

# **Air Quality Criteria for Lead**

## **Volume II of II**

# **Air Quality Criteria for Lead**

## **Volume II**

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

## PREFACE

National Ambient Air Quality Standards (NAAQS) are promulgated by the United States Environmental Protection Agency (EPA) to meet requirements set forth in Sections 108 and 109 of the U.S. Clean Air Act. Those two Clean Air Act sections require the EPA Administrator (1) to list widespread air pollutants that reasonably may be expected to endanger public health or welfare; (2) to issue air quality criteria for them that assess the latest available scientific information on nature and effects of ambient exposure to them; (3) to set “primary” NAAQS to protect human health with adequate margin of safety and to set “secondary” NAAQS to protect against welfare effects (e.g., effects on vegetation, ecosystems, visibility, climate, manmade materials, etc); and (5) to periodically review and revise, as appropriate, the criteria and NAAQS for a given listed pollutant or class of pollutants.

Lead was first listed in the mid-1970’s as a “criteria air pollutant” requiring NAAQS regulation. The scientific information pertinent to Pb NAAQS development available at the time was assessed in the EPA document *Air Quality Criteria for Lead*, published in 1977. Based on the scientific assessments contained in that 1977 lead air quality criteria document (1977 Lead AQCD), EPA established a 1.5  $\mu\text{g}/\text{m}^3$  (maximum quarterly calendar average) Pb NAAQS in 1978.

To meet Clean Air Act requirements noted above for periodic review of criteria and NAAQS, new scientific information published since the 1977 Lead AQCD was later assessed in a revised Lead AQCD and Addendum published in 1986 and in a Supplement to the 1986 AQCD/Addendum published by EPA in 1990. A 1990 Lead Staff Paper, prepared by EPA’s Office of Air Quality Planning and Standards (OPQPS), drew upon key findings and conclusions from the 1986 Lead AQCD/Addendum and 1990 Supplement (as well as other OAQPS-sponsored lead exposure/risk analyses) in posing options for the EPA Administrator to consider with regard to possible revision of the Pb NAAQS. However, EPA chose not to revise the Pb NAAQS at that time. Rather, as part of implementing a broad 1991 U.S. EPA Strategy for Reducing Lead Exposure, the Agency focused primarily on regulatory and remedial clean-up efforts to reduce Pb exposure from a variety of non-air sources that posed more extensive public health risks, as well as other actions to reduce air emissions.

The purpose of this revised Lead AQCD is to critically assess the latest scientific information that has become available since the literature assessed in the 1986 Lead AQCD/Addendum and 1990 Supplement, with the main focus being on pertinent new information useful in evaluating health and environmental effects of ambient air lead exposures. This includes discussion in this document of information regarding: the nature, sources, distribution, measurement, and concentrations of lead in the environment; multimedia lead exposure (via air, food, water, etc.) and biokinetic modeling of contributions of such exposures to concentrations of lead in brain, kidney, and other tissues (e.g., blood and bone concentrations, as key indices of lead exposure).; characterization of lead health effects and associated exposure-response relationships; and delineation of environmental (ecological) effects of lead. This final version of the revised Lead AQCD mainly assesses pertinent literature published or accepted for publication through December 2005.

The First External Review Draft (dated December 2005) of the revised Lead AQCD underwent public comment and was reviewed by the Clean Air Scientific Advisory Committee (CASAC) at a public meeting held in Durham, NC on February 28-March 1, 2006. The public comments and CASAC recommendations received were taken into account in making appropriate revisions and incorporating them into a Second External Review Draft (dated May, 2006) which was released for further public comment and CASAC review at a public meeting held June 28-29, 2006. In addition, still further revised drafts of the Integrative Synthesis chapter and the Executive Summary were then issued and discussed during an August 15, 2006 CASAC teleconference call. Public comments and CASAC advice received on these latter materials, as well as Second External Review Draft materials, were taken into account in making and incorporating further revisions into this final version of this Lead AQCD, which is being issued to meet an October 1, 2006 court-ordered deadline. Evaluations contained in the present document provide inputs to an associated Lead Staff Paper prepared by EPA's Office of Air Quality Planning and Standards (OAQPS), which poses options for consideration by the EPA Administrator with regard to proposal and, ultimately, promulgation of decisions on potential retention or revision, as appropriate, of the current Pb NAAQS.

Preparation of this document has been coordinated by staff of EPA's National Center for Environmental Assessment in Research Triangle Park (NCEA-RTP). NCEA-RTP scientific staff, together with experts from academia, contributed to writing of document chapters. Earlier

drafts of document materials were reviewed by scientists from other EPA units and by non-EPA experts in several public peer consultation workshops held by EPA in July/August 2005.

NCEA acknowledges the valuable contributions provided by authors, contributors, and reviewers and the diligence of its staff and contractors in the preparation of this document. The constructive comments provided by public commenters and CASAC that served as valuable inputs contributing to improved scientific and editorial quality of the document are also acknowledged by NCEA.

### **DISCLAIMER**

Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use.

**Air Quality Criteria for Lead  
(Second External Review Draft)**

**VOLUME I**

EXECUTIVE SUMMARY .....	E-1
1. INTRODUCTION .....	1-1
2. CHEMISTRY, SOURCES, AND TRANSPORT OF LEAD.....	2-1
3. ROUTES OF HUMAN EXPOSURE TO LEAD AND OBSERVED ENVIRONMENTAL CONCENTRATIONS.....	3-1
4. TOXICOKINETICS, BIOLOGICAL MARKERS, AND MODELS OF LEAD BURDEN IN HUMANS.....	4-1
5. TOXICOLOGICAL EFFECTS OF LEAD IN LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS .....	5-1
6. EPIDEMIOLOGIC STUDIES OF HUMAN HEALTH EFFECTS ASSOCIATED WITH LEAD EXPOSURE .....	6-1
7. ENVIRONMENTAL EFFECTS OF LEAD .....	7-1
8. INTEGRATIVE SYNTHESIS OF LEAD EXPOSURE/HEALTH EFFECTS INFORMATION.....	8-1

**VOLUME II**

CHAPTER 4 ANNEX (TOXICOKINETICS, BIOLOGICAL MARKERS, AND MODELS OF LEAD BURDEN IN HUMANS).....	AX4-1
CHAPTER 5 ANNEX (TOXICOLOGICAL EFFECTS OF LEAD IN LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS).....	AX5-1
CHAPTER 6 ANNEX (EPIDEMIOLOGIC STUDIES OF HUMAN HEALTH EFFECTS ASSOCIATED WITH LEAD EXPOSURE).....	AX6-1
CHAPTER 7 ANNEX (ENVIRONMENTAL EFFECTS OF LEAD).....	AX7-1

## Table of Contents

	<u>Page</u>
PREFACE .....	iii
DISCLAIMER .....	v
List of Tables .....	xi
List of Figures .....	xiii
Authors, Contributors, and Reviewers .....	xv
U.S. Environmental Protection Agency Project Team for Development of Air Quality Criteria for Lead .....	xxi
U.S. Environmental Protection Agency Science Advisory Board (SAB) Staff Office Clean Air Scientific Advisory Committee (CASAC) .....	xxiii
Abbreviations and Acronyms .....	xxv
AX7. CHAPTER 7 ANNEX (ENVIRONMENTAL EFFECTS OF LEAD).....	AX7-1
AX7.1 TERRESTRIAL ECOSYSTEMS.....	AX7-1
AX7.1.1 Methodologies Used in Terrestrial Ecosystems Research.....	AX7-1
AX7.1.1.1 Lead Isotopes and Apportionment .....	AX7-1
AX7.1.1.2 Speciation in Assessing Lead Bioavailability in the Terrestrial Environment.....	AX7-3
AX7.1.1.3 Tools for Bulk Lead Quantification and Speciation.....	AX7-9
AX7.1.1.4 Biotic Ligand Model .....	AX7-18
AX7.1.1.5 Soil Amendments.....	AX7-19
AX7.1.2 Distribution of Atmospherically Delivered Lead in Terrestrial Ecosystems .....	AX7-22
AX7.1.2.1 Speciation of Atmospherically-Delivered Lead in Terrestrial Ecosystems .....	AX7-24
AX7.1.2.2 Tracing the Fate of Atmospherically- Delivered Lead in Terrestrial Ecosystems.....	AX7-31
AX7.1.2.3 Inputs/Outputs of Atmospherically- Delivered Lead in Terrestrial Ecosystems.....	AX7-34
AX7.1.2.4 Resistance Mechanisms .....	AX7-44
AX7.1.2.5 Physiological Effects of Lead .....	AX7-46
AX7.1.2.6 Factors that Modify Organism Response.....	AX7-48
AX7.1.2.7 Summary .....	AX7-55
AX7.1.3 Exposure-Response of Terrestrial Species.....	AX7-57
AX7.1.3.1 Summary of Conclusions from the 1986 Lead Criteria Document.....	AX7-59
AX7.1.3.2 Recent Studies on the Effects of Lead on Primary Producers.....	AX7-61

**Table of Contents**  
(cont'd)

		<u>Page</u>
	AX7.1.3.3	Recent Studies on the Effects of Lead on Consumers..... AX7-64
	AX7.1.3.4	Recent Studies on the Effects of Lead on Decomposers..... AX7-81
	AX7.1.3.5	Summary ..... AX7-86
AX7.1.4		Effects of Lead on Natural Terrestrial Ecosystems ..... AX7-88
	AX7.1.4.1	Effects of Terrestrial Ecosystem Stresses on Lead Cycling..... AX7-89
	AX7.1.4.2	Effects of Lead Exposure on Natural Ecosystem Structure and Function..... AX7-95
	AX7.1.4.3	Effects of Lead on Energy Flows and Biogeochemical Cycling..... AX7-99
	AX7.1.4.4	Summary ..... AX7-105
AX7.2		AQUATIC ECOSYSTEMS..... AX7-106
	AX7.2.1	Methodologies Used in Aquatic Ecosystem Research.... AX7-106
	AX7.2.1.1	Analytical Methods..... AX7-107
	AX7.2.1.2	Ambient Water Quality Criteria: Development ..... AX7-108
	AX7.2.1.3	Ambient Water Quality Criteria: Bioavailability Issues ..... AX7-111
	AX7.2.1.4	Sediment Quality Criteria: Development and Bioavailability Issues.... AX7-113
	AX7.2.1.5	Metal Mixtures..... AX7-116
	AX7.2.1.6	Background Lead..... AX7-117
AX7.2.2		Distribution of Lead in Aquatic Ecosystems ..... AX7-117
	AX7.2.2.1	Speciation of Lead in Aquatic Ecosystems..... AX7-118
	AX7.2.2.2	Spatial Distribution of Lead in Aquatic Ecosystems ..... AX7-122
	AX7.2.2.3	Tracing the Fate and Transport of Lead in Aquatic Ecosystems..... AX7-142
	AX7.2.2.4	Summary ..... AX7-145
AX7.2.3		Aquatic Species Response/Mode of Action..... AX7-146
	AX7.2.3.1	Lead Uptake ..... AX7-146
	AX7.2.3.2	Resistance Mechanisms ..... AX7-152
	AX7.2.3.3	Physiological Effects of Lead ..... AX7-159
	AX7.2.3.4	Factors That Modify Organism Response to Lead ..... AX7-162
	AX7.2.3.5	Factors Associated with Global Climate Change..... AX7-174
	AX7.2.3.6	Summary ..... AX7-174

**Table of Contents**  
(cont'd)

	<u>Page</u>
AX7.2.4 Exposure/Response of Aquatic Species.....	AX7-175
AX7.2.4.1 Summary of Conclusions From the Previous Criteria Document.....	AX7-175
AX7.2.4.2 Recent Studies on Effects of Lead on Primary Producers.....	AX7-177
AX7.2.4.3 Recent Studies on Effects of Lead on Consumers.....	AX7-183
AX7.2.4.4 Recent Studies on Effects of Lead on Decomposers.....	AX7-193
AX7.2.4.5 Summary .....	AX7-193
AX7.2.5 Effects of Lead on Natural Aquatic Ecosystems .....	AX7-194
AX7.2.5.1 Case Study: Coeur d'Alene River Watershed .....	AX7-196
AX7.2.5.2 Biotic Condition.....	AX7-198
AX7.2.5.3 Summary .....	AX7-209
REFERENCES .....	AX7-211

## List of Tables

		<u>Page</u>
AX7-1.1.1	Relative Standard Deviation (RSD) for Lead Isotope Ratios on Selected Mass Spectrometers.....	AX7-2
AX7-1.1.2	National Institute of Standards and Technology Lead SRMs.....	AX7-10
AX7-1.1.3	Characteristics for Direct Speciation Techniques.....	AX7-17
AX7-1.2.1	Tissue Lead Levels in Birds Causing Effects .....	AX7-43
AX7-1.3.1	Plant Toxicity Data Used to Develop the Eco-SSL .....	AX7-62
AX7-1.3.2	Plant Toxicity Data Not Used to Develop the Eco-SSL .....	AX7-63
AX7-1.3.3	Avian Toxicity Data Used to Develop the Eco-SSL .....	AX7-66
AX7-1.3.4	Mammalian Toxicity Data Used to Develop the Eco-SSL.....	AX7-72
AX7-1.3.5	Invertebrate Toxicity Data Used to Develop the Eco-SSL.....	AX7-82
AX7-1.3.6	Invertebrate Toxicity Data Not Used to Develop the Eco-SSL.....	AX7-84
AX7-2.1.1	Common Analytical Methods for Measuring Lead in Water, Sediment, and Tissue .....	AX7-107
AX7-2.1.2	Development of Current Acute Freshwater Criteria for Lead .....	AX7-109
AX7-2.1.3	Recommended Sediment Quality Guidelines for Lead .....	AX7-115
AX7-2.2.1	NAWQA Land Use Categories and Natural/Ambient Classification.....	AX7-125
AX7-2.2.2	Summary Statistics of Ambient and Natural Levels of Dissolved Lead in Surface Water .....	AX7-126
AX7-2.2.3	Summary Statistics of Ambient and Natural Levels of Total Lead in <63 μm Bulk Sediment.....	AX7-127
AX7-2.2.4	Summary Statistics of Ambient and Natural Levels of Lead in Whole Organism and Liver Tissues.....	AX7-136
AX7-2.2.5	Comparison of NCBP and NAWQA Ambient Lead Levels in Whole Organism Tissues .....	AX7-138

**List of Tables**  
(cont'd)

	<u>Page</u>
AX7-2.3.1	Bioconcentration Factors for Aquatic Plants ..... AX7-151
AX7-2.3.2	Bioconcentration Factors for Aquatic Invertebrates ..... AX7-151
AX7-2.4.1	Effects of Lead to Freshwater and Marine Invertebrates..... AX7-185
AX7-2.4.2	Effects of Pb to Freshwater and Marine Fish..... AX7-190
AX7-2.4.3	Nonlethal Effects in Amphibians..... AX7-192
AX7-2.5.1	Ecological Attributed Studies by Maret et al. (2003) in the Coeur d'Alene Watershed..... AX7-197
AX7-2.5.2	Essential Ecological Attributes for Natural Aquatic Ecosystems Affected by Lead..... AX7-200

