 2011). The low shoot-root translocation factors reported for alfalfa (0.17 to 0.43) indicated that plant Pb content was largely contained in root tissue. Businelli et al. (2011) calculated whole-plant Pb BAFs for lettuce, radish, tomato and Italian ryegrass using Pb-spiked soils (average values of 0.025, 0.021, 0.032, and 0.65, respectively). Again, the majority of accumulated Pb was stored in root tissue, with comparatively little translocated to above-ground tissues (Businelli et al., 2011).

Recent research has shown that Pb translocation to stem and leaf tissues does occur at significant rates in some species, including the legume *Sesbania drummondii* (Peralta-Videa et al., 2009) and buckwheat (*Fagopyrum esculentum*) (Tamura et al., 2005). Wang et al. (2006b) noted that Pb soil-to-plant transfer factors were higher for leafy vegetables (Chinese cabbage, pak-choi, and water spinach) than for the non-leafy vegetables tested (towel gourd, eggplant, and cowpea). Tamura et al. (2005) demonstrated that buckwheat is an efficient translocator of Pb. Buckwheat grown in Pb-containing soils collected from a shooting range site (average 1M HCl extractable Pb= 6,643 mg Pb/kg) preferentially accumulated Pb in leaves (8,000 mg Pb/kg) and shoots (4,200 mg Pb/kg), over root tissues (3,300 mg Pb/kg). Although plant growth was unaffected, this level of leaf and shoot accumulation is likely to have significant implications for exposure of herbivores. Similarly, Shaheen and Tsadilas (2009) reported that vegetables (pepper, okra, and eggplant) grown in soils containing 24 to 30 mg Pb/kg total Pb were more likely to accumulate Pb in leaves (range: undetected to 25 mg Pb/kg) rather than in fruits (range: undetected to 19 mg Pb/kg); however, no significant correlation between soil Pb concentration and plant tissue Pb concentration could be established (Shaheen and Tsadilas, 2009). Tobacco plants were also observed to take up significant amounts of Pb into leaf tissue. Field-grown plants in soils containing an average of 19.8 mg Pb/kg contained average lower, middle and upper leaf Pb concentrations of 11.9, 13.3, and 11.6 mg Pb/kg respectively (Zaprjanova et al., 2010). Uptake by tobacco plants was correlated with both total soil Pb concentrations and the mobile Pb fraction (average 3.8 mg Pb/kg soil).

There is broad variability in uptake and translocation among plant species, and interspecies variability has been shown to interact with other factors such as soil type. By studying multiple species in four Pb-Zn mining sites in Yunnan, China, Li et al. (2009d) demonstrated not only significant differences in uptake and translocation among the species studied, but also modification of the effect on species by type of soil. Plants sampled represented nine species from four families—Caryophyllaceae, Compositae, Cruciferae, and Pteridaceae. Overall, soil Pb concentration averaged 3,772 mg Pb/kg dry weight, with the highest site average measured at the Minbingying site (5,330 mg Pb/kg), followed by Paomaping (2,409 mg Pb/kg), Jinding (1,786 mg Pb/kg), and Qilinkeng (978 mg Pb/kg). The highest average shoot Pb concentration (3,142 mg Pb/kg) was detected in *Stellaria vestita* (Caryophyllaceae) collected at Paomaping, while *Sinopteris grevilloides* (Pteridaceae) collected from Minbingying exhibited the lowest shoot Pb concentration (69 mg Pb/kg). A similar trend was detected in root tissues. *S. vestita* root collected from the Paomaping area contained the maximum Pb concentration measured (7,457 mg Pb/kg), while the minimum root Pb levels were measured in *Picris hieraciodides* (Pteridaceae) tissues collected from Jinding. These results indicate significant interspecies differences in Pb uptake, as well as potential soil-specific

differences in Pb bioavailability. *S. vestita*, in particular, was 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 (Li et al., 2009d). Within plant species, the variability in uptake and translocation of Pb may extend to the varietal level. Antonious and Kochhar (2009) determined uptake of soil-associated Pb for 23 unique genotypes from four species of pepper plants (*Capsicum chinense*, *C. frutescens*, *C. baccatum*, and *C. annum*). Soil Pb concentration averaged approximately 0.6 mg Pb/kg dry soil. No Pb was detected in the fruits of any of the 23 genotypes, except two out of seven genotypes of *C. baccatum*, which had 0.9 and 0.8 mg Pb/kg dry weight Pb in fruit.

Recent studies substantiated findings from the 2006 Pb AQCD that plants store a large portion of Pb in root tissue. Pb soil-to-plant transfer factors are higher for leafy vegetables than for the non-leafy vegetables (Wang et al., 2006b) and buckwheat has recently been shown to be an efficient translocator of Pb from soil to above-ground shoots (Tamura et al., 2005).

Field studies carried out in the vicinity of Pb smelters (Hu et al., 2009b) show that Pb may accumulate in shoot tissue through direct stomatal uptake rather than by soil-root-shoot translocation. For instance, Hovmand and Johnsen (2009) determined that about 98% of Pb sequestered in Norway spruce needles and twigs was derived from atmospheric sources, and that less than 2% of Pb was translocated from the roots (Hovmand et al., 2009). Dendrochronology has become more advanced in recent years and is a useful tool for monitoring historical uptake of Pb into trees exposed to atmospheric or soil Pb. Trees accumulate and sequester atmospheric Pb in close correlation with the rate of smelter emissions, although one study indicated that sequestration can lag behind exposure from emissions by 15 years. Pb in the outer woody portion of the tree is more likely the result of direct atmospheric exposure, while Pb in sapwood is more likely a result of soil uptake. This difference provides an important tool for analyzing source apportionment of Pb accumulation in plants (Guyette et al., 1991).

7.2.3.2 Terrestrial 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

Pauget et al. (2011) reported that uptake of Pb from soil by the land snail (*Cantareus asperses*) was most significantly influenced by soil pH and organic matter, as increases in these variables were correlated to decreased Pb bioavailability. *Cantareus asperses* snails exposed to dietary Pb at 3.3, 86, and 154 mg/kg of diet (spiked with Pb sulfate) for up to 64 days were found to assimilate a significant proportion of Pb, and feeding rates were unaffected by the presence of the metal (Beeby and Richmond, 2010). While BCFs for Cd were observed to increase over the 64-day study period, the rate of Pb assimilation remained consistent over time and the authors inferred the absence of a regulatory mechanism for uptake of Pb. The authors speculated that uptake is a function of growth or cell turnover instead. *Helix aspersa* snails rapidly accumulated Pb from contaminated soil (1,212 mg Pb/kg) and from eating contaminated lettuce (approximately 90 mg Pb/kg after 16 weeks' growth on Pb-contaminated soil) during the first 2 weeks of exposure, at which point snail body burdens reached a plateau (Scheifler et al., 2006b). There were no observed effects of Pb exposure or accumulation on survival or growth in *C. asperses* or *H. aspersa*. In another study (Ebenso and Ologhobo, 2009b), juvenile *Achatina achatina* snails confined in cages on former Pb-battery waste dump sites were found to accumulate Pb from both plant and soil sources. Soil Pb concentration averaged 20, 200, and 1,200 mg Pb/kg at the three main waste sites, while leaf tissues of radish (*Raphanus sativus*) grown at these sites averaged 7, 30, and 68 mg Pb/kg dry weight, respectively. Concentration of Pb in snail tissues rose with concentration in both soil and plants, and the authors found that for both sources, a log-log relationship could be estimated with a very close fit (r^2 =0.94 and 0.95, respectively). Pb concentration in snail tissues averaged 12, 91, and 468 mg Pb/kg, respectively, at the three sites, which the authors stipulated were above the maximum permissible concentration of Pb for human consumption of mollusks, mussels, and clams (1.5 µg Pb/g tissue) as determined by the U.K. Food Standards Agency. Pb concentration in snail tissues generally is much lower than that of the soil substrates upon which they were reared, but higher than in other soil-dwelling organisms. De Vaufleury et al. (2006) exposed *Helix aspera* snails to standardized (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 Pb/kg in control organisms. Viscera also exhibited increased Pb levels at the two highest exposures, with measured tissue concentration of 1.2 and 1.1 mg Pb/kg, respectively, as

compared with control tissue Pb levels of 0.4 mg Pb/kg. However, there was no significant increase in snail-tissue Pb concentration when natural soil was used in place of ISO medium, and there was no relationship between soil Pb concentration and snail tissue concentration, strongly suggesting the presence of soil variables that modify bioavailability. Notten et al. (2008) investigated the origin of Pb pollution in soil, plants, and snails by means of Pb isotope ratios. They found that a substantial proportion of Pb in both plants and snails was from current atmospheric exposure.

Finally, a study by Coeurdassier et al. (2007) found that the presence of snails was associated with higher Pb content in earthworms, suggesting that snails themselves may have an effect on bioavailability.

Earthworms

Accumulation studies conducted with *Eisenia* sp. earthworms documented the difficulty of extrapolating accumulation kinetic constants from one soil type to another, and showed that many soil physiochemical properties, including pH, organic matter, and CEC, among others, affect metal bioavailability (Nahmani et al., 2009). Source of Pb, and proportion of soil:leaf litter also affect Pb bioavailability. Bradham et al. (2006) examined the effect of soil chemical and physical properties on Pb bioavailability. *Eisenia andrei* earthworms were exposed to 21 soils with varying physical properties that were freshly spiked with Pb to give a standard concentration of 2,000 mg Pb/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. Garg et al. (2009) reported that the smaller native earthworm *Allolobophora parva* accumulated significantly greater Pb concentrations than *E. fetida*. Subsequently, it was concluded that native earthworm species may exhibit a higher Pb accumulation potential as a result of increased tolerance to the heavy metal (Garg et al., 2009).

Earthworm activity can alter Pb bioavailability and subsequent uptake by earthworms themselves and other organisms. Sizmur and Hodson (2009) speculated that earthworms affect Pb mobility by modifying the availability of cations or anions. The concentration of water-soluble Pb was observed to increase following earthworm (*Lumbricus terrestris*) feeding activity in field-collected soils containing 132.7, 814.9, and 821.4 mg total Pb/kg (calculated BAFs of 0.27, 0.33, and 0.13, respectively) (Alonso-Azcarate et al., 2011). However, Coeurdassier et al. (2007) found that snails did not have a higher Pb content when earthworms were present, and that unexpectedly, Pb was higher in earthworm tissue when snails were present.

Despite significant Pb uptake by earthworms, Pb in earthworm tissue may not be bioavailable to predators. Pb in the earthworm (*Aporrectodea caliginosa*) was determined to be contained largely in the granular fraction (approximately 60% of total Pb), while the remaining Pb body burden was in the tissue, cell membrane, and intact cell fractions (Vijver et al., 2006). However, this may vary by species, as (Li et al., 2008b) found that more than half of the Pb accumulated by *E. fetida* was contained within earthworm tissue and cell membranes. Regardless, Vijver et al. (2006) concluded that only a minority of earthworm-absorbed Pb would be toxicologically available to cause effects in the earthworms or in their predators.

Arthropods

Pb and other metals were analyzed in honeybees (*Apis mellifera*) foraging in sampling sites that included both urban areas and wildlife reserves in central Italy. (Perugini et al., 2011). Pb in whole bees ranged from 0.28 to 0.52 mg Pb/kg with the highest concentration in honeybees caught in hives near an airport. Cicadas pupating in historically Pb-arsenate-treated soils accumulated Pb at concentrations similar to those reported previously for earthworms (Robinson et al., 2007). Likewise, tissue Pb levels measured in Coleoptera specimens collected from areas containing average soil concentration of 45 and 71 mg Pb/kg exhibited a positive relationship with soil Pb

content, although abundance was unaffected (Schipper et al., 2008). By contrast, two grasshopper species inhabiting Pb and Cd-contaminated areas near zinc smelting facilities exhibited different Pb accumulation rates. Locust (*Locusta migratoria*) collected from areas with an average Pb soil concentration of 540mg Pb/kg contained 47 mg Pb/kg, while grasshoppers (*Acrida chinensis*) inhabiting the same area accumulated 93.9 mg Pb/kg (Zhang et al., In Press). This gives respective BAFs of 0.09 and 0.17. Similarly, the Pb sequestration rates that were observed in two woodlouse species, *O. asellus* and *P. scaber*, were species-dependent (Gál et al., 2008). Both species were field collected at Pb-contaminated sites (average concentration, 245 mg Pb/kg dry weight; range, 21-638 mg Pb/kg dry weight), with *O. asellus* Pb levels averaging 43 mg Pb/kg over all sites, while *P. scaber* contained no detectable Pb residues. Pb concentration measured in granivorous rough harvester ants (*Pogonomyrmex rugosus*), in the seeds of some plant species they consume, and in surface soil, were all shown to decline with increasing distance from a former Pb smelter near El Paso, Texas, where soil leachable Pb at the three sites of ant collection ranged from 0.003 to 0.117 mg Pb/kg (Del Toro et al., 2010). Ants accumulated approximately twice as much Pb as was measured in seeds, but the study did not separate the effects of dietary exposure from those of direct contact with soil or respiratory intake.

7.2.3.3 Terrestrial Vertebrates

At the time of the 1977 Pb AQCD few studies of Pb exposure and effects in wild animals other than birds had been conducted. A limited number of rodent trapping studies near roadsides indicated general trends of species differences in Pb uptake and higher concentrations of Pb in habitats adjacent to high-traffic areas (U.S. EPA, 1977). In the 1986 Pb AQCD concentration of Pb in bone tissue was reported for selected herbivore, omnivore and carnivore species [Table 8-2 in (U.S. EPA, 1986b)].

Tissue Pb residues in birds and mammals associated with adverse toxicological effects were presented in the 2006 Pb AQCD. In general, avian blood, liver, and kidney Pb concentrations of 0.2-3 µg Pb/dL, 2-6 mg Pb/kg wet weight, and 2-20 mg Pb/kg wet weight, respectively, were linked to adverse effects. A few additional studies of Pb uptake and tissue residues in birds and mammals conducted since 2006 are reviewed here.

In a study of blood Pb levels in wild Steller's eiders (*Polysticta stelleri*) and black scoters (*Melanitta nigra*) in Alaska, the authors compiled avian blood Pb data from available literature to develop reference values for sea ducks (Brown et al., 2006). The background exposure reference value of blood Pb was <20 µg Pb/dL, with levels between 20 and

59 µg Pb/dL as indicative of Pb exposure. Clinical toxicity was in the range of 60-99 µg Pb/dL in birds while >100 µg Pb/dL results in acute, severe toxicity. In measurement of blood Pb with a portable blood Pb analyzer, only 3% of birds had values indicating exposure and none of the birds had higher blood Pb levels or clinical signs of toxicity. Tissue distribution of Pb in liver, kidney, ovary and testes of rain quail (*Coturnix coramandelica*) following oral dosing of 0.5 mg Pb/kg, 1.25 mg Pb/kg or 2.5 mg Pb/kg Pb-acetate for 21 days indicated that Pb uptake was highest in liver and kidney and low in ovary and testes (Mehrotra et al., 2008). Resident feral pigeons (*Columba livia*) captured in the urban and industrial areas of Korea exhibited increased lung Pb concentration, ranging from 1.6 to 1.9 mg Pb/kg wet weight (Nam and Lee, 2006). However, tissue concentration did not correlate with atmospheric Pb concentration, so the authors concluded that ingestion of particulate Pb (paint chips, cement, etc.) in the urban and industrial areas was responsible for the pigeons' body burden. Similarly, 70% of American woodcock (*Scolopax minor*) chicks and 43% of American woodcock young-of-year collected in Wisconsin, U.S., exhibited high bone Pb levels of 9.6-93 mg Pb/kg dry weight and 1.5-220 mg Pb/kg, respectively, even though radiographs of birds' gastrointestinal tracts revealed no evidence of shot ingestion (Strom et al., 2005). Authors hypothesized that unidentified anthropogenic sources may have caused the observed elevated Pb levels.

In addition to birds, soil-dwelling mammals can also bioaccumulate atmospherically-sourced Pb. Northern pocket gophers (*Thomomys talpoides*) trapped within the Anaconda Smelter Superfund Site were shown to accumulate atmospherically deposited Pb. Gopher liver and carcass Pb concentration averaged 0.3 and 0.4 mg Pb/kg wet weight on low Pb soils (47 mg Pb/kg), 0.4 and 0.9 mg Pb/kg wet weight in medium Pb soils (95 mg Pb/kg) and 1.6 and 3.8 mg Pb/kg wet weight in high Pb soils (776.5 mg Pb/kg) (Reynolds et al., 2006). Likewise, rats trapped in the vicinity of a Kabwe, Zambia Pb-Zn mine had significantly elevated liver and kidney Pb concentrations. Soil Pb concentrations were measured between 9 and 51,188 mg Pb/kg (approximate average of 200 mg Pb/kg dry weight), while rat liver and kidney Pb concentrations ranged between 0.009 and 7.3 mg Pb/kg dry weight and 0.3 and 22.1 mg Pb/kg dry weight, respectively. Consequently, residence in the mining region was correlated to significantly increased Pb body burdens for rats (Nakayama et al., 2011). Angelova et al. (2010) reared rabbits on a fodder mixture containing lead-contaminated rapeseed grown adjacent to a metal works plant. Following a four week exposure, Pb was most heavily concentrated in rabbit kidney tissue (3.9 mg Pb/kg and 1.9 mg Pb/kg, for high and low diet respectively), bone (1.0 and 0.3 mg Pb/kg, respectively), and liver (0.6 and 0.4 mg Pb/kg, respectively). Yucatan micropigs (*Sus scrofa*) and Sprague-Dawley rats (*Rattus norvegicus*) reared on Pb-contaminated soil (5% of 1,000 µg Pb/g soil as dietary component) consumed significantly different amounts of Pb. Over a 30-day period, rats consumed an average of

19.4 mg Pb, while micropig intake averaged 948 mg Pb (Smith et al., 2009a). This resulted in significantly higher Pb accumulation in micropigs, based on liver, blood, kidney and bone Pb concentrations (average concentrations of 1.2, 25, 0.9, and 9 µg Pb/g for micropigs, and 0.2, 7, 0.5, and 1.5 µg Pb/g for rats, respectively).

Casteel et al. (2006) found that bioavailability of Pb from environmental soil samples in swine (*Sus domestica*) depended on Pb form or type, with high absorption of 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).

Consistent with observations in humans, 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 Pb/L (Dauwe et al., 2006). Liver and bone Pb concentration were increased by an approximate factor of three, while Pb concentration in kidney, muscle, and brain tissues were roughly doubled by a Ca-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 organism-level factors reviewed above, understanding the bioavailability of Pb along a simple food chain is essential for determining risk to terrestrial animals. While the bioavailability of ingested soil or particles is relatively simple to measure and model, the bioavailability to secondary consumers of Pb ingested and sequestered by primary producers and primary consumers is more complex. Kaufman et al. (2007) caution that the use of total Pb concentration in risk assessments can result in overestimation of risk to ecological receptors, and they suggest that the bioaccessible fraction may provide a more realistic approximation of receptor exposure and effects. This section reviews recent literature that estimates the bioaccessible fraction of Pb in dietary items of higher order consumers, and various studies suggesting that Pb may be transferred through the food chain but that trophic transfer of Pb results in gradual attenuation, i.e., lower concentration at each successive trophic level.

Earthworm and plant vegetative tissue collected from a rifle and pistol range that contained average soil Pb concentration of 5,044 mg Pb/kg were analyzed for Pb content and used to model secondary bioavailability to mammals (Kaufman et al., 2007).

Earthworms were determined to contain an average of 727 mg Pb/kg, and the Pb content of unwashed leaf tissues averaged 2,945 mg Pb/kg. Canonical correspondence analysis detected no relationship between earthworm and soil Pb concentration, but did show correlation between unwashed vegetation and soil concentration. The authors noted that the relatively high Pb concentration of unwashed as opposed to washed vegetation indicated the potential importance of aerial deposition (or dust resuspension) in determining total vegetative Pb concentration. Based on the mammalian gastric model, they noted that 50% of vegetation tissue Pb and 77% of earthworm tissue Pb was expected to be bioavailable to consumers. The avian gizzard model indicated that 53% of soil Pb and 73% of earthworm Pb was bioaccessible to birds, and, for both mammals and birds, the bioaccessible fraction of Pb was a function of total Pb concentration.

The transfer of Pb from soils contaminated by a Pb-Zn mine was limited along a soil-plant-insect-chicken food chain (Zhuang et al., 2009). In soils averaging 991 mg Pb/kg, plants of the fodder plant *Rumex patientia X tianschanicus* sequestered an average of 1.6 mg Pb/kg wet weight in the shoot tissue, while larvae of the leafworm *Spodoptera litura* accumulated an average Pb concentration of 3.3 mg Pb/kg wet weight *S. litura*-fed chickens (*Gallus gallus domesticus*) accumulated 0.58 mg Pb/kg and 3.6 mg Pb/kg in muscle and liver tissue, respectively, but only liver Pb burden was increased significantly relative to controls. A large proportion of ingested Pb was excreted with the feces. Likewise, 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*), elm leaf blackberry (*Rubus ulmifolius*), and grass (Graminae) tissues collected from mining areas exhibited increased Pb levels (up to 98 mg Pb/kg in grasses and 21 mg Pb/kg in oak) despite the fact that total soil Pb concentrations were not significantly greater than those of the non-mining areas.

Positive relationships were observed between *Cepaea nemoralis* snail tissue Pb levels and Pb concentration measured in *Urtica dioica* leaves in field-collected samples from areas characterized by metal soil contamination (approximately 200 to 400 mg Pb/kg) (Notten et al., 2005). Inouye et al. (2007) found that several invertebrate prey of fence lizards, including *Acheta domestica* crickets, *Tenebrio molitor* beetles, and *P. scaber* isopods, accumulate Pb from dietary exposures (10, 50, 100, 250, 500, 750, and 1,000 mg Pb/kg) lasting between 44 and 72 days. By day 44, Pb body burdens of crickets were 31, 50 and 68 mg Pb/kg (wet weight) at the three highest dietary exposures, respectively. Isopods and beetle larvae accumulated significantly less Pb, with average body burdens of 10, 15, and 14 mg Pb/kg following 56 days of exposure, and 12, 14, and 31 mg Pb/kg following 77 days of exposure, respectively. For all invertebrates tested, Pb was sequestered partly in the exoskeleton, and partly in granules. Exoskeleton Pb may be available to predators, but returns to background level with each shedding, while granular Pb is likely unavailable, at least to other invertebrates (Vijver et al., 2004).

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.

7.2.4 Biological Effects

Various effects can be observed in exposed terrestrial species following uptake and accumulation of Pb. While many of the responses are specific to organism type, induction of antioxidant activities in response to Pb exposure has been reported in plants, invertebrates, and vertebrates. 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 Terrestrial Plants and Lichen

Pb exposure has been linked to decreased photosynthesis in affected plants, significant induction of antioxidant activities, genetic abnormalities, and decreased growth. Induction of antioxidant responses in plants has been shown to increase tolerance to metal soil contamination, but at sufficiently high levels, antioxidant capacity is exceeded, and metal exposure causes peroxidation of lipids and DNA damage, eventually leading to accelerated senescence and potentially death (Stobrawa and Lorenc-Plucinska, 2008).

Effects on Photosystem and Chlorophyll

Photosynthesis and mitosis were recognized as targets of Pb toxicity in plants in the 1977 Pb AQCD and additional effects of Pb on these processes were reported in subsequent Pb AQCDs (U.S. EPA, 2006c, 1986b, 1977). The effect of Pb exposure on the structure and function of plant photosystem II was recently studied in giant duckweed, *Spirodela polyrrhiza* (Ling and Hong, 2009). Although this is an aquatic plant, photosystem II is present in all plants. This finding thus provides support for effects on photosystem II

being the cellular-level mechanism that leads to decreases photosynthesis observed in other plants. The Pb concentration of extracted photosystem II particles was found to increase with increasing environmental Pb concentration, and increased Pb concentration was shown to decrease emission peak intensity at 340 nm, amino acid excitation peaks at 230 nm, tyrosine residues, and absorption intensities. This results in decreased efficiency of visible light absorption by affected plants. The authors theorized that Pb^{2+} may replace either Mg^{2+} or Ca^{2+} in chlorophyll or the oxygen-evolving center, inhibiting photosystem II function through an alteration of chlorophyll structure. Consistently with these results, Wu et al. (2008c) 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. Similarly, the photosynthetic rate of maize (*Zea mays*) seedlings decreased over 21 days exposure to Pb, and measured leaf Pb concentrations in photosynthetically-depressed seedlings ranged from approximately 0.1 to 0.3 mg Pb/g dry weight (Ahmad et al.). Liu et al. (2010a) observed that chlorophyll *a* and *b* content in wheat grown in soils spiked with Pb nitrate rose with length of exposure until 14 days, at which point chlorophyll decreased. At 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 and Tripathi, 2008). Changes in chlorophyll content in response to Pb were also observed in lichen and moss species following exposures intended to simulate atmospheric deposition (Carreras and Pignata, 2007). *Usnae 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. It should be noted that highly productive *Sphagnum* mosses accumulated atmospheric lead at the same rate as slower growing mosses, indicating that moss growth allowed for further lead uptake, rather than a "dilution" effect (Kempter et al., 2010). As compared to other metals, however, Pb caused less physiological damage, which the authors attributed to the metal's high affinity for binding to and sequestration within cell walls (Carreras and Pignata, 2007).

The effect of Pb exposure on chlorophyll content of the moss and liverwort species *Thuidium delicatulum*, *T. sparsifolium*, and *Ptychanthus striatus* was investigated following immersion in six solutions of Pb Nitrate containing from 10^{-10} to 10^{-2} M Pb (Shakya et al., 2008). Both chlorophyll *a* and total chlorophyll content of the mosses (*T. delicatulum* and *T. sparsifolium*) decreased with increasing Pb exposure. For the liverwort, increasing Pb exposure resulted in decreases in content of chlorophyll *a*, chlorophyll *b*, and total chlorophyll. Further, the total chlorophyll content of *Hypnum*

plumaeforme mosses was decreased by 5.8% following exposure to 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 from 20 mg Pb/kg soil to 2,000 mg Pb/kg have been demonstrated in broad bean (*Vicia faba*) (Wang et al., 2010c; Wang et al., 2010b; Wang et al., 2008b) and tomato (*Lycopersicon esculentum*) (Wang et al., 2008a), where they were accompanied up to approximately 500mg Pb/kg by proportional increases in superoxide dismutase (SOD), glutathione, guaiacol peroxidase, and lipid peroxidation, as well as decreases in catalase. Spinach seedlings grown in soil containing six increasing concentrations of Pb from 20 to 520 mg Pb /kg exhibited higher production of reactive oxygen species, increased rates of lipid peroxidation and increased SOD concentrations. Many of these responses persisted for 50 days after germination and growth in the Pb-contaminated soil (Wang et al., 2011a). Similarly, the bryophyte mosses *Hypnum plumaeforme*, *Thuidium cymbifolium*, and *Brachythecium piligerum* exposed to Pb solutions of greater than 0.1 mM Pb for 48 hours exhibited increased production of $\bullet O_2^-$ and H_2O_2, although no single moss species could be identified as most sensitive to Pb exposure (Sun et al., In Press). Increased rates of lipid peroxidation were also observed in Pb-exposed mosses; however, SOD and catalase activity was suppressed or unaffected by Pb.

Reddy et al. (2005) found that horsegram (*Macrotyloma uniflorum*) and bengalgram (*Cicer arietinum*) plants 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., 2010a) grown in soils spiked with Pb nitrate. Evidence of increasing lipid peroxidation (MDA accumulation) with increasing Pb exposure was also found in mosses (Sun et al., 2009) and lichens. Lichens field-collected from the trunks of poplar (*Populus tremula*) trees in eastern Slovakia were chemically analyzed for metal concentration arising from exposure to smelter pollution (Dzubaj et al., 2008). These concentrations (ranging from 13 to 1,523 mg Pb/kg dry weight) were assessed in relation to physiological variables, including chlorophyll *a* and *b*, carotenoids, photosystem II activity, CO_2 gas exchange (respiration), and MDA content. Lichen Pb levels were significantly correlated only with MDA content. Determination of plant chitinase content following exposure to As, Cd and Pb indicated that while levels of these defense proteins were elevated by As and Cd, chitinase levels were not increased following exposure to Pb (Békésiová et al., 2008).

Growth

Evidence of effects of Pb on higher growth processes in terrestrial plants was reported in early NAAQS reviews. Growth effects of Pb on plants in the 1977 Pb AQCD primarily included visible growth responses observed in laboratory studies with plants grown in artificial nutrient culture (U.S. EPA, 1977). No Pb toxicity was observed in plants growing under field conditions at the time of the 1977 Pb AQCD. Indirect effects of Pb on plant growth (i.e., inhibition of uptake of other nutrients when Pb is present in the plant) were also reported in the 1977 Pb AQCD. In the 1986 Pb AQCD mechanisms of Pb effects on growth included reduction of photosynthetic rate, inhibition of respiration, cell elongation, root development or premature senescence (U.S. EPA, 1986b). All of these effects were observed to occur in isolated cells or in plants grown hydroponically in solutions comparable to 1 to 2 µg Pb/g soil or in soils with 10,000 mg Pb/kg or greater (U.S. EPA, 1986b). Pb effects on other plant processes, especially maintenance, flowering and hormone development had not been studied at the time of the 1986 Pb AQCD and remain poorly characterized.

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, when smelter ash–spiked soils containing 1,466 mg Pb/kg (and 18.6 mg Cd/kg) or 7,331 mg Pb/kg (98.0 mg/kg Cd) were used to grow maize (*Zea mays*), effects were seen in growth or chlorophyll production only at the higher concentration (Komarek et al., 2009) . Given the low solubility of smelter ash, these observations are consistent with solubility being a key determinant of bioavailability. Similarly, wheat seedling growth was unaffected when exposed to soil leachate containing up to 686 µg Pb/L for six weeks. Lettuce seedling root growth was negatively correlated to leachate Pb concentration, but this correlation was only significant for week 3 and week 6 measurements. Authors concluded that although the total concentrations of multiple metals in tested soils and leachates exceeded Canadian Environmental Quality Guidelines, no toxic or only slightly toxic effects occurred following exposure to the metal mixture (Chapman et al., 2010). Further, 14-day growth bioassays conducted with lettuce seedlings (*Latuca sativa*) and field-collected Pb-arsenate contaminated soil produced an unbounded NOEC value of 390 mg Pb/kg (and 128 mg As/kg) (Delistraty and Yokel, In Press).

Chinese cabbage (*Brassica pekinensis*) exposed to Pb-containing soils exhibited depressed nitrogen assimilation as measured by shoot nitrite content, nitrate reductase activity, and free amino acid concentration (Xiong et al., 2006). The authors planted germinated cabbage seeds in soils spiked with Pb-acetate to give final soil concentrations of 0.2, 4, and 8 mM Pb/kg dry weight total Pb and collected leaf samples for 11 days. At exposures of 4 and 8 mM Pb/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.

Nitrogen, potassium, and phosphorus concentrations in the shoot and root tissues of four canola cultivars (*Brassica napus*) also decreased as spiked soil Pb concentrations increased from 0 to 90 mg/kg. At the highest soil Pb concentration, nitrogen concentrations were reduced 56% in roots and 58% in shoots versus control levels, while phosphorous concentrations were reduced 37% and 45%, respectively, and potassium content decreased by 42% in both tissues (Ashraf et al., 2011). Cultivation in Pb-spiked soils was also linked to decreased shoot and root biomass (32% and 62%, respectively at 90 mg Pb/kg).

Genetic and Reproductive Effects

Exposure to Pb also resulted in genetic abnormalities, including bridges, condensed bivalents, and laggards, in the meiotic cells of pea plants (*Lathyrus sativus*) (Kumar and Tripathi, 2008). Seeds were germinated in soils amended with Pb nitrate at concentrations of 25, 50, 100, 200, and 300 mg Pb/kg, and concentrations of 100 mg Pb/kg and greater were found to be genotoxic or detrimental to pea viability. Cenkci et al. (2010) exposed fodder turnip (*B. rapa*) to 0.5 to 5 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.

Two genotypes of maize seedlings exhibited a significant and concentration-dependent reduction in seed germination following 7 days of Pb treatment in nutrient solution of 10, 100 and 1,000 µg Pb/L as Pb sulfate (Ahmad et al.). 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 and Tripathi, 2008). Plants exposed to Pb exhibited decreased growth, curling and chlorosis of young leaves, and decreased root growth. In addition, Gopal and Rizvi (2008) showed that Pb exposure increased uptake of phosphorus and iron and decreased sulfur concentration in radish tops.

Interestingly, as in zebra finch (Section 7.2.3.3) Ca was found to moderate the effects of Pb in both monocotyledon and dicotyledon plant seedlings, with tomato (*Lycopersicon esculentum*), rye (Lolium sp.), mustard, and maize plants exhibiting increased tolerance to Pb exposures of 5, 10, and 20 mg Pb/L in the presence of Ca concentration of 1.2 mM and higher (Antosiewicz, 2005).

7.2.4.2 Terrestrial Invertebrates

Exposure to Pb also causes antioxidant effects, reductions in survival and growth, as well as decreased fecundity in terrestrial invertebrates as summarized in the 2006 Pb AQCD. In addition to these endpoints, recent literature also indicates that Pb exposure can cause significant neurobehavioral aberrations, and in some cases, endocrine-level impacts. Second-generation effects have been observed in some invertebrate species.

The morphology of γ-aminobutyric acid (GABA) motor neurons in *Caenorhabditis elegans* nematodes was affected following exposure to Pb nitrate for 24 hours (Du and Wang, 2009). The authors determined that exposures as low as 2.5 µM Pb nitrate 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 et al., 2009c). Neural degeneration, as demonstrated by dorsal and ventral cord gaps and neuronal loss was also more pronounced in young larval *C. elegans* than in older larvae and adults (Xing et al., 2009b). *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 (Wang and Xing, 2008). Chemotaxis toward NaCl, cAMP, and biotin was also decreased in *C. elegans* nematodes exposed to Pb concentration greater than 2.5 µM (Xing et al., 2009a). This evidence suggests that Pb may exert neurotoxic action in invertebrates as it does in vertebrates. However, it is unclear how these behavioral aberrations would affect fitness or survival (Wang and Xing, 2008).

In a study of *C. elegans* exposed to 4 sub-lethal concentrations of Pb nitrate between 25 and 100 µ*M*, Vigneshkumar et al. (In Press) observed upregulation of both catalase and antimicrobial response-related genes. When challenged with addition of a pathogenic strain of *Pseudomonas aeruginosa*, exposed *C. elegans* showed greater resistance to microbial colonization than controls.

Younger individuals also appear to be more sensitive to the reproductive effects of Pb exposure. Guo et al. (2009) showed that concentrations of 2.5, 50, and 100 µM Pb 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 (Wang and 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. Reproductive effects of Pb exposure were also observed in springtails *F. candida* following 10 day exposure to Pb-spiked soils. Egg hatch significantly decreased at concentrations of 1,600 mg Pb/kg dry soil and higher and the EC_{50} for hatching was 2,361 mg Pb/kg dry soils (Xu et al., 2009a).

E. andrei earthworms exposed to 21 different soils, each containing 2,000 mg Pb/kg freshly added Pb, for 28 days exhibited highly variable mortality, ranging from 0% to 100%, (Bradham et al., 2006). Pb body burden of exposed worms ranged from 29 to 782 mg Pb/kg. Internal Pb concentration was also negatively correlated to reproductive

output. CEC and pH were found to be the principal soil characteristics determining the differences in those effects, although the apparent role of CEC may only have been due to its correlation with other soil characteristics. Low soil Pb concentration (5 mg Pb/kg) also decreased the protein content of *E. fetida* earthworms during a 7-day exposure (Li et al., 2009b). Higher Pb concentration had no effect on protein production. However, cellulase activity was increased by the 7-day exposures to Pb at all exposure concentrations (31%, 13%, and 23% of control activity at exposures of 5, 50, and 500 mg Pb/kg, respectively), which the authors reported as an indication of detrimental effects on worm metabolism. By contrast, Svendsen et al. (2007) found that *L. rubellus* earthworms exposed for 42 days to field-collected smelter-polluted soils containing average Pb concentration of 106, 309, and 514 mg Pb/kg dry weight exhibited normal survival and cocoon production rates, even though they accumulated more Pb with increased environmental concentration. The much smaller effect may be explained by the increased aging time undergone by field soil, causing a larger fraction of the total Pb to be complexed and sequestered by organic and inorganic compounds. Similarly, earthworms (*E. fetida*) exposed to field-collected soils with concentrations of Pb and As up to 390 mg/kg and 128 mg/kg, respectively, due to historical treatments of lead-arsenate pesticides, exhibited no change in survival, behavior or morphology (Delistraty and Yokel, In Press). Soil aging (e.g., from of the time of Pb-arsenate applications in 1942 to soil collection in approximately 2009) likely reduced Pb bioavailability to earthworms.

As in plants, induction of metal chelating proteins and antioxidant activity in invertebrates is affected by exposure to Pb. Metallothionein production in earthworms (*Lampito mauritii*) was significantly induced following exposure to Pb-contaminated soil. Tissue metallothionein levels increased after a two week exposure to 75 to 300 mg Pb/kg soil, although by 28 days levels had begun to decrease, perhaps as a result of Pb toxicity (Maity et al., 2011). Further, the induction of antioxidant activity was correlated to standard toxicity measurements in *Theba pisana* snails (Radwan et al., 2010). Topical application of Pb solutions (estimated to be 500 to 2,000 µg Pb per animal) to snails resulted in decreased survival, increased catalase and glutathione peroxidase activities, and decreased glutathione concentration. The 48-hour LD_{50} concentration was determined to be 653 µg per snail, as measured in digestive gland tissue. Snail glutathione content was decreased at exposures of 72.2% of the 48-hour LD_{50} value, while Pb exposure at 40% of the 48-hour LD_{50} value induced catalase and glutathione peroxidase activities.

Dietary exposure to Pb also affected *T. pisana* snail growth. After three weeks on Pb-contaminated diet, snail feeding rates were depressed by all Pb exposures (50 to 15,000 µg Pb/g diet dry weight) (El-Gendy et al., 2011). A five week dietary exposure to 1,000 µg Pb/g and greater resulted in reduced snail growth. Decreased food consumption,

growth, and shell thickness were also observed in juvenile *A. achatina* snails exposed to Pb-contaminated (concentration greater than 134 mg Pb/kg) diet for 12 weeks (Ebenso and Ologhobo, 2009a) . A similar depression of growth was observed in sentinel juvenile *A. achatina* snails deployed at Pb-polluted sites in the Niger Delta region of Nigeria. Although snail mortality was not increased significantly by exposure to soil Pb up to 1,200 mg Pb/kg, a concentration-dependent relationship was established for growth, with significant reduction observed at 12-week exposures to 20 mg Pb/kg (Ebenso and Ologhobo, 2009b). However, consumption of field-collected Pb-polluted *U. dioica* leaves containing 3 mg Pb/kg stopped all reproductive output in *C. nemoralis*. Snails also exhibited diminished food consumption rates when offered leaves with both low (1.5 mg Pb/kg) and high Pb content, but the mechanism of the dietary aversion was not defined (Notten et al., 2006).