## List of Figures

<u>Number</u>		<u>Page</u>
AX7-1.1.1	Relationship of bioaccessibility (low, medium, high) versus speciation as shown with scanning electron micrographs of various Pb-bearing materials.....	AX7-5
AX7-1.1.2	Variation of bioavailability with particle size.....	AX7-7
AX7-1.1.3	Illustration of particle lability and bioavailability at two different sites with similar total Pb concentrations and Pb forms .....	AX7-8
AX7-1.1.4	Scanning electron micrograph of a large native Pb particle showing outer ring of highly bioavailable Pb-chloride and Pb-oxide.....	AX7-8
AX7-1.1.5	Bulk lead versus single species modality.....	AX7-12
AX7-1.2.1	Long-term (1982-1989) annual trends in lead concentrations ( $\mu\text{g/L}$ ) in Lewes, Delaware precipitation .....	AX7-35
AX7-1.3.1	Avian reproduction and growth toxicity data considered in development of the Eco-SSL .....	AX7-69
AX7-1.3.2	Mammalian reproduction and growth toxicity data considered in development of the Eco-SSL .....	AX7-81
AX7-2.2.1	Distribution of aqueous lead species as a function of pH based on a concentration of $1 \mu\text{g Pb/L}$ .....	AX7-119
AX7-2.2.2	Lead speciation versus chloride content .....	AX7-121
AX7-2.2.3	Spatial distribution of natural and ambient surface water/sediment sites .....	AX7-128
AX7-2.2.4	Spatial distribution of natural and ambient liver tissue sample sites.....	AX7-129
AX7-2.2.5	Spatial distribution of natural and ambient whole organism tissue sample sites .....	AX7-130
AX7-2.2.6	Frequency distribution of ambient and natural levels of surface water dissolved lead ( $\mu\text{g/L}$ ). .....	AX7-131
AX7-2.2.7	Spatial distribution of dissolved lead in surface water (N = 3445). .....	AX7-132

**List of Figures**  
(cont'd)

<u>Number</u>		<u>Page</u>
AX7-2.2.8	Frequency distribution of ambient and natural levels of bulk sediment <63 $\mu\text{m}$ total Pb ( $\mu\text{g/g}$ ).....	AX7-134
AX7-2.2.9	Spatial distribution of total lead in bulk sediment <63 $\mu\text{m}$ (N = 1466).....	AX7-135
AX7-2.2.10	Frequency distribution of ambient and natural levels of lead in liver tissue ( $\mu\text{g/g}$ dry weight).....	AX7-137
AX7-2.2.11	Frequency distribution of ambient and natural levels of lead in whole organism tissue ( $\mu\text{g/g}$ dry weight). ....	AX7-137
AX7-2.2.12	Spatial distribution of lead in liver tissues (N = 559). ....	AX7-139
AX7-2.2.13	Spatial distribution of lead in whole organism tissues (N = 332).....	AX7-140
AX7-2.2.14	Lead cycle in an aquatic ecosystem. ....	AX7-143

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

## Abbreviations and Acronyms

$\alpha$ FGF	$\alpha$ -fibroblast growth factor
AA	arachidonic acid
AAL	active avoidance learning
AAS	atomic absorption spectroscopy
ABA	$\beta$ -aminoisobutyric acid
ACBP	Achenbach Child Behavior Profile
ACE	angiotensin converting enzyme
ACh	acetylcholine
AChE	acetylcholinesterase
ACR	acute-chronic ratio
AD	adult
ADC	analog digital converter
ADP	adenosine diphosphate
AE	anion exchange
AEA	<i>N</i> -arachidonylethanolamine
AFC	antibody forming cells
2-AG	2-arachidonylglycerol
A horizon	uppermost layer of soil (litter and humus)
AHR	aryl hydrocarbon receptor
AI	angiotensin I
ALA	*-aminolevulinic acid
ALAD	*-aminolevulinic acid dehydratase
ALAS	aminolevulinic acid synthetase
ALAU	urinary $\delta$ -aminolevulinic acid
ALD	aldosterone
ALS	amyotrophic lateral sclerosis
ALT	alanine aminotransferase
ALWT	albumin weight
AMEM	Alpha Minimal Essential Medium
AMP	adenosine monophosphate
ANCOVA	analysis of covariance
ANF	atrial natriuretic factor
Ang II	angiotensin II

ANOVA	analysis of variance
ANP	atrial natriuretic peptide
AP	alkaline phosphatase
AP-1	activated protein-1
ApoE	apolipoprotein E
AQCD	Air Quality Criteria Document
Arg	arginine
AS52	cells derived from the CHO cell line
ASGP-R	asialyl glycoprotein receptor
AST	aspartate aminotransferase
ASV	anode stripping voltammetry
3-AT	3-aminotriazole; 3-amino triazide
ATP	adenosine triphosphate
ATP1A2	sodium-potassium adenosine triphosphate $\alpha 2$
ATPase	adenosine triphosphatase
ATSDR	Agency for Toxic Substances and Disease Research
AVCD	atrioventricular conduction deficit
AVS	acid volatile sulfide
AWQC	ambient water quality criteria
$\exists$	beta-coefficient; slope of an equation
$\exists$ FGF	$\exists$ -fibroblast growth factor
17 $\exists$ -HS	17 $\exists$ -hydroxysteroid
3 $\exists$ -HSD	3 $\exists$ -hydroxysteroid dehydrogenase
17 $\exists$ -HSDH	17 $\exists$ -hydroxysteroid dehydrogenase
6 $\exists$ -OH-cortisol	6- $\exists$ -hydroxycortisol
B	both
BAEP	brainstem auditory-evoked potentials
BAER	brainstem auditory-evoked responses
BAF	bioaccumulation factor
B cell	B lymphocyte
BCFs	bioconcentration factors
BCS	bovine calf serum
BDNF	brain derived neurotrophic factor
BDWT	body weight changes
BEI	biological exposure index

BFU-E	blood erythroid progenitor
BLL	blood lead level
BLM	biotic ligand model
BM	basement membrane
BMI	body mass index
BDNF	brain-derived neurotrophic factor
BOTMP	Bruinicks-Oseretsky Test of Motor Proficiency
BP	blood pressure
BPb	blood lead concentration
BSA	bovine serum albumin
BSI	Brief Symptom Inventory
BTQ	Boston Teacher Questionnaire
BUN	blood urea nitrogen
bw, b. wt., BW	body weight
C3H10T/12	mouse embryo cell line
C3, C4	complement proteins
CA	chromosome aberration
CA3	cornu ammonis 3 region of hippocampus
<sup>45</sup> Ca	calcium-45 radionuclide
Ca-ATP	calcium-dependent adenosine triphosphate
Ca-ATPase	calcium-dependent adenosine triphosphatase
CaCO <sub>3</sub>	calcium carbonate
CaEDTA	calcium disodium ethylenediaminetetraacetic acid
CAL	calcitonin
CaM	calmodulin
Ca-Mg-ATPase	calcium-magnesium-dependent adenosine triphosphatase
cAMP	cyclic adenosinemonophosphate
CaNa <sub>2</sub> EDTA	calcium disodium ethylenediaminetetraacetic acid
CANTAB	Cambridge Neuropsychological Testing Automated Battery
CAT	catalase; Cognitive Abilities Test
CBCL	Achenbach Child Behavior Checklist
CBCL-T	Total Behavior Problem Score
CBL	cumulative blood lead
CBLI	cumulative blood lead index
CCB	cytochalasin B

CCD	charge-coupled device
CCE	Coordination Center for Effects
CCL	carbon tetrachloride
CCS	cosmic calf serum
C-CV <sub>RSA</sub>	coefficient of component variance of respiratory sinus arrhythmia
Cd	cadmium
<sup>109</sup> Cd	cadmium-109 radionuclide
CdU	urinary cadmium
CEC	cation exchange capacity
CESD, CES-D	Center for Epidemiologic Studies Depression (scale)
GFAP	glial fibrillary acidic protein
CFU-E	colony forming unit blood-erythroid progenitor (cell count)
CFU-GEMM	colony forming unit blood-pluripotent progenitor (cell count)
CFU-GM	blood granulocyte/macrophage progenitor (cell count)
cGMP	cyclic guanosine-3',5'-monophosphate
ChAT	choline acetyltransferase
CHD	coronary heart disease
CHO	Chinese hamster ovary cell line
CI	confidence interval
CLE-SV	competitive ligand-exchange/stripping voltammetry
CLRTAP	Convention on Long-Range Transboundary of Air Pollution
CLS	Cincinnati Lead Study
CMC	criterion maximum concentration
CMI	cell-mediated immunity
CNS	central nervous system
COH	cation-osmotic hemolysis
ConA	concanavalin A
COR	cortisol
CoTx	cotreatment
COX-2	cyclooxygenase-2
CP	coproporphryn
CPT	current perception threshold
cr	creatinine
CRAC	calcium release activated calcium reflex
CREB	cyclic AMP-response element binding protein

CRF	chronic renal failure
CRI	chronic renal insufficiency
CSF	cerebrospinal fluid
CuZn-SOD	copper and zinc-dependent superoxide dismutase
CV	conduction velocity
CVLT	California Verbal Learning Test
CV <sub>R-R</sub>	coefficient of variation of the R-R interval
CYP	cytochrome (e.g., CYP1A, CYP-2A6, CYP3A4, CYP450)
CYP3a11	cytochrome P450 3a11
D	D-statistic
DA	dopamine; dopaminergic
dbcAMP	dibutyryl cyclic adenosine-3',5'-monophosphate
DCV	distribution of conduction velocities
DEAE	diethylaminoethyl (chromatography)
DET	diffusive equilibrium thin films
DEYO	death of young
DFS	decayed or filled surfaces, permanent teeth
dfs	covariate-adjusted number of caries
DG	dentate gyrus
DGT	diffusive gradient thin films
DL	DL-statistic
DMEM	Dulbecco's Minimal Essential Medium
DMEM/F12	Dulbecco's Minimal Essential Medium/Ham's F12
DMFS	decayed, missing, or filled surfaces, permanent teeth
DMPS	2,3-dimercaptopropane 1-sulfonate
DMSA	2,3-dimercaptosuccinic acid
DMT	Donnan membrane technique
DMTU	dimethylthiourea
DNA	deoxyribonucleic acid
DO	distraction osteogenesis
DOC	dissolved organic carbon
DOM	dissolved organic carbon
DOPAc	3,4-dihydroxyphenylacetic acid
DPASV	differential pulse anodic stripping voltammetry
dp/dt	rate of left ventricular isovolumetric pressure

DPPD	<i>N-N</i> -diphenyl- <i>p</i> -phynylene-diamine
DR	drinking water
DSA	delayed spatial alternation
DTC	diethyl dithiocarbomate complex
DTH	delayed type hypersensitivity
DTPA	diethylenetriaminepentaacetic acid
DTT	dithiothreitol
dw	dry weight
E	embryonic day
E <sub>2</sub>	estradiol
EBE	early biological effect
EBV	Epstein-Barr virus
EC	European Community
EC <sub>50</sub>	effect concentration for 50% of test population
eCB	endocannabinoid
ECG	electrocardiogram
Eco-SSL	ecological soil screening level
EDS	energy dispersive spectrometers
EDTA	ethylenediaminetetraacetic acid
EEDQ	<i>N</i> -ethoxycarbonyl-2-ethoxy-1,2-dihydroquinone
EEG	electroencephalogram
EG	egg
EGF	epidermal growth factor
EGG	effects on eggs
EGPN	egg production
EKG	electrocardiogram
electro	electrophysiological stimulation
EM/CM	experimental medium-to-control medium (ratio)
EMEM	Eagle's Minimal Essential Medium
eNOS	endothelial nitric oxide synthase
EP	erythrocyte protoporphyrin
EPA	U.S. Environmental Protection Agency
Epi	epinephrine
EPMA	electron probe microanalysis
EPO	erythropoietin

EPSC	excitatory postsynaptic currents
EPT	macroinvertebrates from the Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) group
ERG	electroretinogram; electroretinographic
ERL	effects range – low
ERM	effects range – median
EROD	ethoxyresorufin- <i>O</i> -deethylase
ESCA	electron spectroscopy for chemical analysis
ESRD	end-stage renal disease
EST	estradiol
ESTH	eggshell thinning
ET	endothelin; essential tremor
ETOH	ethyl alcohol
EXAFS	extended X-ray absorption fine structure
EXANES	extended X-ray absorption near edge spectroscopy
F	F-statistic
F344	Fischer 344 (rat)
FAV	final acute value
FBS	fetal bovine serum
FCS	fetal calf serum
FCV	final chronic value
FD	food
FEF	forced expiratory flow
FEP	free erythrocyte protoporphyrin
FERT	fertility
FEV <sub>1</sub>	forced expiratory volume in one second
FGF	fibroblast growth factor (e.g., $\beta$ FGF, $\alpha$ FGF)
FI	fixed interval (operant conditioning)
FIAM	free ion activity model
FMLP	<i>N</i> -formyl-L-methionyl-L-leucyl-L-phenylalanine
fMRI	functional magnetic resonance imaging
FR	fixed-ratio operant conditioning
FSH	follicle stimulating hormone
FT3	free triiodothyronine
FT4	free thyroxine

FTES	free testosterone
FTII	Fagan Test of Infant Intelligence
FTPLM	flow-through permeation liquid membranes
FURA-2	1-[6-amino-2-(5-carboxy-2-oxazolyl)-5-benzofuranyloxy]-2-(2-amino-5-methylphenoxy) ethane- <i>N,N,N',N'</i> -tetraacetic acid
FVC	forced vital capacity
(-GT	(-glutamyl transferase
G	gestational day
GABA	gamma aminobutyric acid
GAG	glycosaminoglycan
G12 CHV79	cells derived from the V79 cell line
GCI	General Cognitive Index
GD	gestational day
GDP	guanosine diphosphate
GEE	generalized estimating equations
GFAAS	graphite furnace atomic absorption spectroscopy
GFR	glomerular filtration rate
GGT	(-glutamyl transferase
GH	growth hormone
GI	gastrointestinal
GIME-VIP	gel integrated microelectrodes combined with voltammetric in situ profiling
GIS	geographic information system
GLU	glutamate
GMAV	genus mean acute value
GMCV	genus mean chronic value
GMP	guanosine monophosphate
GMPH	general morphology
GnRH	gonadotropin releasing hormone
GOT	aspartate aminotransferase
GP	gross productivity
G6PD, G6PDH	glucose-6-phosphate dehydrogenase
GPEI	glutathione <i>S</i> -transferase P enhancer element
gp91 <sup>phox</sup>	NAD(P)H oxidase
GPT	glutamic-pyruvic transaminase
GPx	glutathione peroxidase