Chronic dietary exposure to Pb was also examined in post-embryonic oribatid mites (*Archegozetes longisetosus*) (Kohler et al., 2005). Both algae and bark samples were soaked in 100 mg/L Pb as Pb nitrate and provided as diet and substrate, respectively, to larval mites. In addition to elevated heat shock proteins (hsp70), 90.8% of the protonymphs exhibited significant leg deformities, including abnormal claws, shortened and thickened legs, and translocated setae. Although not specifically discussed, it is very likely that these deformities would decrease mite mobility, prey capture, and reproductive viability. While there is some evidence that oribatid mites exhibit Pb avoidance behavior, this response may not significantly reduce Pb exposure and effects. Although soil-inhabiting mites (*Oppia nitens*) were observed to avoid high Pb concentrations, the EC_{50} for this behavior was approximately five times higher than the chronic EC_{50} for reproduction (8,317 and 1,678 mg Pb/kg, respectively) (Owojori et al., 2011). Consequently, it is unlikely that oribatid mites will avoid soils containing toxic Pb concentrations.

Lock et al. (2006) compared the toxicity of both laboratory-spiked soils and field-collected Pb-contaminated soils to springtails (*F. candida*). The 28-day EC_{50} values derived for *F. candida* ranged from 2,060 to 3,210 mg Pb/kg in leached and unleached Pb-spiked soils, respectively, whereas field-collected soils had no significant effect on springtail reproduction up to (but not including) 14,436 mg Pb/kg (Lock et al., 2006). Consequently, leaching soils prior to use in bioassays had only a slight effect on Pb toxicity to resident springtails, and did not provide an appropriate model for field-weathered, Pb-contaminated soils. This indicates that physiochemical factors other than leaching may be more important determinants of Pb bioavailability. A 4-week exposure to Pb-amended soils containing up to 3,200 mg Pb/kg had no significant effect on *Sinella curviseta* springtail survival or reproduction (Xu et al., 2009b).

Carabid beetles (*Pterostichus oblongopunctatus*) inhabiting soils contaminated by pollution from a Pb-Zn smelter (containing 136 to 2,635 mg Pb/kg) were field-collected and then laboratory-reared for two generations (Lagisz and Laskowski, 2008). While fecundity was positively correlated to soil metal concentration (e.g., more eggs were produced by females collected from contaminated areas), the hatching rate of eggs diminished with increasing soil metal contamination. For the F1 generation, females produced by parents inhabiting highly polluted areas exhibited decreased body mass. The authors stated that these results indicate that invertebrates inhabiting metal- (or Pb-) contaminated soils could face "significantly altered life-history parameters." Similarly, aphids (*Brevicoryne brassicae*) reared on cabbage and radish plants exposed to 0.068 mg Pb daily exhibited altered development and reproduction when compared to those reared on non-exposed plants. Development time was increased by approximately two days, which led to a reduction in relative fecundity (Gorur, 2007). Although the authors noted that study exposures were greater than what would be expected in naturally polluted areas, Pb exposure under field conditions could alter invertebrate life history patterns.

Several studies suggest that Pb may disrupt hormonal homeostasis in invertebrates. Shu et al. (2009) reported that vitellogenin production in both male and female *S. litura* moths was disrupted following chronic dietary exposure to Pb. Adult females reared on diets containing 25, 50, 100, or 200 mg Pb/kg exhibited decreased vitellogenin mRNA induction, and vitellogenin levels 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 and Li, 2009). Common deformities were swollen head and head helices, while head bending was also recorded in some cases. These deformities were observed following exposures to concentration below the 14-day LC_{50} (3,207 mg Pb/kg) and below the concentration at which weight was diminished (2,800 mg Pb/kg). Experimentation with the model organism Drosophila indicates that Pb exposure may increase time to pupation and decrease pre-adult development, both of which are endocrine-regulated (Hirsch et al., 2010).

7.2.4.3 Terrestrial Vertebrates

Pb poisoning is one of the earliest recognized toxicoses of terrestrial vertebrates, occurring primarily through the ingestion of spent shot by birds. While the focus of the ISA is on more environmentally relevant exposures, studies of Pb poisoning provide historical context for the review. The widespread nature of this toxicosis was first noticed

in American waterfowl around the turn of the last century (see (Jones, 1939) for an historical summary). Wetmore (1919) demonstrated that Pb shot caused the observed effects and described in detail the species affected, associated symptoms, and additional factors involved. By 1959, the estimated annual loss of waterfowl to Pb poisoning was 2−3 percent of the fall population (Bellrose, 1959). Smaller numbers of shorebirds and upland game birds were also found poisoned by Pb (Locke and Thomas, 1996).

The first reported Pb poisoning of a bald eagle (*Haliaeetus leucocephalus*) was described by Mulhern et al. (1970), and subsequently several hundred bald eagle lead poisonings were diagnosed throughout the U.S. prior to the ban on use of Pb shot for waterfowl hunting (Kramer and Redig, 1997). Eagles and other raptors are poisoned by consuming Pb pellets imbedded in the flesh of ducks or upland prey species and may also be exposed to other sources of Pb, such as fishing sinkers and weights (Kramer and Redig, 1997). The use of Pb shot for waterfowl hunting was banned in 1991 due to the poisoning of bald eagles, which had been previously added to the endangered species list and were specially protected under the Bald Eagle Protection Act of 1940.

Anderson et al. (2000) reported that by 1997, mallard (*Anas platyrhynchus*) deaths from Pb poisoning in the Mississippi flyway were reduced by 64 percent, and ingestion of toxic pellets had declined by 78 percent. They estimated the ban prevented approximately 1.4 million duck deaths in the first 6-year period. However, Pb exposure remains widespread in bald eagles, although blood lead concentrations have significantly decreased (Kramer and Redig, 1997). The endangered California condor (*Gymnogyps californianus*) also continues to have significantly elevated blood Pb levels as well as Pb-associated mortality resulting from exposure to ammunition fragments contained in food items (Cade, 2007; Church et al., 2006). Although there is a significant amount of information on Pb tissue residues of mammals, there are very few reports of Pb poisoning; exceptions are reports of Pb poisoned bats in a cave in the southern U.S. and small mammals in the vicinity of several smelters (Shore and Rattner, 2001).

At the time of the 1977 Pb AQCD few studies of the effects of exposure to Pb had been conducted in wild animals other than birds, and the majority of those studies were of direct poisoning (U.S. EPA, 1977). Several studies of domestic animals grazing near Pb smelters indicated that horses are more susceptible than cattle to chronic Pb exposure although the findings were not conclusive due to the presence of other metals. Delta-aminolevulinic acid dehydratase (ALAD) was recognized as a sensitive indicator of Pb exposure in rats and waterfowl. In the 1986 Pb AQCD, additional effects of Pb on small mammals and birds were reported. According to the 2006 Pb AQCD, commonly observed effects of Pb on avian and mammalian wildlife include decreased survival, reproduction, and growth, as well as effects on development and behavior. More recent

experimental data presented here expand and support these conclusions, and also indicate that Pb can exert other effects on exposed terrestrial vertebrates, including alteration of hormones and other biochemical variables.

Since the 2006 Pb AQCD, there is additional evidence for hematological effects of Pb exposure in terrestrial vertebrates. Red-backed salamanders (*Plethodon cinereus*) exposed to Pb-amended soils (553, 1,700, 4,700, and 9,167 mg Pb/kg) by Bazar et al. (2010) exhibited lowered appetite and decreased white blood cell counts at the two highest concentrations, but tolerated field-collected, aged soils containing Pb concentrations of up to 16,967 mg Pb/kg with no significant deleterious effects. The white blood cell count of adult South American toads, (*Bufo arenarum)* was also decreased by weekly sublethal i.p. injections of Pb-acetate at 50 mg Pb/kg body weight (Chiesa et al., 2006). The toads also showed altered serum profiles and increased number of circulating blast cells. Final toad blood Pb levels were determined to be 8.6 mg Pb/dL, although it is unclear whether this is representative of Pb concentrations observed in field *B. arenarum* populations exposed to Pb. The authors suggested that, based on these findings, long-term environmental exposure to Pb could affect toad immune response. In western fence lizards (*S. occidentalis*), sub-chronic (60-day) dietary exposure to 10 to 20 mg Pb/kg per day resulted in significant sublethal effects, including decreased cricket consumption, decreased testis weight, decreased body fat, and abnormal posturing and coloration (Salice et al., 2009). Long-term dietary Pb exposures are thus likely to decrease lizard fitness.

Even in cases of high environmental Pb exposures, however, linking Pb body burdens to biological effects can be difficult. Pb 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 et al., 2006a). 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 times higher in the mining region than in reference areas during mining operations, and 1.7 higher five years after cessation of mining operations. The authors noted that Pb deposition in the mining region remained elevated even after mining operations ceased, and that stable Pb isotope analysis suggested that smelter Pb remained available to pied flycatcher through the transfer of historically deposited Pb in soil to prey items.

Berglund (Berglund et al., 2010) also analyzed ALAD activity in pied flycatchers at the later period, and found that it was 46% lower at the mine site. Beyer et al. (2004) observed that elevated blood Pb levels in several types of birds inhabiting the Tri-State Mining District (Oklahoma, Kansas, Missouri) were correlated with decreases in ALAD activity. Based on reduction in ALAD activity, robins (*Turdus migratorius*) were most sensitive to Pb exposure (35% reduction), followed by cardinals (*Cardinalis cardinalis*), waterfowl, and bobwhite quail (*Colinus virginianus*) (40%, 41%, and 56% reductions, respectively). Eagle owl (*Bubo bubo*) nestlings living in a historical mining area in Spain also exhibited elevated blood Pb levels (average 8.61 µg/dL as compared to an average reference area value of 3.18 µg/dL), and this was correlated to an approximate 60% reduction in ALAD activity (Gómez-Ramírez et al., 2011). Hansen et al. (2011a) determined that ground-feeding songbirds were frequently exposed to Pb within the Coeur d'Alene, ID mining region. Robins, in particular, were significantly likely to exhibit blood Pb levels in the clinical and severe clinical poisoning ranges (50 to 100 µg/dL and >100 µg/dL, respectively). Ingested soil Pb accounted for almost all of the songbirds' exposure to Pb, with Pb exposure correlated with estimated soil ingestion rates (20% for robins, 17% for song sparrows, and 0.7% for Swanson's thrushes, *Catharus ustulatus*). More than half of the robins and song sparrows from all contaminated sites and more than half of the Swainson's thrushes from highly contaminated sites showed at least 50% inhibition of ALAD. The highest hepatic Pb concentration of 61 mg/kg (dry weight) was detected in a song sparrow (Hansen et al., 2011a).

Blood Pb was significantly elevated in waterfowl in the Lake Coeur d'Alene areas of Blackwell Island and Harrison Slough (mean sediment concentrations of 679 and 3,507 mg Pb/kg dry weight, respectively). Twenty-seven percent of the waterfowl sampled in the Blackwell Island region had blood Pb concentrations suggestive of severe clinical poisoning (average concentration =0.17 µg Pb/g); in the Harrison Slough, 60% of sampled waterfowl had highly elevated blood Pb levels that exceeded the severe clinical poisoning threshold (average concentration= 2,2 µg Pb/g) (Spears et al., 2007). The level

of corticosteroid hormones in field populations of white stork nestlings (*Ciconia ciconia*) in a mining area affected by Pb and other metals was positively correlated with blood Pb levels (Baos et al., 2006). The effect was more pronounced for single nestlings than for multiple-chick broods. Surprisingly, average blood Pb levels in chicks inhabiting reference areas was 91 µg Pb/L (± 51), which was higher than blood Pb levels from the mining area (44 ± 34 µg Pb/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 i.p. injection of 100 mg Pb/kg body weight, to produce feather Pb concentration approximately equivalent to those observed in wild gulls. Pb-exposed gulls exhibited abnormal behaviors, including decreased walking and food begging, erratic behavioral thermoregulation, and diminished recognition of caretakers. Interestingly, subchronic exposure of Japanese quail (*Coturnix coturnix japonica*) to 5,000 and 50,000 µg Pb/L in drinking water caused an increase in their immune response. Exposed quail exhibited significantly lower rates of death or health effects (including septicemia, perihepatitis, and pericarditis among others) than control animals following infection with *E.coli*, and the incidence of infection-related effects was dependent on Pb exposure (Nain and Smits, 2011). These observations contrast with immunotoxicology results in mice reported in Section 5.6.4.1.

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 20,000 µg Pb/L 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 affect several mammalian species. Young adult rats reared on a diet containing 1,500 mg Pb/kg Pb-acetate for 50 days demonstrated less plasticity in learning than non-exposed rats (McGlothan et al., 2008), indicating that Pb exposure caused significant alteration in neurological function. Yu et al. (2005) showed that dietary Pb exposure affected both the growth and endocrine function of gilts (*S. domestica*). Consumption of 10 mg Pb/kg diet resulted in lower body weight and food intake after 120 days of dietary exposure; Pb exposure decreased final weight by 8.2%, and average daily food intake of Pb-exposed pigs was decreased by 6.8% compared to control intake. Additionally, concentration of estradiol, luteinizing hormone, and pituitary growth hormone were decreased (by 12%, 14%, and 27% versus controls, respectively), while blood Pb level was increased by 44% to an average 2.1 µg/dL. In cattle grazing near Pb-Zn smelters in India, blood Pb levels were positively

correlated with plasma levels of the thyroid hormones thyroxine (T4) and tri-iodothyronine (T3) and the hepatic biomarkers alanine transaminase and aspartate transaminase (Swarup et al., 2007). Total lipids, total protein and albumin levels were decreased in the same animals. Rodriguez-Estival et al. (2011) determined that red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) inhabiting a Pb-contaminated mining area in Spain exhibited increased liver and bone Pb concentrations (geometric means of 0.35 and 0.46 µg Pb/g for red deer, and 0.81 and 7.36 µg Pb/g for wild boar, respectively). These tissue concentrations were correlated to a significant decrease in red deer glutathione production, but corresponded to an increase in wild boar glutathione (Rodríguez-Estival et al., 2011). Authors proposed that the different antioxidant responses may be indicative of different Pb susceptibilities in the two species.

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 the red deer from the Pb-contaminated mining area of Spain studied by Rodriguez-Estival et al. (2011) was shown to be altered, with 11% and 15% reductions in spermatozoa and acrosome integrity observed in male deer from the mining area compared with those residing in reference areas (Reglero et al., 2009b).

7.2.5 Exposure and Response of Terrestrial Species

Evidence regarding exposure-response relationships and potential thresholds for Pb effects on terrestrial populations can inform determination of standard levels that are protective of terrestrial ecosystems. Given that exposure to Pb may affect biota at the organism, population, or community level, determining the rate and concentration at which these effects occur is essential in predicting the overall risk to terrestrial organisms. This section updates available information derived since the 2006 Pb AQCD, summarizing several dose-response studies with soil invertebrates. 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., 2009b). Five levels of Pb soil concentration were prepared for the acute 14-day study via spiking with Pb nitrate—0, 300, 711, 1,687, and 2,249 mg Pb/kg, while soil concentration of 0, 355, 593, 989, and 1,650 mg Pb/kg were used in chronic (56-day) earthworm bioassays. A 14-day acute LC_{50} of 2,490 mg Pb/kg was determined from the dose-response relationship, while the approximate 56-day NOEC (no observed effect concentration) and EC_{50} values were about 400 mg Pb/kg and 1,000 mg Pb/kg, respectively. Currie et al. (2005) observed mortality of *E. fetida* after 7 and 14 days in spiked field soil at seven levels of Pb (0 to 10,000 mg Pb/kg). They reported LC_{50} values of 2,662 mg Pb/kg at 7 days and 2,589 mg Pb/kg at 14 days or 2,827 mg Pb/kg at both 7 and 14 days, depending on the number of worms in the experimental enclosure.

Other studies have shown no correlation between Pb concentration in either earthworm tissue or soil, and earthworm survival rate. Although the Pb content of *E. fetida* held in metal-contaminated soils containing between 9.7 and 8,600 mg Pb/kg was positively correlated with Pb concentration of fully aged soil collected from disused mines, there was no statistical relationship with earthworm survival during a 42-day exposure period (Nahmani et al., 2007). However, Pb concentration in soil leachate solution was significantly correlated with decreased earthworm survival and growth (linear regression: $R^2 = 0.64$, $p < 0.0001$). The 42-day Pb EC_{50} for *E. fetida* growth was 6,670 mg Pb/kg.

Langdon et al. (2005) exposed three earthworm species (*E. andrei*, *L. rubellus*, and *A. caliginosa*) to Pb nitrate-amended soils at concentrations of 1,000 to 10,000 mg Pb/kg to determine species variability in uptake and sensitivity. Twenty-eight-day LC_{50} values for the three species were 5,824 mg Pb/kg, 2,867 mg Pb/kg, and 2,747 mg Pb/kg, respectively, indicating that *L. rubellus* and *A. caliginosa* are significantly more vulnerable to Pb contamination than *E. andrei*, a common laboratory species. This is comparable to previous findings by Spurgeon et al. (1994) who reported 14-day LC_{50} of 4,480 mg Pb/kg and 50-day LC_{50} of 3,760 mg Pb/kg for *E. fetida*, another standard laboratory test species. In the more recent study of *E. fetida* sensitivity summarized above, Jones (2009b) reported LC_{50} values for *E. fetida* that are similar to those for *L. rubellus* and *A. caliginosa*. It is likely that these apparent species differences are a result of differential bioavailability of the Pb in test soils. However, the Pb body burden of all

three species in the study by Langdon et al. (2005) increased with increasing environmental concentration, and there were no species differences in Pb tissue content. When given a choice between treated and untreated soils, all worm species exhibited significant avoidance of Pb-contaminated soils, and altering pH (and, consequently, Pb bioavailability) had no impact on avoidance (Langdon et al., 2005). Field earthworms may thus be able to reduce their exposure to Pb through behavior.

Reproductive success of other soil invertebrates is impacted by Pb. The organismal and population-level responses of the springtail *Paronychiurus kimi* to Pb were determined by Son et al. (2007) using artificial soils, following the 1999 ISO methodology. The 7-day Pb LC_{50} was determined to be 1,322 mg Pb/kg dry weight, while the 28-day reproduction EC_{50} was established as 428 mg Pb/kg. The intrinsic rate of population increase was lower at a Pb soil concentration of 1,312 mg Pb/kg, and the authors estimated that, at this level, *P. kimi* populations would be extirpated. The authors noted that, in this case, the reproductive endpoint overestimated the population-level risk for *P. kimi* springtails exposed to Pb, and proposed that more specific measures of population-level endpoints (such as the reduction in intrinsic rate of increase) be used to determine risk to populations. Menta et al. (2006) showed that a nominal soil concentration of 1,000 mg Pb/kg decreased the reproductive output of two collembolans, *Sinella coeca* and *F. candida*. Pb 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. The hatching success of *F. candida* eggs was diminished by 10 day exposure to Pb-spiked soils; the 10-day EC_{50} for hatching success was reported as 2,361 mg/kg Pb (Xu et al., 2009d). However, authors noted that egg development was more sensitive to Cu and Zn exposure, and by comparison, was less susceptible to Pb.

In addition to species variability, physical and chemical factors affecting Pb bioavailability were also demonstrated to significantly influence the toxicity of Pb to terrestrial species. As noted previously in Section 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 BLM proposed by Smolders et al. (2009), have proven difficult to develop due to active physiological properties of soil organisms affecting either uptake (such as

root phytochelatins) or sequestration of Pb (such as granule formation in root tissues and earthworms, or substitution of Pb for calcium in bones).

7.2.6 Community and Ecosystem Effects

A study reviewed in the 1977 Pb AQCD provided evidence for Pb effects on forest-nutrient cycling and shifts in community composition. Reduced arthropod density, biomass and richness were observed in the vicinity of a smelting complex in southeastern Missouri where Pb, Cd, Zn and Cu were measured in the litter layer and soil (U.S. EPA, 1977; Watson et al., 1976). In the 1986 Pb AQCD it was reported that Pb at environmental concentrations occasionally found near roadsides and smelters (10,000 to 40,000 µg Pb/g dry weight [mg Pb/kg]) can eliminate populations of bacteria and fungi on leaf surfaces and in soil. At soil concentrations of 500 to 1,000 µg Pb/g (mg Pb/kg) or higher, populations of plants, microorganisms, and invertebrates may shift toward Pb-tolerant populations of the same or different species (U.S. EPA, 1986b).

According to the 2006 Pb AQCD, 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, 2006c). More recent literature explored the interconnected effects of Pb contamination on soil bacterial and fungal community structure, earthworms, and plant growth, in addition to impacts on soil microbial community function.

Inoculation of maize plants with *Glomus intraradices* arbuscular mycorrhizal fungi isolates decreased Pb uptake from soil, resulting in lower shoot Pb concentration and increased plant growth and biomass (Sudova and Vosatka, 2007). Similarly, Wong et al. (2007) showed that the presence of arbuscular mycorrhizal fungi improved vetiver grass (*Vetiveria zizanioides*) growth, and while Pb uptake was stimulated at low soil concentration (10 mg Pb/kg), it was depressed at higher concentration (100 and 1,000 mg Pb/kg). Bojarczuk and Kieliszewska-Rokicka (2010) found that the abundance of ectomycorrhizal fungi was negatively correlated with the concentration of metals, including Pb, in the leaves of silver birch seedlings. Arbuscular mycorrhizal fungi may thus protect plants growing in Pb-contaminated soils. Microbes too may dampen Pb uptake and ameliorate its deleterious effects: biomass of plants grown in metal-contaminated soils (average Pb concentration 24,175 mg Pb/kg dry weight) increased with increasing soil microbial biomass and enzymatic activity (Epelde et al., 2010). However, above certain Pb concentration, toxic effects on both plants and microbial

communities may prevent these ameliorating effects. R.Y. Yang et al. (2008b) found that both the mycorrhizal colonization and the growth of *Solidago canadensis* were negatively affected by soil Pb contamination. They suggested that, more generally, Pb-mediated alterations in plant-fungal dynamics may be the cause of ecological instability in terrestrial vegetative communities exposed to metals.

The presence of both earthworms and arbuscular mycorrhizal fungi decreased the mobility of Pb in mining soils undergoing phytoremediation (Ma et al., 2006). Inoculation with both earthworms and fungi increased plant growth at sites contaminated with mine tailings compared to that observed at sites with 75% less Pb contamination. Most likely, this was a result of the decrease in bioavailable (DTPA-extractable and ammonium acetate-extractable) Pb to 17% to 25% of levels in areas without the earthworm and arbuscular mycorrhizal fungi amendments. The presence of earthworms in metal-contaminated soils decreased the amount of water-soluble Pb (Sizmur and Hodson, 2008), but despite this decrease, ryegrass accumulated more Pb from earthworm-worked soils than soils without worms present. Sizmur and Hodson speculated that increased root dry biomass may explain the increased uptake of Pb in the presence of earthworms. However, Sizmur et al. (2011) found that the presence of anecic (deep-burrowing) earthworms (*L. terrestris*) increased soil leachate Pb concentrations by 190%. The authors observed that worms promoted a faster breakdown of organic matter, which caused a decrease in soil pH and a concurrent increase in Pb solubility. As a result, ryegrass (*L. perenne*) accumulated a greater amount of Pb in systems with earthworms (Sizmur et al., 2011). Further, the presence of earthworms (*Lumbricus terrestris*) was found to increase Pb concentrations in both maize and barley, although growth of these species was unaffected (Ruiz et al., 2011). Authors noted that worm activity increased Pb extraction yields by factors of 4.4 and 7.6, for barley and maize. By contrast, Coeurdassier et al. (2007) found that Pb was higher in earthworm tissue when snails were present, but that snails did not have a higher Pb content when earthworms were present.

Microbial communities of industrial soils containing Pb concentrations of 61, 456, 849, 1,086, and 1,267 mg Pb/kg dry weight were also improved via revegetation with native plants, as indicated by increased abundances of fungi, actinomycetes, gram-negative bacteria, and protozoa, as well as by enhanced fatty acid concentration (Zhang et al., 2006). Increased plant diversity ameliorated the effects of soil Pb contamination (300 and 600 mg Pb/kg) on the soil microbial community (Yang et al., 2007).

The effect of Pb on microbial community function has been quantified previously using functional endpoints such as respiration rates, fatty acid production, and soil acid phosphatase and urease activities, which may provide an estimate of ecological impacts separate from microbial diversity and abundance measurements. Most studies of metal-

induced changes in microbial communities have been conducted using mixtures of metals. However, Akerblom et al. (2007) tested the effects of six metals (Cr, Zn, Mo, Ni, Cd, and Pb) individually. All tested metals had a similar effect on the species composition of the microbial community. Exposure to a high Pb concentration (52 mg Pb/kg) also negatively affected respiration rates. Total phospholipid fatty acid content was determined to negatively correlate with increasing Pb exposure, indicating alteration of the microbial community. When Yang et al. (2006) compared the microbial properties of metal-contaminated urban soils to those of rural soils, significant differences were detected in basal community respiration rates and microbial abundance. The urban soils studied contained multiple metal contaminants, but microbial biomass was the only measured endpoint to be significantly and negatively correlated to Pb concentration. Similarly, the fungal community in a naturally Pb-enriched forest in Norway exhibited differences in community composition and abundance when compared with other, low Pb sites. The number of colony–forming fungal units was diminished by soil Pb, and was approximately 10 times lower in the highest Pb soil concentration (~4.5 mg/g dw). Further, only one fungus species was isolated from both high Pb and control soils, indicating highly divergent communities; species diversity was also reduced by high soil Pb concentrations (Baath et al., 2005). These studies suggest that anthropogenic Pb contamination may affect soil microbial communities, and alter their ecological function. However, (Khan et al., 2010c) reported that it is possible for indicators of microbial activity to recover after an initial period depression. (Khan et al., 2010c) found that following a 2-week exposure to three levels of Pb (150, 300, and 500 mg Pb/kg), the number of culturable bacteria at the highest exposure concentration tested was decreased. Acid phosphatase and urease levels (measures of soil microbial activity) decreased significantly, but they had recovered by the ninth week. Another study (Bamborough and Cummings, 2009) reported that no changes in bacterial and actinobacterial diversity in metallophytic soils containing 909 to 5,280 mg Pb/kg (43 to 147 mg Pb/kg bioavailable Pb (as defined by the study authors).Soil bacteria community structure and basal respiration rates were examined in natural soils with pH values ranging from 3.7 to 6.8 (Lazzaro et al., 2006). Six soil types of differing pH were treated with Pb nitrate concentrations of 0.5, 2, 8, and 32 mM. Basal respiration was decreased 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 contamination was also demonstrated to reduce phenol oxidase activity in several type of soils; concentrations between 5 and 50 nM Pb

significantly decreased phenol oxidase activity in all soils tested, while 400 nM and greater completely arrested phenol oxidase activity in one soil tested (a high pH sandy loam) (Carine et al., 2009). Carine (2009) suggested that the decreased soil enzymatic activity resulted from changes in the microbial community following Pb exposure. Pb concentrations between 50 and 500 mg Pb/kg significantly reduced microbial abundance and diversity, and also resulted in lower soil phosphatase, urease, and dehydrogenase activities (Gao et al., 2010b). Further, the weekly soil carbon dioxide evolution rate was significantly reduced by concentrations of 5, 10, and 50 mg Pb/g, which also indicated decreased microbial respiration and adverse effects on the microbial community (Nwachukwu and Pulford, 2011). Gai et al. (2011) examined the microbial activity of three soils via microcalorimetric methods following Pb exposure. They noted an increase in activity immediately following Pb application (giving 10, 20, 40, 80, and 160 µg Pb/g), and theorized that this was a result of rapid mortality of sensitive microbial species, followed by a concurrent proliferation of Pb-tolerant microorganisms. As Pb concentrations increased, however, the calculated microbial growth rate constant decreased, indicating a suppression of microbial activity (Gai et al., 2011). Authors also noted a strong correlation between microcalorimetry estimates and the number of colony forming units isolated from soil samples.

Pb exposure negatively affected the prey capture ability of certain fungal species. Nematophagous fungi are important predators of soil-dwelling nematodes, collecting their prey with sticky nets, branches, and rings. The densities of traps they constructed decreased in soils treated with 0.15 mM Pb chloride (Mo et al., 2008). This suppression caused a subsequent reduction in fungal nematode capturing capacity, and could result in increased nematode abundance.

In a study of microbial communities and enzyme activity, Vaisvalavicius et al. (Vaisvalavicius et al., 2006) observed that high concentration of soil metals were linked to a significant reduction in soil microorganism abundance and diversity. Soil columns spiked with Cu, Zn, and Pb-acetate (total Pb concentration of 278 to 838 mg Pb/kg, depending on depth) exhibited a 10- to 100-fold decrease in microbial abundance, with specific microbe classes (e.g., actinomycetes) seemingly more affected than others (Vaisvalavicius et al., 2006). Concurrently, decreases in soil enzymatic activity were also observed, with saccharase activity decreased by 57–77%, dehydrogenase activity by 95–98%, and urease activity 65–97%. Although this suggests that Pb contamination may alter the nutrient cycling capacity of affected soil communities, it is difficult to separate the impact of Pb in this study from the contributions of Cu and Zn that were also added. In contrast, Zeng et al. (2007) reported that soil concentrations of 300 mg Pb/kg and less stimulated soil enzymatic activity. Both urease and dehydrogenase levels were increased and rice dry weight was unaffected by concentrations of 100 and 300 mg Pb/kg.

However, at 500 mg Pb/kg, both rice and soil enzyme activities and microbial biomass were decreased suggesting impacts at the community level for the soil-rice system. The authors proposed that these concentrations could be considered the critical Pb concentration in rice paddy systems (Zeng et al., 2007).

The microbial communities of soils collected from a Pb-Zn mine and a Pb-Zn smelter were significantly affected by Pb and other metals (e.g., Cd) (Hu et al., 2007b). At a mine site, Pb concentration of 57 to 204 mg Pb/kg and Cd concentration of 2.4 to 227 mg Cd/kg decreased the number of bacteria-forming colonies extracted from soils. Principal component analysis of microbial community structure demonstrated that different communities were associated with different metal soil concentration. Similarly, soil microbial communities exposed to metal contamination from a smelter site (soil Pb concentration ranging from 30 to 25,583 mg Pb/kg dry weight) showed decreased bacterial functional diversity (although fungal functional diversity increased) and no effects on soil respiration rates were observed (Stefanowicz et al., 2008). This led the authors to conclude that bacterial diversity is a more sensitive endpoint and a better indicator of metal exposure than fungal diversity or microorganism activity. In a similar study, Kools et al. (2009) showed that soil ecosystem variables measured after a 6-month exposure to metal-contaminated soil indicated that Pb concentration (536 or 745 mg Pb/kg) was an important driver of soil microbial species biomass and diversity.

Pb-resistant bacterial and fungal communities were extracted regularly from soil samples at a shooting range site in southern Finland (Hui et al., 2009). While bioavailable Pb concentration averaged 100 to 200 mg Pb/kg as determined by water extraction, the total Pb concentrations measured on site were 30,000 to 40,000 mg Pb/kg. To determine Pb tolerance, bacterial colonies extracted and cultured from shooting range and control soils were grown on media containing either 0.4 or 1.8 mM Pb. While bacteria isolated from control soil did not proliferate on high-Pb media, shooting-range soil microbe isolates grew on high-Pb media and were deemed Pb tolerant. The authors noted that bacterial species common in control samples were not detected among the Pb-tolerant species isolated from shooting-range soils. They speculated that if long-term exposure to minimally bioavailable Pb can alter the structure of soil decomposer communities, decomposition rates could be altered. However, this would require that the microbial ecosystem decomposing function be altered along with structure, and the authors provided no evidence for alteration of function.

Microbial communities associated with habitats other than soils are also affected by exposure to atmospherically deposited Pb. Alder (*Alnus nepalensis*) leaf microorganism populations were greater in number at non-affected sites than at sites adjacent to a major Indian highway with increased Pb pollution (Joshi, 2008). The density, species richness,

and biomass of testate amoebae communities grown on *Sphagnum fallax* mosses were significantly decreased following moss incubation in Pb solutions of either 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 by a reduction of available earthworm biomass.

During the past 5 years, there has been increasing interest in the effects of Pb and other metals on the functional aspects of soil microbial communities. Most studies show that Pb decreases diversity and function of soil microorganisms. However, in an example of ecological mutualism, plant-associated arbuscular mycorrhizal fungi were found to protect the host plant from Pb uptake, while fungal viability is protected by the host plants. Similarly, soil microbial communities (bacterial species as well as fungi) in Pb-contaminated soils are improved by revegetation. A few studies have reported on effects of Pb to populations of soil invertebrates. They demonstrated that Pb can decrease earthworm population density, although not to levels that would result in local extinction. There have been no recently reported studies on the potential effects of Pb on terrestrial vertebrate populations or communities, or possible indirect effects through reduction of prey items such as earthworms.

7.2.7 Critical Loads in Terrestrial Systems

The general concept and definition of critical loads is introduced in Section 7.1.3 of this chapter [also see Section 7.3 of the 2006 Pb AQCD (U.S. EPA, 2006c)]. An international workshop was conducted in 2005 on the development of critical loads for metals and other trace elements (Lofts et al., 2007). Among the findings of the workshop it was reported that soil transport and transformation processes are key in controlling the fate of metals and trace elements, thus their importance in the input-output mass balance needs to be considered. The degree to which these processes are understood and can be

quantified varies. Complexation, sorption, ion exchange and precipitation are well understood in the lab, but to a lesser extent in the field (Lofts et al., 2007). Slower processes of weathering and fixation are less well understood or studied than leaching (Lofts et al., 2007).

As noted in previous section, soil pH and organic matter influence Pb availability. De Vries et al. (2007) demonstrate that critical limits, measured as critical reactive metal content, can significantly vary between soil types that differ in pH and organic matter. Critical limits of Pb increased from 30 to 64 (mg Pb/kg) over a pH range of 4-7 when soil organic matter content was 5%, while these limits increased from 187 to 400 (mg Pb/kg) over the same pH range when organic content was 80%. These implications suggest that critical limits increase with increasing soil organic matter. This has important consequences for forest soils because many are covered by an organic layer where roots, fungi and other microorganisms are located. Baath (1989) evaluated the effects of organic matter on critical limits for microorganisms, measured via enzyme synthesis, litter decomposition and soil respiration. Results indicate critical limits are up to four times higher in the organic (135 to 976 mg Pb/kg) than the mineral soil layer (32 to 690 mg Pb/kg) at hazardous concentration ranging from 5-50% of species. In general, De Vries et al. (2007) found support that ecotoxicological critical limits in European soils for Pb decrease with increasing pH.

Several methods are routinely used for Pb risk assessment of terrestrial animals. Buekers et al. (2009) proposed the use of a Tissue Residue Approach as a risk estimation method for terrestrial vertebrates that eliminates the need for quantitative estimation of food intake or Pb species bioavailability. Blood Pb no observed effect concentration (NOEC) and lowest observed effects concentration (LOEC) data derived from 25 studies examining the effects of Pb exposure on growth, reproduction, and hematological endpoints were used to construct a series of species sensitivity distributions for mammals and birds. They also used the HC5 criterion (5th percentile of species NOEC values for collection of species) proposed by Aldenberg and Slob (1993). For mammals, the HC5 values obtained ranged from 11 to 18 µg Pb/dL blood; HC5 values for birds ranged from 65 to 71 µg Pb/dL. The authors proposed the use of 18 and 71 µg Pb/dL as critical threshold values for mammals and birds respectively, which are below the lowest NOEC for both data sets used, and are above typical background Pb values. It is difficult to determine environmental Pb toxicity given the variation of physiochemical and soil properties that alter bioavailability and toxicity. This variability makes it difficult to extrapolate between areas. Furman et al. (2006) proposed the use of a physiologically based extraction test to predict risks posed to waterfowl from environmental Pb contamination. The extraction process was modeled after gastric and intestinal conditions of waterfowl, and was used to gauge the bioavailability of Pb from freshly amended and

aged contaminated soils. The concentration of Pb extracted through the use of the physiologically based extraction test was demonstrated to be significantly correlated to Pb tissue concentration in waterfowl exposed via in vivo studies of the same soils.

There are few critical loads for Pb reported for terrestrial ecosystems in the U.S.; however, work has been conducted in Europe. Given that local conditions (including historic loading, soil transport and transformation processes) are key elements to critical load calculation the utility of critical loads that are developed from other countries for application to U.S. ecosystems is unclear. The most recent European publications on Pb critical loads include assessments of the U.K., Netherlands and Italy. Hall et al. (2006) used the critical load approach to conduct a national risk assessment of atmospheric Pb deposition for the U. K. While specific regions were determined to have low critical load values for Pb (central England, 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 and Groenenberg, 2009). Similarly, although Pb soil concentrations in the Bologna Province of Italy were far below concentrations harmful to soil organisms, current atmospheric Pb deposition rates suggest that critical load exceedances are likely in the future, unless annual Pb emissions are decreased (Morselli et al., 2006).

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, 2006c). Preference is given to studies using the most bioavailable form of Pb, to derive conservative values. Soil concentration protective of avian and mammalian diets are calculated by first converting dietary concentration to dose (mg/kg body weight per day) for the critical study, then using food (and soil) ingestion rates and conservatively derived

uptake factors to calculate soil concentration that would result in unacceptable dietary doses. This frequently results in Eco-SSL values below the average background soil concentration [19 mg Pb/kg dry weight (U.S. EPA, 2005b, 2003b)], as is the case with Pb for birds. The Pb Eco-SSL was completed in March 2005 and has not been updated since. Values for terrestrial birds, mammals, plants, and soil invertebrates are 11, 56, 120, and 1,700 mg Pb/kg soil (dry weight), respectively.

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 Pb/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 Pb/kg Pb-spiked soil (Xu et al., 2009b). Mammalian NOEC values expressed as blood Pb levels were shown to vary by a factor of 8, while avian blood NOECs varied by a factor of 50 (Buekers et al., 2009). Age at exposure, in particular, may affect sensitivity to Pb. For instance, earlier instar *C. elegans* were more likely than older individuals to exhibit neurobehavioral toxicity following Pb exposure (Xing et al., 2009c), and also demonstrated more pronounced neural degeneration than older larvae and adults (Xing et al., 2009b).