GRO	growth
GRP78	glucose-regulated protein 78
GSD	geometric standard deviation
GSH	reduced glutathione
GSIM	gill surface interaction model
GSSG	glutathione disulfide
GST	glutathione- <i>S</i> -transferase
GSTP	placental glutathione transferase
GTP	guanosine triphosphate
GV	gavage
H <sup>+</sup>	acidity
<sup>3</sup> H	hydrogen-3 radionuclide (tritium)
HA	humic acid; hydroxyapatite
Hb	hemoglobin
HBEF	Hubbard Brook Experimental Forest
HBSS	Hank's Balanced Salt Solution
HCG; hCG	human chorionic gonadotropin
Hct	hematocrit
HDL	high-density lipoprotein (cholesterol)
HEP	habitat evaluation procedure
HET	Binghamton heterogeneous stock
HFPLM	hollow fiber permeation liquid membranes
Hgb	hemoglobin
HGF	hepatocyte growth factor
HH	hydroxylamine hydrochloride
H-H	high-high
HHANES	Hispanic Health and Nutrition Examination Survey
H-L	high-low
HLA	human leukocyte antigen
H-MEM	minimum essential medium/nutrient mixture–F12-Ham
HMP	hexose monophosphate shunt pathway
HNO <sub>3</sub>	nitric acid
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
HOME	Home Observation for Measurement of Environment
HOS TE	human osteosarcoma cells

HPLC	high-pressure liquid chromatography
H <sub>3</sub> PO <sub>4</sub>	phosphoric acid
HPRT	hypoxanthine phosphoribosyltransferase (gene)
HR	heart rate
HSI	habitat suitability indices
H <sub>2</sub> SO <sub>4</sub>	sulfuric acid
HSPG	heparan sulfate proteoglycan
Ht	hematocrit
HTC	hepatoma cells
hTERT	catalytic subunit of human telomerase
HTN	hypertension
IBL	integrated blood lead index
IBL H WRAT-R	integrated blood lead index H Wide Range Achievement Test-Revised (interaction)
ICD	International Classification of Diseases
ICP	inductively coupled plasma
ICP-AES	inductively coupled plasma atomic emission spectroscopy
ICP-MS, ICPMS	inductively coupled plasma mass spectrometry
ID-MS	isotope dilution mass spectrometry
IFN	interferon (e.g., IFN-())
Ig	immunoglobulin (e.g., IgA, IgE, IgG, IgM)
IGF-1	insulin-like growth factor 1
IL	interleukin (e.g., IL-1, IL-1 $\beta$ , IL-4, IL-6, IL-12)
ILL	incipient lethal level
immuno	immunohistochemical staining
IMP	inosine monophosphate
iNOS	inducible nitric oxide synthase
i.p., IP	intraperitoneal
IPSC	inhibitory postsynaptic currents
IQ	intelligence quotient
IRT	interresponse time
ISEL	in situ end labeling
ISI	interstimulus interval
i.v., IV	intravenous
IVCD	intraventricular conduction deficit

JV	juvenile
KABC	Kaufman Assessment Battery for Children
KTEA	Kaufman Test of Educational Achievement
KXRF, K-XRF	K-shell X-ray fluorescence
LA	lipoic acid
LB	laying bird
LC	lactation
LC <sub>50</sub>	lethal concentration at which 50% of exposed animals die
LC <sub>74</sub>	lethal concentration at which 74% of exposed animals die
LD <sub>50</sub>	lethal dose at which 50% of exposed animals die
LDH	lactate dehydrogenase
LDL	low-density lipoprotein (cholesterol)
L-dopa	3,4-dihydroxyphenylalanine (precursor of dopamine)
LE	Long Evans (rat)
LET	linear energy transfer (radiation)
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
LN	lead nitrate
L-NAME	L-N <sup>G</sup> -nitroarginine methyl ester
LOAEL	lowest-observed adverse effect level
LOEC	lowest-observed-effect concentration
LOWESS	locally weighted scatter plot smoother
LPO	lipoperoxide
LPP	lipid peroxidation potential
LPS	lipopolysaccharide
LT	leukotriene
LT <sub>50</sub>	time to kill 50%
LTER	Long-Term Ecological Research (sites)
LTP	long term potentiation
LVH	left ventricular hypertrophy
μPIXE	microfocused particle induced X-ray emission
μSXRF	microfocused synchrotron-based X-ray fluorescence
MA	mature
MA-10	mouse Leydig tumor cell line
MANCOVA	multivariate analysis of covariance

MAO	monoamine oxidase
MATC	maximum acceptable threshold concentration
MDA	malondialdehyde
MDA-TBA	malondialdehyde-thiobarbituric acid
MDCK	kidney epithelial cell line
MDI	Mental Development Index (score)
MDRD	Modification of Diet in Renal Disease (study)
MEM	Minimal Essential Medium
MG	microglobulin
Mg-ATPase	magnesium-dependent adenosine triphosphatase
MiADMSA	monoisoamyl dimercaptosuccinic acid
Mi-DMSA	mi monoisoamyl dimercaptosuccinic acid
MK-801	NMDA receptor antagonist
MLR	mixed lymphocyte response
MMSE	Mini-Mental State Examination
MMTV	murine mammary tumor virus
MN	micronuclei formation
MND	motor neuron disease
MNNG	<i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine
MPH	morphology
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MROD	methoxyresorufin- <i>O</i> -demethylase
MRS	magnetic resonance spectroscopy
MS	mass spectrometry
MSCA	McCarthy Scales of Children's Abilities
mSQGQs	mean sediment quality guideline quotients
MT	metallothionein
MVV	maximum voluntary ventilation
MW	molecular weight (e.g., high-MW, low-MW)
N, n	number of observations
N/A	not available
NAAQS	National Ambient Air Quality Standards
NAC	<i>N</i> -acetyl cysteine
NAD	nicotinamide adenine dinucleotide

NADH	reduced nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NAD(P)H, NADPH	reduced nicotinamide adenine dinucleotide phosphate
NADS	nicotinamide adenine dinucleotide synthase
NAF	nafenopin
NAG	<i>N</i> -acetyl- $\beta$ -D-glucosaminidase
Na-K-ATPase	sodium-potassium-dependent adenosine triphosphatase
NAWQA	National Water-Quality Assessment
NBT	nitro blue tetrazolium
NCBP	National Contaminant Biomonitoring Program
NCD	nuclear chromatin decondensation (rate)
NCS	newborn calf serum
NCTB	Neurobehavioral Core Test Battery
NCV	nerve conduction velocity
ND	non-detectable; not detected
NDI	nuclear division index
NE	norepinephrine
NES	Neurobehavioral Evaluation System
NF- $\kappa$ B	nuclear transcription factor- $\kappa$ B
NGF	nerve growth factor
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute for Standards and Technology
NK	natural killer
NMDA	<i>N</i> -methyl-D-aspartate
NMDAR	<i>N</i> -methyl-D-aspartate receptor
NMR	nuclear magnetic resonance
NO	nitric oxide
NO <sub>2</sub>	nitrogen dioxide
NO <sub>3</sub>	nitrate
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOM	natural organic matter

NORs	nucleolar organizing regions
NOS	nitric oxide synthase; not otherwise specified
NO <sub>x</sub>	nitrogen oxides
NP	net productivity
NPSH	nonprotein sulfhydryl
NR	not reported
NRC	National Research Council
NRK	normal rat kidney
NS	nonsignificant
NSAID	non-steroidal anti-inflammatory agent
NT	neurotrophin
NTA	nitriлотriacetic acid
O <sub>2</sub>	oxygen
ODVP	offspring development
OH	hydroxyl
7-OH-coumarin	7-hydroxy-coumarin
1,25-OH-D, 1,25-OH D <sub>3</sub>	1,25-dihydroxyvitamin D
24,25-OH-D <sub>3</sub>	24,25-dihydroxyvitamin D
25-OH-D <sub>3</sub>	25-hydroxyvitamin D
8-OHdG	8-hydroxy-2'-deoxyguanosine
O horizon	forest floor
OR	odds ratio; other oral
OSWER	Office of Solid Waste and Emergency Response
P, p	probability value
P300	event-related potential
P450 1A1	cytochrome P450 1A1
P450 1A2	cytochrome P450 1A2
P450 CYP3a11	cytochrome P450 3a11
PAD	peripheral arterial disease
PAH	polycyclic aromatic hydrocarbon
PAI-1	plasminogen activator inhibitor-1
PAR	population attributable risk
Pb	lead
<sup>203</sup> Pb	lead-203 radionuclide
<sup>204</sup> Pb, <sup>206</sup> Pb, <sup>207</sup> Pb, <sup>208</sup> Pb	stable isotopes of lead-204, -206, -207, -208, respectively

$^{210}\text{Pb}$	lead-210 radionuclide
$\text{Pb}(\text{Ac})_2$	lead acetate
PbB	blood lead concentration
$\text{PbCl}_2$	lead chloride
$\text{Pb}(\text{ClO}_4)_2$	lead chlorate
PBG-S	porphobilinogen synthase
PBMC	peripheral blood mononuclear cells
$\text{Pb}(\text{NO}_3)_2$	lead nitrate
PbO	lead oxides (or litharge)
PBP	progressive bulbar paresis
PbS	galena
PbU	urinary lead
PC12	pheochromocytoma cell
PCR	polymerase chain reaction
PCV	packed cell volume
PDE	phosphodiesterase
PDGF	platelet-derived growth factor
PDI	Psychomotor Development Index
PEC	probable effect concentration
PEF	expiratory peak flow
PG	prostaglandin (e.g., $\text{PGE}_2$ , $\text{PGF}_2$ ); prostate gland
PHA	phytohemagglutinin A
Pi	inorganic phosphate
PIXE	particle induced X-ray emission
PKC	protein kinase C
pl NEpi	plasma norepinephrine
PMA	progressive muscular atrophy
PMN	polymorphonuclear leucocyte
PMR	proportionate mortality ratio
PN	postnatal (day)
P5N	pyrimidine 5'-nucleotidase
PND	postnatal day
p.o., PO	per os (oral administration)
POMS	Profile of Mood States
ppb	parts per billion

ppm	parts per million
PPVT-R	Peabody Picture Vocabulary Test-Revised
PRA	plasma renin activity
PRL	prolactin
PROG	progeny counts or numbers
PRR	prevalence rate ratio
PRWT	progeny weight
PST	percent transferrin saturation
PTH	parathyroid hormone
PTHrP	parathyroid hormone-related protein
PVC	polyvinyl chloride
PWM	pokeweed mitogen
PRWT	progeny weight
QA/QC	quality assurance/quality control
Q/V	flux of air (Q) divided by volume of culture (V)
r	Pearson correlation coefficient
R <sup>2</sup>	multiple correlation coefficient
r <sup>2</sup>	correlation coefficient
<sup>226</sup> Ra	most stable isotope of radium
R/ALAD	ratio of ALAD activity before and after reactivation
RAVLT	Rey Auditory Verbal Learning Test
<sup>86</sup> Rb	rubidium-86 radionuclide
RBA	relative bioavailability
RBC	red blood cell; erythrocyte
RBF	renal blood flow
RBP	retinol binding protein
RBPH	reproductive behavior
RCPM	Ravens Colored Progressive Matrices
REL	rat epithelial (cells)
REP	reproduction
RHIS	reproductive organ histology
<sup>222</sup> Rn	most stable isotope of radon
RNA	ribonucleic acid
ROS	reactive oxygen species
ROS 17.2.8	rat osteosarcoma cell line

RPMI 1640	Roswell Park Memorial Institute basic cell culture medium
RR	relative risk; rate ratio
RT	reaction time
RSEM	resorbed embryos
RSUC	reproductive success (general)
RT	reproductive tissue
ΣSEM	sum of the molar concentrations of simultaneously extracted metal
SA7	simian adenovirus
SAB	Science Advisory Board
SAM	<i>S</i> -adenosyl-L-methionine
SBIS-4	Stanford-Binet Intelligence Scale-4th edition
s.c., SC	subcutaneous
SCAN	Test for Auditory Processing Disorders
SCE	selective chemical extraction; sister chromatid exchange
SCP	stripping chronopotentiometry
SD	Sprague-Dawley (rat); standard deviation
SDH	succinic acid dehydrogenase
SDS	sodium dodecyl sulfate; Symbol Digit Substitution
SE	standard error; standard estimation
SEM	standard error of the mean
SES	socioeconomic status
sGC	soluble guanylate cyclase
SH	sulfhydryl
SHBG	sex hormone binding globulin
SHE	Syrian hamster embryo cell line
SIMS	secondary ion mass spectrometry
SIR	standardized incidence ratio
SLP	synthetic leaching procedure
SM	sexually mature
SMAV	species mean acute value
SMR	standardized mortality ratio
SNAP	Schneider Neonatal Assessment for Primates
SNP	sodium nitroprusside
SO <sub>2</sub>	sulfur dioxide
SOD	superoxide dismutase

SOPR	sperm-oocyte penetration rate
SPCL	sperm cell counts
SPCV	sperm cell viability
SQGs	sediment quality guidelines
SRA	Self Reported Antisocial Behavior scale
SRD	Self Report of Delinquent Behavior
SRIF	somatostatin
SRM	Standard Reference Material
SRT	simple reaction time
SSADMf	Social Security Administration Death Master File
SSB	single-strand breaks
SSEP	somatosensory-evoked potential
StAR	steroidogenic acute regulatory protein
STORET	STORage and RETrieval
SVC	sensory conduction velocity
SVRT	simple visual reaction time
T	testosterone
TA	tail
TABL	time-averaged blood lead
T&E	threatened and endangered (species)
TAT	tyrosine aminotransferase
TB	tibia
TBARS	thiobarbituric acid-reactive species
TBPS	Total Behavior Problem Score
TCDD	methionine-choline-deficient diet
T cell	T lymphocyte
TCLP	toxic characteristic leaching procedure
TE	testes
TEC	threshold effect concentration
TEDG	testes degeneration
TEL	tetraethyl lead
TES	testosterone
TEWT	testes weight
TF	transferrin, translocation factor
TG	6-thioguanine