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 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 (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, beech, and conifer forests. The Pine Barrens in southern New Jersey (also known as the Pinelands) is an example of a highly vulnerable ecosystem: it is a dense coniferous (pine) forest with acidic, sandy, nutrient poor soil. As agricultural areas are taken out of production and revert to old fields and eventually forests, their vulnerability to Pb is likely to increase as a result of decreasing OM and acidification of soils (from discontinuation of fertilizing and liming). On the other hand, increasing density of native or invasive plants with associated arbuscular mycorrhizal fungi will likely act to ameliorate some of the effects of Pb (see previous discussion of studies by Sudova and Vostka (2007) and Wong et al. (2007). It is, however, difficult to categorically state that certain plant or soil invertebrate communities are more vulnerable to Pb than others, as the available toxicity data have not yet been standardized for differences in bioavailability (because of use of different Pb salts, different soil properties, and different lengths of aging of soil prior to testing), nutritional state, or organism age, or other interacting factors. Data from field studies are complicated by the co-occurrence of other metals and alterations of pH, such as acidification from SO_2 in smelter emissions, which are almost universal at sites of high Pb exposure, especially at mine or smelter sites. However, because plants primarily sequester Pb in the roots, uptake by soil invertebrates is the most likely pathway for Pb exposure of higher trophic level organisms. Invertivores are likely at higher risk than herbivores. In fact, estimations of Pb risk at a former Pb smelter in northern France indicated that area Pb concentration presented the greatest threat to insectivorous bird and mammal species, but only minimal risk to soil invertebrate and herbivorous mammals (Fritsch et al., 2010). By extension, birds and mammals in ecosystems with a richer biodiversity of soil invertebrates may be more vulnerable to Pb than those in ecosystems with fewer invertebrates (e.g., arid locations). Regardless, the primary determinant of terrestrial ecosystem vulnerability is soil geochemistry, notably pH, CEC, and amount of OM.

7.2.10 Ecosystem Services

Pb deposited on the surface of, or taken up by organisms has the potential to alter the services provided by terrestrial biota to humans. There are no publications at this time that specifically focus on the ecosystem services affected by Pb in terrestrial systems. 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. Honeybees are important for provisioning services such as pollination and production of honey. They can be exposed to atmospheric Pb by direct deposition or through Pb associated with plants, water or soil. In a study of heavy metals in honeybees in central Italy, there was a statistically significant difference in Pb between bees collected in wildlife reserves compared to bees collected in urban areas with the highest concentration of Pb detected from bees caught in hives near an airport (Perugini et al., 2011). In a review of the effects of metals on insect behavior, ecosystem services provided by insects such as detritus reduction and food production for higher trophic levels were evaluated by considering changes in ingestion behavior and taxis (Mogren and Trumble, 2010). Pb was shown in a limited number of studies to affect ingestion by insects. Crickets (Chorthippus spp) in heavily contaminated sites reduced their consumption of leaves in the presence of increasing cadmium and Pb concentrations (Migula and Binkowska, 1993). Decreased feeding activity in larval and adult Colorado potato beetle (*Leptinotarsa decemlineata*) were observed as a result of dietary exposures of Pb and Cu (Kwartirnikov et al., 1999), while no effects were found in ingestion studies of Pb with willow leaf beetle, *Lochmaea caprae* (Rokytova et al., 2004) mottled water hyacinth weevil, *Neochetina eichhorniae* (Kay and Haller, 1986) and hairy springtail, *Orchesella cincta* (Van Capelleveen et al., 1986).

Soil health for agricultural production and other soil-associated ecosystem services is dependent upon the maintenance of four major functions: carbon transformations, nutrient cycles, soil structure maintenance, and the regulation of diseases and pests and

these parameters may be altered by metal deposition (Kibblewhite et al., 2008). Pb impacts to terrestrial systems reviewed in the previous sections provide evidence for impacts to supporting, provisioning, and regulating ecosystem services provided by soils. For example, earthworms were shown to impact soil metal mobility and availability, which in turn resulted in changes to microbial populations (biodiversity), pH, dissolved organic carbon, and metal speciation (Sizmur and Hodson, 2009), all of which may directly affect soil fertility.

Pb is bioaccumulated in plants, invertebrates and vertebrates inhabiting terrestrial and aquatic systems that receive Pb from atmospheric deposition. This represents a potential route for Pb mobilization into the food web or into food products. For example, Pb bioaccumulation in leaves and roots of an edible plant may represent an adverse impact to the provisioning of food, an essential ecosystem service. Although there is no consistent evidence of trophic magnification there is substantial evidence of trophic transfer. It is through consumption of Pb-exposed prey or Pb-contaminated food that atmospherically deposited Pb reaches species that may have very little direct exposure to it.

There is limited evidence of Pb impacts to plant productivity. Productivity of gray birch (*Betula populifolia*) was impaired in soils with elevated As, Cr, Pb, Zn and V (Gallagher et al., 2008). Tree growth measured in both individuals and at the assemblage level using satellite imagery and field spectrometry was significantly decreased with increasing metal load in soil.

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: Causal Determinations for Ecological Effects of Lead for a synthesis of all evidence dating back to the 1977 Pb AQCD considered in determining causality.

7.2.11.1 Biogeochemistry and Chemical Effects

The amount of Pb dissolved in soil pore water determines the impact of soil Pb on terrestrial ecosystems to a much greater extent than the total amount present. It has long been established that the amount of Pb dissolved in soil solution is controlled by at least six variables: (1) solubility equilibria; (2) adsorption-desorption relationship of total Pb with inorganic compounds; (3) adsorption-desorption reactions of dissolved Pb phases on soil OM; (4) pH; (5) CEC; and (6) aging. Since 2006, further details have been contributed to the understanding of the role of pH, CEC, OM, and aging. Smolders et al.

(2009) demonstrated that the two most important determinants of 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. Studies found that in trees, Pb that is taken up through the roots is then generally translocated from the roots to other parts. However, multiple new studies showed that in trees, the proportion of Pb that is taken up through the leaves is likely to be very substantial. One study attempted to quantify it, and suggested that 50% of the Pb contained in Scots Pine in Sweden is taken up directly from the atmosphere. 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.

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 Pb/kg wet weight Pb in the lungs, while in Wisconsin 70% of American woodcock chicks and 43 % of young-of-year had elevated bone Pb (9.6 to 93 mg Pb/kg dry weight in chicks, 1.5 to 220 mg Pb/kg dry weight in young-of-year). None of the locations for these studies was in proximity to 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 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 levels of biological organization include several studies of the ameliorative effects of mycorrhizal fungi on plant growth, attributed to decreased uptake of Pb by plants, although both mycorrhizal fungus and plant were negatively affected. The presence of both earthworms and mycorrhizal fungi decreased solubility and mobility of Pb in soil in one study, but the presence of earthworms was associated with higher uptake of Pb by plants in another. The presence of snails increased uptake of Pb by earthworms, but not vice-versa. Most recently published research on community and ecosystem effects of Pb has focused on soil microbial communities, which have been shown to be impacted in both composition and activity. Many recent studies have been conducted using mixtures of metals, but have tried to separate the effects of individual metals when possible. One study compared the effects of 6 metals individually (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

Over the longer term, terrestrial systems may be more affected particularly by those metals with a long soil residence time, such as Pb. 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.

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 within the U.S.

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, 2006c) on the effects of Pb on freshwater and marine ecosystems. Freshwater and marine/estuarine systems are considered separately due to differences in Pb speciation, bioavailability of Pb, and salinity as modifying factors for Pb toxicity. 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 lifestage (e.g., amphibians). Pb from atmospheric sources can be directly deposited over a water surface or enter aquatic systems through runoff from terrestrial systems. Pb loadings to aquatic ecosystems, especially freshwater systems, are primarily derived from the runoff and erosional transport from terrestrial systems (U.S. EPA, 2006c). Wet and dry deposition of Pb to land and water (Section 3.3.1.2), Pb in runoff (Section 3.3.2.4) and Pb in water and sediment (Section 3.3.2.3) are considered in Section 3.3 Fate and Transport of Pb. The flux of Pb in aquatic ecosystems is therefore influenced by the dynamic physical and chemical interactions within a watershed. Data on ambient Pb concentrations in rain, snow, natural waters, and sediment are summarized in Section 3.6.

7.3.2 Biogeochemistry and Chemical Effects of Pb in Freshwater and Saltwater Systems

Quantifying Pb speciation in aquatic environments is critical for determining the toxicity of the metal to aquatic organisms. As reviewed in the 2006 Pb AQCD 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 Fe and Mn oxides. For instance, in neutral to acidic aquatic environments, Pb is typically present as $PbSO_4$, $PbCl_4$, Pb^{2+}, cationic forms of Pb hydroxide, and ordinary hydroxide [$Pb(OH)_2$], 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 Ca and Mg 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. Sediment quality criteria have not been established, although the EPA has developed methods based on equilibrium partitioning theory to estimate sediment benchmarks for Pb and a few other metals (U.S. EPA, 2005d). The approach is based on the ratio of the sum of simultaneously extracted metals and amount of AVS, adjusted for the fraction of organic carbon present in the sediments, and is reviewed in detail in the 2006 Pb AQCD (U.S. EPA, 2006c). It is important to note that this method cannot accurately predict which sediments are toxic or which metal is the primary risk driver.

A more detailed understanding of the biogeochemistry of Pb in aquatic systems (both the water column and sediments) is critical to accurately predicting toxic effects of Pb to aquatic organisms. It should be recognized, however, that in addition to exposure via sediment and water, chronic exposures to Pb also include dietary uptake, even though the toxicokinetics of this exposure pathway are not yet well understood in aquatic organisms and the influence of the bioavailability factors described above is unknown. Furthermore, changes in environmental factors that reduce the bioaccessible Pb fraction can result in either sequestration in sediments or subsequent release as mobile, bioaccessible forms.

This section provides updated information about the influence of chemical parameters that affect Pb bioaccessibility in the aquatic environment (in sediments and the water column).

Several models are available for estimating the speciation of dissolved Pb. These models were tested by Balistrieri and Blank (2008) by comparing the speciation of dissolved Pb in aquatic systems affected by historical mining activities with that predicted by several models, including Windermere humic aqueous model (WHAM VI), non-ideal competitive absorption Donnan-type model (NICA-Donnan), and Stockholm humic model (SHM). Accurate prediction of labile Pb concentrations was achieved only with SHM, although other metal concentrations were better described by the WHAM model. Whereas both WHAM VI and NICA-Donnan predicted that the bulk of Pb contamination would be complexed with 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 and 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 and Batley, 2007). However, physical disturbance, pH change, and even the biota themselves also alter sediment binding or release of Pb. Atkinson et al. (2007) studied the effects of pH on sequestration or release of Pb from sediments. Although high and circumneutral water pH (8.1 and 7.2) did not affect the release of sequestered Pb from sediments, lowering the pH to 6 increased the concentration of Pb in overlying waters from less than 100 µg Pb/L to 200-300 µg Pb/L. Physical sediment disturbance also increased the amount of sediment-bound Pb released into the aqueous phase. When Pb-contaminated sediment was physically disturbed, the dissolved oxygen content of the overlying water was observed to significantly impact Pb mobilization, with greater Pb mobilization at lower dissolved oxygen levels (3 to 9 mg/L O_2) (Atkinson et al., 2007). In addition, although Pb concentrations in the sediments of a mine-impacted wetland in Hezhang, China, were determined to be strongly associated with organic/sulfide and residual fractions (e.g., 34 to 82% of total Pb), the presence of aquatic macrophytes altered the Pb speciation, increasing the fraction of Pb bound to Fe-Mn oxides (42% to 47% of total Pb) (Bi et al., 2007). This phenomenon was investigated in greater depth by Sundby et al. (2005), who determined that release of oxygen from

macrophyte roots resulted in the oxidation of sediment-bound Pb, leading to the release of bioaccessible Pb fractions (Sundby et al., 2005).

7.3.2.1 Other Metals

Multiple metals are present simultaneously in many aquatic environments and may interact with one another influencing Pb uptake and toxicity. Interactions of Pb with other metals were reviewed in the 2006 Pb AQCD and new evidence in this ISA supports previous findings of altered bioavailability associated with metal mixtures. Komjarova and Blust (2008) looked at the effect of the presence of Cd^{2+} on the uptake of Pb by the freshwater cladoceran *Daphnia magna*. While Pb uptake rates were not affected by Cu, Ni or Zn, enhanced Pb accumulation was observed in the presence of 0.2 µM Cd. The highest Pb 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). Evidence for the presence of Pb influencing the uptake of other metals was observed in the marine bivalves *Macomona liliana* and *Austrovenus stutchburyi*. Significantly, more Zn bioaccumulated in the presence of Pb in these mussels than with Zn alone following a 10-day exposure to spiked sediments (Fukunaga and Anderson, 2011).

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, MT, U.S. They concluded that the fraction of Pb associated with Fe-oxides

was most frequently transferred to biofilms and the other biological components of the sampled systems (Farag et al., 2007). Consequently, an increase in the Pb Fe-oxide fraction could signify a potential increase in the bioaccessible pool of Pb. The authors also noted that this fraction may promote downstream transport of Pb contamination. Ancion et al. (2010) investigated whether urban runoff metal contaminants could modify biofilm bacterial community structure and diversity and therefore potentially alter the function of biofilms in stream ecosystems. They found that accumulation rates for metals in biofilm were maximal during the first day of exposure and then decreased with time. Equilibrium between metal concentrations in the water and in the biofilm was reached for all metals after 7-14 days of exposure. The affinity of the biofilm for Pb was, however, much greater than for Cu and Zn. With respect to recovery, the release of metals was slow and after 14 days in clean water 35% of Pb remained in the biofilm. By retaining and releasing such metal pollutants, biofilms may play a key role in determining both the concentration of the dissolved metals in the water column and the transfer of the metals to invertebrates and fish grazing on them. An enrichment factor of 6,000:1 for Pb between the biofilm and the water was measured after 21 days exposure to synthetic urban runoff. The relatively slow release of such metal may greatly influence the transfer of Pb to organisms feeding on the biofilms. This may be of particular importance during storm events when large amounts of Pb are present in the urban runoff. It was suggested that biofilms constitute an integrative indicator of metal exposure over a period of days to weeks.

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., 2011b).

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., 2011a; Mager et al., 2010). In a 7-day chronic study with *C. dubia*, DOM protected against toxicity while water hardness was not protective (Mager et al., 2011a). The specific composition of DOM has been shown to affect the bioaccessibility of environmental Pb. Humic acid-rich DOM resulted in decreased free Pb ion concentration when compared to systems containing DOM with high concentrations of polysaccharides (Lamelas and Slaveykova, 2008). When the sequestering abilities of various components of DOM were compared, humic acid again was shown to be most efficient at reducing the Pb free ion concentration, followed by fulvic acid, alginic acid, polygalacturonic acid, succinoglycan, and xanthan (Lamelas et al., 2005). Lamelas et al. (2009) considered the effect of humic acid on Pb(II) uptake by freshwater algae taking account of kinetics and cell wall speciation. The uptake flux was described by a Michaelis-Menten type equation. Comparison of Cu(II), Cd(II) and Pb(II) uptake by green freshwater algae, *Chlorella Kessleri*, in the presence of either citric acid or humic acid was made. The uptake fluxes, percentage adsorbed and percentage internalized for Cu and Cd were identical in the presence of either citric or humic acid. In contrast, however, there was a ten-fold increase in the respective values for Pb. The increase in adsorbed Pb was attributed to the increase in adsorption sites from the adsorbed humic acid on the surface of the algae. Two hypotheses were considered to explain the increase in internalized Pb and the internalization flux: (1) direct interaction of Pb-humic acid complexes with the internalization sites, and (2) uptake of Pb(II) after dissociation from the Pb-humic acid complex. The authors favor the former hypothesis but no evidence is presented for the proposed ternary Pb-humic acid-internalized site complexes, nor is there an explanation as to why this behavior is not observed for Cd or Cu.

There is evidence, however, that DOC/DOM does not have the same effect on free Pb ion concentration in marine systems as in freshwater systems. No correlation was observed between DOM concentration or composition and Pb toxicity when examined using the sea urchin *Paracentrotus lividus* embryo-larval bioassay (Sanchez-Marin et al., 2010b). For marine invertebrates, the presence of humic acid increased both the uptake and toxicity of Pb, despite the fact that a larger fraction of Pb is complexed with humic acid (25 to 75%). Although the authors could not provide a precise explanation for this, they theorized that in marine environments, addition of humic acid could induce and enhance uptake of Pb via membrane Ca^{2+} channels (Sanchez-Marin et al., 2010a). This mechanism was observed in the marine diatom *Thalassiosira weissflogii*, in that humic acids absorbed to cell surfaces increased metal uptake; however, water column Pb-humic acid associations did appear to reduce free Pb ion concentrations (Sanchez-Marin et al., 2010a). Formation of a ternary complex that is better absorbed by biological membranes

was another proposed mechanism that could describe the increased bioaccessibility to marine invertebrates of Pb bound to humic acid (Sánchez-Marín et al., 2007).

Sanchez-Marin et al. (2011) subsequently have shown that different components of DOM have different effects on Pb bioavailability in marine systems. Their initial research using commercially-derived humic acid found that increasing humic acid concentrations increased Pb uptake by mussel gills and increased toxicity to sea urchin larvae in marine environments (Sánchez-Marín et al., 2007). In contrast, a subsequent investigation found that fulvic acid reduced Pb bioavailability in marine water (Sánchez-Marín et al., 2011). The contradictory effects of different components of DOM on marine bioavailability likely reflect their distinct physico-chemical characteristics. More hydrophobic than fulvic acid, humic acid may adsorb directly with cell membranes and enhance Pb uptake through some (still unidentified) mechanism (Sánchez-Marín et al., 2011).

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 *C. kesslerii* algal surfaces, and that the presence of Pb sorbed to humic acid did not interfere with humic acid-algae complexation. The authors concluded that humic acids bound to algae acted as additional binding sites for Pb, thus increasing the concentrations associated with the algal fraction (Lamelas and Slaveykova, 2007).

Based on the above, the recent literature indicates the existence of a number of deviations from current models used to predict bioaccessibility of Pb. In marine aquatic systems, for instance, surface water DOM was found to increase (rather than decrease) uptake of Pb by fish gill structures, potentially through the alteration of membrane Ca-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) adequately predicted Pb bioaccessibility.

7.3.2.5 Sulfides

In sediments, Pb bioavailability is further influenced by sulfides. In the presence of sulfides, most of the reactive metal in sediments will form insoluble metal sulfide that is not bioavailable for uptake by benthic organisms. Acid volatile sulfide (AVS) has been used to predict the toxicity of Pb and other metals in sediments (Ankley et al., 1996; Di Toro et al., 1992) and in the development of sediment quality criteria (Section 7.3.3). The role of sulfides in the flux of Pb from sediments is discussed further in Section 3.3.2.3.

7.3.3 Introduction to Bioavailability and Biological Effects of Pb in Freshwater Ecosystems

Freshwater ecosystems across the U.S. encompass many habitats including ponds, streams, rivers, wetlands and lakes. Representative median and range of Pb concentrations in surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L), sediments (median 28 µg Pb/g dry weight, range 0.5 to 12,000 µg Pb/g dry weight) and fish tissues (median 0.59 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 reported in the previous 2006 Pb AQCD (U.S. EPA, 2006c). Additional information on ambient Pb levels in waters, sediments and biota is presented in Section 3.6.5 and Table 2-1 including new data from the Western Airborne Contaminants Assessment Project (WACAP) on Pb in environmental media and biota from remote ecosystems in the Western U.S. WACAP assessed concentrations of semi-volatile organic compounds and metals in up to seven ecosystem components (air, snow, water, sediment, lichen, conifer needles and fish) in watersheds of eight core national parks during a multi-year project conducted from 2002-2007 (Landers et al., 2008). The goals of the study were to assess where these contaminants were accumulating in remote ecosystems in the Western U.S., identify ecological receptors for the pollutants, and to determine the source of the air masses most likely to have transported the contaminants to the parks.

The 2006 Pb AQCD 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 in freshwater were released in 1985 (U.S. EPA, 1985) by the EPA Office of Water which employed empirical regressions between observed toxicity and water hardness to develop hardness-dependent equations for acute and chronic criterion. These criteria are published pursuant to Section 304(a) of the Clean Water Act and provide guidance to states and tribes to use in adopting water quality standards for the protection of aquatic life and human health in surface water. The ambient water quality criteria for Pb are expressed as a criteria maximum concentration (CMC) for acute toxicity and criterion continuous concentration (CCC) for chronic toxicity (U.S. EPA, 2010b). In freshwater, the CMC is 65 µg Pb/L and the CCC is 2.5 µg Pb/L at a hardness of 100 mg/L.

The 2006 Pb AQCD summarized two approaches for establishing sediment criteria for Pb based on either bulk sediment or equilibrium partitioning (Section 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, 2005d). The equilibrium partitioning approach considers bioavailability by relating sediment toxicity to pore water concentration of metals. The amount of simultaneously extracted metal (SEM) is compared with the metals extracted via AVS since metals that bind to AVS (such as Pb) should not be toxic in sediments where AVS occurs in greater quantities than SEM.

Since the 2006 Pb AQCD both of these methods, for estimating sediment criteria for metals, they have continued to be used and refined. The SEM approach was further refined in the development of the sediment BLM (Di Toro et al., 2005). The BLM is discussed further in Sections 7.2.3 and 7.3.4. Comparison of empirical approaches with AVS-SEM in metal contaminated field sediments shows that samples where either method predicted there should be no toxicity due to metals, no toxicity was observed in chronic amphipod exposures (Besser et al., 2009; MacDonald et al., 2009). However, when the relationship between invertebrate habitat (epibenthic and benthic) and environmental Pb bioaccumulation was investigated, De Jonge et al. (2010) determined that different environmental fractions of Pb were responsible for invertebrate uptake and exposure. Pb uptake by benthic invertebrate taxa was not significantly correlated to AVS

Pb levels, but rather to total sediment concentrations (De Jonge et al., 2009). Conversely, epibenthic invertebrate Pb body burdens were better correlated to AVS concentrations, rather than total Pb sediment concentrations (De Jonge et al., 2010).

In the following sections, new information since the 2006 Pb AQCD on Pb in aquatic ecosystems will be presented. Throughout the sections, brief summaries of conclusions from the 1977 Pb AQCD, the 1986 Pb AQCD and 2006 Pb AQCD are included where appropriate. The sections are organized to consider uptake of Pb and effects at the species level, followed by community and ecosystem level effects. Freshwater ecosystem effects are considered first, followed by corresponding sections on effects in saltwater ecosystems. New research on the bioavailability and uptake of Pb into freshwater organisms including plants, invertebrates and vertebrates is presented in Section 7.3.4 Effects of Pb on the physiology of freshwater fauna and biota (Section 7.3.5) are followed with data on exposure and response of freshwater organisms (Section 7.3.6). Responses at the community and ecosystem levels of biological organization are reviewed in Section 7.3.7 followed by a brief consideration of critical loads in freshwater systems (Section 7.3.8), and characterization of sensitivity and vulnerability of ecosystem components (Section 7.3.9). Corresponding sections for saltwater ecosystems include bioavailability (Section 7.3.11), biological effects of Pb in saltwater (Section 7.3.12) and exposure and response of saltwater species (Section 7.3.13). Community and ecosystem level effects in saltwater are considered in Section 7.3.14, followed by characterization of sensitivity and vulnerability in saltwater species (Section 7.3.15). Finally, the effects of Pb on ecosystem services associated with aquatic environments are discussed in Section 7.3.16 and an overall summary of aquatic effects is presented in Section 7.3.17.

7.3.4 Bioavailability in Freshwater Systems

Bioavailability was defined in the 2006 Pb AQCD as "the proportion of a toxin that passes a physiological membrane (the plasma membrane in plants or the gut wall in animals) and reaches a target receptor (cytosol or blood)." In 2007, EPA took cases of bioactive adsorption into consideration and revised the definition of bioavailability as "the extent to which bioaccessible metals absorb onto, or into, and across biological membranes of organisms, expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time and under defined conditions" (Fairbrother et al., 2007).

The bioavailability of metals varies widely depending on the physical, chemical, and biological conditions under which an organism is exposed (U.S. EPA, 2007c). The bioavailability of a metal is also dependent upon the bioaccessible fraction of metal. The

bioaccessible fraction of a metal is the portion (fraction or percentage) of environmentally available metal that actually interacts at the organism's contact surface and is potentially available for absorption or adsorption by the organism (U.S. EPA, 2007c). The processes for evaluating bioavailability and bioaccessibility are presented in Figure 7-1 and in Section 7.2.3. In brief, trace metals, and their complexes, must first diffuse from the external medium to the surface of the organism (mass transport). Metal complexes may dissociate and re-associate in the time that it takes to diffuse to the biological surface. These processes are considered further in Chapter 3. To have an effect on the organism, metals must then react with a sensitive site on the biological membrane (adsorption/desorption processes), often but not necessarily followed by biological transport (internalization). Any of these processes may be the rate limiting step for the overall biouptake process. Internalization is, however, the key step in the overall biouptake process. Although the transport sites often have a high affinity for required metals they do not always have high selectivity and so a toxic metal may bind to the site of an essential metal with a similar ionic radius or co-ordination geometry, e.g., Pb^{2+}, Cd^{2+} and Zn^{2+} are similar to Ca^{2+}. At the molecular level, there are three major classes of transition metal transporter: P-type ATPases, Zn regulated transporter/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.

Routes of Exposure

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 physiological effects (Luoma and Rainbow, 2005). Pb accumulation rates are controlled, in part, by metabolic rate. Other factors that influence bioavailability of Pb to organisms in aquatic systems are reviewed in Section 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:

1) Recent information on bioavailability and uptake in algae, plants, invertebrates and vertebrates from freshwater systems are reviewed with summary material from the 2006 Pb AQCD where appropriate.

2) An overview of the BLM is presented as the most widely used method for predicting both the bioaccessible and bioavailable fractions of Pb in the aquatic environment. This is followed by a discussion of

3) 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.

BLM Models

In addition to the biogeochemical effects that govern the environmental pool of accessible Pb, reactions of Pb with biological surfaces and membranes determines the

bioavailability and uptake of the metal by aquatic organisms. The BLM (Figure 7-2) predicts both the bioaccessible and bioavailable fraction of Pb in the aquatic environment, and can be used to estimate the importance of environmental variables such as DOC in limiting uptake by aquatic organisms (Alonso-Castro et al., 2009). The BLM integrates the binding affinities of various natural ligands in surface waters and the biological uptake rates of aquatic organisms to determine the site-specific toxicity of the bioavailable fraction.

In the 2006 Pb AQCD, limitations of the use of BLM in developing air quality criteria were recognized including the focus of this model on acute endpoints and the absence of consideration of dietary uptake as a route of exposure. Atmospheric deposition of Pb to aquatic systems and subsequent effects on ecosystem receptors is likely characterized as a chronic, cumulative exposure rather than an acute exposure. Recommendations from the 2006 Pb AQCD included developing both chronic toxicity BLMs and BLMs that consider the dietary route of Pb uptake. The EPA recently incorporated the BLM into the Framework for Metal Risk Assessment (U.S. EPA, 2007c) and has published an ambient freshwater criteria document for Cu based on the BLM model (U.S. EPA, 2007a). This section reviews the literature from the past 5 years on applications of the BLM to predicting bioavailability of Pb to aquatic organisms. However, the primary focus of initial BLMs has been acute toxicity endpoints for fish and invertebrates following gill or cuticular uptake of metals.

Di Toro et al. (2005) constructed BLMs for metals exposure in sediments, surface water, and sediment pore water to determine how to most accurately predict the toxicity of metals-contaminated sediments. Results from models were compared with literature-derived acute toxicity values for benthic and epibenthic invertebrates to establish the accuracy of the developed models. Although the models tended to overestimate the toxicity of aqueous and sediment-bound Pb in freshwater environments, it was determined that the model significantly underestimated Pb toxicity to marine invertebrates (Di Toro et al., 2005). This may be because pore water metal concentrations were not modeled. Consequently, these results may suggest that either 1) mobilization of Pb concentrations from sediments into pore water is greater in marine environments, or 2) marine invertebrates are significantly more sensitive to Pb exposures than are freshwater species.

A number of deviations from results predicted by Pb exposure models (such as the BLM) were documented by Ahlf et al. (2009). They highlighted that uptake of metals by sediment-dwelling bivalves was significantly greater than predicted, because bivalves accumulate Pb from multiple sources not included in the model, such as ingestion of algae, bacteria, and colloidal matter. Species-specific dietary assimilation of ingested

particulate-bound metals is also likely to play a role in the toxicity of Pb to aquatic organisms, yet insufficient data are available to permit modeling of this additional factor (Ahlf et al., 2009). The authors outlined the need for additional data in developing bioavailability models for chronic metal exposures. As recent evidence suggests that the hydrophobic DOC fraction (e.g., humic and fulvic acids) sequesters the greatest fraction of Pb in aquatic systems (Pernet-Coudrier et al., 2011), understanding the influence of this adsorption on Pb toxicity is critical for the prediction of chronic aquatic Pb toxicity. For instance, although the presence of humic acid is considered to reduce the bioavailable fraction of metals in surface water, green algae uptake and biosorption of metals, including Pb, was actually increased by humic acid. The authors determined that humic acid bound to algal surfaces served to increase the total number of metal binding sites over those afforded solely by the algal surface (Lamelas and Slaveykova, 2007). This highlights the complexity of modeling chronic metals bioavailability through multiple exposure routes, as humic acid would decrease gill or cuticular uptake of metals from the water column, but could potentially enhance dietary exposure by increasing algal metal content. Slaveykova and Wilkinson (2005) also noted that humic acid is likely to interact with other biological membranes and alter their permeability to metals, especially in acidic environments. Further, they observed that increased surface water temperatures 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 and Wilkinson, 2005). Despite this, the authors noted that, in most cases, the BLM could predict acute metals toxicity with a reasonable degree of accuracy.

7.3.4.1 Freshwater Plants and Algae

In the 1977 Pb AQCD, the root system of plants was recognized as the major route of uptake for Pb (U.S. EPA, 1977). 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. Plants that are hyperaccumulators of Pb and other metals may be used for phytoremediation at highly contaminated sites and there is a large body of literature on uptake of very high concentrations of metals by different species, however, this chapter focuses on

environmentally relevant concentrations of Pb and also those studies with doses or exposures in the range of one or two orders of magnitude above current or ambient conditions, as described in the Preamble**.** Uptake and translocation studies of Pb in plants and algae reviewed in the 1977 Pb AQCD and the 2006 Pb AQCD indicated that plants tend to sequester larger amounts of Pb in their roots than in their shoots. Recent studies on bioavailability of Pb to plants support the findings of the previous Pb AQCDs and provide additional evidence for species-dependent differences in responses to Pb in water and sediments.

The microalgae *Spirulina platensis* was demonstrated to accumulate Pb from Zarrouk culture medium, with 2.7, 6.9, 19, 45 and 145 µg Pb/mg accumulated at high Pb concentrations of 5,000, 10,000, 30,000, 50,000 and 100,000 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. 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* (Chen et al., 2010c). The authors noted that, in the case of Cu-Pb binary exposures, uptake rates of Pb exhibited complex non-linear dynamics in other aquatic organisms as well. When exposed to water concentrations of up to 100 µmol Pb/L, floating (non-rooted) coontail plants (*Ceratophyllum demersum*) accumulated an average Pb concentration of 1,748 mg Pb/kg after 7 days, although this was not significantly higher than levels accumulated in the first day of exposure (Mishra et al., 2006b). Induction of the antioxidant system improved the tolerance of the aquatic plant *Najas indica* for bioaccumulated Pb, allowing for increased biomass and the potential to accumulate additional Pb mass. High Pb accumulation (3,554 mg Pb/kg dry weight tissue following a 7-day exposure to 100 µmol Pb/L) was considered to be a function of plant morphology; as a submerged, floating plant, *N. indica* provides a large surface area for the absorption of Pb (Singh et al., 2010). Pb bioaccumulation in curly pondweed (*Potamogeton crispus*) was observed to be dose-dependent, with plants accumulating 3.3, 5.5, 15.4, and 23.6 µg Pb/g, at aquatic concentrations of 10, 20, 30, 40, and 50 mM Pb (Xu et al., 2011).

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 BCFs for Pb were all less than one, indicating that Pb uptake, although high, was less efficient than for other metals present (Ha et al., 2009).

Aquatic plants inhabiting a wetland containing an average sediment Pb concentration of 99 mg Pb/kg exhibited variable Pb tissue concentrations, but these do not appear to be related to macrophyte type (e.g., submerged, floating, emergent, etc.). Consequently, the authors concluded that uptake of Pb by aquatic plants appears to be dependent on species, at the exclusion of habitat or type. For instance, among the submerged plant species, *Ceratophyllum demersum* accumulated the greatest amount of Pb (22 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 and Greger, 2006).

Dwivedi et al. (2008) reared nine different species of aquatic plants in a fly-ash contaminated medium containing approximately 7 mg Pb/kg dry weight. Not only did species exhibit different Pb accumulation efficiencies but they also compartmentalized sequestered Pb differently. The submerged macrophyte *Hydrilla verticillata* accumulated the greatest amount of Pb (approximately 180 mg Pb/kg dry weight tissue), but Pb was sequestered solely in the shoot tissue. In contrast, other plant species accumulated between 15 and 100 mg Pb/kg dry weight (*Ranunculus scloralus* and *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 and Mushrifah, 2009).

Lemna sp., a free floating macrophyte, incubated in a water extract of waste ash containing 19 µg Pb/L accumulated 3.5 mg Pb/kg dry weight over 7 days of exposure. Slight toxic effects, including suppression of growth, were observed over this exposure period, but this may have been a result of exposures to multiple metals in the water extract, including Cr, Mn, Cu, and Zn (Horvat et al., 2007). Lemna sp. was also demonstrated to be effective in the biosorption of Pb from solution, even in the presence of sediments (1 g per 700 mL water). Over 7 days of exposure to 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 and 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 and 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 and Lo Giudice, 2010). Conversely, the semi-aquatic plant *Ammania baccifera*, grown in mine tailings containing 35 to 78 mg Pb/kg, did not accumulate analytically detectable levels of Pb in either root or shoot tissues, despite the fact that other metals (Cu, Ni, Zn) were bioaccumulated (Das and Maiti, 2007). This would indicate that at low/moderate environmental Pb concentrations, some plant species may not bioaccumulate significant (or measurable) levels of Pb.

The average concentration of Pb in the tissues of rooted aquatic macrophytes (*Callitriche verna*, *P. natans*, *C. demersum*, *Polygonum amphibium*, *Veronica beccabunga*) collected from two metals-polluted streams in Poland (average sediment concentration 38 to 58 mg Pb/kg) was less than 30 mg Pb/kg. Pb bioaccumulation in plants was significantly correlated with sediment Pb concentrations (Samecka-Cymerman and Kempers, 2007). A similar significant correlation was established between reed sweet grass root Pb concentration and sediment Pb concentrations (Skorbiowicz, 2006).

Pb tissue concentrations of aquatic plants *P. australis* and *Ludwigia prostrata* collected from wetlands containing an average of 52 mg Pb/kg in surficial sediments were

predominantly in root tissues, indicating poor translocation of Pb from roots. In the former, Pb decreased from an average of 37 mg Pb/kg in roots to 17, 14, and 12 mg Pb/kg in rhizome, stem and leaf tissues, respectively, while *L. prostrata* Pb tissue concentrations decreased from 77 mg Pb/kg in fibrous root to 7 and 43 mg Pb/kg in stem and leaf tissues (Yang et al., 2008a). The authors proposed that this diminished transfer ability explained the relatively low BCFs for Pb uptake in these two species, when compared with those of other metals.

Despite no significant seasonal effect on surface water Pb concentrations, shining pondweed (*Potamogeton lucens*), a rooted aquatic macrophyte grown in an urbanized metal-contaminated lake in Turkey, exhibited seasonal alterations in Pb tissue concentrations. Average 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 Pb/kg dry weight in autumn (Duman et al., 2006). No differences were detected in stem Pb concentrations between spring and summer (approximately 4 mg Pb/kg dry weight), but stem Pb concentrations were found to be significantly higher in autumn (6 mg Pb/kg dry weight). In the same system, *P. australis* plants accumulated the most Pb during winter: 103, 23, and 21 mg Pb/kg dry weight in root, rhizome, and shoot tissue, respectively, in sediments containing 13 mg Pb/kg dry weight. By contrast, *Schoenoplectus lacustris* accumulated maximum rhizome and stem Pb concentrations of 5.1 and 7.3 mg Pb/kg dry weight in winter, but sequestered the greatest amount of Pb in root tissues during the spring (30 mg Pb/kg dry weight) at a comparable sediment concentration, 18 mg Pb/kg dry weight (Duman et al., 2007). The authors suggest that this indicated that metal uptake was regulated differently between species.

Tree species that inhabit semi-aquatic environments have also been shown to absorb Pb from Pb-contaminated sediments. Bald-cypress trees (*Taxodium distichum*) growing in sediments of a refinery-impacted bayou in Louisiana accumulated significantly greater amounts of Pb than did trees of the same species growing in bankside soil, despite the lower Pb concentrations of sediments. Bankside soils contained greater than 2,700 mg Pb/kg versus concentrations of 10 to 424 mg Pb/kg in sediments, yet Pb concentrations in trees averaged 4.5 and 7.8 mg Pb/kg tissue, respectively (Devall et al., 2006). The authors theorized that Pb was more readily released from sediments and that soil dispersion to the swamp sediments provides additional, if periodic, loads of Pb into the system. Willow seedlings planted in Pb-contaminated sediment were more effective at removing Pb from the media than a diffusive gradient in thin film technique predicted (Jakl et al., 2009). The authors proposed that the plant's active mobilization of nutrients from soil during growth also resulted in increased Pb uptake and sequestration.

Given that sediments are a significant sink for Pb entering aquatic systems, it is not surprising that rooted macrophytes bioaccumulate significant quantities of the metal. Although there are some similarities to Pb accumulation observed in terrestrial plants (e.g., preferential sequestration of the metal in root tissue), Pb appears to be more bioavailable in sediment than it is in soil. This may be a result of differences in plant physiology between aquatic and terrestrial plants (e.g., more rapid growth or more efficient assimilation of nutrients and ions from a water-saturated medium). While rooted macrophytes are likely to be chronic accumulators of Pb sequestered in sediments, aerial deposition of Pb into aquatic systems may result in pulsed inputs of labile Pb that would be available for uptake by floating macrophytes and algae.

7.3.4.2 Freshwater Invertebrates

Uptake and subsequent bioaccumulation of Pb in freshwater invertebrates varies greatly between species and across taxa as previously characterized in the 2006 Pb AQCD. This section expands on the findings from the 1986 Pb AQCD and 2006 Pb AQCD on bioaccumulation and sequestration of Pb in aquatic invertebrates. In the case of invertebrates, Pb can be bioaccumulated from multiple sources, including the water column, sediment, and dietary exposures, and factors such as proportion of bioavailable Pb, lifestage, age, and metabolism can alter the accumulation rate. In this section, new information on Pb uptake from freshwater and sediments by invertebrates will be considered, followed by a discussion on dietary and water routes of exposure and factors that influence species-specific Pb tissue concentrations such as invertebrate habitat and functional feeding group.

In a new uptake study in freshwater mussels available since the 2006 Pb AQCD, the Eastern elliptio mussel (*Elliptio complanata*) was shown to accumulate Pb rapidly from water and then reach an equilibrium with exposure level and tissue concentration by two weeks following exposures to 1, 4, 14, 57 or 245 µg Pb/L as Pb-nitrate (Mosher et al., In Press). Tissue concentrations of Pb increased at an exposure-dependent rate for the first 14 days and then did not change significantly for the remainder of the 28-day exposure although mussels continued to accumulate Pb. At the end of the exposure period, average Pb in tissue ranged from 0.33 to 898 µg Pb/g. The authors concluded that the mussels were likely eliminating Pb via pseudo feces and through storage of Pb in shell.

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., 2008a). Vink (2009) studied six river systems and found that, for a range of metals, uptake by benthic organisms (the oligochaete, Limnodrilus (Family Tubificidae) and the midge, *C. riparius*) from the sediment pore water (as compared with surface water) was observed only occasionally, and solely for Pb. The physiological mechanisms of Pb uptake are still unclear but it is suggested that uptake and elimination of Pb obey different mechanisms than for other heavy metals.

The 2006 Pb AQCD recognized the potential importance of the dietary uptake pathway as a source of Pb exposure for invertebrates. Specifically, in a study with the freshwater amphipod *Hyalella azteca,* dietary exposure was found to contribute to the chronic toxicity of Pb, while acute toxicity was unaffected (Besser et al., 2004). Since the 2006 Pb AQCD, additional studies have considered the relative importance of water and dietary uptake of Pb in aquatic invertebrates. A stable isotope technique was used to simultaneously measure uptake of environmentally relevant concentrations of Pb (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 and Blust, 2009a). *D. magna* accumulated the metal from both sources, but the relative proportion of uptake from each source changed over the exposure period. After the first day of exposure, 12% of accumulated Pb was determined to have been absorbed from dietary (algal) sources, but this percentage decreased by day four of exposure to 4%. Pb absorbed from water exposure only resulted in Daphnia body burdens of approximately 300 µmol Pb/kg dry weight, and was similar to the amount absorbed by algae (Komjarova and Blust, 2009a). In a comparison of dietary and waterborne exposure as sources of Pb to aquatic invertebrates, no correlation between Pb uptake and dietary exposure was observed in the amphipod *H. azteca* (Borgmann et al., 2007).

Stable isotope analysis was to used measure uptake and elimination simultaneously in net-spinning caddisfly larvae (Hydropsyche sp.) exposed to aqueous Pb concentrations of 0.2 to 0.6 µg Pb/L for 18 days (Evans et al., 2006). The measured uptake constant for Pb in this study was 7.8 g/dry weight·day and the elimination rate constant of 0.15/day for Pb-exposed larvae was similar in both presence and absence of the metal in the water. Measured tissue concentrations ranged from approximately 15 to 35 µg Pb/g. Hydropsychid Pb BCFs 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).

Recent reports on Pb distribution in freshwater organisms generally support the findings of the 2006 Pb AQCD that Pb is primarily sequestered in the gills, hepatopancreas, and

muscle. Uptake of Pb by the crayfish *Cherax destructor* exposed to 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). 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).

When the relationship between invertebrate habitat (epibenthic and benthic) and environmental Pb bioaccumulation was investigated, De Jonge et al. (2010) determined that different environmental fractions of Pb were responsible for invertebrate uptake and exposure. Pb uptake by benthic invertebrate taxa was not significantly correlated to AVS Pb levels, but rather to total sediment concentrations (De Jonge et al., 2009). Conversely, epibenthic invertebrate Pb body burdens were better correlated to AVS concentrations, rather than total Pb sediment concentrations (De Jonge et al., 2010). For instance, the biologically available Pb (e.g., bound to metal-rich granules or metallothioneins) accumulated by the oligochaete *Tubifex tubifex* was determined to correlate with sediment SEM-AVS Pb concentrations (De Jonge et al., 2011). Similarly, Desrosiers et al. (2008) reported that Pb accumulation by chironomid larvae from St. Lawrence river sediments was significantly correlated to both total Pb and reactive Pb sediment concentrations.

Both inter- and intra-specific difference in Pb uptake and bioaccumulation may occur in macroinvertebrates of the same functional-feeding group. Cid et al. (2010) reported significant differences in Pb bioaccumulation between field collected *Ephoron virgo* mayflies and Hydro psyche sp, caddisflies, with only the mayfly exhibiting increased Pb tissue concentrations when collected from Pb-contaminated sites; the caddisfly Pb tissue concentrations were similar between reference and Pb-contaminated areas. The authors also examined the lifestage specific accumulation of Pb for *E. virgo* mayflies, and although there was no statistical difference in Pb tissue concentrations between different lifestages, Pb bioaccumulation did change as mayflies aged (Cid et al., 2010).

Reported BAF values for Pb in aquatic invertebrates from the 2006 Pb AQCD ranged from 499 to 3,670 [Table AX7-2.3.2 (U.S. EPA, 2006c)]. Since the 2006 Pb AQCD, additional BAF values have been established for invertebrates in field studies which tend to be higher than BCF values calculated in laboratory exposures (Casas et al., 2008; Gagnon and Fisher, 1997). A complicating factor in establishing BAF values is that laboratory studies usually assess uptake in water-only or sediment only exposures while field studies take into account dietary sources of Pb as well as waterborne Pb resulting in BAF values that are frequently 100-1,000 times larger than BCF values for the same

metal and species (DeForest et al., 2007). The EPA Framework for Metals Risk Assessment states that the latest scientific data on bioaccumulation do not currently support the use of BCFs and BAFs when applied as generic threshold criteria for the hazard potential of metals (U.S. EPA, 2007c).

7.3.4.3 Freshwater Vertebrates

Uptake of Pb by vertebrates considered here includes data from fish species as well as a limited amount of new information on amphibians and aquatic mammals. 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 freshwater is reviewed below, followed by studies on dietary uptake as a route of Pb exposure. Next, tissue accumulation patterns in fish species are reported with special consideration of the anterior intestine as a newly identified target of Pb from dietary exposures.

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).

Pb in fish is primarily found in bone, gill, blood, kidney and scales (Spry and Wiener, 1991). Since the 2006 Pb AQCD, multiple studies on uptake of Pb from water by fathead

minnow and subsequent tissue distribution have been conducted. Spokas et al. (2006) showed that Pb accumulates to the highest concentration in gill when compared to other tissues over a 24 day exposure. This pattern was also observed in larval fathead minnows exposed to 26 µg Pb/L for 10-30 days, where gill exhibited the highest Pb concentration compared to carcass, intestine, muscle and liver (Grosell et al., 2006b). 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. (2006b) 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 and 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). In contrast to uptake in teleosts, in Pb-uptake studies with the Chondrostei fish Chinese Sturgeon (*Acipenser sinensis*), muscle tissue accumulated higher levels of Pb than gills (Hou et al., 2011).

Sloman et al. (2005) investigated the uptake of Pb in dominant-subordinate pairings of rainbow trout exposed to 46 µg/L or 325 µg/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 and 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 (*Carassius 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 and Sherriff, 2008). Pb was primarily sequestered in skin, gill, and bone tissues, but was also detected at elevated levels in muscle and liver tissues, as well as in eggs. Two fish species (*Labeo rohita* and *Ctenopharyngodon idella*) collected from the Upper Lake of Bhopal, India with average Pb concentration 30 µg Pb/L in the water column contained elevated Pb tissue concentrations (Malik et al., 2010). However, while liver and kidney Pb concentrations were similar between the two species (1.5 and 1.1 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., 2006b) 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., 2006b). 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 2006 Pb AQCD, several studies have focused on dietary uptake of Pb in teleosts. Metals have been shown to assimilate differently in tissues depending on the exposure route (Meyer et al., 2005; Rozon-Ramilo et al., In Press). 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 et al., 2009b). 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 and Wood, 2006). Pb accumulation in the gut was followed by bone, kidney, liver, spleen, gill, carcass, brain and white muscle (Alves and Wood, 2006). Ojo and Wood (2007) investigated the bioavailability of ingested Pb within different compartments of the rainbow trout gut using an in vitro gut sac technique. Although a significant increase in Pb uptake was observed in the mid-intestines, this was determined to be much lower than Pb uptake rates via gill surfaces. However, given that intestinal uptake rate for Pb did not significantly differ from those derived for essential metals (e.g., Cu, Zn, and Ni), this uptake route is likely to be significant when aqueous Pb concentrations are low and absorption via gill surfaces is negligible (Ojo and Wood, 2007).

Following a chronic 63-day dietary exposure to Pb, male zebrafish had significantly increased Pb body burdens, but did not exhibit any significant impairment when compared with controls. Fish were fed diets consisting of field-collected *Nereis diversicolor* oligochaetes that contained 1.7 or 33 mg Pb/kg dry weight. This resulted in a daily Pb dose of either 0.1 or 0.4 mg Pb/kg (Boyle et al., 2010). At the end of the exposure period, tissue from male fish reared on the high-Pb diet contained approximately 0.6 mg Pb/kg wet weight, as compared with approximately 0.48 mg Pb/kg wet weight in the low-Pb dietary exposure group. Pb level was elevated in female fish fed the high-Pb diet, but not significantly so.

Ciardullo et al. (2008) examined bioaccumulation of Pb in rainbow trout tissues following a 3-year chronic dietary exposure to the metal. Diet was determined to contain 0.19 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 from dietary sources, toxicity does not correlate with dietary exposure, but does correlate with gill accumulation from waterborne exposure (Alves et al., 2006).

Comparison of uptake rates across the gut and gill have shown that transporter pathways in the gill have a much higher affinity for Pb than do similar pathways in the gut (Ojo and Wood, 2007).

Since the 2006 Pb AQCD, several field studies have considered Pb uptake and bioaccumulation in fish as a tool for environmental assessment. Pb tissue concentrations were elevated in several species of fish exposed in the field to Pb from historical mining waste, and blood Pb concentrations were highly correlated with elevated tissue concentrations, suggesting that blood sampling may be a useful and potentially non-lethal monitoring technique (Brumbaugh et al., 2005).

This review of the recent literature indicates that the primary and most efficient mode of Pb absorption for freshwater fish is assimilation of labile Pb via gill surfaces; recent research indicates that chronic dietary Pb exposure may result in some Pb bioaccumulation although it is not the predominant route of exposure. Nevertheless, if benthic invertebrates comprise a large portion of fish diets in chronically contaminated systems, assimilated Pb loads may be significant. This was demonstrated by Boyle et al. (2010), who showed that laboratory diets consisting of less than one third field-collected Pb-contaminated invertebrates were sufficient to raise fish tissue Pb levels. However, data from field sites suggest that fish accumulation of Pb from dietary sources is highly variable and may be strongly dependent on the physiology of individual species and absorption capacities.

Amphibians

Since the 2006 Pb AQCD, there are a few new field measurements and laboratory-based studies that consider uptake of Pb in amphibians. Whole body Pb measured in three species of field-collected tadpoles in the Mobile-Tensaw River Delta in Alabama averaged 1.19 µg Pb/g dry weight in *Rana clamitans*, 0.65 µg Pb/g dry weight in *Rana catesbeiana* and 1.32 µg Pb/g dry weight in *Hyla cinerea* (Albrecht et al., 2007). Blood-Pb levels in Ozark hellbenders (*Cryptobranchus alleganiensis alleganiensis*), a candidate species for the Endangered Species Act, ranged from 0.04 to 0.06 µg/g dry whole blood weight, in three rivers in Missouri (Huang et al., 2010). In the same study, Pb-blood levels were measured from Eastern hellbenders (*Cryptobranchus alleganiensis bishopi*), a species of concern, collected from four rivers and ranged from 0.075 to 0.088 µg Pb/g dry whole blood weight.

In a chronic laboratory-based study with tadpoles of the Northern Leopard frog (*Rana pipiens*), Pb tissue concentrations were evaluated following exposures to 3, 10, and 100 µg Pb/L from embryo to metamorphosis. The tadpole tissue concentrations ranged

from 0.1 to 224.5 mg Pb/kg dry mass and were positively correlated to Pb concentrations in the water (Chen et al., 2006b). Dose-dependent bioaccumulation of Pb was observed in the livers of tadpoles of the African clawed frog (*Xenopus laevis*) exposed to concentrations ranging from 0.001 to 30 mg Pb/L (2.91 to 114.5 Pb µg/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 sphenocephala*) tadpoles exposed to Pb in sediment (45 to 7,580 mg Pb/kg dry weight) with corresponding pore water concentrations of 123 to 24,427 µg Pb/L from embryonic stage to metamorphosis (Sparling et al., 2006). There was 100% mortality at 3,940 mg Pb/kg and higher. In all body residues analyzed there was a significant positive correlation between Pb in sediment and Pb in sediment pore water. Concentrations of Pb in liver were similar to results with whole body and bodies without liver indicating that Pb is not preferentially sequestered in liver.

Reptiles

New field surveys of Pb in water snakes since the 2006 Pb AQCD indicate that Pb is bioaccumulated in several species. Water snakes spend time in terrestrial and aquatic habitats and could potentially be exposed to atmospherically deposited-Pb in both environments. Average Pb levels in whole body samples of Eastern Ribbon Snakes (*Thamnophis sauritus*) collected from the Mobile-Tensaw River, a large watershed that drains more than 75% of Alabama were 0.35 ± 0.12 µg Pb/g dry weight) (Albrecht et al., 2007). Burger et al. (2007) measured Pb levels in blood, kidney, liver, muscle and skin from water snakes, *Nerodia sepedon*, collected from an urban/suburban canal in New Jersey. Pb was highest in skin (0.467 µg Pb/g wet weight) followed by kidney (0.343 µg Pb/g wet weight) blood (0.108 µg Pb/g wet weight), muscle (0.103 µg Pb/g wet weight) and liver (0.063 µg Pb/g wet weight). No interspecies differences were observed in blood Pb (range 0.04 to 0.1 µg Pb/g) from field-collected banded water snakes (*Nerodia fasciata*), brown water snakes (*N. taxsipilota*) and cottonmouth (*Agkistrodon piscivorus*) from a reference area and an area contaminated by chemical and radiation releases from the 1950's to the 1980's at the Department of Energy Savannah River site in South (Burger et al., 2006). Cottonmouth and brown water snake from the exposed site had significantly higher levels of Pb in tail muscle when compared to the reference creek.

Mammals

Pb bone levels in Eurasian otters (*Lutra lutra*) measured in dead individuals collected in southwest England fell by 73% between 1992 and 2004 (Chadwick et al., 2011). Annual mean bone Pb levels were 446 µg Pb/kg in 1992 and 65 µg Pb/kg in 2004. The 73%

decline of Pb in otter bones from 1992 to 2004 was found to coincide with legislative controls on Pb emissions implemented in the U.K. starting in 1986. A positive correlation with stream sediment Pb and bone Pb was also observed in this study. The strength of this correlation decreased with increasing Ca in streams.

7.3.4.4 Food Web

In the 2006 Pb AQCD, trophic transfer of Pb through aquatic food chains was considered to be negligible (U.S. EPA, 2006c). Measured concentrations of Pb in the tissues of aquatic organisms were found to be generally higher in algae and benthic organisms and lower in higher trophic-level consumers indicating that Pb was bioaccumulated but not biomagnified (U.S. EPA, 2006c; Eisler, 2000). New literature since the 2006 Pb AQCD provides evidence of the potential for Pb to be transferred in aquatic food webs. Other studies indicate Pb is decreased with increasing trophic level. This section incorporates recent literature on transfer of Pb through freshwater aquatic food chains including the application of stable isotope techniques to trace the accumulation and dilution of metals through producers and consumers.

Pb was transferred through at least one trophic level in El Niagara reservoir, Aguascalientes, Mexico, a freshwater ecosystem that lacks fishes (Rubio-Franchini et al., 2008). Pb was measured in sediment, water, and zooplankton samples of this freshwater system. BAFs were calculated for predatory and grazing zooplanktonic species. The BAF of the rotifer *A. brightwellii* (BAF 49,300) was up to four times higher than the grazing cladocerans *D. similis* (BAF 9,022) and *M. micrura* (BAF 8,046). According to the authors, since *M. micrura* are prey for *A. brightwellii* this may explain the biomagnifications of Pb observed in the predatory rotifer and provides evidence that Pb biomagnifies at intermediate trophic levels.

The relative contribution of water and food as source of trace metals including Pb was investigated in the larvae of the alderfly *Sialis velata* (Croisetiere et al., 2006). Its prey, the midge (*C. riparius*) was reared in the laboratory and then exposed to trace elements in a metal-contaminated lake for one week prior to being fed to *S. velata*. During the one-week exposure period of *C. riparius* to the contaminated water, five of six trace elements, including Pb, reached steady state within *C. riparius*. Alderfly larvae were held in the lab in uncontaminated lake water and feed one of the treated *C. riparius* per day for up to six days to measure Pb uptake via prey. A separate group of alderfly larvae were exposed directly to the contaminated lake water for six days and fed uncontaminated *C. riparius* while a third group was exposed to Pb via prey and water. Trace metal concentrations in *S. velata* that consumed contaminated *C. riparius* increased significantly compared to *S.*

velata in water-only exposures. Food was concluded to be the primary source of Pb (94%) to these organisms, not Pb in the water.

The trophic transfer of Pb from the sediment dwelling polychaete worm *N. diversicolor* to the invertebrate polychaete predator *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 (Besser et al., 2007). In addition, Pb concentrations in invertebrates (snails, crayfish, and other benthos) were negatively correlated with Pb concentrations in detritus, periphyton, and algae. Fish tissue concentrations, however, were consistently correlated only with detritus Pb concentrations (Besser et al., 2007).

Other studies have traced Pb in freshwater aquatic food webs and have found no evidence of biomagnification of Pb with increasing trophic level. Watanabe et al. (2008) observed decreasing Pb concentrations through a stream macroinvertebrate food web in Japan from producers to primary and secondary consumers. In a Brazilian freshwater coastal lagoon food chain, Pb was significantly higher in invertebrates than in fishes (Pereira et al., 2010).

Introduction of exotic species into an aquatic food web may alter Pb concentrations at higher tropic levels. In Lake Erie, the invasive round goby (*Neogobius melanostomus*) and the introduced zebra mussel (*Dreissena polymorpha*) have created a new benthic pathway for transfer of Pb and other metals (Southward Hogan et al., 2007). The goby is a predator of the benthic zebra mussel, while the endemic smallmouth bass (*Micropterus dolomieui*) feed on goby. Since the introduction of goby into the lake, total Pb concentrations have decreased in bass. The authors attribute this decrease of Pb in bass to changes in food web structure, changes in prey contaminant burden or declines in sediment Pb concentrations.

7.3.5 Biological Effects of Pb in Freshwater Systems

This section focuses on the studies of biological effects of Pb on freshwater algae, plants, invertebrates, fish and other biota with an aquatic lifestage (e.g., amphibians) published since the 2006 Pb AQCD. Key studies from the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD on biological effects of Pb are summarized where appropriate. Waterborne Pb is highly toxic to aquatic organisms with 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, alterations of blood chemistry, and decreases in enzyme levels. A review of the more recent literature corroborated these findings, and added information about induction of oxidative stress by Pb, alterations in chlorophyll, and changes in production and storage of carbohydrates and proteins.

New studies available since the 2006 Pb AQCD further consider effects of Pb on reproduction and development, growth and survival of aquatic organisms. Alterations to these endpoints can lead to changes at the community and ecosystem levels of biological organization such as decreased abundance, reduced taxa richness, and shifts in species composition. In this ISA, effects on reproduction, growth and survival are reported in additional species with some effects occurring in sensitive biota at or near ambient levels of Pb. Because this review is focused on effects of Pb, studies reviewed for this section include only those for which Pb was the only, or primary, metal to which the organism was exposed. Areas of research not addressed here include literature related to exposure to Pb from ingestion of shot or pellets. Biological effects of Pb on freshwater algae and plant species are considered below, followed by information on effects on freshwater invertebrates and vertebrates.

7.3.5.1 Freshwater Plants and Algae

The toxicity of Pb to algae and plants has been recognized in earlier agency reviews of this metal. In the 1977 Pb AQCD, differences in sensitivity to Pb among different species of algae were observed and concentrations of Pb within the algae varied among genera and within a genus (U.S. EPA, 1977). The 1986 Pb AQCD (U.S. EPA, 1986b) reported that some algal species (e.g., Scenedesmus sp.) were found to exhibit physiological changes when exposed to high Pb concentrations in situ. The observed changes included increased numbers of vacuoles, deformations in cell organelles, and increased autolytic activity. Effects of Pb on algae reported in the 2006 Pb AQCD included decreased growth, deformation and disintegration of algae cells, and blocking of the pathways that lead to pigment synthesis, thus affecting photosynthesis. Observations in additional algal species since the 2006 Pb AQCD support these findings and indicate that Pb exposure is associated with oxidative stress.

The effect of Pb exposure on the structure and function of plant photosystem II was studied in giant duckweed, *S. polyrrhiza* (Ling and Hong, 2009). The Pb concentration of extracted photosystem II particles was found to increase with increasing 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.

Pb exposure in microalgae species has been linked to several effects, including disruption of thylakoid structure and inhibition of growth in both *Scenedesmus quadricauda* and *Anabaena flos-aquae* (Arunakumara and Zhang, 2008). Arunakumara et al. (2008) determined the effect of aqueous Pb on the algal species *S. platensis* using solutions of Pb-nitrate. Exposures at 5,000 µg Pb/L stimulated 10-day algal growth, growth was inhibited at higher concentrations of 10,000 30,000 50,000 and 100,000 µg Pb/L by 5, 40, 49, and 78%, respectively. In addition to growth inhibition, algal chlorophyll *a* and *b* content were significantly diminished at the three highest Pb exposures (Arunakumara et al., 2008). Although no specific morphological abnormalities were linked to Pb exposure, filament breakage was observed in *S. platensis* at Pb concentrations >50,000 µg Pb/L. Since the 2006 Pb AQCD, the production of reactive oxygen species following Pb exposure has been measured directly in cells of the freshwater algae *Chlamydomonas reinhardtii* at environmentally relevant concentrations of Pb (0.1 to 250 nmol/L) with the greatest response at 3.15 times more stained cells compared to the control sample (Szivak et al., 2009).

At the time of the 1977 Pb AQCD, there was limited information available on Pb effects on aquatic macrophytes. For plants in general, Pb was recognized to affect photosynthesis, mitosis, and growth, however, the majority of studies reporting Pb toxicity were not conducted with plants grown under field conditions (U.S. EPA, 1977). The mechanism for Pb inhibition of photosynthesis was further elucidated in the 1986 Pb AQCD. Additional evidence of Pb effects on plant growth was also observed, however, the available studies were conducted under laboratory conditions at concentrations that exceeded Pb levels in the environment except near smelters or roadsides (U.S. EPA, 1986b). In the 1986 Pb AQCD, EC_{50} values for plant growth were available for several aquatic plants with the lowest EC_{50} of 1,100 µg Pb/L in *Azolla pinnata* exposed to Pb-nitrate for 4 days. Effects of Pb on metabolic processes in aquatic plants reviewed in the 2006 Pb AQCD included nitrate uptake, nitrogen fixation, ammonium uptake and carbon fixation at concentrations of 20,000 µg Pb/L and higher.

New information is available on Pb effects on oxidative stress endpoints such as changes in antioxidant enzymes, lipid peroxidation and reduced glutathione in aquatic plant, algae, and moss species exposed to Pb. 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 1,000 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 1,000 10,000 and 100,000 µg Pb/L (Dogan et al., 2009). This, along with the induction of some antioxidant systems and the reduction of growth at the highest two exposures, indicated that exposure to the metal caused significant stress, and that toxicity increased with exposure. In addition, native *Myriophyllum quitense* exhibited elevated antioxidant enzyme activity (glutathione-S-transferase, glutathione reductase, peroxidase) following transplantation in anthropogenically polluted areas containing elevated Pb concentrations. These were correlated with sediment Pb concentrations in the range of 5 to 23 mg Pb/g dry weight (Nimptsch et al., 2005).

Since the 2006 Pb AQCD, toxicity and oxidative stress were also observed in coontail (*C. demersum*) rooted aquatic macrophytes following 7-day 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 (Mishra et al., 2006b). Similarly, curly pondweed (*P. crispus*) exposed to lead (10 to 50 mM) exhibited increased generation of reactive oxygen species (Xu et al., 2011).

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 (50,000 to 300,000 µg Pb/L); total phenols also increased with increasing Pb exposure. Authors noted that this species exhibited similar responses under extreme temperatures, drought, and disease. According to Odjegba and Fasidi (2006), 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 and 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 freshwater algae *Scenedesmus vacuolatus* were significantly increased over that of non-exposed algal cultures (Le Faucheur et al., 2006). The 72-hour Pb exposure also significantly reduced *S. vacuolatus* growth, and of all the metals tested (Cu, Zn, Ni, Pb, Ag, As, and Sb), Pb was determined to be the most toxic to the algae species. In the algae *Chlamydomonas reinhardtii*, phytochelatin concentrations were lower than intracellular Pb and not sufficient to bind to accumulated metal following 72-hour exposure (Scheidegger et al., 2011).

In addition to oxidative stress responses, there is new information since the 2006 Pb AQCD on growth effects observed at high concentrations of Pb. 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 20,000 µg 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 5,000 and 10,000 µg Pb/L, while exposure to 15,000 µg Pb/L was associated with notable plant mortality (Hurd and 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). 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).

All of the observed effects on aquatic macrophytes and algae occur at concentrations not typically encountered in surface waters.

7.3.5.2 Freshwater Invertebrates

Few studies on biological effects of Pb in freshwater invertebrates had been conducted at the time of the 1977 Pb AQCD. One study reported an effect on reproduction in *Daphnia magna* at 30 µg Pb/L (U.S. EPA, 1977). In the 1986 Pb AQCD, increased mortality was observed in the freshwater snail *Lymnaea palustris* as low as 19 µg Pb/L and reproductive impairment was reported as low as 27 µg Pb/L for Daphnia sp. Endpoints of effects of Pb on aquatic invertebrates reviewed in the 2006 Pb AQCD included metabolism, reproduction, growth, and survival. Pb was recognized to be more toxic in longer-term exposures than shorter-term exposures with chronic toxicity thresholds for reproduction in water fleas (*D. magna*) ranging as low as 30 µg Pb/L. In aquatic invertebrates, Pb has also been shown to affect stress responses and osmoregulation. New evidence that supports previous findings of Pb effects on reproduction and growth in invertebrates is reviewed here as well as limited studies on changes in gene expression and behavioral effects associated with Pb exposure.

Recent literature strengthens the evidence indicating that Pb affects enzymes and antioxidant activity in aquatic invertebrates. These alterations at the suborganismal level may serve as biomarkers for effects at the organism level and higher. In invertebrate species that have hemoglobin, ALAD activity can be measured as a biomarker for Pb exposure. In the freshwater mussel *B. glabrata* and the freshwater oligochaete *Lumbriculus variegatus* a significant negative correlation between whole body tissue ALAD enzyme activity and increasing Pb was observed following 48 hour exposure to varying concentrations of the metal (Aisemberg et al., 2005). The concentration at which 50% of enzyme inhibition was measured was much lower in *B. glabrata* (23 to 29 µg Pb/L) than in *L. variegatus* (703 µg Pb/L). A significant negative correlation was also observed between ALAD activity and metal accumulation by the organisms. Sodium and potassium-ATPase (NA, K-ATPase) activity in gills of Eastern elliptio mussels was

significantly reduced following a 28-day exposure to 57 µg Pb/L and 245 µg Pb/L (Mosher et al., In Press). A significant reduction in Na^{2+} and significant increase in Ca^{+} in hemolymph was only observed at the highest concentration.

Studies of stress responses to Pb in invertebrates, conducted since the 2006 Pb AQCD, include induction of heat shock proteins and depletion of glycogen reserves. 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.

The effect of Pb on the osmoregulatory response has been studied since 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.

Recently, genomic-level responses to Pb-exposure have been observed in Daphnia. The effects of Pb on *D. magna* hemoglobin gene expression was measured in daphnids exposed for 24 hours to 25, 250, or 2,500 µg Pb/L as Pb-nitrate (Ha and Choi, 2009). A significant induction in all four genes tested was observed at the highest concentration with a two-fold change in two of the genes. Hemoglobin expression was also significantly elevated above the controls in one gene at 25 µg Pb/L and in two genes at 250 µg Pb/L although this expression was less than two-fold when compared to the controls. Changes in gene expression can lead to changes at the whole organism level although these links are frequently not established.

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. Feeding rate of the blackworm *L. variegatus* was significantly suppressed by day 6 of a 10 day sublethal test in Pb-spiked sediments (Penttinen et al., 2008) as compared to feeding rates at the start of the experiment. However, this decrease of approximately 50% of the initial feeding rate was also observed in the controls; therefore it is likely caused by some other factor other than Pb exposure. Aqueous soil leachates containing multiple metals, including Pb, had no effect on *D. magna* mobility. Authors noted that although concentrations (13 to 686 µg Pb/L) exceeded Canadian Environmental Quality Guidelines, no significant correlation could be established between Pb exposure and *D. magna* mobility; in fact, the cladocerans were more sensitive to Fe and Al in the leachate than to Pb (Chapman et al., 2010).

New evidence of reproductive and developmental effects of Pb on freshwater invertebrates available since the 2006 Pb AQCD include data from previously untested species as well as further characterization of reproductive effects in commonly tested organisms such as Daphnia sp. 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. 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. In a 21-day reproductive test in *D. magna* the number of neonates born per female was significantly reduced at 25, 250, and 2,500 µg Pb/L (Ha and Choi, 2009). *C. dubia* reproduction was also impacted by a seven-day exposure to 50 to 500 µg Pb/L. Both DOC, and, to a lesser degree, alkalinity were observed to ameliorate the effects of Pb on *C. dubia* reproduction. As DOC increased from 100 µmol C/L to 400 and 600 µmol C/L, the calculated mean EC_{50} values for *C. dubia* reproduction increased from approximately 25 µg Pb/L to 200 µg Pb/L and greater than 500 µg Pb/L, respectively (Mager et al., 2011a).

Reproductive variables including average lifespan, rate of reproduction, generation time and rate of population increase were adversely affected in the rotifer *Brachionus patulus* under conditions of increasing turbidity and Pb concentration (Garcia-Garcia et al., 2007).

In larvae of the mosquito, C*ulex quinquefasciatus*, exposed to 50 µg Pb/L, 100 µg Pb/L or 200 µg Pb/L, Pb-nitrate exposure was found to significantly reduce hatching rate and egg-production at all concentrations and larval emergence rate at 200 µg Pb/L

(Kitvatanachai et al., 2005). Larval emergence rates of 78% (F0), 86% (F1) and 86% (F2) were observed in the control group while emergence rates decreased in each generation 46% (F0), 26% (F1) and 58% (F2) in mosquitoes reared in a concentration of 200 µg Pb/L. The time to first emergence also increased slightly to 10 days in the Pb-exposed group as compared to the control group where emergence was first observed on day 9. In the F2 generation of parents exposed to 200 µg Pb/L, the ratio of female to male offspring was 3.6:1.0. No effects were observed on oviposition preference of adult females, larval weight or larval deformation.

As noted in the 2006 Pb AQCD, Pb exposure negatively affects the growth of aquatic invertebrates. Some studies reviewed in the previous Pb AQCD suggested that juveniles do not discriminate between the uptake of essential and non-essential metals (Arai et al., 2002). In new literature, the freshwater pulmonate snail *Lymnaea stagnalis* has been identified as a species that is extremely sensitive to Pb exposure. Growth of juveniles was inhibited at EC_{20} <4 µg Pb/L. (Grosell and Brix, 2009; Grosell et al., 2006a). In *L. stagnalis* exposed to 18.9 µg/L Pb for 21 days, Ca^{2+} influx was significantly inhibited and model estimates indicated 83% reduction in growth of newly hatched snails after 30 days at this exposure concentration (Grosell and Brix, 2009). The authors speculate that the high Ca^{2+} demand of juvenile *L. stagnalis* for shell formation and interference of the Ca^{2+} uptake pathway by Pb result in the sensitivity of this species.

In a study of the combined effects of temperature (22^0C or 32^0C), Pb concentration (50, 100 and 200 µg Pb/L) and presence of a competitor, the population growth rate of two freshwater rotifer species, *Brachionus havanaensis* and *B. rubens*, as measured by quantifying the number of live rotifers for 15 days responded to presence of stressors (Montufar-Melendez et al., 2007). At the lowest temperature, *B. rubens* suppressed population growth of *B. havanaensis* at 50 µg Pb/L and higher and *B. rubens* population growth did not increase at any Pb concentration at 32^0C, a temperature more suited for *B. havanaensis*. In situ toxicity testing with the woodland crayfish (*Orconectes hylas*) indicated that crayfish survival and biomass were significantly lower in streams impacted by Pb mining and that concentrations of Pb and other metals in water, detritus, macroinvertebrates, fish and crayfish were significantly higher at mining sites (Allert et al., 2009a).

Although Pb is known to cause mortality when invertebrates are exposed at sufficiently high concentrations, species that are tolerant of Pb may not exhibit significant mortality even at high concentrations of Pb. 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 (185,000 µg Pb/L) (Tollett et al., 2009). Other species are more sensitive to Pb in the environment and these responses are reviewed in Section 7.3.6.

7.3.5.3 Freshwater Vertebrates

The 1977 Pb AQCD reported on Pb effects to domestic animals, wildlife and aquatic vertebrates. The available Pb studies were from exposure to Pb via accidental poisoning or ingestion of Pb shot. Studies on aquatic vertebrates reviewed in the 1986 Pb AQCD were limited to hematological, neurological and developmental responses in fish. In the 2006 Pb AQCD, effects on freshwater vertebrates included new data for fish specifically considering the effects of water quality parameters on toxicity, as well as limited information on sensitivity of aquatic stages of frogs and turtles to Pb. Biological effects of Pb on freshwater fish that have been studied since the 2006 Pb AQCD report are reviewed here, and limited new evidence of Pb effects on amphibians are considered. This section also presents 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 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.

Freshwater Fish

Evidence of toxicity of Pb and other metals to freshwater fish goes back to early observations whereby contamination of natural areas by Pb mining lead to extirpation of fish from streams (U.S. EPA, 1977). At the time of the 1977 Pb AQCD, documented effects of Pb on fish included mucous secretion, anemia, functional damage to inner organs, physical deformities and growth inhibition. Additionally, the role of temperature, pH, hardness and other water quality parameters on Pb toxicity was discussed in the 1977 Pb AQCD. The 1986 Pb AQCD reported that hematological and neurological responses were the most commonly observed effects in fish and the lowest exposure concentration causing either hematological or neurological effects was 8 µg Pb/L. These findings were additionally supported in the 2006 Pb AQCD, where observed effects of Pb on fish included inhibition of heme formation, alterations in brain receptors, effects on blood chemistry, and decreases in some enzyme activities (U.S. EPA, 2006c). Functional responses resulting from Pb exposure included increased production of mucus, changes in growth patterns, and gill binding affinities. According to Eisler (2000) and reviewed in

the 2006 Pb AQCD, the general symptoms of Pb toxicity in fish include production of excess mucus, lordosis, anemia, darkening of the dorsal tail region, degeneration of the caudal fin, destruction of spinal neurons, ALAD inhibition, growth inhibition, renal pathology, reproductive effects, growth inhibition and mortality.

Evidence of Pb effects on fish available since the 2006 Pb AQCD generally support the findings in previous Pb reviews and elucidate the mechanisms of Pb-associated toxicity on some physiological responses. At the sub-organism level, new information on Pb effects on DNA, specific enzymes, ionoregulation and other biochemical responses is presented followed by a discussion of new information on organism-level endpoints including behavior and reproduction. Since the 2006 Pb AQCD evidence of direct interaction of Pb with fish DNA has become available as well as additional studies on the genotoxic effects of Pb exposure to fish. Hong et al. (2007a) observed covalent binding of Pb with kidney DNA from silver crucian carp (*Carassius auratus gibelio*) though extended X-ray absorption fine structure spectroscopy. In the freshwater fish *Prochilodus lineatus*, blood, liver, and gill cells were sampled from fish treated with nominal concentration of 5,000 µg Pb/L as Pb-nitrate for 6, 24 and 96-hours and then DNA damage was assessed by comet assay (Monteiro et al., 2011). DNA breaks were observed in all cell types after 96-hour exposure.

Upregulation of antioxidant enzymes in fish is a well-recognized response to Pb exposure. Since the last review, additional studies demonstrating antioxidant activity as well as evidence for production of reactive oxygen species following Pb exposure are available. Silver crucian carp injected with 10, 20 or 30 mg Pb/kg wet weight Pb-chloride showed a significant increase in the rate of production of superoxide ion and hydrogen peroxide in liver (Ling and Hong, 2010). In the same fish, activities of liver SOD, catalase, ascorbate peroxidase, and glutathione peroxidase were significantly inhibited. Both glutathione and ascorbic acid levels decreased and malondialdehyde content increased with increasing Pb dosage, suggesting that lipid peroxidation was occurring and the liver was depleting antioxidants. 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, 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 and 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. Serum alanine aminotransferase and aspartate aminotransferase activities were elevated in Nile tilapia exposed to 50 µg Pb/L in 4 and 21 day aqueous exposures while elevations in alkaline phosphatase and lactate dehydrogenase were only observed at 21 days (Fırat et al., In Press). In another study with Nile tilapia, Pb had no effect on glutathione measured in liver, gill, intestine, muscle and blood and liver metallothionein levels following a 14 day exposure to 5, 10, and 20 µM concentrations of Pb nitrate (Atli and Canli, 2008).

Metabolic enzyme activity in teleosts has also been measured following dietary exposures. Alves and Wood (2006) in a 42 day chronic dietary Pb study with 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, aspartate transaminase, and lactate dehydrogenase activities significantly decreased in kidney in a concentration-dependent manner (Dai et al., 2009a) and increased in liver with increasing concentration of dietary Pb. In a subsequent study using the same exposure paradigm, the digestive enzymes amylase, trypsin and lipase in tilapia were inhibited by dietary Pb in a concentration-dependent manner (Dai et al., 2009b). 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.

Pb was shown to inhibit hepatic cytochrome P450 in carp (*C. 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.

In environmental assessments of metal-impacted habitats, ALAD is a recognized biomarker of Pb exposure (U.S. EPA, 2006c). For example, lower ALAD activity has been significantly correlated with elevated blood Pb concentrations in wild caught fish from Pb-Zn mining areas although there are differences in species sensitivity (Schmitt et al., 2007b; Schmitt et al., 2005). Suppression of ALAD activity in brown trout transplanted to a metal contaminated stream was linked to Pb accumulation on gills and in liver in a 23 day exposure (Heier et al., 2009). 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), $CaSO_4$ (37 µg 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).