TGF	transforming growth factor
TH	tyrosine hydroxylase
<sup>232</sup> Th	stable isotope of thorium-232
TLC	Treatment of Lead-exposed Children (study)
TNF	tumor necrosis factor (e.g., TNF- $\alpha$ )
TOF	time-of-flight
tPA	plasminogen activator
TPRD	total production
TRH	thyroid releasing hormone
TRV	toxicity reference value
TSH	thyroid stimulating hormone
TSP	triple-super phosphate
TT3	total triiodothyronine
TT4	serum total thyroxine
TTES	total testosterone
TTR	transthyretin
TU	toxic unit
TWA	time-weighted average
TX	tromboxane (e.g., TXB <sub>2</sub> )
U	urinary
<sup>235</sup> U, <sup>238</sup> U	uranium-234 and -238 radionuclides
UCP	urinary coproporphyrin
UDP	uridine diphosphate
UNECE	United Nations Economic Commission for Europe
Ur	urinary
USFWS	U.S. Fish and Wildlife Service
USGS	United States Geological Survey
UV	ultraviolet
V79	Chinese hamster lung cell line
VA	Veterans Administration
VC	vital capacity; vitamin C
VDR	vitamin D receptor
VE	vitamin E
VEP	visual-evoked potential
VI	variable-interval

vit C	vitamin C
vit E	vitamin E
VMA	vanilmandelic acid
VMI	Visual-Motor Integration
VSM	vascular smooth muscle (cells)
VSMC	vascular smooth muscle cells
WAIS	Wechsler Adult Intelligence Scale
WDS	wavelength dispersive spectrometers
WHO	World Health Organization
WISC	Wechsler Intelligence Scale for Children
WISC-R	Wechsler Intelligence Scale for Children-Revised
WO	whole organism
WRAT-R	Wide Range Achievement Test-Revised
WT	wild type
WTHBF-6	human liver cell line
ww	wet weight
XAFS	X-ray absorption fine structure
XANES	X-ray absorption near edge spectroscopy
XAS	X-ray absorption spectroscopy
XPS	X-ray photoelectron spectroscopy
X-rays	synchrotron radiation
XRD	X-ray diffraction
XRF	X-ray fluorescence
ZAF	correction in reference to three components of matrix effects: atomic number (Z), absorption (A), and fluorescence (F)
ZnNa <sub>2</sub> DTPA	zinc disodium diethylenetriaminepentaacetic acid
ZnNa <sub>2</sub> EDTA	zinc disodium ethylenediaminetetraacetic acid
ZPP	zinc protoporphyrin

## **AX7. CHAPTER 7 ANNEX - ENVIRONMENTAL EFFECTS OF LEAD**

### **AX7.1 TERRESTRIAL ECOSYSTEMS**

#### **AX7.1.1 Methodologies Used in Terrestrial Ecosystems Research**

The distribution of Pb throughout the terrestrial ecosystem, via aerial deposition, has been discussed throughout this document. Its further impacts on soil, sediment, and water provide numerous pathways that may promote unacceptable risk to all levels of biota. Stable isotopes of Pb have been found useful in identifying sources and apportionment to various sources. One of the key factors affecting assessment of risk is an understanding, and perhaps quantification, of bioavailability. Therefore, the bioavailability of Pb is a key issue to the development of NAAQS. However, the discussion of all methods used in characterizing bioavailability is beyond the scope of this chapter. The following topics are discussed in this chapter.

- Lead Isotopes and Apportionment
- Methodologies to determine Pb speciation
- Lead and the Biotic Ligand Model (BLM)
- In situ methods to reduce Pb bioavailability

##### **AX7.1.1.1 Lead Isotopes and Apportionment**

Determination of the extent of Pb contamination from an individual source(s) and its impact are of primary importance in risk assessment. The identification of exposure pathway(s) is fundamental to the risk analysis and critical in the planning of remediation scenarios.

Although societies have been consuming Pb for nearly 9,000 years, production of Pb in the United States peaked in 1910 and 1972, at approximately 750 and 620 kt/year, respectively (Rabinowitz, 2005). The diversity of potential Pb sources (fossil fuel burning, paint pigments, gasoline additives, solders, ceramics, batteries) and associated production facilities (mining, milling, smelting-refining) make fingerprinting of sources difficult. (See Chapter 2 and its Annex for additional information on sources.) Therefore, dealing with multiple sources (point and nonpoint), a reliable and specific fingerprinting technique is required. It has been well established (Sturges and Barrie, 1987; Rabinowitz, 1995) that the stable isotope composition of

Pb is ideally suited for this task. Lead isotopic ratio differences often allow multiple sources to be distinguished, with an apportionment of the bulk Pb concentration made to those sources.

Lead has four stable isotopes:  $^{204}\text{Pb}$ ,  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ , and  $^{208}\text{Pb}$  in natural abundances of 1.4, 24.1, 22.1, and 52.4%, respectively. The radiogenic  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ , and  $^{208}\text{Pb}$  are produced by radioactive decay of  $^{238}\text{U}$ ,  $^{235}\text{U}$ , and  $^{232}\text{Th}$ , respectively. Thus, the isotopic composition of Pb varies based on the U:Pb and Th:Pb ratios of the original ore's source and age (Faure, 1977). Because of the small fractional mass differences of the Pb isotopes, ordinary chemical and pyrometallurgical reactions will not alter their original composition. Therefore, anthropogenic sources reflect the isotopic composition of the ores from which the Pb originated.

To acquire the Pb isotopes, a sample, generally in aqueous form, is analyzed on an ICP/MS (quadrapole, magnetic sector, or time-of-flight). Studies reviewing the most common analytical and sample preparation procedures include Ghazi and Millette (2004), Townsend et al. (1998), and Encinar et al. (2001a,b). The correction factor for mass discrimination biases is generally made by analyzing the National Institute for Standards and Technology (NIST), Standard Reference Material (SRM) 981 and/or spiked  $^{203}\text{Tl}$  and  $^{205}\text{Tl}$  isotopes (Ketterer et al., 1991; Begley and Sharp, 1997). The overall success of Pb isotope fingerprinting is generally dependent on analysis precision, which in turn depends on the type of mass analyzer used (Table AX7-1.1.1).

**Table AX7-1.1.1. Relative Standard Deviation (RSD) for Lead Isotope Ratios on Selected Mass Spectrometers**

<b>RSD</b>	<b>Quadrapole</b>	<b>Double-Focusing</b>	<b>Single-Focusing Magnetic Sector</b>	<b>High-Resolution Magnetic Sector ICP/MS</b>
$^{204}\text{Pb}:$ $^{206}\text{Pb}$	0.0031	0.0032	0.00053	0.0011
$^{207}\text{Pb}:$ $^{206}\text{Pb}$	0.0032	0.0027	0.00053	0.00048
$^{208}\text{Pb}:$ $^{206}\text{Pb}$	0.0026	0.0024	0.00053	0.00046

An extensive database comprising primarily North American Pb sources can be assembled from Doe and Rohrbough (1977), Doe and Stacey (1974), Doe et al. (1968), Heyl et al. (1974),

Leach et al. (1998), Stacey et al. (1968), Zartman (1974), Cannon and Pierce (1963), Graney et al. (1996), Unruh et al. (2000), James and Henry (1993), Rabinowitz (2005), and Small (1973).

The use of Pb isotopes to quantitatively apportion source contributions follows the simple mixing rule when only two sources are possible (Faure, 1977). Once multiple sources need to be considered, a unique solution can no longer be calculated (Fry and Sherr, 1984). Phillips and Gregg (2003) have designed a model to give feasible source contributions when multiple sources are likely. Many studies have demonstrated the usefulness of this apportionment technique. Media of all types have been studied: water (Flegal et al., 1989a,b; Erel et al., 1991; Monna et al., 1995), ice (Planchon et al., 2002), dust (Adgate et al., 1998; Sturges et al., 1993), and soil/sediments (Hamelin et al., 1990; Farmer et al., 1996; Bindler et al., 1999; Haack et al., 2004; Rabinowitz and Wetherill, 1972; Rabinowitz, 2005; Ketterer et al., 2001).

#### **AX7.1.1.2 Speciation in Assessing Lead Bioavailability in the Terrestrial Environment**

One of the three processes defined by the National Research Council in its review on bioavailability (NRC, 2002) is “contaminant interactions between phases”, more commonly referred to as “speciation.”

A wide variety of analytical (XRD, EPMA, EXAFS, PIXE, XPS, XAS, SIMS) and chemical speciation modeling (SOILCHEM, MINTEQL, REDEQL2, ECOSAT, MINTEQA2, HYDRAQL, PHREEQE, WATEQ4F) tools have been used to characterize a metal’s speciation as it is found in various media. Currently, for risk assessment purposes (not considering phytotoxicity), where large sites with numerous media, pathways, and metals must often be characterized in a reasonable time frame, electron microprobe analysis (EMPA) techniques provide the greatest information on metal speciation. Other techniques such as extended X-ray absorption fine structure (EXAFS) and extended X-ray absorption near edge spectroscopy (EXANES) show great promise and will be important in solving key mechanistic questions. In the case of phytotoxicity, the speciation of metals by direct measurement or chemical models of pore-water chemistry is most valuable. Further work needs to be done in developing analytical tools for the speciation of the methyl-forming metals (Hg, As, Sb, Se, and Sn) in soils and sediments.

### ***Concept***

For a given metal or metalloid (hereafter referred to as metal), the term speciation refers to its chemical form or species, including its physicochemical characteristics that are relevant to bioavailability. As a result of the direct impact these factors often have on a metal's bioavailability, the term "bioaccessibility" has been adopted to define those factors.

### ***Speciation Role***

The accumulation of metals in the lithosphere is of great concern. Unlike organic compounds, metals do not degrade and, thus, have a greater tendency to bioaccumulate. It is now well accepted that knowledge of the bulk, toxic characteristic leaching procedure (TCLP), or synthetic leaching procedure (SLP) concentrations for any metal is not a controlling factor in understanding a metal's environmental behavior or in developing remedies for its safe management. Although these tests are essential to site characterization and management, they offer no insight into risk assessment. Rather, it is the metal's bioavailability (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]), which plays a significant role in the risk assessment of contaminated media.

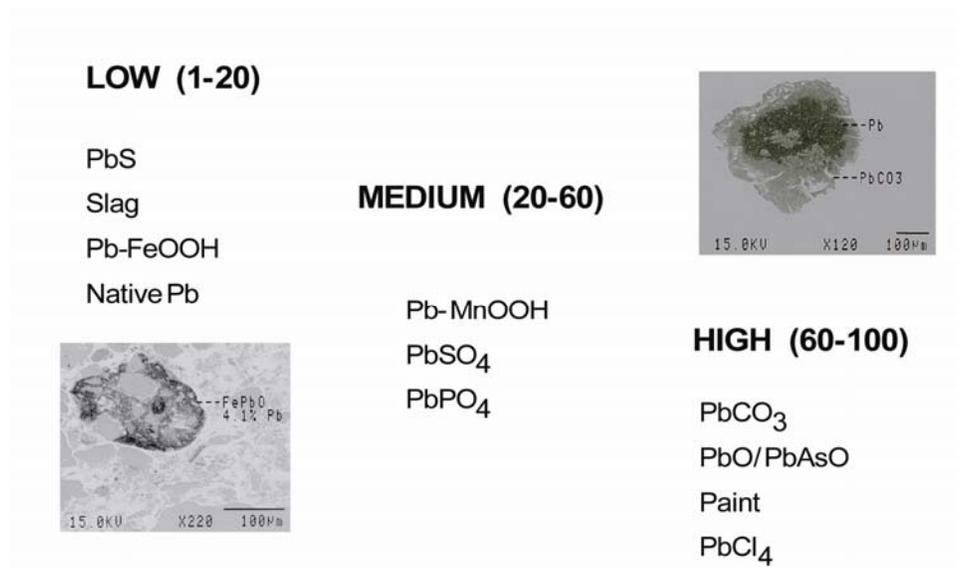
The NRC review (NRC, 2002) on bioavailability defined bioavailability processes in terms of three key processes:

- contaminant interactions between phases (association-dissociation/bound-released),
- transport of contaminants to organism, and
- passage across a physiological membrane.

As mentioned previously, the first process is more commonly referred to as speciation. The speciation of a toxic metal in the environment is a critical component of any ecosystem health risk assessment. Four important toxicologic and toxicokinetic determinants relating speciation to bioavailability are the (1) chemical form or species, (2) particle size of the metal form, (3) lability of the chemical form, and (4) source.

### ***Chemical Form of Species***

The solid phase in a medium controls the activity of a metal in solution, whether the solution is surface, ground, or pore water or GI fluids, and plays a profound role in metal bioavailability. This is perhaps best illustrated by in vivo and in vitro results for many of the common Pb-bearing minerals (Drexler, 1997) (Figure AX7-1.1.1). The metal species found in media are often diverse, and data suggest that their bioavailability may be significantly influenced by site-specific variations within these identified metal species (Davis et al., 1993; Ruby et al., 1992; Drexler and Mushak, 1995).