In fish, changes in blood chemistry associated with Pb exposure were noted in the 2006 Pb AQCD and limited new literature since the last Pb review support these findings. 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. In traira exposed to dietary doses (21 µg Pb/g via prey [Astyanax sp.]) for five days, there were no significant changes to leukocytes or hemoglobin concentration and volume (Oliveira Ribeiro et al., 2006). Significant differences in area, elongation and roundness of erythrocytes were observed in the Pb-exposed individuals using light microscopy image analysis.

Disruption of ionoregulation is one of the major modes of action of Pb toxicity. The gill has long been recognized as a target of Pb in teleosts. Acute Pb toxicity at the fish gill primarily involves disruption of Ca homeostasis as previously characterized in the 2006 Pb AQCD (Rogers and Wood, 2004; Rogers and Wood, 2003). In addition to this mechanism, Pb was found to induce ionoregulatory toxicity at the gill of rainbow trout through a binding of Pb with Na-K, ATPase and rapid inhibition of carbonic anhydrase activity thus enabling noncompetitive inhibition of Na^+ and Cl^- influx (Rogers et al., 2005). Alves et al. (2006) administered a diet of three concentrations of Pb (7, 77 and 520 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 and Blust, 2009b).

Long-term exposures of Pb can impact gill structure and function. Histopathological observations of gill tissue in the catfish (*C. gariepinus*) following an 8-week aqueous exposure to 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 tubular 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 1,200 µg Pb/L as Pb nitrate. Rates of Na^+ and Cl^- excretion decreased by 30% by 48 hours while Mg excretion increased two-to-three fold by 96 hours. Urine flow rate was not altered by Pb exposure, although urinary Pb excretion rate was significantly increased. After 24 hours of Pb exposure, the urine excretion rate of Ca^+ increased significantly by approximately 43% and remained elevated above the excretion rate in the control group for the duration of the exposure. Glomerular filtration rate significantly decreased only during the last 12 hours of the exposure. Ammonia excretion rate increased significantly at 48 hours as urine pH correspondingly decreased. At the end of the experiment glucose excretion was significantly greater in Pb-exposed fish. Although the exposures in this study approached the 96-hour LC_{50}, nephrotoxic effects of Pb indicate the need to consider additional binding sites for this metal in the development of biotic ligand modeling (Patel et al., 2006). Additional evidence for Pb effects on ion levels were observed in serum of Nile tilapia; Na^+ and Cl^- were decreased and K^+ levels were elevated following a 21 day exposure to 50 µg Pb/L (Fırat et al., In Press).

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, 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).

Evidence from the 2006 Pb AQCD and earlier Pb reviews indicate that Pb can impair both cognitive and motor function in fish. Reduced locomotion and foraging ability were observed in Chinese sturgeon juveniles exhibiting abnormal body curvature following exposure to either 800 or 1,600 µg Pb/L for 112 days (Hou et al., 2011). These chondrostei fish gradually recovered from deformities during a depuration period and were able to swim and forage effectively 6 weeks after transfer into clean water.

Since the 2006 Pb AQCD, several studies integrating behavioral and physiological measures of Pb toxicity have been conducted on fish. Sloman et al. (2005) investigated the effect of Pb on hierarchical social interactions and the corresponding monoaminergic profiles in rainbow trout. Trout were allowed to establish dominant-subordinate relationships for 24 hours, and then were exposed to 46 µg Pb/L or 325 µg Pb/L (Pb-nitrate) for 48 hours to assess effects on behavior and brain monoamines. In non-exposed fish, subordinate individuals had higher concentrations of circulating plasma cortisol and telencephalic 5-hydroxyindoleacetic acid/5-hydroxytryptamine (serotonin) (5-HIAA/5-HT) ratios. In the high concentration of Pb, there was significant uptake of Pb into gill, kidney and liver when compared with the control group and dominant fish appeared to have elevated hypothalamic 5-HIAA/5HT ratios. Uptake of Pb into the liver was higher in subordinate fish when compared to the dominant fish. No significant differences were observed in cortisol levels or behavior after metal exposure.

Mager et al. (2010) conducted prey capture assays with 10 day old fathead minnow larvae born from adult fish exposed to 120 µg Pb/L for 300 days, then subsequently tested in a breeding assay for 21 days. The time interval between 1st and 5th ingestion of 10 prey items (*Artemia nauplii*) was used as a measure of behavior and motor function of offspring of Pb-exposed fish. Larvae were offered 10 Artemia and the number ingested within 5 minutes was scored. The number of larvae ingesting 5 Artemia decreased within

the time period in offspring of Pb-exposed fish as compared to the control group, leading the authors to suggest this behavior is indicative of motor/behavioral impairment.

In another study with fathead minnows, swimming performance measured as critical aerobic swim speed was significantly impaired in minnows in 24-hour acute (139 µg Pb/L) and chronic 33 to 57 day (143 µg Pb/L) exposures, however, no significant difference in swim speed was observed in chronic exposures to 33 µg Pb/L (Mager and Grosell, 2011).

Zebrafish embryos exposed to low concentrations of Pb (10nM or 30 nM) until 24 hours post-fertilization and then subsequently tested as larvae or adult fish exhibited behavioral disruptions in response to mechanosensory and visual stimuli (Rice et al., 2011). Startle response time in larvae measured as maximum head turn velocity and escape time decreased in a concentration-dependent pattern following a directional, mechanical stimulus (tapping). The pattern of escape swimming was altered in larvae of Pb-exposed embryos compared to controls. In adult fish hatched from Pb-exposed embryos (30 nM), visual response to a rotating black bar against a white background (ability to detect contrast) was significantly degraded. In another study with zebrafish embryos exposed to higher concentration of Pb (200,000 nM) from 0 to 6 days post hatch, swim movements and escape action were also significantly slower than the control group (Dou and Zhang, 2011).

Reproductive and developmental effects of Pb in fish have been reported for several decades. In the 1977 Pb AQCD, second generation brook trout (*Salvelinus fontinalis*) exposed to 235 or 474 µg Pb/L were shown to develop severe spinal deformities (scoliosis) (U.S. EPA, 1977). Pb concentration of 120 µg Pb/L produced spinal curvature in rainbow trout (*Oncorhynchus mykiss*) and spinal curvatures were observed in developing eggs of killifish as reviewed in the 1986 Pb AQCD (U.S. EPA, 1986b). Limited new studies on reproductive effects of Pb in fish from oocyte formation to spawning are available. The effects of metals on embryonic stage of fish development in *C. carpio* and other species were reviewed in Jezierska et al. (2009) and included developmental abnormalities during organogenesis as well as embryonic and larval malformations. The authors concluded that the initial period of embryonic development, just after fertilization, and the period of hatching are the times at which developing embryos are most sensitive to metals. A significant concentration-dependent increase in morphological malformations was observed in African catfish embryos exposed to 100 µg Pb/L, 300 µg Pb/L or 500 µg Pb/L Pb-nitrate from 6 hours post-fertilization to 168 hours post-fertilization (Osman et al., 2007b). Hatching was delayed with increasing Pb concentration and hatch success of the embryos decreased from 75% in the controls to 40% in the group exposed to 500 µg Pb/L. Chinese sturgeon exposed to nominal

concentrations of 200 µg Pb/L, 800 µg Pb/L or 1,600 µg Pb/L for 112 days (96 hour post-fertilized eggs through juvenile stages) exhibited body curvatures in the two highest concentrations (Hou et al., 2011). During a 42 day depuration period in clean water following exposure, the degree of curvature in affected individuals decreased with decreasing tissue concentrations of Pb.

Reproductive 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 and endocrine effects of Pb have also been observed at the cellular level in fish, 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).

There is also evidence for alterations in steroid levels associated with Pb exposure in other species of fish. Carp (*Cyprinus carpio*) exposed for 35 days to nominal concentration of 410 µg Pb/L experienced altered plasma cortisol and prolactin levels. Plasma cortisol levels significantly increased throughout the study period while plasma

prolactin increased up to day 14 and then declined and was not significantly different from controls by the end of the experiment (Ramesh et al., 2009). Cortisol levels were significantly decreased in Nile tilapia exposed to 50 µg Pb/L for 4 days but were followed by a return to control levels at 21 days of exposure (Fırat et al., In Press). Vitellogenin was significantly induced in juvenile goldfish (*Carassius auratus*) following 96-hour exposure to 1 x 10^{-9} and 1 x10^{-10} M Pb when compared to control fish (Isidori et al., 2010). In the same study, estrogenicity of Pb was detected in vitro using a proliferation assay with estrogen receptor-positive human MCF-7 cells.

Reduction of growth in fish was noted as an effect of Pb exposure in the 2006 Pb AQCD. New studies available since the 2006 Pb AQCD do not present consistent evidence of growth reduction in fish associated with Pb. 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. (2006b) 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., 2006b). Fathead minnows exposed to 33 µg Pb/L to test swimming performance had significantly greater body length and body mass compared to control fish following a mean Pb exposure duration of 41 days (range 33 to 57 days) (Mager and Grosell, 2011). In 30 day chronic tests in which a range of pH values (6.4, 7.5 and 8.3) were tested with low (25-32 µg Pb/L), intermediate (82-156 µg Pb/L) and high (297-453 µg Pb/L) concentrations of Pb, Mager et al. (2011b) did not observe growth impairment in fathead minnows at environmentally relevant concentrations of Pb.

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 et al., 2009b). Growth and survival were not significantly affected in juvenile rainbow trout, fathead minnow and channel catfish (*Ictalurus punctatus*) fed a live diet of *L. variegatus* contaminated with Pb (850-1,000 µg Pb/L·g dry mass for 30 days. (Erickson et al., 2010). Two 60-day early lifestage tests with rainbow trout showed differences in LOEC for reduced growth (Mebane et al., 2008). In the first test, a 69 day exposure, the LOECs for mortality and reduced growth were the same (54 µg Pb/L). In the second test, a 62 day exposure of Pb to rainbow trout, the LOEC for fish length was 18 µg Pb/L with an EC_{20} of >87 µg Pb/L. Faster growth rates were associated with lower whole-body trace element concentrations in salmon (*Salmo salar*) across several streams in New Hampshire and Massachusetts, U.S., regardless of whether accumulation was from prey items or from water (Ward et al., 2010). In sites where conditions in the streams were conducive to rapid salmon growth, Pb concentrations were 86% lower than in streams where salmon were smaller.

Amphibians

Amphibians move between terrestrial and aquatic habitats and can therefore be exposed to Pb both on land and in water. The studies reviewed here are all aquatic or sediment exposures. Biological effects of Pb on amphibians in terrestrial exposure scenarios are reviewed in Sections 7.2.3.3 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, 2006c). 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 (Chen et al., 2006b). In this chronic study, the concentrations represent the range of Pb found in surface freshwaters across the U.S. The lowest concentration of 3 µg Pb/L approaches the EPA chronic criterion for Pb of 2.5 µg Pb/L at a hardness of 100 mg/L or 4.5 µg Pb/L at a hardness of 170 mg/L (U.S. EPA, 2002c). 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 absorption for 30 days (Wang and Jia, 2009). All doses significantly increased MDA, a product of oxidative stress, and glutathione levels were elevated in all but the lowest treatment group. In the same study, damage to DNA assessed by DNA tail length showed effects at >200 µg Pb/L and DNA tail movement showed effects at >400 µg Pb/L. The authors concluded that the effects on endpoints of oxidative stress and DNA damage detected in testes indicated a possible reproductive effect of Pb to black-spotted frogs.

The genotoxic potential of Pb to larvae of the frog (*X. laevis*) was assessed by determining the number of micronucleated erythrocytes per thousand (MNE) following a 12 day exposure (Mouchet et al., 2007). The lowest Pb concentrations with *X. laevis* (10 and 100 µg Pb/L) did not exhibit genotoxic effects while both 1,000 and 10,000 µg Pb/L significantly increased MNE to 14 and 202, respectively compared to the control (6 MNE). In another chronic genotoxic study, erythrocytic micronuclei and erythrocytic nuclear abnormalities were significantly increased with increasing Pb concentrations (700 µg Pb/L , 1,400 µg Pb/L , 14,000 µg Pb/L, 70,000 µg Pb/L) during 45, 60, and 75 day exposures of tadpoles *Bufo raddei* (Zhang et al., 2007b). The authors noted that the erythrocytic micronuclei and erythrocytic nuclear abnormalities frequencies generally decreased with increasing exposure time and that this may be indicative of regulation of genotoxic factors by tadpoles.

Birds

As reviewed in Koivula and Eeva (2010) measurement of enzymes associated with oxidative stress in birds is a well-established biomarker of exposure to metals, however, little is known about the effects of this stress response in wild populations or at higher levels of ecological organization. Changes in ALAD activity and other oxidative stress biomarkers at low levels of Pb exposure were recently documented in mallards and coots (*Fulica atra*) from a lagoon in Spain impacted by Pb shot (Martinez-Haro et al., 2011). ALAD ratio in mallards decreased linearly with blood Pb levels between 6 and 40 µg

Pb/dL, and at Pb levels of < 20 µg Pb/dL effects on several antioxidant enzymes were observed in coots. Although the primary route of exposure to the birds was via ingestion of Pb shot, effects were observed lower than 20 µg Pb/dL, the background level frequently applied to Pb exposures in birds (Martinez-Haro et al., 2011; Brown et al., 2006).

Consideration of toxicity of Pb to vertebrate embryos that develop surrounded by a protective egg shell has been expanded since the 2006 Pb AQCD. Pb treatment of mallard duck (*Anas platyrhynchos*), eggs by immersion in 100 µg Pb/L for 30 minutes on day 0 of development did not increase malformations or mortality of embryos (Kertész and Fáncsi, 2003). However, immersion of eggs in 2,900 µg Pb/L under the same experimental conditions resulted in increased rate of mortality and significant malformations including hemorrhages of the body, stunted growth, and absence of yolk sac circulatory system (Kertesz et al., 2006). The second study was conducted to emulate environmental levels of Pb following a dam failure in Hungary.

7.3.6 Exposure and Response of Freshwater Species

Evidence regarding exposure-response relationships and potential thresholds for Pb effects on aquatic populations can inform determination of standard levels that are protective of aquatic ecosystems. The Annex of the 2006 Pb AQCD (U.S. EPA, 2006c) summarized data on exposure-response functions for invertebrates (Table AX7-2.4.1) and fish (Table AX7-2.4.2). The recent exposure-response studies in this section expand on the findings from the 2006 Pb AQCD with information on newly-tested organisms (including microalgae, invertebrate, amphibian and fish species). Overall, new data for freshwater invertebrates generally supports the previous finding of sensitivity of juvenile lifestages and indicates some effects of Pb observed in some species at environmentally relevant concentrations.

The aquatic freshwater microalgae *Scenedesmus obliquus* was significantly more sensitive to Pb exposure than *Chlorella vulgaris* algae, although these authors stated that both appeared to be very tolerant of the heavy metal. Laboratory 48-hour standard toxicity tests were performed with both of these species and respective EC_{50} values of 4,000 and 24,500 µg Pb/L for growth as measured by cell division rate were derived (Atici et al., 2008). The aquatic macrophyte *Lemna minor* (duckweed) exhibited a EC_{50} for growth inhibition of 6,800 µg Pb/L in a 4-day exposure and 5,500 µg Pb/L for a 7-day exposure to a range of Pb concentrations from 100 to 10,000 µg Pb/L (Dirilgen, 2011). Experiments with the blue-green algae *Spirulina platensis* produced a LC_{50} value of 75,300 µg Pb/L (95% CI: 58.5, 97.0) (Arunakumara et al., 2008).

In the 2006 Pb AQCD, effects of Pb-exposure in amphipods (*H. azteca*) and water fleas (*D. magna*) were reported at concentrations as low as 0.45 µg Pb/L. Effective concentrations for aquatic invertebrates were found to range from 0.45 to 8,000 µg Pb/L. Since the 2006 Pb AQCD, recent studies have identified the freshwater snail *L. stagnalis* as a species that is extremely sensitive to Pb exposure (Grosell and Brix, 2009; Grosell et al., 2006a). Growth of juvenile *L. stagnalis* was inhibited below the lowest concentration tested resulting in an EC_{20} of < 4 µg Pb/L. In the same study, the NOEC was 12 µg Pb/L and the LOEC was 16 µg Pb/L. In contrast, freshwater juvenile ramshorn snails *M. cornuarietis* were less sensitive to Pb with the same LOEC for hatching rate and $LC_{50,}$ calculated to be about 10,000 µg Pb/L (Sawasdee and Köhler, 2010).

Additional studies on Pb effects in aquatic invertebrates published since the 2006 Pb AQCD have indicated differences in sensitivity of different lifestages of aquatic organisms to Pb. 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 (Wang et al., 2010e). The 96-hour EC_{50} values for the 5-day-old *L. siliquoidea* in two separate toxicity tests were 142 and 298 µg Pb/L (mean EC_{50} 220 µg Pb/L) in contrast to older juveniles (2 months old) with an EC_{50} >426 µg/L. The 24-hour median effect concentration for glochidia (larvae) of *L. siliquoidea* in 48-hour acute toxicity tests was >299 µg/L. A 28 day exposure chronic value of 10 µg Pb/L was obtained from 2-month-old *L. siliquoidea* juveniles, and was the lowest genus mean chronic value ever reported for Pb (Wang et al., 2010e). A 96-hour test on newly transformed juveniles was also conducted on *Lampsilis rafinesqueana* (Neosho mucket), a mussel that is a candidate for the endangered species list. The EC_{50} for this species was 188 µg Pb/L.

Different lifestages of chironomids have been shown to have varying sensitivity to Pb exposure in several studies available since the 2006 Pb AQCD. The acute toxicity of Pb to first-instar *C. riparius* larvae was tested in soft water, with hardness of 8 mg/L as $CaCO_3$ (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., 2006a). The EC_{20} for reduced growth and emergence of the midge *Chironomus dilutus* was 28 µg Pb/L, observed in a 55-day exposure, while the same species had a 96-hour LC_{50} of 3,323 µg Pb/L (Mebane et al., 2008). In fourth instars of the freshwater midge larvae *Chironomus javanus* the 24, 48, 72 and 96 hour LC_{50}'s were 20,490, 6,530, 1,690 and 720 µg Pb/L, respectively (Shuhaimi-Othman et al., In Press). This was

comparable to the 96-hour LC_{50} (420 µg Pb/L) in *Chironomus plumosus (*Vedamanikam and Shazilli, 2008a*).*

Cladocerans are commonly tested aquatic organisms, with data from three species: *D. magna*, *D. pulex* and *Ceriodaphnia dubia*, representing approximately 70% of available metal toxicological literature on this group (Wong et al., 2009). Recent studies have been conducted with *C. dubia* and acute toxicity values for other cladocerans as well as sublethal endpoints for *D. magna* are available. In a series of 48 hour acute toxicity tests with *C. dubia* conducted in a variety of natural waters across North America, LC_{50} values ranged from 29 to 180 µg Pb/L and were correlated with DOC (Esbaugh et al., 2011). Median lethal concentrations for *Moina micrura* (LC_{50} 690 µg Pb/L), *Diaphanosoma birgei* (LC_{50} 3,160 µg Pb/L), and *Alona rectangular* (LC_{50} 7,000 µg Pb/L) indicate differences in sensitivity to Pb in these freshwater cladocerans 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). The EC_{50} for swimming inhibition in neonate *D. magna* exposed to Pb-nitrate for 24 hours was 18,153 µg Pb/L (Ha and Choi, 2009).

Rotifers are among the most sensitive aquatic genera to Pb with wide variation in LC_{50} values reported between species (Pérez-Legaspi and Rico-Martínez, 2001). For example, in the rotifer genus *Lecane*, a 22-fold difference in LC_{50} values was observed in 48-hour exposure to Pb between *L. hamata, L. luna* and *L. quadridentata*. (Pérez-Legaspi and Rico-Martínez, 2001). *L. luna* was most sensitive to Pb toxicity with a 48-hour LC_{50} of 140 µg Pb/L. In a 48-hour toxicity test with the rotifer *Brachionus calyciflorus*, an NOEC (194 µg Pb/L), a LOEC (284 µg Pb/L), and an EC_{20} of 125 µg Pb/L was established for this species (Grosell et al., 2006a). The freshwater rotifer *Euchlanis dilatata* 48 hour LC_{50} was 35 µg Pb/L using neonates hatched from asexual eggs (Arias-Almeida and Rico-Martínez, 2011). In contrast, for rotifer *Brachionus patulus* neonates, the 24-hour LC_{50} was 6,150 µg Pb/L (Garcia-Garcia et al., 2007) .

Exposure-response assays on other freshwater species have been conducted since the 2006 Pb AQCD. The 24-hour LC_{50} for larvae of *C. quinquefasciatus* mosquitoes was 180 µg Pb/L (Kitvatanachai et al., 2005). A 48-hour LC_{50} of 5,200 µg Pb/L was observed in water-only exposures of the blackworm *Lumbriculus variegatus* (Penttinen et al., 2008). In the mayfly *Baetis tricaudatus*, the 96-hour LC_{50} was 664 µg Pb/L (Mebane et al., 2008). An EC_{20} value of 66 µg Pb/L was derived for *B. tricaudatus* by quantifying the reduction in the number of molts over a 10-day exposure to Pb (Mebane et al., 2008). The number of molts was significantly less than the control (average of 14 molts over 10 days) at concentrations of 160 µg Pb/L and higher with the lowest number of molts (average of 5.3 molts over 10 days) observed in the highest concentration (546 µg Pb/L).

In the freshwater ostracod *Stenocypris major*, the 96-hour LC_{50} was 526.2 µg Pb/L (Shuhaimi-Othman et al., In Press). In another freshwater crustacean, the prawn *Macrobrachium lancesteri*, the 96-hour LC_{50} was 35 µg Pb/L in soft water (<75 mg/L as $CaCO_3$) (Shuhaimi-Othman et al., 2011).

In the studies reviewed for the 2006 Pb AQCD, freshwater fish demonstrated adverse effects at concentrations ranging from 10 to >5,400 µg Pb/L, generally depending on water quality parameters (e.g., pH, hardness, salinity) (U.S. EPA, 2006c). Pb tended to be more toxic in longer-term exposures and correlated to Pb-uptake in tissues. Table AX7-2.4.2 of the 2006 Pb AQCD summarizes effects of Pb to fish. A series of studies published since the 2006 Pb AQCD have been conducted and have further elucidated the influence of water chemistry parameters on Pb uptake and toxicity in fathead minnow resulting in additional dose-response data for this species. Grosell et al. (2006a) 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., 2006b).

Mager et al. (2010) conducted 300-day chronic toxicity tests at 35 and 120 µg Pb/L with fathead minnow under conditions of varied DOC and alkalinity to assess the effects of these water quality parameters on fish growth and Pb-uptake. In additional tests with fathead minnow, Mager et al. (2011b) conducted both 96-hour acute and 30-day chronic tests to further characterize Ca^{2+}, DOC, pH, and alkalinity values on Pb toxicity. Increased Ca^{2+}, DOC and $NaHCO_3$ concentration afforded protection to minnows in acute studies. The role of pH in Pb toxicity is complex and likely involves Pb speciation and competitive interaction of H^+ with Pb^{2+} (Mager et al., 2011b). In a series of 96-hour acute toxicity tests with fathead minnow conducted in a variety of natural waters across North America, LC_{50} values ranged from 41 to 3,598 µg Pb/L and no Pb toxicity occurred in three highly alkaline waters (Esbaugh et al., 2011).

In the 2006 Pb AQCD, fish lifestage was recognized as an important variable in determining the sensitivity of these organisms to Pb. New data available since the 2006 Pb AQCD support the findings of increased sensitivity of juvenile fish to Pb when compared to adults. Acute (96-hour) and chronic (60-day) early-lifestage test exposures were conducted with rainbow trout to develop ACR's for this species (Mebane et al., 2008). Two early-lifestage chronic tests were conducted, the first with an exposure range

of 12-384 µg Pb/L (69 days) at 20 mg $CaCO_3$/L water hardness and the second with an exposure range of 8 to 124 µg Pb/L (62 days) and a water hardness of 29 mg $CaCO_3$/L. In the 69-day test, the following chronic values were observed for survival: NOEC=24 µg Pb/L, maximum acceptable toxicant concentration=36 µg Pb/L, EC_{10}=26 µg Pb/L, EC_{20}=34 µg Pb/L, and LC_{50}=55 µg Pb/L. Results from the 62-day test, with fish length as the endpoint, were NOEC=8 µg Pb/L, MATC=12 µg Pb/L, EC_{10}=7µg Pb/L, EC_{20}=102 µg Pb/L and LC_{50}=120 µg Pb/L. In acute tests with rainbow trout run concurrently with the chronic tests, 96-hour LC_{50} values were 120 and 150 µg Pb/L, respectively. Data from this study resulted in ACR's for trout lower than previously reported. The low ACR values were due to the acute tests which produced LC_{50} values that were 10 to 25 times lower than earlier studies with trout (Mebane et al., 2008). The authors speculated that the lower LC_{50} values were due to the age of the fish used in the study (two to four week old fry) and that testing with larger and older fish may not be protective of more sensitive lifestages.

There have been only a few 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 Pb/kg dry weight) with corresponding sediment pore water concentrations from 123 to 24,427 µg Pb/L from embryonic stage to metamorphosis exhibited concentration-dependent effects on survival (Sparling et al., 2006). The LC_{50} value for Pb in sediment was 3,738 mg Pb/kg, which corresponds to 12,539 µg Pb/L in sediment pore water. In the same study, concentration-dependent effects on skeletal development were observed. The 40 day-EC_{50} for deformed spinal columns in the tadpoles was 1,958 mg Pb/kg (corresponding to 6,734 µg Pb/L sediment pore water) and the 60 day-EC_{50} was 579 mg Pb/kg (corresponding to 1,968 µg Pb/L sediment pore water) (Sparling et al., 2006). 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).

7.3.7 Freshwater Community and Ecosystem Effects

As discussed in the 1986 Pb AQCD and the 2006 Pb AQCD, exposure to Pb is likely to have impacts in aquatic environments via effects at several levels of ecological organization (organisms, populations, communities, or ecosystems). These effects resulting from toxicity of Pb would be evidenced by changes in species composition and richness, in ecosystem function, and in energy flow. The 2006 Pb AQCD concluded that, in general, there was insufficient information available for single materials in controlled

studies to permit evaluation of specific impacts on higher levels of organization (beyond the organism). Furthermore, Pb rarely occurs as a sole contaminant in natural systems making the effects of Pb difficult to ascertain. New information on effects of Pb at the population, community and ecosystem level is reviewed below.

In laboratory studies reviewed in the 2006 Pb AQCD and this ISA, Pb exposure has been demonstrated to alter predator-prey interactions, as well as feeding and avoidance behaviors. In aquatic ecosystems there are field studies reviewed in the 1977 Pb AQCD, the 1986 Pb AQCD, the 2006 Pb AQCD and this ISA on reductions of species abundance, richness or diversity particularly in benthic macroinvertebrate communities coexisting with other metals where the sources of Pb were from mining or urban effluents. Additionally, field studies have linked Pb contamination to reduced primary productivity and respiration, and to altered energy flow and nutrient cycling. However, because of the complexity inherent in defining such effects, there are relatively few available population, community, or ecosystem level studies that conclusively relate Pb exposure to aquatic ecosystem effects. In addition, most of the available work is related to point-source Pb contamination, with very few studies considering the effects of diffuse Pb pollution.

Microcosm evaluations of the effects of Pb on aquatic cyanobacteria communities indicated that exposure to 25 mM Pb reduced bacterial biomass and diversity. After one week of Pb exposure, total bacteria biomass was reduced from an average of 9.3 mg carbon/cm^3 sediment to 1.3 mg carbon/cm^3 (Burnat et al., 2009). Pb exposure impacted individual cyanobacteria species differently, with *Microcoleus* sp. experiencing a greater decrease in abundance than *Halomicronema*-like cyanobacteria.

Both plant species and habitat type were determined to be factors affecting the rate of Pb accumulation from contaminated sediments. While the rooted aquatic plant *E. canadensis* was observed to accumulate the highest concentrations of Pb, the authors concluded that submerged macrophytes (versus emergent plants) as a group were the most likely to accumulate Pb and other heavy metals (Kurilenko and Osmolovskaya, 2006). This would suggest that certain types of aquatic plants, such as rooted and submerged species, may be more susceptible to aerially-deposited Pb contamination, resulting in shifts in plant community composition as a result of Pb pollution.

Alteration of macrophyte community composition was demonstrated in the presence of elevated surface water Pb concentrations at three lake sites impacted by mining effluents (Mishra et al., 2008). A total of 11 species of macrophytes were collected. Two sites located 500 m and 1,500 m downstream from the mining point-source (study sites 2 and 3) exhibited similar dissolved Pb concentrations (78 to 92 µg Pb/L, depending on season) and contained six and eight unique macrophyte species, respectively. The site nearest the

discharge point of the mine effluent (study site 1) had the highest Pb concentrations (103 to 118 µg Pb/L) and the lowest number of resident macrophyte species; these included *E. crassipes*, *L. minor*, *Azolla pinnata* and *S. polyrrhiza*. Based on analysis of plant tissue Pb concentrations, the authors theorized that certain species may be more able to develop Pb tolerant eco-types that can survive at higher Pb concentrations (Mishra et al., 2008).

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.

In field studies available for certain freshwater habitats, exposure to Pb has been shown to result in significant alterations of invertebrate communities. Macroinvertebrate community structure in mine-influenced streams was determined to be significantly correlated to Pb sediment pore water concentrations. Multiple invertebrate community indices, including Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa richness, Missouri biotic index, and Shannon-Wiener diversity index, were integrated into a macroinvertebrate biotic condition score (Poulton et al., 2010). These scores were determined to be significantly lower at sample sites downstream from mining sites where Pb pore water and bulk sediment concentrations were elevated. Sediment Pb, Cd, and Zn levels were inversely correlated to mussel taxa richness in the Spring River basin encompassing sites in Kansas, Missouri and Oklahoma overlapping a former Pb and Zn mining and processing area (Angelo et al., 2007). In sites upstream of the mining area, 21 to 25 species of mussels were present whereas in sites downstream, only 6 to 8 species were observed.

Rhea et al. (2006) examined the effects of multiple heavy metals in the Boulder River, 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 and Nabelkova, 2005). In contrast, benthic predators and collector-filterers accumulated significantly lower metals concentrations. Consequently, it is likely that sediment-bound Pb contamination would differentially affect members of the benthic invertebrate community, potentially altering 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. brightwellii* collected from a Pb-impacted freshwater reservoir in Mexico, contained 384 ng Pb/mg and exhibited a water-to-tissue BCF of 49,344. The authors theorized that Pb biomagnification may have been observed in this case because the cladoceran *M. micrura* is both a known Pb accumulator and a favorite prey item of the rotifer (Rubio-Franchini et al., 2008). They showed that *M. micrura* had twice the Pb body burden of *D. similis*, another grazing cladoceran species present in the reservoir. These two species exhibited average Pb tissue concentrations of 57 and 98 ng Pb/mg, respectively, with respective water column BCFs of 9,022 and 8,046. Conversely, an examination of the simultaneous uptake of dissolved Pb by the algae *P. subcapitata* and the cladoceran *D. magna* suggests that the dietary exposure route for the water column filter-feeder is minor. Although Pb accumulated in the algal food source, uptake directly from the water column was determined to be the primary route of exposure for *D. magna* (Komjarova and Blust, 2009c).

For many invertebrate species, sediment Pb concentrations may be the most important driver in determining Pb uptake. For instance, while Hg and Cd body burdens in lentic invertebrates were affected by lake ecological processes (e.g., eutrophication), a similar effect was not observed for Pb concentrations in crayfish tissue, despite a high variability between sites. 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 field survey of fishes in the Viburnum Trend Pb-Zn mining district in southeast Missouri available since the 2006 Pb AQCD found that species richness and species density of riffle-swelling benthic fishes were negatively correlated with metal concentrations in pore water and in fish in mining impacted streams (Allert et al., 2009b). Density of Ozark sculpin (*Cottus hypselurus*) and banded sculpin (*Cottus carolinae*) were positively correlated with distance from mining sources.

In addition to the ecological effects discussed above, there is additional evidence that Pb exposure could alter bacterial infection (and potentially disease transmission) in certain fish species. Following 96-hour exposures to 4,000 µg Pb/L, bacterial density in *Channa punctatus* fish was observed to be significantly altered when compared to non-exposed fish. Bacteria population densities in fish spleen, gills, liver, kidneys and muscle tissues were higher following Pb exposure, with bacterial abundance in the gills too numerous to quantify (Pathak and Gopal, 2009). In addition, bacteria inhabiting Pb-exposed fish were more likely to exhibit antibacterial resistance than colonies isolated from non-exposed fish. Although the mechanism remains unknown, this study suggests that Pb exposure may increase the likelihood of infection in fish, potentially affecting fish abundance and recruitment.

In summary, despite the fact that alterations of macrophyte communities may be highly visible effects of increased sediment Pb concentrations, several recently published papers propose that ecological impacts on invertebrate communities are also significant, and can occur at environmental Pb concentrations lower than those required to impact plant communities. High sediment Pb concentrations were linked to shifts in amphipod communities inhabiting plant structures, and potentially to alterations in ecosystem nutrient processing through selective pressures on certain invertebrate functional feeding groups (e.g., greater bioaccumulation and toxic effects in collector-gatherers versus predators or filter-feeders). Increased sediment pore water Pb concentrations were demonstrated to likely be of greater importance to invertebrate communities, as well. Interestingly, recent research also suggests that Pb exposure can alter bacterial infestations in fish, increasing both microbial density and resilience, and potentially increasing the likelihood of serious disease outbreak.

7.3.8 Critical Loads in Freshwater Aquatic Systems

The general concept and definition of critical loads is introduced in Section 7.1.3 of this chapter [also see Section 7.3 of the 2006 Pb AQCD (U.S. EPA, 2006c)]. Critical load

values are linked to critical limits of Pb for endpoints/receptors of interest in the ecosystems, such as blood Pb. Some important critical limits for lead in aquatic ecosystems are discussed in this section along with information on aquatic critical loads for Pb.

Unit World Models (UWM) have been used to calculate critical loads for metals in aquatic ecosystems. These models couple an ecotoxicity model, the BLM, to a speciation/complexation model, the Windermere Humic Adsorption Model (WHAM), then to the multi-species fate model, TRANsport-SPECiation (TRANSPEC). Gandhi et al. (In Press) apply the UWM to estimate speciation/complexation, fate and critical loads using lakes of three different trophic status. A high percentage of colloidal-bound Pb was found in the eutrophic and mesotrophic lakes (75-80%) vs. the oligotrophic lakes (2%), owing the high affinity of Pb to DOM. Pb concentrations were lowest for mesotrophic and highest for oligotrophic systems. Critical loads were not calculated for Pb; however, for the other metals tested the critical load was lowest in the oligotrophic and highest in the eutrophic systems.

A critical load of 39.0 g $Pb/m^2 \cdot yr$ was calculated for a generalized lake in the Sudbury area of the Canadian Shield using TICKET-UWM based on acute toxicity data for *D. magna*. (Farley et al., 2011). The model was set up to calculate critical loads of metals by specifying free metal ion activity or the critical biotic ligand concentration. This critical load for Pb was an order of magnitude higher than for Cu, Ni and Zn and the authors attribute this difference to the strong binding of Pb to particulate organic matter and the sequestration of $PbCO_3$ in sediment. Refer to Section 7.3.6 of the 2006 Pb AQCD for additional discussion of critical loads of Pb in aquatic systems.

7.3.9 Characterization of Sensitivity and Vulnerability in Freshwater Systems

Data from the literature indicate that exposure to Pb may affect survival, reproduction, growth, metabolism, and development in a wide range of freshwater aquatic species. Often, species differences in metabolism, sequestration, and elimination rates control relative sensitivity and vulnerability of exposed organisms. Diet and lifestage at the time of exposure also contribute significantly to the determination of sensitive and vulnerable populations and communities. Further, environmental conditions in addition to those discussed as affecting bioavailability (Sections 7.3.3 and 7.3.4) 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 lifestage as other important determinants of differential sensitivity to Pb.

7.3.9.1 Seasonally-Affected Physiological Changes

A study by Duman et al. (2006) identified species and seasonal effects of Pb uptake in aquatic plants. *P. australis* accumulated higher root Pb concentrations than *S. lacustris*. Additionally, the *P. australis* Pb accumulation factor was significantly higher during the winter versus other seasons, while the Pb accumulation factor for *S. lacustris* was greatest in spring and autumn. The Pb accumulation factor for a third species, *P. lucens*, was greatest in autumn (Duman et al., 2006). Most significantly, these changes in bioaccumulation were not linked with biomass increases, indicating that species-dependent seasonal physiological changes may control Pb uptake in aquatic macrophytes (Duman et al., 2007). Significant interspecies differences in Pb uptake were observed for plants representing the same genus (Sargassum), indicating that uptake of Pb by aquatic plants also may be governed by highly species-dependent factors (Jothinayagi and Anbazhagan, 2009).

Heier et al. (2009) established the speciation of Pb in water draining from a shooting range in Norway and looked at the time dependent accumulation in brown trout. They found that 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.

7.3.9.2 Increased Nutrient Uptake

Singh et al. (2010) proposed that metal-resistant plants have the capacity to not only up-regulate antioxidant synthesis, but also have the ability to increase nutrient consumption and uptake to support metal sequestration and detoxification via production of antioxidants (Singh et al., 2010). Therefore, it is likely that such plant species would be significantly less susceptible to Pb exposure than those species without those abilities.

7.3.9.3 Temperature and pH

Water temperature also appears to affect the toxicity of Pb to aquatic organisms, with higher temperatures leading to greater responses. Pb toxicity to crayfish increased 7 to

10% when the water temperature was increased by 4 °C, and by 14% when the temperature increased by 7 °C. The authors determined that the increased toxicity was a result of the negative impact of Pb on crayfish respiration, which was exacerbated by the lower dissolved oxygen concentrations at higher water temperatures (Khan et al., 2006). In a study of the combined effects of temperature and Pb concentration on two freshwater rotifer species, *Brachionus havanaensis* and *B. rubens*, population growth was measured in three concentrations of Pb (50, 100 and 200 µg Pb/L) for 15 days at either 22^0C or 32^0C (Montufar-Melendez et al., 2007). At 22^0C, population growth of *B. havanaensis* was suppressed by *B. rubens* regardless of Pb treatment. At the higher temperature, there was no population increase of *B. rubens* at any Pb concentration. In the controls, population growth rates of *B. havanaensis, but not B. rubens, increased with an increase in temperature. These studies highlight the role of temperature in Pb toxicity in organisms adapted to low temperatures.*

The sequestration ability of *L. minor* macrophytes was similarly impacted by increased surface water temperature; plants absorbed a maximum Pb concentration of 8.6 mg /g at 30 °C, while uptake at 15 °C was only 0.3 mg/g (Uysal and Taner, 2009). Decreased pH was also demonstrated to increase the uptake of environmental Pb in aquatic plants (Wang et al., 2010a; Uysal and Taner, 2009). 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.

7.3.9.4 Lifestage

It is clear that certain stages of a life cycle are more vulnerable to Pb. A comparison of *C. riparius* Pb LC_{50} values derived from toxicity tests with different instars indicates a significant effect of lifestage on Pb sensitivity for aquatic invertebrates. 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} values derived using later instar larvae—350,000 and 200,000 µg Pb/L, respectively. This disparity would suggest that seasonal co-occurrence of aquatic Pb contamination and sensitive early instars could have significant population-level impacts (Bechard et al., 2008). Similarly, Wang et al. (2010e) demonstrated that the newly transformed juvenile mussels, *L. siliquoidea* and *L. rafinesqueana*, at 5 days old were more sensitive to Pb exposure than were glochidia or two to six month- old juveniles, suggesting that Pb exposure at particularly sensitive lifestages could have a significant influence on population viability (Wang et al., 2010e).

Evidence for differences in susceptibility to Pb at distinct lifestages is also available for freshwater fish. In chronic (60-day) early-lifestage test exposures conducted with rainbow trout to develop ACR's for this species the study resulted in ACR's for rainbow trout lower than previously reported due to the acute tests which produced LC_{50} values that were 10 to 25 times lower than earlier studies with trout. (Mebane et al., 2008). The authors speculated that the lower LC_{50} values were due to the age of the fish used in the study (two to four week old fry) and that testing with larger and older fish may not be protective of more sensitive lifestages. Post-hatching stages of the African catfish were more sensitive than the embryonic stage to Pb-exposure and the authors attributed this apparent protective effect to the presence of a hardened chorion in embryos (Osman et al., 2007a).

7.3.9.5 Species Sensitivity

Species-specific Ca requirements have 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 and 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., 2006a).

There is some indication that molting may comprise an additional sequestration and excretion pathway for aquatic animals exposed to Pb (Soto-Jiménez et al., 2011b; Mohapatra et al., 2009; Tollett et al., 2009; Bergey and Weis, 2007). Libellulidae dragonfly nymphs (Tollett et al., 2009) have been shown to preferentially sequester Pb in exoskeleton tissue. Consequently, aquatic arthropod species and those species that shed their exoskeleton more frequently may be able to tolerate higher environmental Pb concentrations than non-arthropods or slow-growing molting species, as this pathway allows them to effectively lower Pb body burdens.

In contrast, the effect of Pb exposure on fish bacterial loads demonstrated by Pathak and Gopal (2009) suggest that infected fish populations may be more at risk to the toxic effects of Pb than healthier species. Aqueous Pb was demonstrated to both increase bacteria density in several fish organs and to improve the likelihood of antibacterial resistance (Pathak and Gopal, 2009).

Tolerance to prolonged Pb exposure may develop in aquatic invertebrates and fish. Multi-generational exposure Pb appears to confer some degree of metal tolerance in

invertebrates such as *C. plumosus* larvae; consequently, previous population Pb exposures may decrease species' susceptibility to Pb contamination (Vedamanikam and Shazilli, 2008b). However, the authors noted that metal tolerant larvae were significantly smaller than larvae reared under clean conditions, and that transference of Pb-tolerant *C. plumosus* larvae to clean systems resulted in a subsequent loss of tolerance. Evidence of acclimation to elevated Pb in fathead minnow was suggested in the variations in ionoregulatory parameters that were measured on day 10 and 30 in fish exposed to 115 µg Pb/L for 30 days. At the end of the experiment, whole body Ca^{2+} was elevated while Na^{+} and K^{+} recovered from elevated levels at 30 days (Grosell et al., 2006b).

A series of species sensitivity distributions constructed by Brix et al. (2005) in freshwater systems indicated that sensitivity to Pb was greatest in crustacean species, followed by coldwater fish, and warmwater fish and aquatic insects, which exhibited a similar sensitivity. Further, analysis of both acute and chronic mesocosm data sets indicated that Pb-contaminated systems exhibited diminished species diversity and taxa richness following both types of exposure (Brix et al., 2005). Wong et al. (2009) constructed Pb species sensitivity distributions for both cladoceran and copepod freshwater species. A comparison of the two curves indicated that cladoceran species, as a group, were more sensitive to the toxic effects of Pb than were copepods, with respective hazardous concentration values for 5% of the species (HC5) values of 35 and 77 µg Pb/L. This difference in sensitivities would indicate that cladoceran species are more likely to be impacted at lower environmental Pb concentrations than copepods, potentially altering community structures or ecosystem functions (Wong et al., 2009).

7.3.9.6 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 sensitive to effects of Pb include habitats that are acidified due to atmospheric deposition of pollutants, runoff from mining activities or lakes and streams with naturally occurring organic acids. Hence, fish and

invertebrate species endemic to such systems may be more at risk from Pb contamination than corresponding species in other habitats.

7.3.10 Introduction to Bioavailability and Biological Effects of Pb in Saltwater Ecosystems

Saltwater ecosystems include salt marsh, estuaries, beaches and other coastal areas that may receive Pb contributions from direct atmospheric deposition and/or via runoff from terrestrial systems. A range of 0.005-0.4 µg Pb/L for saltwater was reported in by Leland and Kuwabara (1985) and 0.01 to 27 µg Pb/L by Sadiq (1992) with the higher values associated with sites involving human activity. Levels of Pb in the North Atlantic and North Pacific surface waters ranged from 0.005 to 0.05 µg Pb/L but the range of values in coastal waters and estuaries were approximately equal to the range of Pb in freshwater. Additional information on Pb levels in water is available in Section 3.6. The 2006 Pb AQCD provided an overview of regulatory considerations for water and sediments in addition to consideration of biological effects and major environmental factors that modify the response of marine organisms to Pb exposure. Regulatory guidelines for Pb in saltwater have not changed since the 2006 Pb AQCD and are summarized below. This section is followed by new information on bioavailability and biological effects of Pb in saltwater since the 2006 Pb AQCD.

The most recent ambient water quality criteria (AWQC) for Pb in saltwater were released in 1985 (U.S. EPA, 1985) by the EPA Office of Water which employed empirical regressions between observed toxicity and water hardness to develop hardness-dependent equations for acute and chronic criteria. These criteria are published pursuant to Section 304(a) of the Clean Water Act and provide guidance to states and tribes to use in adopting water quality standards for the protection of aquatic life and human health in surface water. The ambient water quality criteria for Pb are currently expressed as a criteria maximum concentration (CMC) for acute toxicity and criterion continuous concentration (CCC) for chronic toxicity (U.S. EPA, 2010b). In saltwater, the CMC is 210 µg Pb/L and the CCC is 8.1 µg Pb/L. The 2006 Pb AQCD summarized two approaches for establishing sediment criteria for Pb based on either bulk sediment or equilibrium partitioning as reviewed in this ISA in Section 7.3.2.

In the following sections, new information available since the 2006 Pb AQCD on Pb in marine and estuarine ecosystems will be presented. Throughout the sections, brief summaries of conclusions from the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD are included where appropriate. The sections are organized to consider uptake of

Pb and effects at the species level, followed by community and ecosystem level effects. New research on the bioavailability and uptake of Pb into saltwater organisms including plants, invertebrates and vertebrates is presented in Section 7.3.11. Effects of Pb on the physiology of marine fauna and biota (Section 7.3.12) are followed with data on exposure and response of saltwater organisms (Section 7.3.13). Responses at the ecosystem level of biological organization are reviewed in Section 7.3.14 followed by characterization of sensitivity and vulnerability of ecosystem components (Section 7.3.15).

7.3.11 Bioavailability of Pb in Saltwater Systems

Bioavailability was defined in the 2006 Pb AQCD as "the proportion of a toxin that passes a physiological membrane (the plasma membrane in plants or the gut wall in animals) and reaches a target receptor (cytosol or blood)". In 2007, EPA took cases of bioactive adsorption into consideration and revised the definition of bioavailability as "the extent to which bioaccessible metals absorb onto, or into, and across biological membranes of organisms, expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time and under defined conditions" (Fairbrother et al., 2007).

Factors affecting bioavailability of Pb to marine organisms are the same as those in freshwater systems. However, although routes of exposure and physiological mechanisms for storage and excretion influence uptake of metals by all organisms, they may be different in marine organisms, particularly for ion transport mechanisms (Niyogi and Wood, 2004). Marine environments are characterized by higher levels of ions, such as Na^{+}, Ca^{2+}, and Mg^{2+}, which compete for potential binding sites on biotic ligands such as gills, thereby generally reducing the effective toxicity of metal ions as compared to freshwater environments. However, because the concentrations of these ions are relatively constant, bioavailability may be more predictable in marine systems than in freshwater systems, varying mostly with amount and type of dissolved organic matter. BLMs (Figure 7-2) now being developed for marine organisms are functionally similar to those applied to freshwater organisms (Section 7.3.4).

Although in freshwater systems the presence of humic acid is considered to reduce the bioavailable fraction of metals in freshwater, there is evidence that DOC/DOM does not have the same effect on free Pb ion concentration in marine systems (see Section 7.3.2.4 for detailed discussion). For the sea urchin *P. lividus*, the presence of humic acid increased both the uptake and toxicity of Pb possibly by enhancing uptake of Pb via membrane Ca^{2+} channels (Sanchez-Marin et al., 2010a). This also was observed in the marine diatom *Thalassiosira weissflogii*, where humic acids absorbed to cell surfaces

increased metal uptake (Sanchez-Marin et al., 2010a). Formation of a ternary complex that is better absorbed by biological membranes was another proposed mechanism that could describe the increased bioavailability to marine invertebrates of Pb bound to humic acid (Sánchez-Marín et al., 2007).

Sanchez-Marin et al. (2011) subsequently have shown that different components of DOM have different effects on Pb bioavailability in marine systems. Their initial research using commercially-derived humic acid found that increasing humic acid concentrations increased Pb uptake by mussel gills and increased toxicity to sea urchin larvae in marine environments (Sánchez-Marín et al., 2007). In contrast, a subsequent investigation found that fulvic acid reduced Pb bioavailability in marine water (Sánchez-Marín et al., 2011). The contradictory effects of different components of DOM on marine bioavailability likely reflect their distinct physico-chemical characteristics. More hydrophobic than fulvic acid, humic acid may adsorb directly with cell membranes and enhance Pb uptake through some (still unidentified) mechanism (Sánchez-Marín et al., 2011). Pb AVS-measurements were also determined to accurately predict uptake by mussels (*Mytilus* sp.) in the presence of 2.5 to 20 mg/L fulvic acid (Sánchez-Marín et al., 2011). However, the effects of DOM on Pb bioavailability to mussels were underpredicted by AVS lead concentration measurements, potentially as a result of adsorption of DOM-Pb complexes.

Based on the above, BLMs used to predict bioavailability of Pb to aquatic organisms (Di Toro et al., 2005), may require modifications for application to marine organisms. Of particular importance is the finding that in marine aquatic systems, surface water DOM was found to increase (rather than decrease) uptake of Pb by fish gill structures, potentially through the alteration of membrane Ca channel permeability. Veltman et al. (2010) proposed an integrating BLM and bioaccumulation models in order to more accurately predict metal uptake by fish and invertebrates, and calculated metal absorption efficiencies for marine fish species from both types of models. They noted that affinity constants for Ca, Cd, Cu, Na, and Zn were highly similar across different aquatic species, including fish and invertebrates (Veltman et al., 2010). These findings suggest that the BLM can be integrated with bioaccumulation kinetics to account for both environmental chemical speciation and biological and physiological factors in both marine and freshwater systems.

7.3.11.1 Saltwater Plants and Algae

In the 1977 Pb AQCD, the cordgrass *Spartina alterniflora* was found to reduce by a small amount the quantity of Pb in sediments (U.S. EPA, 1977). Limited data on marine algal species reviewed in the 1986 Pb AQCD and 2006 Pb AQCD provided additional

evidence for Pb uptake. New data available since the 2006 Pb AQCD includes Pb bioaccumulation studies conducted with five species of marine algae, (*Tetraselmis chuii*, *Rhodomonas salina*, *Chaetoceros* sp., *Isochrysis galbana* and *Nannochloropsis gaditana*). In this study it was demonstrated that bioaccumulation rates varied with species. *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).

New uptake studies of Pb in plants associated with marine environments are also available. The roots of two salt marsh species, *Sarcocornia fruticosa* and *Spartina maritima*, significantly accumulated Pb, to maximum concentrations of 2,870 mg Pb/kg and 1,755 mg Pb/kg, respectively (Caetano et al., 2007). Roots had similar isotopic signature to those of sediments in vegetated zones indicating that Pb uptake by plants reflects the input in sediments. BCFs for Pb in root tissue from mangrove tree species range between 0.09 and 2.9, depending on the species and the habitat, with an average BCF of 0.84. The average BCF for mangrove species leaf tissue was considerably less (0.11), as these species are poor translocators of Pb (MacFarlane et al., 2007).

7.3.11.2 Saltwater Invertebrates

Uptake and subsequent bioaccumulation of Pb in marine invertebrates varies greatly between species and across taxa as previously characterized in the 2006 Pb AQCD. This section expands on the findings from the 2006 Pb AQCD on bioaccumulation and sequestration of Pb in saltwater invertebrates. In the case of invertebrates, Pb can be bioaccumulated from multiple sources, including the water column, sediment, and dietary exposures, and factors such as proportion of bioavailable Pb, lifestage, age, and metabolism can alter the accumulation rate. In this section, new information on Pb uptake and subsequent tissue and subcellular distribution will be considered, followed by a discussion on dietary and water routes of exposure and strategies for detoxification of Pb in marine invertebrates.

The gills were the main sites of Pb accumulation in pearl oyster, *Pinctada fucata* followed by mantle, in 72-hour exposures to 103.5 µg Pb/L (Jing et al., 2007). Following a 10 day exposure to 2,500 µg Pb/L as Pb nitrate, accumulation of Pb was higher in gill than digestive gland of *Mytilus edulis*: after a 10 day depuration, Pb content was decreased in the gills and digestive gland of these mussels (Einsporn et al., 2009). In blue crabs, *Callinectes sapidus*, collected from a contaminated and a clean estuary in New

Jersey, U.S., the hepatopancreas was found to be the primary organ for Pb uptake (Reichmuth et al., 2010).

There is more information now on the cellular and subcellular distribution of Pb in invertebrates than there was at the time of writing the 2006 Pb AQCD. Specifically, localization of Pb at the ultrastructural level has been assessed in the marine mussel (*M. edulis*) through an antibody-based detection method (Einsporn et al., 2009; Einsporn and Koehler, 2008). Dissolved Pb was detected mainly within specific lysosomal structures in gill epithelial cells and digestive gland cells and was also localized in nuclei and mitochondria. Transport of Pb is thought to be via lysosomal granules associated with hemocytes (Einsporn et al., 2009). In the digestive gland of the variegated scallop *(Chlamys varia*), Pb was also mainly bound to organelles, (66% of the total metal burden) (Bustamante and Miramand, 2005). In the digestive gland of the cephalopod *Sephia officinalis*, (cuttlefish) most of the Pb was found in the organelles (62%) (Bustamante et al., 2006). In contrast, only 7% of Pb in the digestive gland of the octopus (*Octopus vulgaris*) was associated with the fraction containing nuclei, mitochondria, lysosome and microsomes: the majority of Pb in this species was found in cytosolic proteins (Raimundo et al., 2008).

Metian et al. (2009) investigated the uptake and bioaccumulation of ^{210}Pb in variegated scallop and king scallop to determine the major accumulation route (seawater or food) and then assess subsequent tissue distribution. Dietary Pb from phytoplankton in the diet was poorly assimilated (<20%) while more than 70% of Pb in seawater was retained in the tissues. In seawater, ^{210}Pb was accumulated more rapidly in variegated scallop than king scallop and soft tissue distribution patterns differed between the species. Variegated scallop accumulated Pb preferentially in the digestive gland (50%) while in king scallop, Pb was equally distributed in the digestive gland, kidneys, gills, gonad, mantle, intestine, and adductor muscle with each tissue representing 12-30% of ^{210}Pb body load. An additional test with Pb-spiked sediment in *king scallop* showed low bioaccumulation efficiency of Pb from spiked sediment.

Recently, several studies have attempted to establish biodynamic exposure assessments for various contaminants. In an in situ metal kinetics field study with the mussel *M. galloprovincialis*, simultaneous measurements of metal concentrations in water and suspended particles with mussel biometrics and physiological indices were conducted to establish uptake and excretion rates in the natural environment (Casas et al., 2008). The mean logarithmic ratio of metal concentration in mussels (ng/kg of wet-flesh weight) to metal concentration in water (ng/L) was found to be 4.3 in *M. galloprovincialis*, based on the rate constants of uptake and efflux in a series of transplantation experiments between

contaminated and clean environments. Equilibrium concentrations of Pb in mussels leveled out at approximately 30 days with a concentration of 6.7 mg Pb/kg.

The protective barrier against Pb toxicity formed by the egg structure in some invertebrates was recognized in the 2006 Pb AQCD. Consideration of toxicity of Pb to embryos that develop surrounded by a protective egg shell has been expanded since the 2006 Pb AQCD. In a study with cuttlefish (*S. officinalis*) eggs, radioisotopes were used to assess the permeability of the egg to Pb at low exposure concentrations (^{210}Pb activity concentration corresponding to 512 µg/L Pb) (Lacoue-Labarthe et al., 2009). Retention and diffusion properties of the cuttlefish egg change throughout the development of the embryo and since the eggs are fixed on substrata in shallow coastal waters they may be subject to both acute and chronic Pb exposures. In the radiotracer experiments, ^{210}Pb was never detected in the internal compartments of the egg during the embryonic development stage, while concentrations in the eggshell increased throughout the 48 day exposure. These results are consistent with a study of cuttlefish eggs collected from natural environments in which Pb was only detected in the eggshell. These studies indicate that the cuttlefish egg provides a protective barrier from Pb toxicity (Miramand et al., 2006).

Aquatic invertebrate strategies for detoxifying Pb were reviewed in the 2006 Pb AQCD and include sequestration of Pb in lysosomal-vacuolar systems, excretion of Pb by some organisms, and deposition of Pb to molted exoskeleton. Molting of the exoskeleton can result in depuration of Pb from the body (see Knowlton et al. (1983) and Anderson et al. (1997), as cited in the 2006 Pb AQCD). New research has provided further evidence of depuration of Pb via molting in invertebrates. Mohapatra et al. (2009) observed that Pb concentrations in body tissues were lower in the newly molted mud crabs (*Scylla serrata*) than in the pre-molt, hard-shelled crabs. However, the carapace of hard shelled crabs had lower concentrations of Pb than the exuvium of the soft shell crabs, leading the authors to speculate that some of the metal might be partially excreted during the molting process, rather than entirely through shedding of the previous exoskeleton. Bergey and Weis (2007) showed that differences in the proportion of Pb stored in exoskeleton and soft tissues changed during intermolt and immediate postmolt in two populations of fiddler crabs (*Uca pugnax*) collected from New Jersey. One population from a relatively clean estuary eliminated an average of 56% of Pb total body burden during molting while individuals from a site contaminated by metals eliminated an average of 76% of total Pb body burden via this route. Pb distribution within the body of crabs from the clean site shifted from exoskeleton to soft tissues prior to molting. The authors observed the opposite pattern of Pb distribution in fiddlers from the contaminated site where larger amounts of Pb were depurated in the exoskeleton. The exact dynamics of Pb depuration through molting in crabs are thus still not completely characterized.

7.3.11.3 Saltwater Vertebrates

Saltwater Fish

In comparison to freshwater fish, fewer studies have been conducted on Pb uptake in marine fish. Since marine fish drink seawater to maintain osmotic homeostasis, Pb can be taken up via both gills and intestine (Wang and Rainbow, 2008). Pb was significantly accumulated in gill, liver, plasma, kidney, rectal gland, intestine, skin, and muscle of the elasmobranch spotted dogfish (*Scyliorhinus canicula*) exposed to 2,072 µg Pb/L for one week (De Boeck et al., 2010). In contrast to Pb distribution patterns in freshwater teleosts, high Pb concentrations were present in this species in the skin and rectal gland. Egg cases of the spotted dogfish exposed to ^{210}Pb in seawater for 21 days, accumulated radiolabeled Pb rapidly and the metal was subsequently detected in embryos indicating the permeability of shark eggs to Pb in coastal environments (Jeffree et al., 2008). A study of Pb bioaccumulation in five marine fish species (*Chloroscombrus chrysurus*, *Sardinella aurita*, *Ilisha africana*, *Galeoides decadactylus*, *Caranx latus*) found that *C. chrysurus* was an especially strong bioaccumulator, yielding Pb concentrations of 6 to 10 mg Pb/kg (Gnandi et al., 2006). However, C. *chrysurus* metal content was not correlated to the Pb concentrations along the mine tailings gradient from which they were collected (8.5 and 9.0 µg Pb/L for minimum and maximum tissue concentrations, respectively). This lack of correlation was also observed for fish species that were considered to be weaker Pb bioaccumulators, indicating that unidentified sources of Pb (e.g., in sediments or in dietary sources) may be contributing to Pb uptake by marine fish.

In grunt fish *H. scudderi*, exposed to Pb via dietary uptake through a simulated marine food chain, mean total Pb body burden increased from 0.55 to 3.32 µg Pb/g in a 42-day feeding study (Soto-Jiménez et al., 2011a). Pb was accumulated to the highest relative concentration in liver with less than 3% of total Pb accumulated in gills. Most of the Pb based on total body mass was accumulated in skeleton, skin, scales and muscle.

The 2006 Pb AQCD considered detoxification mechanisms in fish including mucus production and Pb removal by shedding of scales in which Pb is chelated with keratin. Since the 2006 review, additional Pb detoxification mechanisms in marine fish have been further elucidated. Mummichog (*Fundulus heteroclitus*) populations in metal-polluted salt marshes in New York exhibited different patterns of intracellular partitioning of Pb although body burden between sites was not significantly different (Goto and Wallace, 2010). Mummichogs at more polluted sites stored a higher amount of Pb in metal rich granules as compared to other detoxifying cellular components such as heat-stable proteins, heat-denaturable proteins and organelles.

Marine Mammals

Studies that consider uptake of Pb in aquatic mammals are limited. Kannan et al. (2006) compared trace element concentrations in livers of free-ranging sea otters (*Enhydra lutris nereis*) found dead along the California coast. They detected Pb in all individuals sampled (N=80) in a range of 0.019 to 1.06 µg Pb/g. The otters were classified by cause of death (infectious causes, non-infectious causes, emaciated condition) and trace element patterns of tissue distribution were compared. Livers from emaciated otters had significantly elevated levels of Pb compared to non-diseased individuals.

7.3.11.4 Marine Food Web

As discussed in Section 7.3.4.4 trophic transfer of Pb through aquatic food chains was considered to be negligible in the 2006 Pb AQCD (U.S. EPA, 2006c). Measured concentrations of Pb in the tissues of aquatic organisms were found to be generally higher in algae and benthic organisms and lower in higher trophic-level consumers, indicating that Pb was bioaccumulated but not biomagnified (U.S. EPA, 2006c; Eisler, 2000). New literature since the 2006 Pb AQCD provides evidence of the potential for Pb to be transferred in marine food webs while other studies indicate Pb is decreased with increasing trophic level. This section incorporates recent literature on transfer of Pb through aquatic marine chains.

In a dietary study using environmentally realistic concentrations of Pb in prey through four levels of a simplified marine food chain, biological responses including decreased growth and survival and changes in behavior were observed at different trophic levels. However, the concentration of Pb did not increase along the trophic gradient (Soto-Jiménez et al., 2011a; Soto-Jiménez et al., 2011b). The base of the simulated food chain was the microalgae *Tetraselmis suecica* (phytoplankton) grown in 20 µg Pb/L. Pb-exposed cultures of *T. suecica* had significantly less cell divisions per day (growth), biomass and total cell concentrations than control microalgae at 72 hours of exposure. The microalgal cultures were then fed to *Artemia franciscana* (crustacean, brine shrimp) which were then fed to *Litopenaeus vannamei* (crustacean white shrimp) and finally to *Haemulon scudderi* (fish, grunt). Effects on behavior, growth and survival were observed in shrimp and in grunt fish occupying the intermediate and top levels of the simulated marine food chain. The authors speculate that the species used in the simulated food chain were able to regulate and eliminate Pb (Soto-Jiménez et al., 2011a).

Partial evidence for biomagnification was observed in a subtropical lagoon in Mexico with increases of Pb concentration occurring in 14 of the 31 (45.2%) of trophic interactions considered (Ruelas-Inzunza and Páez-Osuna, 2008). The highest rate of

transference of Pb as measured in muscle tissue occurred between the prey species white shrimp (*Litopenaeus vannimei*) and mullet (*Mugil cephalus*) to pelican (*Pelecanus occidentalis*).

Other studies have traced Pb in aquatic food webs and have found no evidence of biomagnification of Pb with increasing trophic level. In the southeastern Gulf of California, Mexico, Pb was not positively transferred (biomagnification factor <1) through primary producers (seston, detritus) and 14 consumer species in a lagoon food web (Jara-Marini et al., 2009). In a planktonic food web in Bahia Blanca estuary, Argentina, Pb levels in macrozooplankton and mesozooplankton exhibited temporal fluctuations, however no biomagnification was observed between mesozooplankton and macrozooplankton (Fernández Severini et al., 2011). It is important to note, however, that even in the absence of biomagnification, aquatic organisms can bioaccumulate relatively large amounts of metals and become a significant source of dietary metal to their predators (Fairbrother et al., 2007; Reinfelder et al., 1998).

7.3.12 Biological Effects of Pb in Saltwater Systems

This section focuses on the studies of biological effects of Pb on marine and estuarine algae, plants, invertebrates, fish and mammals published since the 2006 Pb AQCD. Key studies from the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD on biological effects of Pb are summarized where appropriate. Biological effects of Pb on saltwater algae and plant species are considered below, followed by information on effects on marine invertebrates and vertebrates. In general, Pb toxicity to saltwater organisms is less well characterized than toxicity of Pb in freshwater ecosystems due to the fewer number of available studies on marine species. Because this review is focused on effects of Pb, studies reviewed for this section include only those for which Pb was the only, or primary, metal to which the organism was exposed.

7.3.12.1 Saltwater Algae and Plants

New evidence in this ISA on toxicity of Pb to marine algae indicates that species exhibit varying sensitivities to Pb in saltwater. Pb tested at concentrations up to 10 µmol/L did not affect photosynthetic activity in seven species of marine macroalgae (*Ascophyllum nodosum, Fucus vesiculosus, Ulva intestinalis, Cladophora rupestris, Chondrus crispus, Palmaria palmate, Polysiphonia lanosa*) as measured by pulse amplitude modulation chlorophyll fluorescence yield although Pb was readily accumulated by these species (Baumann et al., 2009). The lowest 72-hour EC_{50} for growth inhibition reported for

marine algae was 105 µg Pb/L in *Chaetoceros* sp (Debelius et al., 2009). In a recent review of the production of phytochelatins and glutathione by marine phytoplankton in response to metal stress, Kawakami et al. (2006) included several studies in which Pb exposure was shown to induce glutathione and phytochelatin at high concentrations in a few species.

7.3.12.2 Saltwater Invertebrates

No studies with marine invertebrates were reviewed in the 1977 Pb AQCD or the 1986 Pb AQCD. Effects of Pb on marine invertebrates reported in the 2006 Pb AQCD included impacts on embryo development in bivalves with an EC_{50} of 221 µg Pb/L for embryogenesis, gender differences in sensitivity to Pb in copepods and increasing toxicity with decreasing salinity in mysids. As in freshwater invertebrates, the antioxidant system, survival, growth and reproduction are affected by Pb in marine organisms. In aquatic invertebrates, Pb has also been shown to affect stress responses and osmoregulation. New evidence on reproduction and growth in invertebrates is also reviewed here.

Recent literature strengthens the evidence indicating that Pb affects enzymes and antioxidant activity in marine invertebrates. 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 subsequently decreased. Phenoloxidase activity was depressed compared with controls and remained significantly lower than control after 72 hours of exposure to Pb. Increased SOD activity was observed in the mantle but decreased with time, although always remaining higher than in the control animals. (Jing et al., 2007). Activity of Se-dependent glutathione peroxidase did not change with Pb exposure. SOD, catalase, and glutathione peroxidase were significantly reduced at environmentally relevant concentrations of Pb (2 µg Pb/L as measured in Bohai Bay, China) in the digestive gland of the bivalve *Chlamys farreri* (Zhang et al., 2010c). 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 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 and MDA levels were increased in mantle and gill (Vlahogianni and Valavanidis, 2007). Catalase activity was decreased in the mantle of

these mussels but fluctuated in their gills, as compared with the control group. In the bivalve *C. farreri* exposed to Pb, there was induction of lipid peroxidation measured as MDA of 24% and a 37% reduction in 7-ethoxyresorufin-o-deethylase (EROD) activity when compared to controls (Zhang et al., 2010c). In red fingered marsh crab, *Parasesarma erythrodactyla*, collected from sites along an estuarine lake in New South Wales, Australia, elevated glutathione peroxidase activity was correlated with individuals with higher metal body burdens (MacFarlane et al., 2006).

ALAD is a recognized biomarker of exposure across a wide range of taxa including bacteria (Korcan et al., 2007), invertebrates and vertebrates. Since the 2006 Pb AQCD, there are additional studies measuring changes in ALAD activity in field-collected bivalves and crustaceans. In the bivalve *Chamelea gallina* collected from the coast of Spain, ALAD inhibition was greater with higher concentrations of Pb measured in whole tissue (Kalman et al., 2008). In another study conducted in Spain, ALAD activity was negatively correlated with total Pb concentration in seven marine bivalves (*C. gallina*, *Mactra corallina*, *Donax trunculus*, *Cerastoderma edule*, *M. galloprovincialis*, *Scrobicularia plana* and *Crassostrea angulata*). However, the authors of this study indicated the need to consider variability of responses between species when using ALAD as a biomarker for Pb (Company et al., 2011). Pb content varied significantly among species and was related to habitat (sediment versus substrate) and feeding behavior.

Behavioral responses of aquatic invertebrates to Pb reviewed in the 2006 Pb AQCD included avoidance. A limited number of new studies have considered additional behavioral endpoints in marine organisms. Valve closing speed was used as a measure of physiological alterations due to Pb exposure in the Catarina scallop (Sobrino-Figueroa and Caceres-Martinez, 2009). The average valve closing time increased from under one second in the control group to 3 to 12 seconds in juvenile scallops exposed to 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.

Since the 2006 Pb AQCD, limited studies on marine invertebrates have indicated effects of Pb on reproduction. In a long term (approximately 60 days) sediment multigenerational bioassay with the estuarine-sediment dwelling amphipod *Elasmopus laevis*, onset to reproduction was significantly delayed at 118 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 and time of first offspring production was also reduced with increasing Pb concentration in sediment above 118 µg Pb/g. The authors indicate that this concentration is below the current marine sediment regulatory guideline for Pb (218 µg Pb/g sediment) (NOAA,

1999) and that reproductive endpoints are more sensitive than survival. 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).

As noted in the 2006 Pb AQCD and supported by new studies reviewed in this ISA, Pb exposure negatively affects the growth of aquatic invertebrates. Wang et al., (2009d) observed growth of embryos of the Asian Clam (*Meretrix meretrix*) was significantly reduced by Pb with an EC_{50} of 197 µg/L. In juvenile Catarina scallop, *Argopecten ventricosus*, exposed to Pb for 30 days, the EC_{50} for growth was 4,210 µg Pb/L (Sobrino-Figueroa et al., 2007). Rate of growth of the deposit feeding *Capitella* sp. polychaetes decreased significantly with increasing concentrations of Pb associated with sediment (Horng et al., 2009).

Although Pb is known to cause mortality when invertebrates are exposed to sufficiently high concentrations, some species may not exhibit significant mortality even at high concentrations. In a 10-day Pb-spiked sediment exposure (1,000 mg Pb/kg), 100% of individuals of the Australian estuarine bivalve *Tellina deltoidalis* survived (King et al., 2010). In the deposit feeding *Capitella* sp., polychaetes, exposure to varying concentrations of Pb associated with sediment up to 0.41 µmol/g had no effect on survival (Horng et al., 2009). Other species are more sensitive to Pb in the environment and these responses are reviewed in Section 7.3.13.

7.3.12.3 Saltwater Vertebrates

Saltwater Fish

There is a dearth of information in previous Pb AQCDs on Pb effects in saltwater fish. New data available since the 2006 Pb AQCD includes a study with a marine elasmobranch. De Boeck et al. (2010) exposed the spotted dogfish (*S. canicula*) to 2,072 µg Pb/L for one week and measured metallothionein induction in gill and liver tissue, and the electrolytes Na, K, Ca and Cl, in plasma. No effects were observed in Pb-

exposed fish for any of the physiological variables measured in this study, although Pb was detected in all organs (De Boeck et al., 2010).

Since the 2006 Pb AQCD, several studies integrating behavioral and physiological measures of Pb toxicity have been conducted on marine fish. The ornate wrasse (*Thalassoma pavo*) was exposed 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.

Additional behavioral studies in fish consider effects of dietary Pb. The grunt fish *H. scudderi*, occupying the top level of a simulated marine food chain, exhibited lethargy and decreased food intake during the last week of a 42-day feeding study (Soto-Jiménez et al., 2011a). The fish were fed white shrimp exposed to Pb via brine shrimp that were in turn fed microalgae cultured at an environmentally-relevant concentration of 20 µg Pb/L. The authors noted a few of the fish exposed to Pb via dietary transfer through the food chain were observed surfacing and speculated that this behavior was air breathing as a response to stress.

Limited new studies on reproductive effects of Pb in marine fish are available. Decreased oocyte diameter and density in the toadfish (*Tetractenos glaber*) were associated with elevated levels of Pb in the gonad of field-collected fish from contaminated estuaries in Sydney, Australia (Alquezar et al., 2006). The authors state this is suggestive of a reduction in egg size which ultimately may lead to a decline in female reproductive output.

Mammals

Although Pb continues to be detected in tissues of marine mammals in U.S. coastal waters (Bryan et al., 2007; Stavros et al., 2007; Kannan et al., 2006) few studies exist that consider biological effects associated with Pb exposure. Pb effects on immune variables, including cell viability, apoptosis, lymphocyte proliferation, and phagocytosis were tested in vitro on phagocytes and lymphocytes isolated from the peripheral blood of bottlenose dolphin (*Tursiops truncates*) (Cámara Pellissó et al., 2008). No effects on viability of immune cells, apoptosis, or phagocytosis were observed in 72 hour exposure to

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.

7.3.13 Exposure and Response of Saltwater Species

Evidence regarding exposure-response relationships and potential thresholds for Pb effects on aquatic populations can inform determination of standard levels that are protective of aquatic ecosystems. The Annex of the 2006 Pb AQCD (U.S. EPA, 2006c) summarized data on exposure-response functions for invertebrates (Table AX7-2.4.1) (Table AX7-2.4.2). The recent exposure-response studies reviewed in this section expand on earlier findings with information on microalgal and invertebrate species.

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.

Other studies of marine invertebrates published since the 2006 Pb AQCD have indicated differences in sensitivity of different lifestages of aquatic organisms to Pb. In a series of seawater and sediment exposures using adult and juvenile amphipods *Melita plumulosa*, juveniles were more sensitive to Pb than adults (King et al., 2006). In the seawater-only exposures, the 96-hour LC_{50} for adults was 3,000 µg Pb/L and 1,520 µg Pb/L for juveniles. Ten-day exposures of juveniles 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. 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 and Valavanidis, 2007).

Since the 2006 Pb AQCD, Pb toxicity to larval stages of marine species has been assessed at sublethal and lethal concentrations. The effective concentrations at which Pb resulted in 50% of abnormal embryogenesis of the Asian clam (*M. meretrix*) was 297 µg Pb/L. The 96-hour LC_{50} for larvae of the same species was 353 µg Pb/L (Wang et al., 2009d). In comparison, juvenile Catarina scallop (*A. ventricosus*) had a LC_{50} of 830 µg Pb/L in a 96-hour exposure (Sobrino-Figueroa et al., 2007). 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 and 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.

7.3.14 Community and Ecosystem Effects in Saltwater Systems

As discussed in the 1986 Pb AQCD and the 2006 Pb AQCD, exposure to Pb is likely to have impacts in aquatic environments via effects at several levels of ecological organization (organisms, populations, communities, or ecosystems). But fewer studies explicitly consider community and ecosystem-level effects in marine and brackish waters than in freshwater. Reduced species abundance and biodiversity of protozoan and meiofauna communities were observed in laboratory microcosm studies with marine water and marine sediments reviewed in the 2006 Pb AQCD as summarized in Table AX7-2.5.2 (U.S. EPA, 2006c). In a laboratory study with larval mummichogs reviewed in the 2006 Pb AQCD, feeding and predator avoidance behaviors were altered in this marine fish species following a 4 week exposure to Pb. Observations from field studies reviewed in the 2006 Pb AQCD included findings of a negative correlation between Pb and species richness and diversity indices of macroinvertebrates associated with estuary sediments and changes in species distribution and abundance in fish, crustaceans and macroinvertebrates correlated with Pb levels in marine sediments. The 2006 Pb AQCD concluded that, in general, information from controlled studies for single pollutants was insufficient to permit evaluation of specific impacts on higher levels of organization (beyond the organism). In studies from natural saltwater ecosystems, Pb rarely occurs as a sole contaminant in saltwater ecosystems making its effects difficult to ascertain. New information on effects of Pb at the population, community and ecosystem level in coastal ecosystems is reviewed below.

The faunal composition of seagrass beds in a Spanish coastal saltwater lagoon was found to be impacted by Pb in sediment, plants, and biofilm (Marín-Guirao et al., 2005).

Sediment Pb concentrations ranged from approximately 100 to 5,000 mg Pb/kg and corresponding biofilm concentrations were 500 to 1,600 mg Pb/kg, with leaf concentrations up to 300 mg Pb/kg. Although multiple community indices (abundance, Shannon-Wiener diversity, Simpson dominance index) did not vary from site to site, multivariate analysis and similarity analysis indicated significant differences in macroinvertebrate communities between sites with different sediment, biofilm, and leaf Pb concentrations. Differences were largely attributable to three amphipod species (*Microdeutopus* sp., *Siphonoecetes sabatieri*, *Gammarus* sp.). This indicates that, although seagrass abundance and biomass were unaffected by Pb exposure, organisms inhabiting these plants still may be adversely impacted.

Caetano et al. (2007) investigated the mobility of Pb in salt marshes using total content and stable isotope signature. They found that roots had similar isotopic signature to sediments in vegetated zones indicating that Pb uptake by plants reflects the input in sediments. At one site, there was a high anthropogenic Pb content while at the other natural mineralogical sources dominated. The roots of *S. fruticosa* and *S. maritima* significantly accumulated Pb, having maximum concentrations of 2,870 mg Pb/kg and 1,755 mg Pb/kg, respectively, indicating that below-ground biomass played an important role in the biogeochemical cycling of Pb.