**Figure AX7-1.1.1. Relationship of bioaccessibility (low, medium, high) versus speciation as shown with scanning electron micrographs of various Pb-bearing materials.**

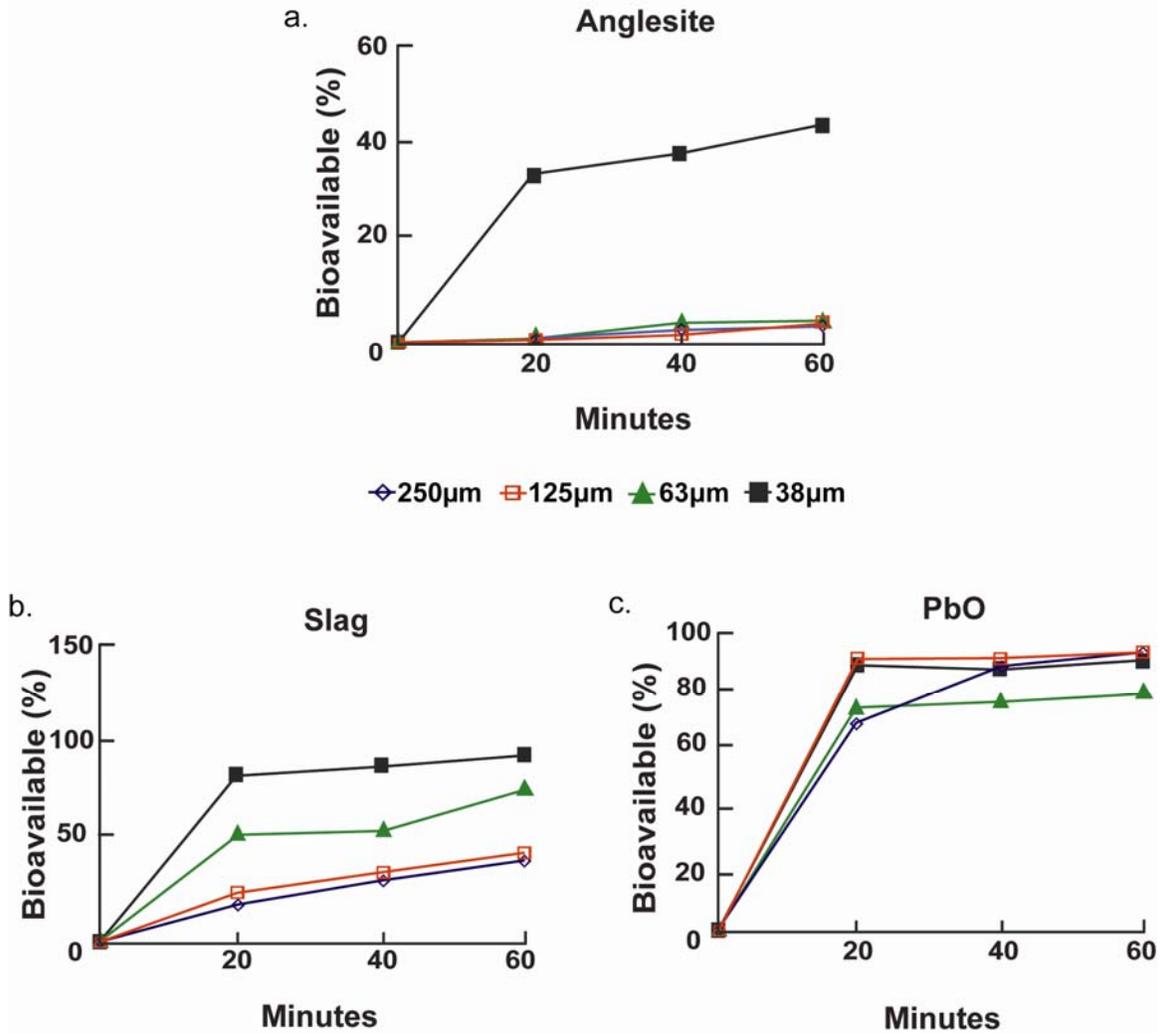
### ***Particle Size of Metal Species***

Particle size of a metal form is an important factor in the mobilization of the metal, primarily because as size decreases, the surface area of the particle increases, thereby increasing solubility. Thus, although solubility is not the only control for bioavailability, an increase in bioavailability has been directly attributed to a decrease in particle size: Barltrop and Meek

(1979) observed that “the smaller the lead particle, the higher blood lead level.” Similar observations were made by Healy et al. (1992) using an in vitro dissolution technique. Drexler (1997) presented in vitro results on numerous Pb-bearing phases ranging in particle size from 35 to 250  $\mu\text{m}$ . While all phases studied showed increased bioavailability with decreasing particle size, more significantly, not all forms showed the same degree or magnitude of change (Figure AX7-1.1.2). Atmospheric particles are generally found to occur in bimodal populations: fine; 0.1 to 2.5  $\mu\text{m}$  and coarse; 2.5 to 15  $\mu\text{m}$ . This distribution is both a function of the transport mechanism and emission source. Although the upper size limit for particles that can be suspended in air is about 75  $\mu\text{m}$  (Cowherd et al., 1974), other means of mechanical entrainment (saltation, and creep) can transport particles as large as 1000  $\mu\text{m}$ , supporting the importance of fugitive emissions on media contamination. In addition, particle size can change post depositional, as soluble forms re-precipitate or sorb onto other surfaces. Limited data are available on the particle-size of discrete Pb phases from multimedia environments. One example is the study by Drexler, 2004 at Herculaneum, Missouri. At this site, galena (PbS) was the dominant Pb species with mean particle-size distributions of 4, 6, and 14  $\mu\text{m}$  in PM<sub>10</sub> filters, house dust, and soils, respectively. These findings support the conclusion that aerial transport was the primary mechanism for Pb deposition in residential yards. Finally, such laboratory data have been supported by extensive epidemiologic evidence, enforcing the importance of particle size (Bornschein et al., 1987; Brunekreef et al., 1983; Angle et al., 1984).

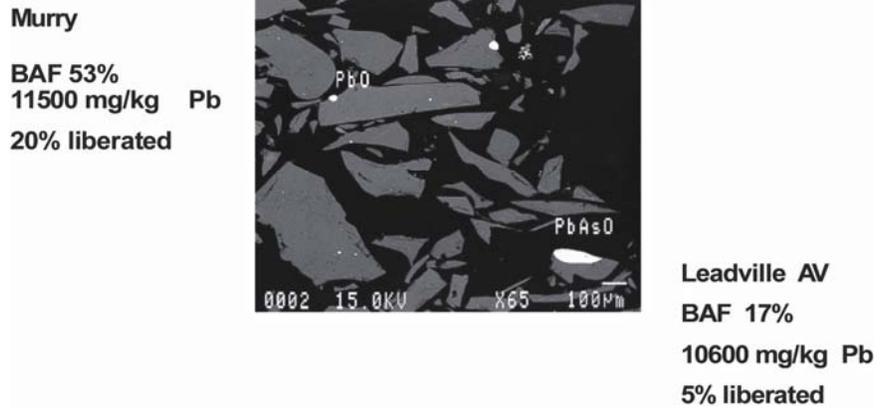
### ***Particle Lability***

The impact on bioavailability of a metal particle's lability (its associations within the medium matrix) is not well documented, but it follows the premise put forth by many of the developing treatment technologies regarding its being bound or isolated from its environment. Data from several EPA Superfund sites and the Region VIII swine study (U.S. Environmental Protection Agency, 2004a) suggest that matrix associations, such as liberated versus enclosed, can play an important part in bioavailability. As illustrated in Figure AX7-1.1.3, two different media with similar total Pb concentrations and Pb forms (slag, Pb-oxide, and Pb-arsenate) exhibit significantly different bioavailabilities. In the Murray, UT sample (bioaccumulation factor [BAF] = 53%), a greater fraction of the more bioavailable Pb-oxides are liberated and not enclosed in the less-soluble glass-like slag as observed in the Leadville, CO sample

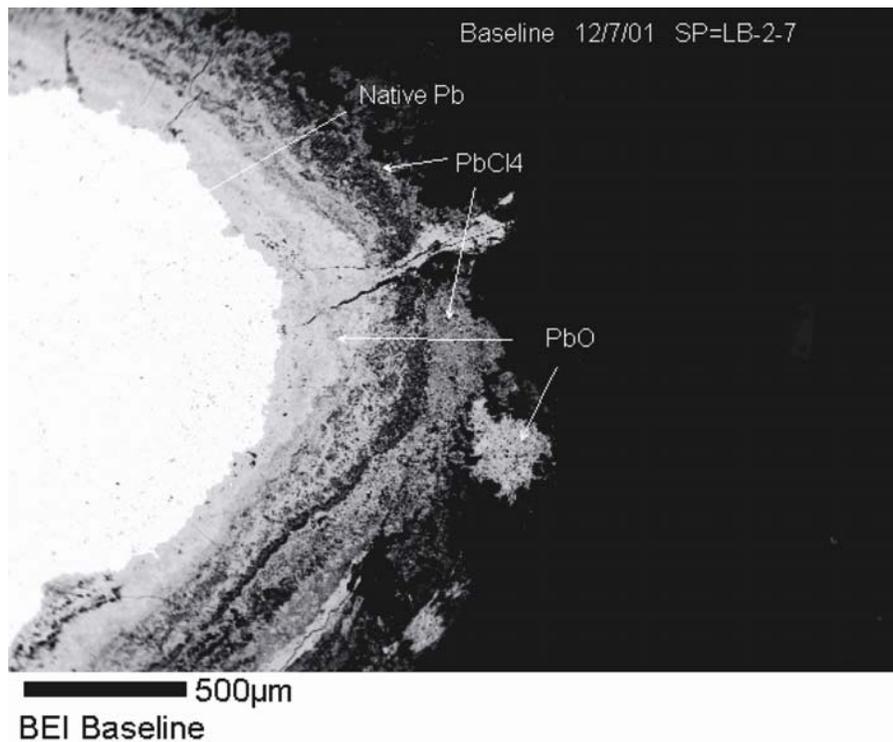


**Figure AX7-1.1.2. Variation of bioavailability with particle size.**

(BAF = 17%). Other evidence is more empirical, as illustrated in Figure AX7-1.1.4, where a large particle of native Pb is shown to have developed a weathering ring of highly bioavailable Pb-chloride and Pb-oxide. Such observations can be useful in understanding the mechanistic phenomena controlling bioavailability. In addition, they will aid in developing and validating models to predict metal-environment interactions.



**Figure AX7-1.1.3. Illustration of particle lability and bioavailability at two different sites with similar total Pb concentrations and Pb forms.**



**Figure AX7-1.1.4. Scanning electron micrograph of a large native Pb particle showing outer ring of highly bioavailable Pb-chloride and Pb-oxide.**

### ***Source***

Although the source of a metal is not directly related to bioavailability, it plays an important role in risk assessment with the evaluation of metal (1) pathways, (2) background, and (3) apportionment. It is important to understand a metal's pathway before any remedial action can be taken; otherwise, recontamination of the primary pathway and reexposure can occur. Knowledge of background is important, as an action level cannot be established below natural background levels.

### ***Plants***

When considering the bioavailability of a metal to plants from soils and sediments, it is generally assumed that both the kinetic rate of supply and the speciation of the metal to either the root or shoot are highly important. In soils and sediments generally, only a small volume of water is in contact with the chemical form, and although the proportion of a metal's concentration in this pore water to the bulk soil/sediment concentration is small, it is this phase that is directly available to plants. Therefore, pore water chemistry (i.e., metal concentration as simple inorganic species, organic complexes, or colloid complexes) is most important.

Tools currently used for metal speciation for plants include (1) in-situ measurements using selective electrodes (Gundersen et al., 1992; Archer et al., 1989; Wehrli et al., 1994); (2) in-situ collection techniques using diffusive equilibrium thin films (DET) and diffusive gradient thin films (DGT) followed by laboratory analyses (Davison et al., 1991, 1994; Davison and Zhang, 1994; Zhang et al., 1995); and (3) equilibrium models (SOILCHEM) (Sposito and Coves, 1988).

### **AX7.1.1.3 Tools for Bulk Lead Quantification and Speciation**

#### ***Bulk Quantification***

The major analytical methods most commonly used for bulk analyses outlined in the 1986 Lead ACQD included:

- Atomic Absorption Spectrometry (AAS)
- Emission Spectrometry (Inductively coupled plasma/atomic emission spectrometry)
- X-ray Fluorescence (XRF)
- Isotope Dilution Mass Spectrometry (ID/MS)

- Colorimetric
- Electrochemical (anodic stripping voltametry and differential pulse polarography).

The choice of analytical method today for bulk quantification is generally ICP/AES or ICP/MS (U.S. Environmental Protection Agency, 2001). Since 1986, numerous SRMs have been developed for Pb (Table AX7-1.1.2), and several significant technological improvements have been developed.

**Table AX7-1.1.2. National Institute of Standards and Technology Lead SRMs**

<b>NIST SRM</b>	<b>Medium</b>	<b>Mean Pb mg/kg</b>
2710	Soil	5532
2711	Soil	1162
2709	Soil	18.9
2587	Soil (paint)	3242
2586	Soil (paint)	432
2783	Filter (PM <sub>2.5</sub> )	317
1648	Urban particulate	6550
1649a	Urban dust	12,400
2584	Indoor dust	9761
2583	Indoor dust	85.9
1515	Apple leaves	0.47
1575	Pine needles	0.167

Modern spectrometry systems have replaced photomultiplier tubes with a charge-coupled device (CCD). The CCD is a camera that can detect the entire light spectrum (>70,000 lines) from 160 to 785 nm. This allows for the simultaneous measurement of all elements, as well as any interfering lines (a productivity increase), and increases precision. The detection limit for Pb in clean samples can now be as low as 40 ppb.

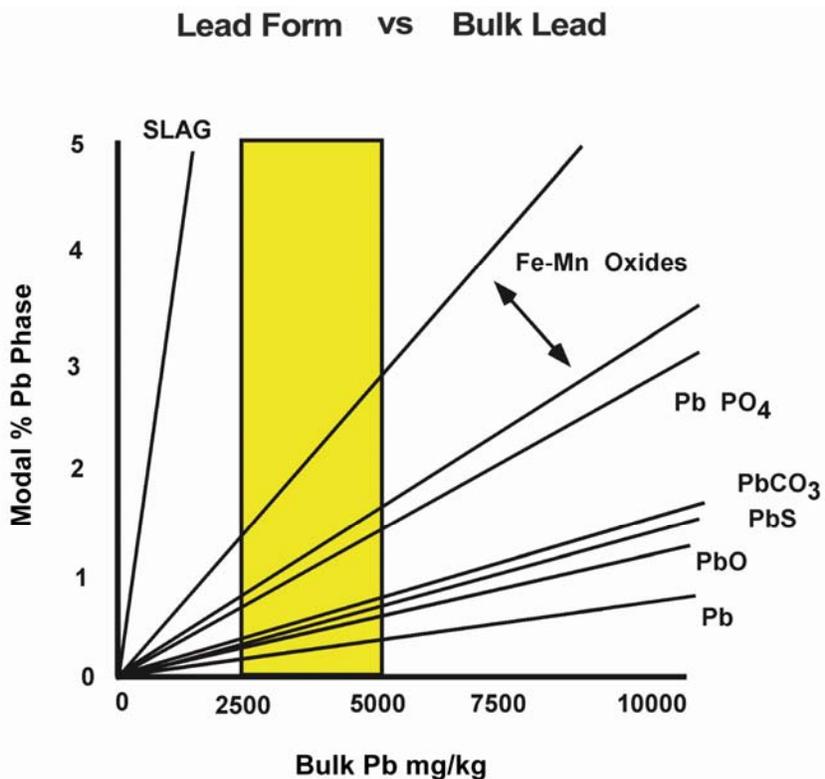
Modern ICP/AES systems offer a choice of either axial viewed plasma (horizontal), which provides greater sensitivity (DL= 0.8 µg Pb/L), or radial (vertical) viewed plasma, which performs best with high total dissolved samples (DL = 5.0 µg Pb/L).

The development of reaction or collision cells have expanded the capabilities of ICP/MS and lowered detection limits for many elements that were difficult to analyze because of interferences such as Se, As, Ti, Zn, Ca, Fe, and Cr. The cells provide efficient interference (isobaric, polyatomic, and argide) removal independent of the analyte and sample matrix by using various reaction gases (H<sub>2</sub>, He, NH<sub>3</sub>), eliminating the need for interference correction equations.

### ***Speciation Tools***

A wide variety of analytical and chemical techniques have been used to characterize a metal's speciation (as defined above) in various media (Hunt et al., 1992; Manceau et al., 1996, 2000a; Welter et al., 1999; Szulczewski et al., 1997; Isaure et. al., 2002; Lumsdon and Evans, 1995; Gupta and Chen, 1975; Ma and Uren, 1995; Charlatchka et al., 1997). Perhaps the most important factor that one must keep in mind in selecting a technique is that, when dealing with metal-contaminated media, one is most often looking for the proverbial “needle in a haystack.” Therefore, the speciation technique must not only provide the information outlined above, but it must also determine that information from a medium that contains very little of the metal. As illustrated in Figure AX7-1.1.5, for a Pb-contaminated soil, less than 1% (modally) of a single species can be responsible for a bulk metal's concentration above an action level. This factor is even more significant for other metals (i.e., As, Cd, or Hg) where action levels are often below 100 mg/kg.

Of the techniques tested (physicochemical, extractive, and theoretical), the tools that have been used most often to evaluate speciation for particle-bound metal include X-ray absorption spectroscopy (XAS), X-ray diffraction (XRD), particle induced X-ray emission (PIXE and µPIXE), electron probe microanalysis (EPMA), secondary ion mass spectrometry (SIMS), X-ray photoelectron spectroscopy (XPS), sequential extractions, and single chemical extractions. The tools that have been used most often to evaluate speciation for metal particles in solution include the following computer-based models: MINTEQL, REDEQL2, ECOSAT, MINTEQA2, HYDRAQL, PHREEQE, and WATEQ4F. These tools are briefly described below.



**Figure AX7-1.1.5. Bulk lead versus single species modality. Shaded area represents typical soil-lead concentrations found at remediation sites in the western United States.**

*Particle-Bound Metal*

Direct Approaches

Over the past decade, numerous advances in materials science have led to the development of a wide range in analytical tools for the determination of metal concentration, bonding, and valance of individual particles on a scale that can be considered useful for the speciation of environmentally important materials (i.e., soils, wastes, sediments, and dust). This review will provide the reader with a brief description of these techniques, including their benefits, limitations (cost, availability, sample preparation, resolution), and usability as well as references to current applications. Although most of these tools are scientifically sound and offer important information on the mechanistic understanding of metal occurrence and behavior, only a few currently provide useful information on metal bioavailability at a “site” level. However, one may still find other techniques essential to a detailed characterization of a selected

material to describe the chemical or kinetic factors controlling a metal's release, transport, and/or exposure.

*X-Ray absorption Spectroscopy (XAS).* X-ray absorption spectroscopy (XAS) is a powerful technique using the tunable, monochromatic (white light) X-rays produced by a synchrotron (2-4 GeV) to record oscillations in atomic absorption within a few 100 GeV of an element's absorption edge. Spectra provide information on both chemical state and atomic structure. Measurements are theoretically available for all elements and are not surface-sensitive nor sample-sensitive (i.e., gases, liquids, solids, and amorphous materials are testable).

High-energy spectra within 30 eV of the edge, termed XANES (X-ray absorption near edge structure spectroscopy (Fendorf et al., 1994; Maginn, 1998), are particularly suited for determination and quantification (10 to 100 ppm) of metal in a particular oxidation state (Szulczewski et al., 1997; Shaffer et al., 2001; Dupont et al., 2002). The lower-energy spectra persist some 100 eV above the edge. These oscillations are termed EXAFS (extended X-ray absorption fine structure) and are more commonly used for speciation analyses (Welter et al., 1999; Manceau et al., 1996, 2000a; Isaure et al., 2002).

The main limitations to XAS techniques are (1) the lack of spatial resolution; (2) XAS techniques provide only a weighted average signal of structural configurations, providing information on the predominant form of the metal, while minor species, which may be more bioavailable, can be overlooked; (3) access to synchrotrons is limited and the beam time required to conduct a site investigation would be prohibitive; (4) a large spectral library must be developed; (5) generally, poor fits to solution models are achieved when the compound list is large; and (6) high atomic number elements have masking problems based on compound density.

*X-Ray Diffraction (XRD).* In X-ray diffraction, a monochromatic Fe, Mo, Cr, Co, W, or Cu X-ray beam rotates about a finely powdered sample and is reflected off the interplanar spacings of all crystalline compounds in the sample, fulfilling Bragg's law ( $n\lambda = 2d\sin\theta$ ). The identification of a species from this pattern is based upon the position of the lines (in terms of  $\theta$  or  $2\theta$ ) and their intensities as recorded by an X-ray detector. The diffraction angle ( $2\theta$ ) is determined by the spacing between a particular set of atomic planes. Identification of the species is empirical, and current available databases contain more than 53,000 compounds.

If a sample contains multiple compounds, interpretation becomes more difficult and computer-matching programs are essential. In some instances, by measuring the intensity of the

diffraction lines and comparing them to standards, it is possible to quantitatively analyze crystalline mixtures; however, if the species is a hydrated form or has a preferred orientation, this method is only semiquantitative at best. Since this technique represents a bulk analysis, no particle size or lability information can be extracted from the patterns.

*Particle Induced X-Ray Emission (PIXE and  $\mu$ PIXE).* Particle induced X-ray emission (PIXE) uses a beam,  $\sim 4 \mu\text{m}$  in diameter, of heavy charged particles (generally He) to irradiate the sample. The resulting characteristic X-rays are emitted and detected in a similar manner as XRF, using Si-Li detectors. Particles generated from a small accelerator or cyclotron, with a potential of 2 to 4 MeV, are commonly used. Detection limits on the order of 1 mg/kg are achieved on thin-film samples. Disadvantages to its use for speciation include (1) only a small volume of material can be analyzed (1 to 2 mg/cm<sup>2</sup>); (2) no particle size information is provided; (3) peak overlaps associated with Si-Li detectors limit identification of species; (4) limited availability; and (5) high cost. For a further review of PIXE analysis and applications, see Maenhaut (1987).

*Electron Probe Microanalysis (EPMA).* Electron probe microanalysis uses a finely focused (1  $\mu\text{m}$ ) electron beam (generated by an electron gun operating at a 2 to 30 kV accelerating voltage and pico/nanoamp currents) to produce a combination of characteristic X-rays for elemental quantification along with secondary electrons and/or backscatter electrons for visual inspection of a sample. Elements from beryllium to uranium can be nondestructively analyzed at the 50-ppm level with limited sample preparation. X-ray spectra can be rapidly acquired using either wavelength dispersive spectrometers (WDS) or energy dispersive spectrometers (EDS).

With WDS, a set of diffracting crystals, of known d-spacing, revolve in tandem with a gas-filled proportional counter inside the spectrometer housing so that Bragg's law is satisfied and a particular wavelength can be focused. Photon energy pulses reflecting off the crystal are collected for an individual elemental line by the counter as a first approximation to concentration. For quantitative analysis, these intensities are compared to those of known standards and must be corrected for background, dead time, and elemental interactions (ZAF) (Goldstein et al., 1992). ZAF correction is in reference to the three components of matrix effects: atomic number (Z), absorption (A), and fluorescence (F).

With EDS, a single Si-Li crystal detector is used in conjunction with a multichannel analog-digital converter (ADC) to sort electrical pulses (with heights approximately proportional to the quantum energy of the photon that generated them), producing a spectrum of energy (wavelength) versus counts. The net area under a particular peak (elemental line) is proportional to its concentration in the sample. For quantitative analyses, corrections similar to WDS analysis must be performed. Although EDS detectors are more efficient than WDS, detection limits are significantly greater (~1000 ppm), because of elevated backgrounds and peak overlaps. For speciation analysis, the EDS system must NEVER be used as the primary detector, as numerous errors in species identification are often made. These are generally the result of higher-order X-ray line overlaps.

This technique has been routinely used for site characterizations (Linton et al., 1980; Hunt et al., 1992; Camp, Dresser, and McKee (CDM), 1994; U.S. Environmental Protection Agency, 2002). Currently this technique offers the most complete data package on metal speciation than any of the other tools. The method is relatively fast and inexpensive, available, and provides all of the required information for bioavailability assessments (i.e., particle size, species, lability, and sourcing). A number of limitations still need to be addressed including: (1) its inability to quickly isolate a statistically significant population of particles in soils with low bulk metal concentrations (<50 mg/kg), meaning that for some metals with low concentrations of concern (i.e., Cd, Mo, Sb, Se), this method may be less useful; (2) the more volatile metals (i.e., Hg, Tl) are often volatilized under the electron beam or lost during sample preparation.

*Secondary Ion Mass Spectrometry (SIMS).* Secondary ion mass spectrometry (also known as ion microprobes or ion probes) is a well-known technique, primarily surface focused, that uses a 0.5 to 20 kV O, Ar, Ga, In, or Cs ion beam in bombarding (sputtering) the surface of a sample while emitting secondary ions that are detected by either quadrupole, time-of-flight (TOF), or magnetic sector mass spectrometers. Sensitivity is very high, in the ppb range for elements hydrogen to uranium, providing quantitative results on elemental or isotopic metals and organic compounds. With the advent of liquid metal (In and Ga) ion beams, beam sizes of less than 1  $\mu\text{m}$  are possible, although 20  $\mu\text{m}$  is more commonly used.

The major disadvantage of SIMS to species identification is that each element or isotope must be tuned and analyzed sequentially. This makes the identification of a metal form highly time-consuming and, thus, the characterization of a multiphase medium impractical.

*X-Ray Photoelectron Spectroscopy (XPS).* X-ray photoelectron spectroscopy or ESCA (electron spectroscopy for chemical analysis, as it was previously known) is a classical surface, 10 to 50 Å in depth, analytical technique for determining qualitative elemental concentrations of elements greater than He in atomic number and provides limited structural and oxidation state information. In XPS, the high-energy (15 kV) electrons are typically produced from a dual-anode (Al-Mg) X-ray tube. The excitation or photoionization of atoms within the near surface of the specimen emit a spectrum of photoelectrons. The measured binding energy is characteristic of the individual atom to which it was bound. Monochromatic sources are often employed to improve energy resolution, allowing one to infer oxidation states of elements or structure of compounds (organic and inorganic) by means of small chemical shifts in binding energies (Hercules, 1970). The major disadvantages of XPS for environmental speciation studies is its poor sensitivity, especially in complex matrices and its large, 100-200 µm, spatial resolution.

The direct speciation techniques discussed above are summarized in Table AX7-1.1.3.

### *Indirect Approaches*

A more indirect approach to speciation than the methods previously described include the functional or operational extraction techniques that have been used extensively over the years (Tessier et al., 1979; Tessier and Campbell, 1988; Gupta and Chen, 1975). These methods use either a single or sequential extraction procedure to release species associated with a particular metal within the media. Single chemical extractions are generally used to determine the bioavailable amount of metal in a functional class: water-soluble, exchangeable, organically bound, Fe-Mn bound, or insoluble.

In a similar approach, sequential extractions treat a sample with a succession of reagents intended to specifically dissolve different, less available phases. Many of these techniques have been proposed, most of which are a variation on the classical method of Tessier et al. (1979), in which metal associated with exchangeable, carbonate-bound, Fe-Mn bound, organically bound, and residual species can be determined. Beckett (1989), Kheboian and Bauer (1987), and Foerstner (1987) provide excellent reviews on the use and abuse of extractions. These

**Table AX7-1.1.3. Characteristics for Direct Speciation Techniques**

<b>Tools</b>	<b>Species Lability</b>	<b>Species Particle Size</b>	<b>Species Valance State</b>	<b>Species Bonding</b>	<b>Species Composition</b>	<b>Species Abundance</b>	<b>Element Specificity</b>	<b>Isotopic Character</b>	<b>Element Sensitivity</b>	<b>Resolution</b>	<b>Availability</b>	<b>Cost</b>
XRD	No	No	No	No	No#	No	No	No	3-4 vol%	Bulk	1	\$
EMPA	Yes	Yes	Yes+	No	Yes	Yes?	B-U	No***	50 ppm	0.5-1 $\mu\text{m}$	2	\$\$
SIMS	No	Yes	No	No	Yes*	Yes**	Li-U	Yes	1 ppb	10 $\mu\text{m}$	4	\$\$\$
XPS	No	No	Yes	Yes	Yes*	Yes**	H-U	No	wt. %	100 $\mu\text{m}$	2	\$\$
XAS	No	No	Yes	Yes	Yes*	Yes**	He-U	No	ppb	2 $\mu\text{m}$	5	\$\$\$\$
PIXIE	No	No	No	No	Yes	Yes**	B-U	No	10 ppm	4 $\mu\text{m}$	4	\$\$\$\$

\* Technique requires each element be tuned and standardized, requiring unreasonable time limits.

\*\* Techniques designed and tested only on simple systems. Multiple species require lengthy analytical times and data reduction.

\*\*\* Limited when combined with ICP/MS/LA.

# Identifies crystalline compounds and stoichiometric compositions only.

? Technique has limitations based on particle counting statistics.

+ Valance determined by charge balance of complete analyses.

techniques can be useful in a study of metal uptake in plants, where transfer takes place predominately via a solution phase. However, one must keep in mind that they are not “selective” in metal species, give no particle size information and, above all, these leachable fractions have never been correlated to bioavailability.

*Solution Speciation Using Computer-Based Models.* Computer-based models are either based upon equilibrium constants or upon Gibb’s free energy values in determining metal speciation from solution chemistry conditions (concentration, pH, Eh, organic complexes, adsorption/desorption sites, and temperature). Both approaches are subject to mass balance and equilibrium conditions. These models have undergone a great deal of development in recent years, as reliable thermodynamic data has become available and can provide some predictive estimates of metal behavior. A good review of these models and their applications is provided by Lumsdon and Evans (1995).

Speciation can be controlled by simple reactions; however, in many cases, particularly in contaminated media, their state of equilibrium and reversibility are unknown. In addition, these models suffer from other limitations such as a lack of reliable thermodynamic data on relevant species, inadequacies in models to correct for high ionic strength, reaction kinetics are poorly known, and complex reactions with co-precipitation/adsorption are not modeled.

The first limitation is perhaps the most significant for contaminated media. For example, none of the models would predict the common, anthropogenic, Pb phases, i.e., paint, solder, and slag.

#### **AX7.1.1.4 Biotic Ligand Model**

The biotic ligand model (BLM) is an equilibrium-based conceptual model that has been incorporated into regulatory agencies guidelines (including the EPA) to predict effects of metals primarily on aquatic biota and to aid in the understanding of their interactions with biological surfaces (see Annex Section AX7.2.1.3).

Because of assumed similarities in mechanisms of toxicity between aquatic and terrestrial organisms, it is likely that the BLM approach as developed for the aquatic compartment may also be applicable to the terrestrial environment. Recent research has been directed toward extending the BLM to predict metal toxicity in soils (Steenbergen et al., 2005). Steenbergen et al. (2005) pointed out that, until recently, the BLM concept has not been applied to predict toxicity to soil

organisms. The authors believe there may be two reasons for this. First, metal uptake routes in soils are generally more complex than those in water, because exposure via pore water and exposure via ingestion of soil particles may, in principle, both be important. Second, it remains very difficult to univariately control the composition of the soil pore water and the metal concentrations in the pore water, due to re-equilibration of the system following modification of any of the soil properties (including addition of metal salts).