In a laboratory microcosm experiment with spiked sediments collected from the Swartkop River estuary, South Africa, the effects of Pb (3 to 6,710 µg/Pb g sediment dry weight) on meiobenthos was tested alone and in combination with Cu, Fe, and Zn (Gyedu-Ababio and Baird, 2006). Total meiofauna density decreased after 32 days in Pb-treated sediments (range 3 to 5 taxa) compared to control (9 taxa). Nematode diversity and community structure was altered with a mean number of 8 genera present in microcosms contaminated with Pb compared to the control with 20 genera, however, the synergistic effect of the four metals on nematode community structure was greater than the individual metals and the effects of Pb could not be distinguished from Cu, Fe and Zn.

7.3.15 Characterization of Sensitivity and Vulnerability in Saltwater Species

Species differences in metabolism, sequestration, and elimination rates have been shown to control relative sensitivity and vulnerability of exposed organisms and effects on survival, reproduction, growth, metabolism, and development. Diet and lifestage at the time of exposure also contribute significantly to the determination of sensitive and vulnerable populations and communities. Further, environmental conditions in addition to those discussed as affecting bioavailability may also alter Pb toxicity. The 2006 Pb

AQCD reviewed the effects of genetics, age, and body size on Pb toxicity. While genetics appears to be a significant determinant of Pb sensitivity, effects of age and body size are complicated by environmental factors that alter metabolic rates of saltwater organisms. A review of the more recent literature corroborated these findings, and identified seasonal physiological changes and lifestage as other important determinants of differential sensitivity to Pb.

7.3.15.1 Seasonally Affected Physiological Changes

Couture et al. (2010) investigated seasonal and decadal variations in Pb sources to mussels (*M. edulis*) from the French Atlantic shoreline. Pb concentrations in the mussels were 5-66 times higher than the natural background value for the north Atlantic. The $^{206}Pb/^{207}Pb$ signature indicated that the bioaccumulated Pb was anthropogenic in origin. The signature was not, however, the same as that emitted in western Europe, as a result of leaded gasoline combustion, although that was a major emission source to the atmosphere during a large part of the study period (1985-2005). Instead, it was most similar to that of Pb released into the environment from wastewater treatment plants, municipal waste incinerators and industries such as metal refineries and smelters. Thus continental runoff rather than atmospheric deposition was identified as the main source of Pb to the French coastal area. The strong seasonal variations in $^{206}Pb/^{208}Pb$ were used to conclude that resuspension of Pb triggered by high river runoff events was a key factor affecting bioaccumulation of Pb in *M. edulis*.

In another 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. In contrast, levels of Pb and other metals were highest in summer and lowest in winter in oysters *Crassostrea corteziensis* collected from Sonora, Mexico (García-Rico et al., 2010).

Carvalho et al. (2011) measured ^{210}Pb in *M. galloprovincialis* sampled at coastal locations in Portugal and noted that the apparent seasonal fluctuation in radionuclide concentrations in mussel soft tissues was mostly attributable to changes in physiological condition (i.e., fat content , gonadal development) and not to radionuclide body burden fluctuation. The authors caution that since concentrations of contaminants are dependent

upon tissue composition, corrections for mussel physiological condition are need to compare results from different seasons and different locations.

7.3.15.2 Lifestage

Lifestages of the marine polychaete *H. elegans* including embryogenesis, sexual maturation, and offspring development were shown to be differentially affected by Pb exposure. Pb water concentrations of 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 offset (Gopalakrishnan et al., 2007). Further, given that the adult lifestage is sedentary, reduction of the mobile early lifestage as a result of Pb exposures may disproportionally affect sessile polychaetes. For instance, larval settlement was significantly reduced at Pb exposures of 50 µg Pb/L and greater (Gopalakrishnan et al., 2008).

7.3.15.3 Species Sensitivity

Both inter- and intra-specific differences in Pb uptake and bioaccumulation may occur in macroinvertebrates of the same functional feeding group. Data from 20 years of monitoring of contaminant levels in filter-feeding mussels of the *Mytilus* species and *Crassostrea virginica* oysters in coastal areas of the U.S. through the National Oceanic and Atmospheric Administration (NOAA) Mussel Watch program indicate that Pb is on average three times higher in mussels than in oysters (Kimbrough et al., 2008). Limpet (Patella sp.) from the Lebanese Coast had Pb BAF values ranging from 2,500 to 6,000 and in the same field study Pb BAF values for a mussel (*Brachidontes variabilis*) ranged from 7,500-8,000 (Nakhle et al., 2006).

There is some indication that molting may comprise an additional sequestration and excretion pathway for aquatic animals exposed to Pb (Soto-Jiménez et al., 2011b; Mohapatra et al., 2009; Tollett et al., 2009; Bergey and Weis, 2007). Crab species *U. pugnax* (Bergey and Weis, 2007) and *Scylla serrata* (Mohapatra et al., 2009), and white shrimp *L. vannamei* (Soto-Jiménez et al., 2011b) have been shown to sequester Pb preferentially in exoskeleton tissue, where it is later shed along with other tissue. Consequently, aquatic arthropod species and those species that shed their exoskeleton more frequently may be able to tolerate higher environmental Pb concentrations than non-arthropods or slow-growing molting species, as this pathway allows them to effectively lower Pb body burdens.

Some tolerant species of fish (e.g., mummichog) have the ability to sequester accumulated Pb in metal-rich granules or heat-stable proteins (Goto and Wallace, 2010). Fish with such abilities are more likely to thrive in Pb-contaminated environments than other species.

7.3.16 Ecosystem Services Associated with Freshwater and Marine Systems

Pb deposited on the surface of, or taken up by organisms has the potential to alter the services provided by aquatic biota to humans. At this time, a few publications address Pb impacts on ecosystem services associated with aquatic systems, mostly 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 explicitly consider the impact of Pb and other heavy metals on ecosystem services provided by salt marsh (Gedan et al., 2009) and estuaries (Smith et al., 2009b). These systems are natural sinks for metals and other contaminants. Pb can be toxic to salt marsh plant species and decaying plant detritus may result in resuspension of Pb into the aquatic food chain (Gedan et al., 2009). Salt marsh and estuaries provide habitat and breeding areas for both terrestrial and marine wildlife and are locations for bird watching. Using a modeling approach designed to assess the degree of risk of Pb and Hg to wading birds in estuarine habitats in the U.K., the authors found a high probability that Pb poses an ecologically relevant risk to dunlin, *Calidris alpina* (Smith et al., 2009b). However, the authors noted that a major source of uncertainty in this study was the NOAEL values for Pb.

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 and Dillon, 2007). Several studies have addressed the response of natural wetlands to Pb (Odum, 2000; Gambrell, 1994). Recent reviews of pollution

control (Mander and Mitsch, 2009) or removal of metals (Marchand et al., 2010) by constructed wetlands and phytoremediation of metals by wetland plants (Rai, 2008) indicate that these systems can remove Pb from the aquatic environment and are important for water quality, sediment stabilization, nutrient cycling and shelter for aquatic biota. The use of plants as a tool for immobilization of Pb and other metals from the environment is not limited to wetland species. Recent advances in the phytoremediation of metals are reviewed in Dickinson et al. (2009).

The impact of Pb on ecological services provided by specific components of aquatic systems has been considered in a limited number of studies. Recent research has suggested that dietary Pb (i.e., Pb adsorbed to sediment, particulate matter, and food) may contribute to exposure and toxicity in primary and secondary order consumers (including humans). Aquatic fauna can take up and bioaccumulate metals. If the bioaccumulating species is a food source, the uptake of metals may make it toxic or more dangerous for people or other wildlife to consume. For example, oysters and mussels bioaccumulate Pb from anthropogenic sources, including atmospheric deposition, and are a food source that is widely consumed by humans and wildlife (Couture et al., 2010). Their capacity to bioaccumulate Pb makes them good bioindicators of environmental contamination and they have been used as monitors of coastal pollutants by the NOAA Mussel Watch program since 1986. Although bioaccumulation may render aquatic fauna toxic to consumers, bioaccumulation is a way to sequester the metals and remove them from waters and soils. Sequestration for this purpose is itself an ecosystem service and has been quantified. For example, the total ecological services value of a constructed intertidal oyster (*Crassostrea* sp.) reef in improving water quality and sequestering metals including Pb was calculated in the Yangtze River estuary to be about $500,000 per year (Quan et al., 2009). 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.

7.3.17 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 Pb AQCD considered in determining causality.

7.3.17.1 Biogeochemistry and Chemical Effects

Atmospherically-derived Pb can enter aquatic ecosystems by direct deposition or via watershed processes. Once the 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.

7.3.17.2 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 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.

Tissue accumulation of Pb via gill and dietary uptake has been further characterized since the 2006 Pb AQCD in aquatic vertebrates and stable isotope techniques have been applied to further elucidate bioaccumulation of Pb. 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 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 and Wallace, 2010). There is more information now on the cellular and subcellular distribution of Pb in invertebrates than there was at the time of writing the 2006 Pb AQCD. Specifically, localization of Pb at the ultrastructural level has been assessed in the marine mussel (*M. edulis*), scallop and cuttlefish and was found to be bound principally to organelles (Einsporn et al., 2009; Einsporn and Koehler, 2008).

There are few new studies on Pb uptake by amphibians and mammals. In 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 bioaccumulated but not biomagnified (U.S. EPA, 2006c; Eisler, 2000). 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).

7.3.17.3 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., 2006a). 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 and Wood, 2004; Rogers and Wood, 2003). In addition to this mechanism, Pb was found to induce ionoregulatory toxicity at the gill of rainbow trout through a binding of Pb with Na-K, ATPase and rapid inhibition of carbonic anhydrase activity thus enabling noncompetitive inhibition of Na^+ and Cl^- influx. Recent studies on fish behavior associated with Pb exposure include decreased prey capture rate (Mager et al., 2010) and swim speed (Mager and Grosell, 2011) in fathead minnow and decline in startle response and visual contrast in zebrafish (Rice et al., 2011).

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, 2006c). 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.

7.3.17.4 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 a commonly measured endpoint 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 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 and Brix, 2009; Grosell et al., 2006a). 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 (Wang et al., 2010e). In a series of 48 hour acute toxicity tests with the cladoceran *C. dubia* conducted in a variety of natural waters across North America, LC_{50} values ranged from 29 to 180 µg Pb/L and were most significantly influenced by DOC and water ionic strength (Esbaugh et al., 2011).

In the 2006 Pb AQCD, effects were reported 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, effects in fish observed in recent studies fall within the range of concentrations observed in the previous Pb AQCD.

7.3.17.5 Community and Ecosystem Effects

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. One ecological effect reported in previous Pb AQCDs is a shift in community composition in Pb-impacted habitats toward 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 and 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 freshwater 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.

7.3.17.6 Critical Loads, Sensitivity and Vulnerability

In the short term, metal emissions generally have greater 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.

Recent studies have identified seasonally-affected physiological changes and lifestage 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 effects.

7.4 Causal Determinations for Ecological Effects of Lead

This section presents key conclusions regarding causality for welfare effects of Pb (Table 7-2). Evidence considered in establishing causality was drawn from recent studies summarized in this ISA, integrated with findings presented in the 1977 (U.S. EPA, 1977), 1986 (U.S. EPA, 1986a) and 2006 Pb AQCDs (U.S. EPA, 2006c) for Pb. The causal statements for terrestrial and aquatic effects are arranged according to ecologically meaningful levels of biological organization (organism, population, community, ecosystem). As recognized in EPA's Framework for Ecological Risk Assessment (U.S. EPA, 1992), and in the adverse outcome pathway (AOP) framework (Ankley et al., 2010) endpoints that are measured at one level of organization may be related to an endpoint at a higher level. The AOP conceptual framework was proposed to link mechanistic data from initiating events at the molecular level, through a series of higher order biological responses, to survival and to developmental and reproductive endpoints that can be used in ecological risk assessment, i.e., at the population level and higher. Fecundity, growth,

and survival are organism-level attributes that lead to population-level (e.g., abundance, production, extirpation), community-level (taxa richness, relative abundance) and ecosystem-level responses (Ankley et al., 2010; Suter et al., 2005). In the case of Pb, physiological stress, hematological effects and neurobehavioral alterations are likely to decrease the overall fitness of an organism, even though their connection to effects at higher levels of biological organization may not have been characterized. Furthermore, the effects of Pb on ecosystems necessarily begin with some initial effects at the molecular level of specific organisms within the ecosystem (U.S. EPA, 1986b). There are many different molecular and cellular level effects, and chronic toxicity of Pb in ecosystems is thus likely attained through multiple modes of action.

Relevant sources of information on ecological effects of Pb include controlled exposures in the laboratory, microcosm experiments and field observations. Controlled exposure studies in laboratory or small-to medium-scale field settings provide the most direct evidence for causality, but their scope of inference may be limited. In contrast, microcosms and field studies where exposure is not controlled also include potentially confounding factors (e.g., other metals, environmental conditions), thus increasing the uncertainty in associating effects with exposure to Pb specifically. The vast majority of available Pb studies are laboratory toxicity tests on single species, in which an organism is exposed to a known concentration of Pb and the effect on a specific endpoint is evaluated. Potential effects of Pb at the population, community and ecosystem level are often, of necessity, inferred from organism-level studies (U.S. EPA, 2006c). Considering the various types of ecological evidence available, effects determined to be causal at the organism or species level of biological organization contribute to the body of evidence for causal effects at the community and ecosystem levels of biological organization. For some ecological endpoints, support for causality is additionally supported by toxicological findings reviewed in the chapters of the ISA that evaluate evidence for human health effects associated with Pb exposure, particularly when a common mode of action is documented.

For aquatic biota, generally, the number of studies available on freshwater organisms is greater than on saltwater organisms, covering more taxa as well as more endpoints. The causal determinations for aquatic biota are, therefore, based primarily on evidence from freshwater species. When available, data from saltwater ecosystems are discussed separately under the appropriate causal endpoint. For most of the endpoints under consideration, evidence is inadequate to establish causality in saltwater species. When sufficient evidence is available for marine organisms, data on concentrations at which effects are observed are presented.

Table 7-2 Summary of Pb causal determinations for plants, invertebrates and vertebrates

Effect	Terrestrial	Aquatic[a]
Physiological Stress-All organisms	Causal	Causal
Hematological Effects-Invertebrates	Inadequate	Causal
Hematological Effects-Vertebrates	Causal	Causal
Neurobehavioral Effects-Invertebrates and Vertebrates	Likely Causal	Likely Causal
Developmental and Reproductive Effects-Plants	Inadequate	Inadequate
Developmental and Reproductive Effects-Invertebrates and Vertebrates	Causal	Causal
Growth-Plants	Causal	Causal
Growth-Invertebrates	Inadequate	Causal
Growth-Vertebrates	Inadequate	Inadequate
Survival-Plants	Inadequate	Inadequate
Survival- Invertebrates and Vertebrates	Causal	Causal
Community and Ecosystem Level Effects	Likely Causal	Likely Causal

[a]Causal determinations for aquatic biota are based primarily on evidence from freshwater organisms.

Characterization of environmental concentrations of Pb in terrestrial and aquatic systems is important for identification of levels of exposure to organisms. Information on ambient Pb concentrations in non-air media and biota is reported in Section 3.6 and Table 2-1. A survey of 1,319 locations in the conterminous U.S. found an average background soil concentration of 19.3 mg Pb/kg , with a 95th percentile of 50 mg Pb/kg, and 16 locations between 100 and 700 mg Pb/kg (Shacklette and Boerngen, 1984). The U.S.EPA's Eco-SSL Guidance documents (2007d, 2003b) augmented these data with observations from an additional 13 studies conducted between 1982 and 1997, most of them limited to one state. The national average of state-level soil averages was 18.9 mg Pb/kg. The range of the state mean soil Pb concentrations was from 5 mg Pb/kg to 38.6 mg Pb/kg. Representative median and range of Pb concentrations in surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L) and sediments (median 28 µg Pb/g dry weight, range 0.5 to 12,000 µg Pb/g dry weight) in the U.S. based on a synthesis of NAWQA data was reported in the previous 2006 Pb AQCD (U.S. EPA, 2006c). The range of Pb levels in saltwater are available from several studies although the values are not specific to the U.S. A range of 0.005-0.4 µg Pb/L for salt water was reported by Leland and Kuwabara (1985) and 0.01 to 27 µg Pb/L by Sadiq (1992). In general, Pb in seawater is higher in coastal areas and estuaries since these locations are closer to sources of Pb contamination and loading from terrestrial systems (Sadiq, 1992).

The level at which Pb elicits a specific effect is difficult to establish in terrestrial and aquatic systems due the influence of other environmental variables on Pb bioavailability and toxicity and substantial species differences in Pb sensitivity. In the 1977 Pb AQCD,

no correlation could be established between toxic effects in invertebrates, fish, birds and small mammals and environmental concentrations of Pb. At the time of the 1986 Pb AQCD additional data were available on toxicity but there was still little information on the levels of Pb that can cause toxic effects in small mammals or birds (U.S. EPA, 1986b). In the 2006 Pb AQCD several studies on effects of Pb exposure on natural ecosystem structure and function advanced the characterization of Pb levels in the environment near point sources (i.e., smelters, mining). According to the 2006 Pb AQCD, natural terrestrial ecosystems near significant Pb point sources exhibited a number of ecosystem-level effects, including decreased species diversity, changes in floral and faunal community composition, and decreasing vigor of terrestrial vegetation. These findings are summarized in Table AX7-2.5.2 of the Annex to the 2006 Pb AQCD (U.S. EPA, 2006c). The 2006 Pb AQCD concluded that, in general, there was insufficient information available for single materials in controlled studies to permit evaluation of specific impacts on higher levels of organization (beyond the organism). Furthermore, Pb rarely occurs as a sole contaminant in natural systems making the effects of Pb difficult to ascertain. New information available since the 2006 Pb AQCD includes additional field studies in both terrestrial and aquatic ecosystems, but the connection between air concentration and ecosystem exposure continues to be poorly characterized for Pb and the contribution of atmospheric Pb to specific sites is not clear.

The key conclusions and causal determinations for welfare effects of Pb are presented in ascending order of the level of ecological organization to which they apply, moving from responses in organisms (physiological stress, hematological effects, neurobehavioral effects) to endpoints relevant to population, community, and ecosystem-level processes (development and reproduction, growth, survival). They focus on studies where effects of Pb exposure are observed at or near ambient levels of Pb and studies generally within the range of one to two orders of magnitude above current conditions were also considered in the body of evidence. Studies at higher concentrations were used to the extent they informed our understanding of modes of action and illustrated the wide range of sensitivity to Pb across taxa.

7.4.1 Causal Determinations for Lead in Terrestrial Systems

7.4.1.1 Physiological Stress-Terrestrial Biota

Induction of enzymes associated with oxidative stress response in terrestrial biota is a recognized effect of Pb exposure (U.S. EPA, 2006c). Several studies from the 2006 Pb AQCD in birds and plants provide evidence that Pb induces lipid peroxidation, however,

exposures in these studies were higher than would be found generally in the environment (U.S. EPA, 2006c). Building on the body of evidence presented in the 2006 Pb AQCD, recent studies provide evidence of upregulation of antioxidant enzymes and increased lipid peroxidation associated with Pb exposure in many species of plants, invertebrates and vertebrates. In plants, increases of antioxidant enzymes with Pb exposure occur in some terrestrial species at concentrations approaching the average Pb concentrations in U.S. soils (18.9 mg Pb/kg). For example, in a series of studies Wang et al. observed increases in reactive oxygen species with increasing exposure to Pb from 20 mg Pb/kg soil to 2,000 mg Pb/kg in broad bean (*Vicia faba*) (Wang et al., 2010c; Wang et al., 2010b; Wang et al., 2008b) and tomato (*Lycopersicon esculentum*) (Wang et al., 2008a), where they were accompanied up to approximately 500mg Pb/kg by proportional increases in SOD, glutathione, guaiacol peroxidase, and lipid peroxidation, as well as decreases in catalase. Spinach seedlings grown in soil containing six increasing concentrations of Pb from 20 to 520 mg Pb/kg exhibited higher production of reactive oxygen species, increased rates of lipid peroxidation and increased SOD concentrations. (Wang et al., 2011a). Markers of oxidative damage are also observed in terrestrial invertebrates, including snails and earthworms, and in terrestrial mammals. Across these biota, there are differences in the induction of antioxidant enzymes that appear to be species-dependent.

The oxidative stress responses associated with Pb exposure in terrestrial biota are consistent with responses in aquatic organisms (Section 7.4.2.1), in experimental animal studies, and in humans (Section 5.2.4). This oxidative stress is characterized by increased presence of reactive oxygen species and membrane and lipid peroxidation that can promote tissue damage, cytotoxicity, and dysfunction. Increases in reactive oxygen species are often followed by a compensatory and protective upregulation in antioxidant enzymes, such that this upregulation is itself indicative of oxidative stress conditions. Continuous production of reactive oxygen species may overwhelm this defensive process, leading to further oxidative stress and injury.

Upregulation of antioxidant enzymes and increased lipid peroxidation are considered reliable biomarkers of stress, and provide evidence that Pb exposure induces a stress response in those organisms which may itself increase susceptibility to other stressors and reduce individual fitness. Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and physiological stress in terrestrial plants, invertebrates and vertebrates.

7.4.1.2 Hematological Effects-Terrestrial Biota

Hematological responses are commonly reported effects of Pb exposure in vertebrates in terrestrial systems. In the 1977 Pb AQCD, ALAD was recognized as the most sensitive indicator of Pb exposure in rats (U.S. EPA, 1977). Furthermore, inhibition of ALAD was associated with death of waterfowl following ingestion of Pb shot. In the 1986 Pb AQCD, decreases in red blood cell ALAD activity were documented in birds and mammals near a smelter (Beyer et al., 1985). Additional evidence for effects on blood parameters and their applicability as biomarkers of Pb exposure in terrestrial birds and mammals were presented in the 2005 Ecological Soil Screening Levels for Lead, the 2006 Pb AQCD and the current ISA (U.S. EPA, 2006c, 2005b). Field studies available since the 2006 Pb AQCD include evidence for elevated blood Pb levels correlated with decreased ALAD activity in songbirds and owls living in historical mining areas (Gómez-Ramírez et al., 2011; Hansen et al., 2011a).

This evidence is strongly coherent with evidence from aquatic biota (Section 7.4.2.2) and observations from human epidemiologic and animal toxicology studies showing that exposure to Pb induces effects on hematological endpoints, including altered heme synthesis mediated through decreased ALAD and ferrochelatase activities, decreased red blood cell survival and function, and increased red blood cell oxidative stress. Taken together, the overall weight of human epidemiologic and animal toxicological evidence is sufficient to conclude that a causal relationship exists between exposure to Pb and hematological effects in humans and in laboratory animals (Section 5.7). Based on observations in terrestrial birds and mammals and additionally supported by observations in aquatic organisms, and toxicological and epidemiological findings in laboratory animals and humans evidence is sufficient to conclude that there is a causal relationship between Pb exposures and hematological effects in terrestrial vertebrates. The evidence is **i**nadequate to conclude that there is a causal relationship between Pb exposures and hematological effects in terrestrial invertebrates.

7.4.1.3 Neurobehavioral Effects-Terrestrial Biota

The central nervous system of animals was recognized as a target of Pb toxicity in the 1977 Pb AQCD and subsequent Pb reviews have provided additional supporting evidence of Pb as a neurotoxicant in terrestrial biota (U.S. EPA, 1977). Effects of Pb on neurological endpoints in terrestrial animal taxa include changes in behaviors that may

decrease the overall fitness of the organism such as food consumption, prey capture ability and avoidance behaviors.

Some organisms exhibit behavioral avoidance while others do not seem to detect the presence of Pb (U.S. EPA, 2006c). Decreased food consumption of Pb-contaminated diet has been demonstrated in some invertebrates (snails) and vertebrates (lizards, pigs). Decreased food consumption, growth, and shell thickness were observed in juvenile *A. achatina* snails exposed to Pb-contaminated (concentration greater than 134 mg Pb/kg) diet for 12 weeks (Ebenso and Ologhobo, 2009a). Similarly, feeding rate in *T. pisana* snails was depressed in 3 week dietary exposures of 50 to 15,000 mg Pb/kg (El-Gendy et al., 2011), while other snails exposed to Pb at similar concentrations have shown no effects on feeding rate (Beeby and Richmond, 2010). Consumption of 10 mg/Pb kg diet resulted in lower body weight and food intake after 120 days of dietary exposure in pigs (*S. domestica*) (Yu et al., 2005).

In limited studies available on worms there is evidence that Pb may affect the ability to escape or avoid predation. For example, the motility of nematodes was disrupted in Pb-contaminated soils (Wang and Xing, 2008). 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 and Wang, 2009).

There is limited evidence for additional behavioral endpoints altered by Pb exposure. In a laboratory study, Pb-exposed gull chicks exhibited abnormal behaviors such as decreased walking, erratic behavioral thermoregulation and food begging that could make them more vulnerable in the wild (Burger and Gochfeld, 2005). The chicks were exposed to Pb via injection to reach a Pb concentration in feathers equivalent to Pb levels in feathers of wild gull populations. Lizards exposed to Pb through diet of 10 to 20 mg Pb/kg per day for 60 days in the laboratory exhibited abnormal coloration and posturing behaviors.

These findings in terrestrial biota are coherent with findings from studies in aquatic biota that showed neurobehavioral alterations in various species of fish, and also in some aquatic invertebrates (Section 7.4.2.3). They are also coherent with findings in laboratory animals (Section 5.3) that show that Pb induces changes in learning, memory, attention and motor skills. New behaviors induced by exposure to Pb are detailed in that section, including hyperactivity and mood disorders, effects on visual and auditory sensory systems, changes in structure and function of neurons and supporting cells in the brain, and effects on the blood brain barrier. Mechanisms that include the displacement of physiological cations, oxidative stress and changes in neurotransmitters and receptors are also reviewed. Human epidemiological evidence, based on several cohort and cross-sectional studies in diverse populations provides clear and consistent evidence of

association between blood Pb concentrations and decrements in neurodevelopmental outcomes in young children (Section 5.3). 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). Data from ecological studies are highly coherent with these human health-related data, especially neurobehavioral findings and evidence of structural changes. Overall, the evidence from aquatic and terrestrial systems is sufficient to conclude that there is a likely causal relationship between Pb exposures and neurobehavioral effects in invertebrates and vertebrates in terrestrial ecosystems.

7.4.1.4 Developmental and Reproductive Effects-Terrestrial Biota

In terrestrial biota, evidence from Chapter 7 of this ISA and in previous Pb AQCDs indicates an association between reproductive effects and Pb exposure. Impaired fecundity at the organismal level can result in a decline in abundance and/or extirpation of populations, decreased taxa richness, and decreased relative or absolute abundance at the community level (Suter et al., 2005; U.S. EPA, 2003a). Evaluation of the literature on Pb effects in terrestrial species indicates that exposure to Pb is associated with reproductive effects. Various endpoints have been measured in various taxa of terrestrial organisms to assess the effect of Pb on development, fecundity and hormone homeostasis and although reproductive effects were demonstrated, no single endpoint in a single taxon has been extensively studied.

In terrestrial plants, few studies are available that specifically address reproductive effects of Pb exposure. Two genotypes of maize seedlings exhibited a significant and concentration-dependent reduction in seed germination following 7 days of Pb treatment in nutrient solution of 10, 100 or 1,000 µg Pb/L as Pb sulfate (Ahmad et al.).

In terrestrial invertebrates, Pb can alter developmental timing, hatching success, sperm morphology and hormone homeostasis. However, effects are observed in a limited number of species and at concentrations that typically exceed Pb levels in U.S. soils. In fruit flies, Pb exposure increased time to pupation and decreased pre-adult development at high Pb concentrations (Hirsch et al., 2010). An increase in development time (approximately two days) and a reduction in relative fecundity was observed in aphids feeding on plants exposed to high concentrations of Pb (Gorur, 2007). Hatching success of the collembolan *F. candida* was decreased following 10 day exposure to Pb-spiked

soils (EC_{50} 2,361 mg Pb/kg dry soils) (Xu et al., 2009a). Sperm morphology in Asian earthworms was significantly altered following 2-week exposures to soils containing 1,000, 1,400, 1,800 and 2,500 mg Pb/kg soil (Zheng and Li, 2009). Pb may also disrupt hormonal homeostasis in invertebrates as studies with moths have suggested (Shu et al., 2009). Adult female moths reared on diets containing 25, 50, 100, or 200 mg Pb/kg exhibited decreased vitellogenin mRNA induction, and vitellogenin levels were demonstrated to decrease with increasing Pb exposure. Evidence of multi-generational toxicity effects of Pb is also present in terrestrial invertebrates, specifically springtails, mosquitoes, carabid beetles and nematodes where decreased fecundity in progeny of Pb-exposed individuals was observed. No significant changes to the number of juveniles were noted in the collembolan *Proisotoma minuta* exposed for 42 days to 300, 750, 1,500 or 3,000 mg Pb/kg in acidic sandy loam soil (Nursita et al., 2005) in agreement with a previous study with collembolans in which a NOEC of 2,000 mg Pb/kg and LOEC of 5,000 mg Pb/kg was determined for reproduction (Sandifer and Hopkin, 1996).

In terrestrial vertebrates, there is evidence for reproductive effects associated with Pb exposure in this ISA and previous Pb AQCDs. 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 (U.S. EPA, 2006c). Decreased testis weight was observed in lizards administered a sublethal dose of 10 or 20 mg Pb/kg day by oral gavage for 60 days (Salice et al., 2009). Few studies in the field have addressed reproductive effects of Pb specifically in mammals, due to most available data in wild or grazing animals being from near smelters, where animals are co-exposed to other metals. 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., 2009b).

Evidence from aquatic invertebrates, amphibians, and fish is sufficient to conclude that there is a causal relationship between Pb exposure and reproductive effects in aquatic invertebrates and vertebrates (Section 7.4.2.4). In Chapter 5, evidence obtained using mammals in the context of human health research demonstrates a consistency of adverse effects of Pb on sperm (Section 5.8.2.1) and the onset of puberty in males and females (Sections 5.8.1.3 and 5.8.2.4) with strong evidence from both toxicology and epidemiology studies. Other reproductive endpoints including spontaneous abortions, pre-term birth, embryo development, placental development, low birth weight, subfecundity, hormonal changes, and teratology were also affected, but less consistently (Section 5.8).

The evidence presented in Section 7.4.1.4 is thus highly coherent with evidence from aquatic ecosystems and laboratory animal studies, and sufficient to conclude that there is a causal relationship between Pb exposures and developmental and reproductive effects in terrestrial invertebrates and vertebrates.

In terrestrial plants, the evidence is inadequate to conclude a causal relationship between Pb exposures and plant developmental and reproductive effects.

7.4.1.5 Growth Effects-Terrestrial Biota

Alterations in growth which is an organism-level variable can have impacts at the population, community and ecosystem levels. In terrestrial ecosystems, evidence for effects of Pb on growth is strongest in terrestrial plants, although these effects are typically observed in laboratory studies with high exposure concentrations or in field studies near point sources. Growth effects observed in both invertebrates and vertebrates, where juvenile organisms are generally more sensitive than adults, underscore the importance of lifestage to overall Pb susceptibility.

In terrestrial plants, there is evidence over several decades of research that Pb inhibits photosynthesis and respiration, all of which can reduce the growth of the plant (U.S. EPA, 2006c, 1986a, 1977). 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. Many laboratory toxicity studies report effects on the growth of plants in synthetic growing media, but observed effects typically occur at concentrations higher than the average background concentrations in U.S. soils (19 mg Pb/kg dry weight) (U.S. EPA, 2005b) and there are few field studies. Effects on plant growth can result in reduced productivity and decreased biomass. The 2006 Pb AQCD relied heavily on the Ecological Soil Screening Levels for Lead (U.S. EPA, 2005b) document regarding ecotoxicological effects of Pb for which growth (biomass) was considered the most sensitive and ecologically relevant endpoint for plants.

Evidence for growth effects in terrestrial fauna is sparse. In the 1986 Pb AQCD, a study was reviewed in which the F1 and F2 generations of the springtail *Onychiurus armatus* fed a diet of Pb-exposed fungi (0.008 to 3.1 mg Pb/g) experienced a delay in achieving maximum length (Bengtsson et al., 1983). The authors suggested that the reduced growth may be accompanied by delayed sexual maturity. Reduced growth of garden snail *T. pisana*, was observed following a five week dietary exposure of 1,000 µg Pb/g and greater (El-Gendy et al., 2011). In previous AQCDs, growth effects of Pb have been

reported in birds (changes in juvenile weight gain), at concentrations typically higher than currently found in the environment.

Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and growth effects in terrestrial plants. Evidence is inadequate to establish causal relationship between Pb exposures and growth effects in terrestrial invertebrates and vertebrates.

7.4.1.6 Survival-Terrestrial Biota

The relationship between Pb exposure and survival has been well demonstrated in terrestrial species as presented in Sections 7.2.5 of this ISA and in the previous Pb AQCDs. Pb exposure can either be lethal or produce sublethal effects that diminish survival probabilities. In the 1977 Pb AQCD, deaths from Pb poisoning in domestic animals caused by emissions from stationary sources were reported (U.S. EPA, 1977). Additional studies in the 1986 and 2006 Pb AQCDs and current ISA provide evidence for an exposure-response relationship between Pb exposure and mortality in terrestrial biota. Survival is a biologically important response that may have direct impact on population size. However, the typically reported concentration where there is 50% mortality of test organisms (LC_{50}) is a poor measure for comparing environmental concentrations. LC_{50} is applicable to acute toxicity whereas Pb effects on ecosystem receptors is likely characterized as a chronic, cumulative exposure rather than an acute exposure. The scenario where half of test animals die in a laboratory situation would be unacceptable from an ecological perspective. From the LC_{50} data on Pb in this review and previous Pb AQCDs, a wide range of sensitivity to Pb is evident across taxa and within genera. However, the LC_{50} is usually much higher than current environmental levels of Pb in the U.S, even though physiological dysfunction that adversely impacts the fitness of an organism often occurs well below concentrations that result in mortality. When available, LC_{10}, NOEC or LOEC is reported.

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.

Survival of soil-associated organisms is adversely affected by Pb exposure. In the 1986 Pb AQCD it was reported that Pb at environmental concentrations occasionally found near roadsides and smelters (10,000 to 40,000 µg Pb/g dry weight [mg Pb/kg]) can eliminate populations of bacteria and fungi on leaf surfaces and in soil. Alterations to bacterial and fungal communities can lead to inhibited or delayed decomposition of organic matter and disruption of energy flow through the food chain. 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, 2006c). 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.

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, 2005b). The NOAELs for survival ranged from 3.5 to 3,200 mg Pb/kg-day. The association of Pb exposure with mortality in vertebrates is coherent with observations in aquatic biota and with the observation of consistently positive associations between Pb exposure and mortality observed in human epidemiologic studies (Section 5.4.5).

The evidence is inadequate to conclude that there is a causal relationship between Pb exposures and survival in terrestrial plants.

The evidence is sufficient to conclude that there is a causal relationship between Pb exposures and survival in terrestrial invertebrates and vertebrates.

7.4.1.7 Community and Ecosystem Level Effects-Terrestrial Biota

Most direct evidence of community and ecosystem level effects is from near point sources where Pb concentrations are higher than typically observed environmental concentrations for this metal. Impacts of Pb on terrestrial ecosystems near smelters, mines, and other industrial sources have been studied for several decades. Emissions of Pb from smelting and other industrial activities are accompanied by other trace metals (e.g., Zn, Cu, Cd) and SO_2 that may cause toxic effects independently or in concert with Pb. Those impacts include decreases in species diversity and changes in floral and faunal community composition. Ecosystem-level field studies are complicated by the confounding of Pb exposure with other factors such as the presence of trace metals and acidic deposition and the inherent variability in natural systems. In natural systems, Pb is often found co-existing with other stressors, and observed effects may be due to cumulative toxicity.

In laboratory studies and simulated ecosystems, where it is possible to isolate the effect of Pb, this metal has been shown to alter competitive behavior of species, predator-prey interactions and contaminant avoidance. These dynamics may change species abundance and community structure at higher levels of ecological organization. Uptake of Pb into aquatic and terrestrial organisms and subsequent effects on mortality, growth, physiological stress, blood, neurobehavioral and developmental and reproductive

endpoints at the organism level are expected to have ecosystem-level consequences, and thus provide consistency and plausibility for causality in ecosystem-level effects.

In the 1977 Pb AQCD the potential for Pb to interfere with ecosystem level processes was explored in a detailed review of a study on the effects of Pb on relationships between arthropods and leaf litter decomposition (U.S. EPA, 1977). Reduced arthropod density, biomass and richness were observed in the vicinity of a Pb smelting complex in Missouri. There were also several studies correlating feeding habits, habitat, and Pb concentrations in body tissues reported in the 1977 Pb AQCD, specifically in insects and small mammals indicating that species differences in Pb concentrations are determined in part by trophic position and habitat preference.

In the 1986 Pb AQCD it was reported that Pb at environmental concentrations occasionally found near roadsides and smelters (10,000 to 40,000 µg Pb/g dry weight [mg Pb/kg]) can eliminate populations of bacteria and fungi on leaf surfaces and in soil (U.S. EPA, 1986b). Some key populations of soil microorganisms and invertebrates die off at 1,000 mg Pb/kg soil interrupting the flow of energy through decomposition processes (U.S. EPA, 1986b) and altering community structure. At soil concentrations of 500 to 1,000 µg Pb/g (mg Pb/kg) or higher, populations of plants, microorganisms, and invertebrates may shift toward Pb-tolerant populations of the same or different species (U.S. EPA, 1986b).

The 2006 Pb AQCD reported that decreased species diversity, changes in floral and faunal community composition and decreased vigor of terrestrial vegetation were observed in ecosystems surrounding former smelters including the Anaconda smelter in southwestern Montana (U.S. EPA, 2006c). Several studies in the 2006 Pb AQCD documented reduced organic matter decomposition rates and decreased microbial biomass in areas heavily polluted by metals. Lower abundance and reduced biodiversity of soil invertebrate communities were observed in field surveys in proximity to Pb-point sources.

New evidence since the 2006 Pb AQCD supports previous findings of a link between high concentration of soil metals and substantial changes in soil microorganism community composition, as well as decreased abundance and diversity. In a naturally Pb-enriched forest in Norway, The number of fungal colony forming units was approximately 10 times lower in the highest Pb soil concentration (~4.5 mg Pb/g dry weight) than in control soils (Baath et al., 2005). The composition of the fungal community was drastically altered, with only one species common to both soils, and the number of species present was substantially lower.