Steenbergen et al. (2005) assessed acute copper toxicity to the earthworm *Aporrectodea caliginosa* using the BLM. To overcome the aforementioned problems inherent in soil toxicity tests they developed an artificial flow-through exposure system consisting of an inert quartz sand matrix and a nutrient solution, of which the composition was univariately modified. Thus, the obstacles in employing the BLM to terrestrial ecosystems seem to be surmountable, and future research may provide useful information on Pb bioavailability and toxicity to terrestrial organisms.

#### **AX7.1.1.5 Soil Amendments**

The removal of contaminated soil to mitigate exposure of terrestrial ecosystem components to Pb can often present both economic and logistic problems. Because of this, recent studies have focused on in situ methodologies to lower soil-Pb RBA (Brown et al., 2003a,b). To date, the most common methods studied include the addition of soil amendments in an effort either to lower the solubility of the Pb form or to provide sorption sites for fixation of pore-water Pb. These amendments typically fall within the categories of phosphate, biosolid, and Al/Fe/Mn-oxide amendments.

##### ***Phosphate Amendments***

Phosphate amendments have been studied extensively and, in some cases, offer the most promising results (Brown et al., 1999; Ryan et al., 2001; Cotter-Howells and Caporn, 1996; Hettiarachchi et al., 2001, 2003; Rabinowitz, 1993; Yang et al., 2001; Ma et al., 1995). Research in this area stems from early work by Nriagu (1973) and Cotter-Howells and Caporn (1996), who pointed out the very low solubilities for many Pb-phosphates ( $K_{sp}$   $10^{-27}$  to  $10^{-66}$ ), particularly chloropyromorphite [ $Pb_5(PO_4)_3Cl$ ]. The quest to transform soluble Pb mineralogical forms into chloropyromorphite continues to be the primary focus of most studies. Sources of phosphorous

have included phosphoric acid ( $H_3PO_4$ ), triple-super phosphate (TSP), phosphate rock, and/or hydroxyapatite (HA). Various studies have combined one or more of these phosphorous sources with or without lime, iron, and/or manganese in an attempt to enhance amendment qualities. Most amendments are formulated to contain between 0.5 and 1.0% phosphorous by weight. They are then either applied wet or dry and then mixed or left unmixed with the contaminated soil. Success of phosphate amendments has been variable, and the degree of success appears to depend on available phosphorous and the dissolution rate of the original Pb species.

A number of potentially significant problems associated with phosphate amendments have been recognized, including both phyto- and earthworm toxicity (Ownby et al., 2005; Cao et al., 2002; and Rusek and Marshall, 2000). Both of these toxicities are primarily associated with very high applications of phosphorous and/or decreased soil pH. Indications of phytotoxicity are often balanced by studies such as Zhu et al. (2004) that illustrate a 50 to 70% reduction in shoot-root uptake of Pb in phosphate-amended soils. Additionally, the added phosphate poses the potential risk of eutrophication of nearby waterways from soil runoff. Finally, Pb-contaminated soils from the extractive metals industry or agricultural sites often have elevated concentrations of arsenic. It has been shown (Impellitteri, 2005; Smith et al., 2002; Chaney and Ryan, 1994; and Ruby et al., 1994) that the addition of phosphate to such soils would enhance arsenic mobility (potentially moving arsenic down into the groundwater) through competitive anion exchange. Some data (Lenoble et al., 2005) indicate that if one could amend arsenic and Pb contaminated soils with iron(III) phosphate this problem can be mitigated, however the increased concentrations of both phosphate and iron still exclude the application when drinking water is an issue.

### ***Biosolid Amendments***

Historically, biosolids have been used in the restoration of coal mines (Haering et al., 2000; Sopper, 1993). More recently, workers have demonstrated the feasibility of their use in the restoration of mine tailings (Brown et al., 2003a), and urban soils (Brown et al., 2003b; Farfel et al., 2005). Mine tailings are inherently difficult to remediate in that they pose numerous obstacles to plant growth. They are most often (1) acidic; (2) high in metal content, thus prone to phytotoxicity; (3) very low in organic content; and (4) deficient in macro- and micronutrients.

Stabilization (i.e., the establishment of a vegetative cover) of these environments is essential to the control of metal exposure or migration from soil/dust and groundwater pathways.

At Bunker Hill, ID, Brown et al. (2003b) demonstrated that a mixture of high nitrogen biosolids and wood pulp or ash, when surface applied at a rate of approximately 50 and 220 tons/ha, respectively, increased soil pH from 6.8 to approximately 8.0, increased plant biomass from 0.01 mg/ha to more than 3.4 tons/ha, and resulted in a healthy plant cover within 2 years. Metal mobility was more difficult to evaluate. Plant concentrations of Zn and Cd were generally normal for the first 2 years of the study; however, Pb concentrations in vegetation dramatically increased two to three times in the first year. Additionally, macronutrients (Ca, K, and Mg) decreased in plant tissue.

Urban soils, whether contaminated from smelting, paint, auto emissions, or industrial activity, are often contaminated with Pb (Agency for Toxic Substances and Disease Registry [ATSDR], 1988) and can be a significant pathway to elevated child blood Pb levels (Angle et al., 1974). Typically, contaminated residential soils are replaced under Superfund rules. However, urban soils are less likely to be remediated unless a particular facility is identified as the contaminate source. Application of biosolids to such soils may be a cost-effective means for individuals or communities to lower Pb RBAs.

A field study by Farfel et al. (2005) using the commercial biosolid ORGO found that, over a 1-year period, Pb in the dripline soils of one residence had reduced RBAs by ~60%. However, soils throughout the remainder of the yard showed either no reduction in RBA or a slight increase. A more complex study was conducted by Brown et al. (2003a) on an urban dripline soil in the lab. The study used an assortment of locally derived biosolids (raw, ashed, high-Fe compost, and compost) with and without lime. All amendments were incubated with approximately 10% biosolids for a little more than 30 days. In vitro and in vivo data both indicated a 3 to 54% reduction in Pb RBA, with the high-Fe compost providing the greatest reduction.

As with phosphate amendments, problems with biosolid application have also been documented. Studies have shown that metal transport is significantly accelerated in soils amended with biosolids (Al-Wabel et al., 2002; McBride et al., 1997, 1999; Lamy et al., 1993; Richards et al., 1998, 2000). Some of these studies indicate that metal concentrations in soil solutions up to 80 cm below the amended surface increased by 3- to 20-fold in concentration up

to 15 years after biosolid application. The increase in metal transport is likely the result of elevated dissolved organic carbon (DOC) in the amended soil. Anodic stripping voltammetry has indicated that very low percentages (2 to 18%) of the soluble metals are present as ionic or inorganic complexes (McBride, 1999; Al-Wabel et al., 2002).

### **AX7.1.2 Distribution of Atmospherically Delivered Lead in Terrestrial Ecosystems**

The 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) contains only a few minor sections that detail the speciation, distribution, and behavior of atmospherically delivered Pb in terrestrial ecosystems. The document concluded that the majority of Pb in the atmosphere at that time was from gasoline consumption: of the 34,881 tons of Pb emitted to the atmosphere in 1984, 89% was from gasoline use and minor amounts were from waste oil combustion, iron and steel production, and smelting. Lead in the atmosphere today, however, is not primarily from leaded-gasoline consumption, but results largely from iron and steel foundries, boilers and process heaters, the combustion of fossil fuels in automobiles, trucks, airplanes, and ships, and other industrial processes (Table 2-8, Polissar et al., 2001; Newhook et al., 2003). The emission source can determine the species of Pb that are delivered to terrestrial ecosystems. For example, Pb species emitted from automobile exhaust is dominated by particulate Pb halides and double salts with ammonium halides (e.g.,  $\text{PbBrCl}$ ,  $\text{PbBrCl}_2\text{NH}_4\text{Cl}$ ), while Pb emitted from smelters is dominated by Pb-sulfur species (Habibi, 1973). The halides from automobile exhaust break down rapidly in the atmosphere, possibly via reactions with atmospheric acids (Biggins and Harrison, 1979). Lead phases in the atmosphere, and presumably the compounds delivered to the surface of the earth (i.e., to vegetation and soils), are suspected to be in the form of  $\text{PbSO}_4$ ,  $\text{PbS}$ , and  $\text{PbO}$  (Olson and Skogerboe, 1975; Clevenger et al., 1991; Utsunomiya et al., 2004).

There are conflicting reports of how atmospherically derived Pb specifically behaves in surface soils. This disagreement may represent the natural variability of the biogeochemical behavior of Pb in different terrestrial systems, the different Pb sources, or it may be a function of the different analytical methods employed. The importance of humic and fulvic acids (Zimdahl and Skogerboe, 1977; Gamble et al., 1983) and hydrous Mn- and Fe-oxides (Miller and McFee, 1983) for scavenging Pb in soils are discussed in some detail in the 1986 Lead AQCD. Nriagu

(1974) used thermodynamics to argue that Pb-orthophosphates (e.g., pyromorphite) represented the most stable Pb phase in many soils and sediments. He further suggested that, because of the extremely low solubility of Pb-phosphate minerals, Pb deposition could potentially reduce phosphorous availability. Olson and Skogerboe (1975) reported that solid-phase  $\text{PbSO}_4$  dominated gasoline-derived Pb speciation in surface soils from Colorado, Missouri, and Chicago, while Santillan-Medrano and Jurinak (1975) suggested that  $\text{Pb}(\text{OH})_2$ ,  $\text{Pb}(\text{PO}_4)_2$ , and  $\text{PbCO}_3$  could regulate Pb speciation in soils. However, insoluble organic material can bind strongly to Pb and prevent many inorganic phases from ever forming in soils (Zimdahl and Skogerboe, 1977).

The vertical distribution and mobility of atmospheric Pb in soils was poorly documented prior to 1986. Chapter 6 of the 1986 AQCD cited a few references suggesting that atmospheric Pb is retained in the upper 5 cm of soil (Reaves and Berrow, 1984). Techniques using radiogenic Pb isotopes had been developed to discern between gasoline-derived Pb and natural, geogenic (native) Pb, but these techniques were mostly applied to sediments (Shirahata et al., 1980) prior to the 1986 Lead AQCD. Without using these techniques, accurate determinations of the depth-distribution and potential migration velocities for atmospherically delivered Pb in soils were largely unavailable.

Several technological advances, combined with the expansion of existing technologies after 1986 resulted in the publication of a large body of literature detailing the speciation, distribution, and geochemical behavior of gasoline-derived Pb in the terrestrial environment. Most notably, the development of selective chemical extraction (SCE) procedures as a rapid and inexpensive means for partitioning Pb into different soil and sediment phases (e.g., Pb-oxides, Pb-humate, etc.) has been exploited by a number of researchers (Tessier et al., 1979; Johnson and Petras, 1998; Ho and Evans, 2000; Scheckel et al., 2003). Also, since 1986, several workers have exploited synchrotron-based XAS in order to probe the electron coordination environment of Pb in soils, organic matter, organisms, and sediments (Manceau et al., 1996; Xia et al., 1997; Trivedi et al., 2003). X-ray absorption studies can be used for the in-situ determination of the valence state of Pb and can be used to quantify Pb speciation in a variety of untreated samples. Biosensors, which are a relatively new technology coupling biological material, such as an enzyme, with a transducer, offer a new, simple, and inexpensive means for quantifying available Pb in ecosystems (Verma and Singh, 2005). Advances in voltammetric, diffusive gradients in

thin films (DGT), and ICP techniques have also increased the abilities of researchers to quantify Pb phases in solutions (Berbel et al., 2001; Scally et al., 2003). In addition to the development of techniques for describing and quantifying Pb species in the soils and solutions, researchers have used radiogenic Pb isotopes ( $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ ,  $^{208}\text{Pb}$ ) to quantify the distribution, speciation, and transport of anthropogenic Pb in soil profiles and in vegetation (Bindler et al., 1999; Erel et al., 2001; Kaste et al., 2003; Klaminder et al., 2005).

Over the past several decades, workers have also developed time-series data for Pb in precipitation, vegetation, organic horizons, mineral soils, and surface waters. Since atmospherically delivered Pb often comprises a significant fraction of the “labile” Pb (i.e., Pb not associated with primary minerals), these data have been useful for developing transport and residence time models of Pb in different terrestrial reservoirs (Friedland et al., 1992; Miller and Friedland, 1994; Johnson et al., 1995a; Wang and Benoit, 1997). Overall, a significant amount of research has been published on the distribution, speciation, and behavior of anthropogenic Pb in the terrestrial environment since 1986. However, certain specific details on the behavior of Pb in the terrestrial environment and its potential effects on soil microorganisms remain elusive.

#### **AX7.1.2.1 Speciation of Atmospherically-delivered Lead in Terrestrial Ecosystems**

##### ***Lead in the Solid Phases***

Lead can enter terrestrial ecosystems through natural rock weathering and by a variety of anthropogenic pathways. These different source terms control the species of Pb that is introduced into the terrestrial environment. While Pb is highly concentrated (percent level) in certain hydrothermal sulfide deposits (e.g., PbS) that are disseminated throughout parts of the upper crust, these occurrences are relatively rare. Therefore, the occurrence of Pb as a minor constituent of rocks (ppm level), particularly granites, rhyolites, and argillaceous sedimentary rocks is the more pertinent source term for the vast majority of terrestrial ecosystems. During the hydrolysis and oxidation of Pb-containing minerals, divalent Pb is released to the soil solution where it is rapidly fixed by organic matter and secondary mineral phases (Kabata-Pendias and Pendias, 1992). The geochemical form of natural Pb in terrestrial ecosystems will be strongly controlled by soil type (Emmanuel and Erel, 2002). In contrast, anthropogenically introduced Pb has a variety of different geochemical forms, depending on the specific source. While Pb in soils from battery reclamation areas can be in the form of  $\text{PbSO}_4$  or  $\text{PbSiO}_3$ , Pb in

soils from shooting ranges and paint spills is commonly found as PbO and a variety of Pb carbonates (Vantelon et al., 2005; Laperche et al., 1996; Manceau et al., 1996). Atmospherically delivered Pb resulting from fossil fuel combustion is typically introduced into terrestrial ecosystems as Pb-sulfur compounds and Pb oxides (Olson and Skogerboe, 1975; Clevenger et al., 1991; Utsunomiya et al., 2004). After deposition, Pb species are likely transformed. Although the specific factors that control the speciation of anthropogenic Pb speciation in soils are not well understood, there are many studies that have partitioned Pb into its different geochemical phases. A thorough understanding of Pb speciation is critical in order to predict potential mobility and bioavailability (see Section AX7.1.1).