The effect of Pb on microbial community function has been quantified previously using functional endpoints such as respiration rates, fatty acid production, and soil acid phosphatase and urease activities, which may provide a better estimate of ecological impacts than microbial diversity or abundance measurements. Studies available since the 2006 Pb AQCD provide further evidence of Pb effects on microbial processes. Pb contamination reduced phenol oxidase activity in several types of soils; concentrations between 5 and 50 nM Pb significantly decreased phenol oxidase activity in all soils tested, while 400 nM and greater completely arrested phenol oxidase activity in one soil tested (a high pH sandy loam) (Carine et al., 2009). Pb concentrations between 50 and 500 mg Pb/kg significantly reduced microbial abundance and diversity, and also resulted in lower soil phosphatase, urease, and dehydrogenase activities (Gao et al., 2010b). When the microbial properties of metal-contaminated urban soils were compared to those of rural soils, significant differences (Sudova and Vosatka, 2007) were detected in basal community respiration rates and microbial abundance (Yang et al., 2006). Gai et al. (2011) examined the microbial activity of three soils via microcalorimetric methods following Pb exposure. They noted an increase in activity immediately following Pb application (giving 10, 20, 40, 80, and 160 mg Pb/kg), and theorized that this was a result of rapid mortality of sensitive microbial species, followed by a concurrent proliferation of Pb-tolerant microorganisms. As Pb concentrations increased, however, the calculated microbial growth rate constant decreased, indicating a suppression of microbial activity (Gai et al., 2011). Akerblom et al. (2007) tested the effects of six metals (Cr, Zn, Mo, Ni, Cd, and Pb) individually. All tested metals had a similar effect on the species composition of the microbial community. Exposure to a high Pb concentration (52 mg Pb/kg) negatively affected respiration rates.

In addition to microbial communities, there is new evidence for effects of Pb on other terrestrial ecosystem components. Increased plant diversity was shown to ameliorate effects of Pb contamination on a microbial community (Yang et al., 2007). The presence of arbuscular mycorrhizal fungi may protect plants growing in Pb-contaminated soils (Bojarczuk and Kieliszewska-Rokicka, 2010; Sudova and Vosatka, 2007). Invertebrates affected by Pb in terrestrial systems may be altering community structure. New evidence since the 2006 Pb AQCD indicates that some species of worms avoid Pb-contaminated soils (Langdon et al., 2005). Reductions in microbial and detritivorous populations can affect the success of their predators (U.S. EPA, 2006c). Following a 28-day exposure to field-collected soils contaminated with metals (including Pb at 426 mg Pb/kg), both population growth and individual growth of the earthworm *L. rubellus* were diminished (Klok et al., 2006). The authors proposed that, although these reductions were unlikely to result in extirpation, avian predators such as the godwit (*Limosa limosa*) that feed heavily on earthworms may be affected by a reduction of available earthworm biomass.

Furthermore, the presence of earthworms increased Pb uptake by plants (Ruiz et al., 2011; Sizmur et al., 2011).

In terrestrial ecosystems, most studies show decreases in microorganism abundance, diversity, and function with increasing soil Pb concentrations in areas near point-sources. Specifically, shifts in nematode communities, bacterial species, and fungal diversity have been observed. Most evidence for Pb toxicity to terrestrial biota is from single-species assays in laboratory studies. Although the evidence is strong for effects of Pb on growth, reproduction and survival in certain species, considerable uncertainties exist in generalizing effects observed under small-scale, particular conditions up to predicted effects at the ecosystem level of biological organization. In many cases it is difficult to characterize the nature and magnitude of effects and to quantify relationships between ambient concentrations of Pb and ecosystem response due to existence of multiple stressors, variability in field conditions, and to differences in Pb bioavailability at that level of organization. However, the cumulative evidence for Pb effects at higher levels of ecological organization is sufficient to conclude that there is a likely causal relationship between Pb exposures and the alteration of species richness, species composition and biodiversity in terrestrial ecosystems.

7.4.2 Causal Determinations for Lead in Aquatic Systems

7.4.2.1 Physiological Stress-Aquatic Biota

Building on the body of evidence presented in the 2006 Pb AQCD (U.S. EPA, 2006c) recent studies provide consistent and coherent evidence of upregulation of antioxidant enzymes and increased lipid peroxidation associated with Pb exposure within one or two orders of magnitude above current or ambient conditions in many species of aquatic plants, invertebrates and vertebrates. A few studies provide evidence of effects at current concentrations of Pb measured in the environment. In plants, increases of antioxidant enzymes with Pb exposure occur in algae, aquatic mosses, and floating and rooted aquatic macrophytes. Most available evidence in aquatic plants for antioxidant responses to Pb are from laboratory studies conducted from 2 to 7 days and at concentrations higher than typically found in the environment. However, data from transplantation experiments from non-polluted to polluted sites indicate that elevated enzyme activities are associated with Pb levels measured in sediments. The macrophyte *Myriophyllum quitense* exhibited elevated antioxidant enzyme activity (glutathione-S-transferase, glutathione reductase, peroxidase) following transplantation in anthropogenically polluted areas containing

elevated Pb concentrations. These were correlated with sediment Pb concentrations in the range of 5 to 23 mg Pb/g dry weight (Nimptsch et al., 2005).

There is considerable evidence for antioxidant activity in response to Pb exposure in aquatic invertebrates, including gastropods, mussels, and crustaceans. Some of the recent evidence for invertebrate antioxidant response indicates effects at Pb levels detected in the environment. For example, SOD, catalase, and glutathione peroxidase activities were significantly reduced in the digestive gland of the marine bivalve *C. farreri* at 2 µg Pb/L (as measured in Bohai Bay, China) (Zhang et al., 2010c). Markers of oxidative damage are also observed in fish, amphibians and mammals in laboratory studies. Across all biota, there are differences in the induction of antioxidant enzymes that appear to be species-dependent.

Additional stress responses to Pb in a few aquatic invertebrates have been reported since the 2006 Pb AQCD, and included elevated heat shock proteins, osmotic stress, lowered metabolism and decreased glycogen levels associated with Pb exposure. Heat shock protein induction has been observed in zebra mussels exposed to 500 µg Pb/L for 10 weeks (Singer et al., 2005). 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 exposed for 14 days to 500 µg Pb/L exhibited a Pb-induced hypometabolism under conditions of environmental hypoxia in the presence of the metal (Morris et al., 2005). Glycogen levels in the freshwater snail *B. glabrata* were significantly decreased following 96-hour exposures at near environmentally relevant concentrations of Pb (50 µg/L and higher) (Ansaldo et al., 2006).

Evidence for stress responses observed in aquatic biota is supported by findings in terrestrial species (Section 7.4.1.1) as well as in humans and experimental animal studies of oxidative stress following impairment of normal metal ion functions (Section 5.2.4). Upregulation of antioxidant enzymes and increased lipid peroxidation are considered to be reliable biomarkers of stress, and provide evidence that Pb exposure induces a stress response in those organisms which may increase susceptibility to other stressors and reduce individual fitness. Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and physiological stress in aquatic plants, invertebrates and vertebrates.

7.4.2.2 Hematological Effects-Aquatic Biota

Hematological responses are commonly reported effects of Pb exposure in aquatic invertebrates and vertebrates. Anemia was recognized as a symptom of chronic Pb poisoning in fish in the 1977 Pb AQCD and has been subsequently reported in various

species using common hematological endpoints (e.g., red blood cell counts, hematocrit, Hb concentrations) (Mager, 2012). In the 1986 Pb AQCD, hematological effects of Pb exposure to fish included a decrease in red blood cells and inhibition of ALAD (U.S. EPA, 1986b). In environmental assessments of metal-impacted habitats, ALAD is a recognized biomarker of Pb exposure (U.S. EPA, 2006c). ALAD activity is negatively correlated with total Pb concentration in bivalves, and lower ALAD activity has been correlated with elevated blood Pb levels in fish as well. Further evidence from the 2006 Pb AQCD and this review of Pb effects on ALAD enzymatic activity, including effects in bacteria, amphibians and additional field and laboratory studies on fish, confirms that the decreased activity in this enzyme is an indicator for Pb exposure across a wide range of taxa. The finding that the hematological system is a target for Pb in natural systems is also supported by some evidence of Pb-induced alterations of serum profiles and changes in white blood cell counts in fish (U.S. EPA, 2006c) and amphibians. This evidence is strongly coherent with evidence from terrestrial organisms (Section 7.4.1.2). It is also coherent with observations from human epidemiologic and animal toxicology studies (Section 5.7) where there is consistent evidence that exposure to Pb induces adverse effects on hematological endpoints, including altered heme synthesis mediated through decreased ALAD and ferrochelatase activities, decreased red blood cell survival and function, and increased red blood cell oxidative stress. 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 (Section 5.7). Based on observations in both terrestrial and aquatic organisms and additionally supported by findings in terrestrial systems and by toxicological and epidemiologic evidence on human health effects, evidence is sufficient to conclude that there is a causal relationship between Pb exposures and hematological effects in invertebrates and vertebrates in aquatic ecosystems.

7.4.2.3 Neurobehavioral Effects-Aquatic Biota

Evidence from laboratory studies and limited data from field studies reviewed in this chapter and in previous Pb AQCDs have shown effects of Pb on neurological endpoints in aquatic animal taxa. These include changes in behaviors that may decrease the overall fitness of the organism such as avoidance responses, decreased ability of an organism to capture prey or escape predators, and alterations in feeding behaviors.

In the 1977 Pb AQCD behavioral impairment of a conditioned response (avoidance of a mild electric shock) in goldfish was observed as low as 70 µg Pb/L (Weir and Hine, 1970). Some organisms exhibit behavioral avoidance while others do not seem to detect the presence of Pb (U.S. EPA, 2006c). Evidence of alteration in behaviors at the level of

the organism is a potential endpoint at the ecological level when its effects on population or community are taken into consideration (U.S. EPA, 2003a).

In the 2006 Pb AQCD several studies were reviewed in which Pb was shown to affect predator-prey interactions, including alteration in prey size choice in juvenile fathead minnows following 2-week pre-exposure to 500 µg Pb/L (Weber, 1996). In limited studies available on worms, snails, tadpoles, hatchling turtles and fish there is evidence that Pb may affect the ability to escape or avoid predation. For example, in the tubificid worm *T. tubifex* the 96 hour EC_{50} for immobilization was 42 µg Pb/L (Khangarot, 1991).

New information since the 2006 Pb AQCD provides further evidence for Pb impacts on behaviors that may affect feeding and predator avoidance in freshwater environments. Prey capture ability was decreased in 10 day old fathead minnows born from adult fish exposed to 120 µg Pb/L for 300 days, then subsequently tested in a 21-day breeding assay (Mager et al., 2010). Zebrafish embryos exposed to low concentrations of Pb (10nM or 30 nM) until 24 hours post-fertilization and then subsequently tested as larvae or adult fish exhibited behavioral deficits in response to mechanosensory and visual stimuli (Rice et al., 2011). Startle response time in larvae measured as maximum head turn velocity and escape time decreased in a concentration-dependent pattern following a directional, mechanical stimulus (tapping). The pattern of escape swimming was altered in larvae of Pb-exposed embryos compared to non-exposed embryos. In adult fish hatched from Pb-exposed embryos (30 nM), visual response to a rotating black bar against a white background (ability to detect contrast) was significantly degraded.

In marine organisms there is evidence for neurobehavioral effects of Pb in a few species. In a study reviewed in the 2006 Pb AQCD, prey capture rate and predator avoidance was affected in mummichogs starting at 300 µg Pb/L (Weis and Weis, 1998). New studies on neurobehavioral endpoints in marine species support previous findings of decreased ability to escape predation associated with Pb exposure. In juvenile Catarina scallops exposed to Pb (40 µg/L to 400 µg/L) for 20 days, the average valve closing time increased from under one second in the control group to 3 to 12 seconds in juvenile scallops A decrease in valve closing speed in these bivalves may impact escape swimming behaviors important for predator avoidance (Sobrino-Figueroa and Caceres-Martinez, 2009). Behavioral effects in grunt fish *H. scudderi*, occupying the top level of a simulated marine food chain included lethargy and decreased food intake in a 42-day feeding study (Soto-Jiménez et al., 2011a). These fish were fed white shrimp exposed to Pb via brine shrimp that were initially fed microalgae cultured at 20 µg Pb/L. In the same study, surfacing, reduction of motility, and erratic swimming were observed in the white shrimp after 30 days of exposure to Pb via diet. The ornate wrasse, *T. 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, however, no changes in feeding activity were noted between non-treated and treated fish.

Findings in laboratory animals and humans support the limited evidence for neurobehavioral effects of Pb in aquatic biota. In studies reviewed in Section 5.3, effects observed in laboratory animals following exposure to Pb include hyperactivity and mood disorders, visual and auditory sensory alterations, and changes in structure and function of neurons and supporting cells in the brain, including effects on the blood brain barrier. Mechanisms that include the displacement of physiological cations, oxidative stress and changes in neurotransmitters and receptors are also reviewed Section 5.3. 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). Human epidemiologic evidence, based on several cohort and cross-sectional studies in diverse populations provides clear and consistent evidence of association between blood Pb concentrations and decrements in neurodevelopmental outcomes in young children (Section 5.3). 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 behaviorally-related endpoints. The evidence presented in sections of this ISA dealing with human health is sufficient to conclude that there is a causal relationship between Pb exposure and neurobehavioral effects (Section 5.3). Evidence in terrestrial ecosystems is not as extensive, but it is highly coherent with findings in aquatic ecosystems. Data related to human health are also highly coherent with aquatic ecosystem data. Overall, the evidence from available studies on neurobehavioral effects of Pb in aquatic systems is limited, but sufficient to conclude that there is a likely causal relationship between Pb exposures and neurobehavioral effects in aquatic invertebrates and vertebrates.

7.4.2.4 Developmental and Reproductive Effects-Aquatic Biota

Evaluation of the findings in previous Pb AQCDs and new literature on Pb effects in aquatic fauna indicates that exposure to Pb is associated with reproductive effects at or near ambient concentrations of this metal in some species. Impaired fecundity at the organismal level can result in a decline in abundance and/or extirpation of populations, decreased taxa richness, and decreased relative or absolute abundance at the community level (Suter et al., 2005; U.S. EPA, 2003a). Various endpoints have been measured in

aquatic organisms to assess the effect of Pb on development, fecundity and hormone homeostasis. However, there are typically only limited studies available from different taxa.

There are no studies reviewed in the ISA or previous Pb AQCDs on development and reproductive effects of Pb in freshwater or saltwater aquatic algae or macrophytes.

Reproductive effects of Pb in freshwater aquatic invertebrates are well-characterized in previous Pb AQCDs and in the current ISA and have been observed at or near current ambient concentrations in some species. In the 1986 Pb AQCD reproductive effects were reported to begin at 19 µg Pb/L for the freshwater snail *Lymnaea palustris* and 27 µg Pb/L for Daphnia sp. (U.S. EPA, 1986b). In the 2006 Pb AQCD the number of neonates per surviving adult was significantly decreased in the amphipod *H. azteca* during chronic 42 day exposures to Pb (Besser et al., 2005). In the group exposed to Pb in water-only exposures, the LOEC for reproductive effects was 16 µg Pb/L while in amphipods receiving both water-borne and dietary Pb the LOEC for reproduction was 3.5 µg Pb/L. Reproductive output in chronic testing with the freshwater rotifer *B. calyciflorus* was measured as total number of individuals and intrinsic growth rate. The EC_{20} for number of rotifers was 125 µg Pb/L and the 48 hour EC_{20} for intrinsic rate of population increase was 307 µg Pb/L with an LOEC of 194 µg Pb/L (Grosell et al., 2006a). In a 7 day exposure of the cladoceran *C. dubia* to 50 to 500 µg Pb/L , increased DOC lead to an increase in mean EC_{50} for reproduction ranging from approximately 25 µg Pb/L to >500 µg Pb/L (Mager et al., 2011a).

Reproductive effects of Pb have also been reported in marine invertebrate species. In the 2006 Pb AQCD embryo development in two commercial bivalves *Ruditapes decussatus* and *M. galloprovincialis* was inhibited by Pb (Beiras and Albentosa, 2003). In *R. decussatus* an EC_{50} range of 156 to 312 µg Pb/L and LOEC of 156 µg Pb/L were observed for inhibition of embryonic development while in *M. galloprovincialis* the EC_{50} was 221 µg Pb/L and the LOEC was 50 µg Pb/L. Larvae of the mussel *M. edulis* were sensitive to Pb exposure with an EC_{50} of 476 µg Pb/L for abnormal development of embryos following 48 hour exposure to Pb during embryogenesis (Martin et al., 1981). In the marine polychaete *H. elegans* an EC_{50} of 261 µg Pb/L was observed for unhatched or abnormal larvae following 20 hour incubation with Pb (Gopalakrishnan et al., 2008). The 1 hour LC_{50} value for Pb toxicity to eggs in the same species was 692 µg Pb/L while the EC_{50} for the metal concentration causing 5% reduction in larval attachment was 100 µg Pb/L. The EC_{50} values for sperm and egg toxicity were 380 and 690 µg Pb/L, respectively. In a multigenerational study with the marine amphipod *E. laevis*, statistically significant delays in onset of reproduction (4 to 8 days), sexual maturation and first offspring were observed at concentrations of 188 µg Pb/g sediment and higher

(Ringenary et al., 2007). The authors indicate that this concentration is below the current sediment regulatory guideline for Pb (218 µg Pb/g sediment) (NOAA, 1999) and that reproductive effects are a more sensitive endpoint than lethality. The LOEC for embryogenesis in the marine bivalve *M. galloprovincialis* was 50 µg Pb/L with an EC_{50} for embryogenesis of 221 µg Pb/L (Beiras and Albentosa, 2003).

In freshwater aquatic vertebrates there is evidence for reproductive and developmental effects of Pb. Pb exposure in frogs has been demonstrated to delay metamorphosis, decrease larval size and produce skeletal malformations. For example, in northern leopard frog *R. pipiens* exposed to nominal concentrations of 100 µg Pb/L from embryonic stage to metamorphosis, maximum swimming speed was significantly slower than other treatment groups and 92% of tadpoles exposed to 100 µg Pb/L had lateral spinal curvature (compared with 6% in the control) (Chen et al., 2006b). Previous Pb AQCDs have reported developmental effects in fish, specifically spinal deformities in brook trout (*Salvelinus fontinalis*) exposed to 119 µg Pb/L for three generations (U.S. EPA, 1977), and in rainbow trout as low as 120 µg Pb/L (U.S. EPA, 1986b). Reproductive behaviors of fathead minnows including reduced time spent in nest preparation by males, increased interspawn periods and reduced oviposition by females was observed following a 4 week exposure to 500 µg Pb/L (Weber, 1993). In the 2006 Pb AQCD, 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 alteration of 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-via consumption of contaminated prey. In one study from a saltwater fish, field collected smooth toadfish (*T. glaber*) from metal contaminated estuaries in Sydney, Australia had elevated Pb levels in gonad and decreased oocyte diameter and density.

Observations of Pb toxicity to reproductive and developmental endpoints in aquatic fauna are further supported by evidence terrestrial invertebrates and vertebrates (Section 7.4.1.4) and from laboratory animals (Section 5.8). Pb appears to affect multiple endpoints associated with reproduction and development in aquatic biota. A few sensitive species have been identified where effects are observed in laboratory studies at concentrations of Pb currently measured in the environment. Overall, there is a dearth of information on reproductive effects of Pb in natural environments, however, the weight of evidence is sufficient to conclude that there is a causal relationship between Pb exposures and developmental and reproductive effects in invertebrates and vertebrates.

In aquatic plants, the evidence is inadequate to conclude a causal relationship between Pb exposures and plant developmental and reproductive effects.

7.4.2.5 Growth Effects-Aquatic Biota

Alterations in the growth of an organism can impact population, community and ecosystem level variables. Growth is a commonly measured endpoint in aquatic plants. Evidence for Pb effects on growth in invertebrates has been observed most extensively in freshwater aquatic taxa, with inhibition in sensitive species occurring at or near the current range of Pb in surface waters. Growth effects observed in both invertebrates and vertebrates underscore the importance of lifestage to overall Pb sensitivity. In general, juvenile organisms are more sensitive than adults.

In the 2006 Pb AQCD, both freshwater algae and plants had EC_{50} values for growth in the range of 1,000 to >100,000 µg Pb/L with minimal inhibition of growth observed as low as 100 µg Pb/L (U.S. EPA, 2006c). The most sensitive aquatic macrophyte reported in the 2006 Pb AQCD was *A. pinnata* with an EC_{50} for relative growth rate of 1,100 µg Pb/L following a 4 day exposure to Pb (Gaur et al., 1994).

Growth effects of Pb on aquatic invertebrates are reviewed in the draft Ambient Aquatic Life Water Quality Criteria for Pb (U.S. EPA, 2008b) and the 2006 Pb AQCD. In the 2006 Pb AQCD, the LOEC for growth of amphipods *H. azteca* in 42-day chronic exposure to Pb was 16 µg Pb/L (Besser et al., 2005). Recent studies provide additional evidence for effects on growth of freshwater aquatic invertebrates at ≤ 10 µg Pb/L. Growth of juvenile freshwater snails *L. stagnalis* was inhibited below the lowest concentration tested resulting in an EC_{20} < 4 µg Pb/L (Grosell and Brix, 2009; Grosell et al., 2006a). In the same study, the NOEC was 12 µg Pb/L and the LOEC was 16 µg Pb/L. The authors indicated that the observed effect level for Pb was very close to the current U.S. EPA water quality criteria for Pb (3.3 µg Pb/L normalized to test water hardness) (Grosell and Brix, 2009). In the freshwater mussel, fatmucket (*L. siliquoidea*) juveniles were the most sensitive lifestage (Wang et al., 2010e). 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. The EC_{20} for reduced growth and emergence of the midge *C. 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 EC_{10} for molting in the mayfly *B. tricaudatus* was 37 µg Pb/L (Mebane et al., 2008). These effects are occurring at or near the current range of Pb concentrations in surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L) (U.S. EPA, 2006c).

Evidence for growth effects of Pb in aquatic vertebrates is limited to a few studies in amphibians and fish. In the 2006 Pb AQCD growth effects of Pb were reported in frogs at concentrations typically higher than currently found in the environment. A new study reviewed in this ISA supports findings of growth effects in frogs and suggests that these effects may be occurring at lower concentrations: the growth rate of tadpoles of the northern leopard frog exposed to 100 µg Pb/L from embryo to metamorphosis was slower than the growth rate of the controls (Chen et al., 2006b). In this study, Pb concentrations in the tissues of tadpoles were quantified and the authors reported that they were within the range of reported tissue concentrations reported in wild-caught populations.

Reports of Pb-associated growth effects in fish are inconsistent and are limited to freshwater species (Mager, 2012). In a review cited in the 2006 Pb AQCD general symptoms of Pb toxicity in fish include growth inhibition (Eisler, 2000) however, other studies with Pb show no growth effect (Mager, 2012). In the studies reviewed for the current ISA no growth effects were observed in fish exposed to Pb via dietary intake. Recent studies with fathead minnows showed significant increases in body length and body mass following chronic low Pb exposure, however, the authors noted that some effects were observed in tanks with high mortality early in the exposure (Mager and Grosell, 2011; Grosell et al., 2006b). Other studies with fathead minnows have shown growth reductions with Pb exposure, however, concentrations of observed effects typically exceeded the 96-hour LC_{50} value (Mager, 2012; Mager et al., 2010; Grosell et al., 2006b). Two 60-day early lifestage tests with rainbow trout showed differences in LOEC for reduced growth (Mebane et al., 2008). In the first test, a 69 day exposure, the LOECs for mortality and reduced growth were the same (54 µg Pb/L). In the second test, a 62 day exposure of Pb to rainbow trout, the LOEC for fish length was 18 µg Pb/L with an EC_{20} of >87 µg Pb/L .

Growth effects associated with exposure to Pb have also been reported in marine species. New studies in marine algae provide evidence for effects of Pb on growth within one to two orders of magnitude of measured levels of Pb in the environment. The lowest 72-hour EC_{50} for growth inhibition reported for marine algae was 105 µg Pb/L in Chaetoceros sp (Debelius et al., 2009). Growth of microalgae *T. suecica* exposed to environmentally relevant concentrations of Pb (20 µg Pb/L) was 40% lower than control cultures (Soto-Jiménez et al., 2011a). The microalgae was the base of a simulated marine food chain including primary, secondary and tertiary level consumers and effects on survival were observed at the higher trophic levels that originated from Pb exposure via consumption of the primary producer. Wang et al., (2009d) observed growth of embryos of the Asian Clam (*M. meretrix*) was significantly reduced by Pb with an EC_{50} of 197 µg Pb/L. In juvenile Catarina scallop, *A. ventricosus*, exposed to Pb for 30 days, the EC_{50} for growth was 4,210 µg Pb/L (Sobrino-Figueroa et al., 2007). Rate of growth of the deposit

feeding polychaete Capitella sp. decreased significantly with increasing concentrations of Pb associated with sediment from polluted estuaries (Horng et al., 2009).

Evidence of effects of Pb exposure on growth in terrestrial plants is highly coherent with evidence from aquatic plants. Evidence is sufficient to conclude that there **is a** causal relationship between Pb exposures and growth effects in aquatic plants and aquatic invertebrates. Evidence is inadequate for a causal relationship between Pb exposures and growth effects in aquatic vertebrates.

7.4.2.6 Survival-Aquatic Biota

The relationship between Pb exposure and survival has been well demonstrated in aquatic species as presented in Section 7.3.5 of this ISA and in the previous Pb AQCDs. Pb exposure can either result in direct lethality or produce sublethal effects that diminish survival probabilities. Survival is a biologically important response that may have a direct impact on population size. However, the concentration typically reported at which there is 50% mortality of test organisms (LC_{50}) is a poor measure for comparing environmental concentrations. LC_{50} is a relevant measure for acute toxicity whereas Pb effects on ecosystem receptors are likely characterized as a chronic, cumulative exposure rather than acute exposure. From the LC_{50} data on Pb in this review and previous Pb AQCDs, a wide range of sensitivity to Pb is evident across taxa and within genera. However, the LC_{50} is usually much higher than current environmental levels of Pb in the U.S, even though physiological dysfunction that adversely impacts the fitness of an organism often occurs at concentrations well below lethal ones. When available, LC_{10}, NOEC or LOEC are therefore reported.

There are no studies reported in the previous Pb AQCDs or the current ISA for aquatic plants that indicate phytotoxicity at current concentrations of Pb in freshwater or saltwater environments.

There is considerable data available on toxicity of Pb to aquatic invertebrates as reviewed in the previous Pb AQCDs and Ambient Water Quality Criteria for Lead (U.S. EPA, 1985). Table AX7-2.4.1 from the 2006 Pb AQCD summarizes LC_{50} data and other endpoints for freshwater and marine invertebrates (U.S. EPA, 2006c). Freshwater invertebrates are generally more sensitive to Pb exposure than other taxa, with survival 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. For example, the freshwater mussel *L. rafinesqueana* (Neosho mucket), is a candidate for the endangered species list. The EC_{50} for foot

movement (a measure of viability) for newly transformed juveniles of this species was 188 µg Pb/L. (Wang et al., 2010e).

Freshwater biota that exhibit sensitivity to Pb in the range of Pb concentrations measured in U.S. waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L (U.S. EPA, 2006c), include some species of gastropods, amphipods, cladocerans and rotifers although the toxicity of Pb is highly dependent upon water quality variables such as DOC, hardness and pH. In the 1986 Pb AQCD, increased mortality was observed in the freshwater gastropod *Lymnaea palutris* at Pb concentration as low as 19 µg Pb/L effectively reducing total biomass production (Borgmann et al., 1978). Toxicity testing with amphipods under various water conditions indicate these organisms are sensitive to Pb at <10 µg Pb/L (U.S. EPA, 2006c) and this ISA). A 7 day LC_{50} of 1 µg Pb/L was observed in soft water with the amphipod *H. azteca* (Borgmann et al., 2005). In this same species, the 96 hour LC_{50} for Pb at pH of 5 was 10 µg Pb/L (Mackie, 1989). In 42-day chronic exposures of *H. azteca* exposed to Pb via water and diet, the LC_{50} was 16 µg Pb/L (Besser et al., 2005). At higher pH and water hardness, amphipods are less sensitive to Pb (U.S. EPA, 2006c). In a series of 48 hour acute toxicity tests with the cladoceran *C. dubia* conducted in a variety of natural waters across North America, LC_{50} values ranged from 29 to 180 µg Pb/L and were most significantly influenced by DOC and water ionic strength (Esbaugh et al., 2011). In the 2006 Pb AQCD the range of 48 hour LC_{50} values for *C. dubia* were reported from 280 to >2,700 µg Pb/L when tested at varying pH levels (U.S. EPA, 2006c). In the rotifer genus *Lecane*, a 22-fold difference in LC_{50} values was observed in 48-hour exposure to Pb between *L. hamata*, *L. luna* and *L. quadridentata*. (Pérez-Legaspi and Rico-Martínez, 2001). *L. luna* was most sensitive to Pb toxicity with a 48-hour LC_{50} of 140 µg Pb/L. In neonate rotifers, *E. dilatata* the 48-hour LC_{50} was 35 µg Pb/L (Arias-Almeida and Rico-Martínez, 2011). Other freshwater 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.

In aquatic vertebrates, there is considerable historic information on Pb toxicity to freshwater fish. Early observations from mining areas where Pb and other metals were present indicated local extinction of fish from streams (U.S. EPA, 1977). The lowest LC_{50} for fish reported in the 1977 Pb AQCD was 1,000 µg Pb/L in soft water for rainbow trout (*Salmo gairdneri*) following 96-hour exposure to Pb (U.S. EPA, 1977). More recently reviewed studies using fish have considered the role of water quality variables on Pb toxicity. Higher toxicity tends to occur in acidic waters where more free-Pb ion is available for uptake. The interactive effects of Pb concentration and water quality variables on toxicity may result in equivalent toxicity for a broad range of Pb concentrations. In a series of 96-hour acute toxicity tests with fathead minnow conducted

in a variety of natural waters across North America, LC_{50} values ranged from 41 to 3,598 µg Pb/L and no Pb toxicity occurred in three highly alkaline waters (Esbaugh et al., 2011). In the 2006 Pb AQCD, the 96-hr LC_{50} values in fathead minnow ranged from 810->5,400 µg Pb/L in varying pH and hardness (U.S. EPA, 2006c). Decreased survival is also a function of age of the fish. Thirty day LC_{50} values for larval fathead minnows ranged from 39 to 1,903 µg Pb/L in varying concentrations of DOC, $CaSO_4$ and pH (Grosell et al., 2006a). In a recent study of rainbow trout fry at 2 to 4 weeks post swim-up, the 96-hour LC_{50} was 120 µg Pb/L at a hardness of 29 mg/L as $CaCO_3$, a value much lower than in testing with older fish (Mebane et al., 2008).

Data on mortality of saltwater species associated with exposure to Pb is limited; however, in general, marine organisms are less sensitive to this metal than freshwater organisms and the highest toxicity is observed in juveniles. In the marine amphipod *Melita plumulosa*, juveniles were more sensitive to Pb than adults in 96 hour seawater-only exposures and 10 day sediment exposures (King et al., 2006). The 96-hour LC_{50} was 1,520 µg Pb/L for juveniles in comparison to adults (3,000 µg Pb/L). Ten-day exposures of juveniles 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 compared to the adult values of 3,560 µg Pb/L. Effects of Pb on survival have been demonstrated though a simulated marine food chain in which the primary producer, the microalgae *T. suecica*, was exposed to 20 µg Pb/L and subsequently fed to brine shrimp *A. franciscana*, (mean Pb content 12 to 15 µg Pb/g) which were consumed by white-leg shrimp *L. vannamei*, itself consumed by grunt fish *H. scudderi* representing the top of the marine food chain (Soto-Jiménez et al., 2011a). Survival of brine shrimp was 25 to 35 % lower than the control and both white shrimp and grunt fish had significantly higher mortalities than controls. Data on Pb toxicity to marine fish is limited. Acute toxicity of Pb to plaice (*Pleuronectes platessa*) ranged from 50 µg Pb/L to 300,000 µg Pb/L depending on the form of Pb (Eisler, 2000). Evidence of effects of Pb on survival in aquatic animals is coherent with evidence in terrestrial ones.

The evidence is inadequate to conclude that there is a causal relationship between Pb exposures and survival in aquatic plants.

The evidence is sufficient to conclude that there is a causal relationship between Pb exposures and survival in aquatic invertebrates and vertebrates.

7.4.2.7 Community and Ecosystem Level Effects-Aquatic Biota

Most direct evidence of community- and ecosystem-level effects in aquatic systems is from near point sources where Pb concentrations are higher than typically observed environmental concentrations for this metal. Impacts of Pb on aquatic habitats that

receive runoff from point sources have been studied for several decades. For aquatic systems, the literature focuses on evaluating ecological stress from Pb originating from urban and mining effluents rather than atmospheric deposition. Ecosystem-level field studies are complicated by the confounding of Pb exposure with other factors such as the presence of trace metals and acidic deposition and by the variability inherent in natural systems. In natural systems, Pb is often found co-existing with other stressors, and observed effects may be due to cumulative toxicity.

In laboratory studies and simulated ecosystems, where it is possible to isolate the effect of Pb, this metal has been shown to alter competitive behavior of species, predator-prey interactions and contaminant avoidance. These dynamics may change species abundance and community structure at higher levels of ecological organization. Uptake of Pb into aquatic and terrestrial organisms and subsequent effects on mortality, growth, physiological stress, blood, neurobehavioral and developmental and reproductive endpoints at the organism level are expected to have ecosystem-level consequences, and thus provide consistency and plausibility for causality in ecosystem-level effects.

In aquatic ecosystems, numerous field studies reviewed in the 2006 Pb AQCD (summarized in Table AX7-2.5.2) and this ISA report reductions of species abundance, richness or diversity. This is particularly the case for benthic macroinvertebrate communities where sources of Pb were mining or urban effluents, and Pb coexisted with other metals. The results often indicate a correlation between the presence of one or more metals and the negative effects observed. For example, in the 2006 Pb AQCD, the Coeur d'Alene River watershed in Idaho, U.S. was used as a case study for Pb effects at the population and community level. Significant negative correlations were observed between Pb in water column and total taxa richness and Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa richness in the river. In a simulated aquatic microcosm a reduction in abundance and richness of protozoan species was observed with increasing Pb concentration from 50 to 1,000 µg Pb/L (Fernandez-Leborans and Antonio-Garcia, 1988).

Since the 2006 Pb AQCD, there is further evidence for effects of Pb in sediment-associated communities and aquatic cyanobacteria 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 and Nabelkova, 2005). Although surface water Pb concentrations in monitored streams were determined to be very low, concentrations of the metal in

sediment were high enough to pose a risk to the benthic community (e.g., 34 to 101 mg Pb/kg). These risks were observed to vary with benthic invertebrate functional feeding group, with collector-gatherer species exhibiting larger body burdens of heavy metals than benthic predators and collector-filterers. Bacterial biomass and diversity of aquatic cyanobacteria communities was reduced in a microcosm following one week exposure to 25 mM Pb (Burnat et al., 2009). Pb exposure impacted individual cyanobacteria species differently, with *Microcoleus* sp. experiencing a greater decrease in abundance than *Halomicronema*-like cyanobacteria.

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) (Mishra et al., 2008). Certain types of plants such as rooted and submerged aquatic plants may be more susceptible to aerially deposited Pb resulting in shifts in Pb community composition. High Pb sediment concentrations are linked to shifts in amphipod communities inhabiting plant structures.

Avoidance response to Pb exposure varies widely in different species and this could affect community composition and structure and species abundance. For example, frogs and toads lack avoidance response while snails and fish avoid higher concentrations of Pb (U.S. EPA, 2006c).

In the Annex to the 2006 Pb AQCD, the Coeur d'Alene River basin in Idaho was presented as a case study for a watershed impacted by Pb and other metals. A significant negative correlation was observed between Pb in water column (0.5 to 30 µg Pb/L) and total taxa richness, EPT taxa richness, and the number of metal-sensitive mayfly species (Maret et al., 2003). Additional lines of evidence including mine density, metal concentrations, and bioaccumulation in caddisfly tissue were included. Since the 2006 Pb AQCD, additional research at this site and model development has resulted in further characterization of the effects of Pb on waterfowl and other biota in this heavily contaminated ecosystem. Mean Pb concentrations in sediment range from 14 to 5,009 mg Pb/kg dry weight (Spears et al., 2007). Modeling of sediment and Pb levels in waterfowl predict a sediment Pb effects range of 147-944 mg Pb/kg dry weight and a mortality effects level of 1,652 mg/kg dry weight (Spears et al., 2007). In a 6-week feeding study with mallard (*Anas platyrhynchos*) ducklings, ingestion of Pb-contaminated sediments from the Coeur d' Alene basin was shown to result in decreased brain growth and altered brain chemistry (Douglas-Stroebel et al., 2004). These findings support previous observations of altered behavior and hematological, hepatotoxic, and histopathological

endpoints in waterfowl from Lake Coeur d'Alene that ingest Pb contaminated sediments and vegetation during feeding.

Fewer studies are available for community and ecosystem effects in saltwater systems. No studies on marine systems were reviewed in the 1977 Pb AQCD or the 1986 Pb AQCD. In a laboratory study with larval mummichogs reviewed in the 2006 Pb AQCD, feeding and predator avoidance behaviors were altered in this marine fish species following a 4 week exposure to Pb (300 to 1,000 µg Pb/L). Observations from field studies reviewed in the 2006 Pb AQCD included findings of a negative correlation between Pb and species richness and diversity indices of macroinvertebrates associated with estuary sediments and changes in species distribution and abundance in fish, crustaceans and macroinvertebrates correlated with Pb levels in marine sediments. In a recent study, significant differences in macroinvertebrate communities associated with seagrass beds were observed between sites with different sediment, biofilm, and leaf Pb concentrations (Marín-Guirao et al., 2005). Sediment Pb concentrations ranged from approximately 100 to 5,000 mg Pb/kg and corresponding biofilm concentrations were 500 to 1,600 mg Pb/kg, with leaf concentrations up to 300 mg Pb/kg. In a laboratory microcosm experiment conducted with estuarine sediments from South Africa, total meiofauna density decreased (range 3 to 5 taxa) after 32 days in Pb-treated (1,886 to 6,710 µg/Pb g sediment dry weight) sediments compared to 9 taxa in the control (3 µg/Pb g sediment dry weight) (Gyedu-Ababio and Baird, 2006).

Most evidence for Pb toxicity to biota is from single-species assays in laboratory studies. Although the evidence is strong for effects of Pb on growth, reproduction and survival in certain species, considerable uncertainties exist in generalizing effects observed under small-scale, particular conditions up to predicted effects at the ecosystem level of biological organization. In many cases it is difficult to characterize the nature and magnitude of effects and to quantify relationships between ambient concentrations of Pb and ecosystem response due to presence of multiple stressors, variability in field conditions and to differences in Pb bioavailability at that level of organization. Nevertheless, evidence of ecosystem effects in aquatic ecosystems is coherent with similar evidence in terrestrial ecosystems, and based on the cumulative evidence from laboratory studies and field data, there is a likely causal relationship between Pb exposures and the alteration of species richness, species composition and biodiversity in aquatic ecosystems.

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