Selective chemical extractions have been employed extensively over the past 20 years for quantifying amounts of a particular metal phase (e.g., PbS, Pb-humate, Pb-Fe/Mn-oxide) present in soil or sediment rather than total metal concentration. Sometimes selective chemical extractions are applied *sequentially* to a particular sample. For example, the *exchangeable* metal fraction is removed from the soil using a weak acid or salt solution (e.g., BaCl<sub>2</sub>), followed immediately by an extraction targeting organic matter (e.g., H<sub>2</sub>O<sub>2</sub> or NaOCl), further followed by an extraction targeting secondary iron oxides (e.g., NH<sub>2</sub>OH·HCl), and finally, a strong reagent cocktail (e.g., HNO<sub>3</sub>-HCl-HF) targets primary minerals. Tessier et al. (1979) developed this technique. More recently, this technique has been modified and developed specifically for different metals and different types of materials (Keon et al., 2001). Alternatively, batch-style selective chemical extractions have been used on soils and sediments to avoid the problems associated with nonselective reagents (Johnson and Petras, 1998). Selective extractions can be a relatively rapid, simple, and inexpensive means for determining metal phases in soils and sediments, and the generated data can be linked to potential mobility and bioavailability of the metal (Tessier and Campbell, 1987). However, some problems persist with the selective extraction technique. First, extractions are rarely specific to a single phase. For example, while H<sub>2</sub>O<sub>2</sub> is often used to remove metals bound to organic matter in soils, others have demonstrated that this reagent destroys clay minerals and sulfides (Ryan et al., 2002). Peroxide solutions may also be inefficient in removing metals bound to humic acids, and in fact could potentially result in the precipitation of metal-humate substances. In addition to the nonselectivity of reagents, significant metal redistribution has been documented to occur during sequential chemical extractions (Ho and Evans, 2000; Sulkowski and Hirner, 2006), and many reagents may not

completely extract targeted phases. While chemical extractions provide some useful information on metal phases in soil or sediment, the results should be treated as “operationally defined,” e.g., “H<sub>2</sub>O<sub>2</sub>-liberated Pb” rather than “organic Pb.”

Lead forms strong coordination complexes with oxygen on mineral surfaces and organic matter functional groups (Abd-Elfattah and Wada, 1981), because of its high electronegativity and hydrolysis constant. Therefore, Pb is generally not readily exchangeable, i.e., the amount of Pb removed from soils by dilute acid or salts is usually less than 10% (Karamanos et al., 1976; Sposito et al., 1982; Miller and McFee, 1983; Johnson and Petras, 1998; Bacon and Hewitt, 2005). Lead is typically adsorbed to organic and inorganic soil particles strongly via inner-sphere adsorption (Xia et al., 1997; Bargar et al., 1997a,b, 1998). Kaste et al. (2005) found that a single extract of 0.02 M HCl removed 15% or less Pb in organic horizons from a montane forest in New Hampshire. The fact that relatively concentrated acids, reducing agents, oxidizing agents, or chelating agents are required to liberate the majority of Pb from soils is used as one line of evidence that Pb migration and uptake by plants in soils is expected to be low.

Lead that is “organically bound” in soils is typically quantified by extractions that dissolve/disperse or destroy organic matter. The former approach often employs an alkaline solution (NaOH), which deprotonates organic matter functional groups, or a phosphate solution, which chelates structural cations. Extractions used to destroy organic matter often rely on H<sub>2</sub>O<sub>2</sub> or NaOCl. Both organic and mineral horizons typically have significant Pb in this soil phase. Miller and McFee (1983) used Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> to extract organically bound Pb from the upper 2.5 cm of soils sampled from northwestern Indiana. They found that organically bound Pb accounted for between 25 and 50% of the total Pb present in the sampled topsoils. Jersak et al. (1997), Johnson and Petras (1998), and Kaste et al. (2005) selectively extracted Pb from spodosols from the northeastern United States. Using acidified H<sub>2</sub>O<sub>2</sub>, Jersak et al. (1997) found that very little (<10 %) of the Pb in mineral soils (E, B, C) sampled from New York and Vermont was organic. Johnson and Petras (1998) used K<sub>4</sub>P<sub>2</sub>O<sub>7</sub> to quantify organically bound Pb in the Oa horizon and in mineral soils from the Hubbard Brook Experimental Forest in New Hampshire. They reported that 60% of the total Pb in the Oa horizon was organic and that between 8 and 17% of the total Pb in the mineral soil was organic. However, in the E, Bh, and Bs1 horizons, organically bound Pb dominated the total “labile” (non-mineral lattice) Pb. Kaste et al. (2005) used selective chemical extractions on organic horizons from montane forests in Vermont and New Hampshire.

They found that repeated extractions with  $\text{Na}_4\text{P}_2\text{O}_7$  removed between 60 and 100% of the Pb from their samples. Caution should be used when interpreting the results of pyrophosphate extractions. Although they are often used to quantify organically bound metals, this reagent can both disperse and dissolve Fe phases (Jeanroy and Guillet, 1981; Shuman, 1982). Acidified  $\text{H}_2\text{O}_2$  has also been reported to destroy and release elements associated with secondary soil minerals (Papp et al., 1991; Ryan et al., 2002).

Aside from organic forms, Pb is often found to be associated with secondary oxide minerals in soils. Pb can be partitioned with secondary oxides by a variety of mechanisms, including (1) simple ion exchange, (2) inner-sphere or outer-sphere adsorption, and (3) co-precipitation and/or occlusion (Bargar et al., 1997a,b, 1998, 1999). As discussed above, very little Pb is removed from soil via dilute acid or salt solutions, so adsorption and co-precipitation are likely the dominant Pb interactions with secondary mineral phases. Reagents used to quantify this phase are often solutions of EDTA, oxalate, or hydroxylamine hydrochloride (HH). Miller and McFee (1983) used an EDTA solution followed by an HH solution to quantify Pb occluded by Fe and Mn minerals, respectively, in their surface-soil samples from Indiana. They reported that approximately 30% of the total soil Pb was occluded in Fe minerals, and 5 to 15% was occluded in Mn phases. In soils from the northeastern United States, Jersak et al. (1997) used various strengths of HH solutions and concluded that negligible Pb was associated with Mn-oxides and that 1 to 30% of the Pb was associated with Fe phases in the mineral soils in their study. Johnson and Petras (1998) reported that no Pb was removed from the Oa horizon at the Hubbard Brook Experimental Forest (HBEF) by oxalate, but that 5 to 15% of the total Pb in mineral soils was removed by this extraction, presumably because it was bound to amorphous oxide minerals. Kaste et al. (2005), however, reported that HH removed 30 to 40% of the Pb from organic horizons in their study. They concluded that Fe phases were important in scavenging Pb, even in soil horizons dominated by organic matter.

Synchrotron radiation (X-rays) allows researchers to probe the electron configuration of metals in untreated soil and sediment samples. This type of analysis has been extremely valuable for directly determining the coordination environment of Pb in a variety of soils and sediments. Since different elements have different electron binding energies ( $E_b$ ), X-rays can be focused in an energy window specific to a metal of interest. In experiments involving XAS, X-ray energy is increased until a rapid increase in the amount of absorption occurs; this absorption edge

represents  $E_b$ . The precise energy required to dislodge a core electron from a metal (i.e.,  $E_b$ ) will be a function of the oxidation state and covalency of the metal. X-ray absorption studies that focus on the location of the absorption edge are referred to as XANES (X-ray absorption near edge structure). In the energy region immediately after the absorption edge, X-ray absorption increases and decreases with a periodicity that represents the wave functions of the ejected electrons and the constructive and destructive interference with the wave functions of the nearby atoms. X-ray absorption studies used to investigate the periodicity of the absorption after  $E_b$  are referred to as EXAFS (extended X-ray absorption fine structure). Since the electron configuration of a Pb atom will be directly governed by its speciation (e.g., Pb bound to organics, Pb adsorbed to oxide surfaces, PbS, etc.) X-ray absorption studies provide a powerful in-situ technique for determining speciation without some of the problems associated with chemical extractions (Bargar et al., 1997a,b, 1998).

Manceau et al. (1996) used EXAFS to study soil contaminated by gasoline-derived Pb in France and found that the Pb was divalent and complexed to salicylate and catechol-type functional groups of humic substances. He concluded that the alkyl-tetravalent Pb compounds that were added to gasoline were relatively unstable and will not dominate the speciation of Pb fallout from the combustion of leaded gasoline. The binding mechanism of Pb to organics is primarily inner-sphere adsorption (Xia et al., 1997). DeVolder et al. (2003) used EXAFS to demonstrate that Pb phases were shifting to the relatively insoluble PbS when contaminated wetland soils were treated with sulfate. Strawn and Sparks (2000) gave evidence that added Pb was adsorbed directly onto organic matter in an untreated silt loam, but in the absence of organic matter (treated with sodium hypochlorite) the added Pb appeared to adsorb instead to some form of  $\text{SiO}_2$ . More recent XAS studies have demonstrated the importance of biomineralization of Pb in soils by bacteria and nematodes (Xia et al., 1997; Templeton et al., 2003a,b; Jackson et al., 2005). Templeton et al. (2003a,b) demonstrated that biogenic precipitation of pyromorphite was the dominant source of Pb uptake by *Burkholderia cepacia* biofilms below pH 4.5. Above pH 4.5, adsorption complexes began to form in addition to Pb mineral precipitation.

In addition to XAS studies of Pb in environmental samples, numerous experimental-based XAS studies have documented in detail the coordination environment of Pb adsorbed to Fe-oxides, Mn-oxides, Al-oxides, and clay minerals (Manceau et al., 1996, 2000a,b, 2002; Bargar et al., 1997a,b, 1998, 1999; Strawn and Sparks, 1999; Trivedi et al., 2003; Chen et al., 2006).

Bargar et al. (1997a) showed that Pb can adsorb to FeO<sub>6</sub> octahedra on three different types of sites: on corners, edges, or faces. Ostergren et al. (2000a,b) showed that the presence of dissolved carbonate and sulfate increased Pb adsorption on goethite. The relative fraction of corner-sharing complexes can be greatly increased by the presence of these ligands, as bridging complexes between the metal and the corners are formed (Ostergren et al., 2000a,b). Furthermore, Elzinga and Sparks showed that the mechanism of Pb sorption to SiO<sub>2</sub> surfaces is pH-dependent. In acid environments (pH < 4) Pb adsorbs largely as an inner-sphere mononuclear complex, but as pH increases, Pb sorption increasingly occurs via the formation of surface-attached covalent polynuclear Pb species.

Recently, Jackson et al. (2005) used microfocused synchrotron-based X-ray fluorescence (ΦSXRF) to detail the distribution of Pb and Cu in the nematode *Caenorhabditis elegans*. They found that, while Cu was evenly distributed throughout the bodies of exposed *C. elegans*, Pb was concentrated in the anterior pharynx region. Microfocused X-ray diffraction indicated that the highly concentrated Pb region in the pharynx was actually comprised of the crystalline Pb mineral, pyromorphite. The authors concluded that *C. elegans* precipitated pyromorphite in the pharynx as a defense mechanism to prevent spreading the toxic metal to the rest of the organism's body. They further suggested that, because of the high turnover rate of nematodes, biomineralization could play an important role in the speciation of Pb in certain soils.

### ***Lead Solid-solution Partitioning***

The concentration of Pb species dissolved in soil solution is probably controlled by some combination of (a) Pb-mineral solubility equilibria, (b) adsorption reactions of dissolved Pb phases on inorganic surfaces (e.g., crystalline or amorphous oxides of Al, Fe, Si, Mn, etc.; clay minerals), and (c) adsorption reactions of dissolved Pb phases on soil organic matter. Dissolved Pb phases in soil solution can be some combination of Pb<sup>2+</sup> and its hydrolysis species, Pb bound to dissolved organic matter, and Pb complexes with inorganic ligands such as Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup>. Alkaline soils typically have solutions supersaturated with respect to PbCO<sub>3</sub>, Pb<sub>3</sub>(CO<sub>3</sub>)<sub>2</sub>(OH)<sub>2</sub>, Pb(OH)<sub>2</sub>, Pb<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>(OH), and Pb<sub>4</sub>O(PO<sub>4</sub>)<sub>2</sub> (Badawy et al., 2002). Pb-phosphate minerals in particular are very insoluble, and calculations based on thermodynamic data predict that these phases will control dissolved Pb in soil solution under a variety of conditions (Nriagu,

1974; Ruby et al., 1994). However, certain chelating agents, such as dissolved organic matter, can prevent the precipitation of Pb minerals (Lang and Kaupenjohann, 2003).

Using a combination of desorption experiments and XAS, EXAFS, and XANES, Rouff et al. (2006) found that aging of Pb-calcite suspensions resulted in changes in the solid-phase distribution of Pb. Increased sorption time can reduce trace metal desorption by enhancing the stability of surface complexes (Rouff et al., 2005) or by mechanisms involving microporous diffusion (Backes et al., 1995), recrystallization-induced incorporation into the solid phase (Ainsworth et al., 1994), and formation and stabilization of surface precipitates (Ford et al., 1999). For adsorption of Pb to hydrous Fe oxides, goethite, and Pb-contaminated soils, aged samples show Pb to be reversibly bound, suggesting Pb adsorption primarily to the substrates' surfaces (Rouff et al., 2006). However, for Pb adsorption to calcite aging played a significant role due to detection of multiple sorption mechanisms, even for short sorption times (Rouff et al., 2006). pH also played a role in Pb sorption. Over long sorption periods (60 to 270 days), slow continuous uptake of Pb occurred at pH 7.3 and 8.2. At pH 9.4, no further uptake occurred with aging and very little desorption occurred. These results show the importance of contact time and pH on Pb solid-phase partitioning, particularly in geochemical systems in which calcite may be the predominant mineralogical constituent.

Soil solution dissolved organic matter content and pH typically have very strong positive and negative correlations, respectively, with the concentration of dissolved Pb species (Sauvé et al., 1998, 2000a, 2003; Weng et al., 2002; Badawy et al., 2002; Tipping et al., 2003). In the case of adsorption phenomena, the partitioning of  $Pb^{2+}$  to the solid phase is also controlled by total metal loading, i.e., high Pb loadings will result in a lower fraction being partitioned to the solid phase. Sauvé et al. (1997, 1998) demonstrated that only a fraction of the total Pb in solution was actually  $Pb^{2+}$  in soils treated with leaf compost. The fraction of  $Pb^{2+}$  to total dissolved Pb ranged from <1 to 60%, depending on pH and the availability of Pb-binding ligands. Nolan et al. (2003) used Donnan dialysis to show that 2.9 to 48.8% of the dissolved Pb was  $Pb^{2+}$  in pore waters of agricultural and contaminated soils from Australia and the United States. In acidic soils, Al species can compete for sites on natural organic matter and inhibit Pb binding to surfaces (Gustafsson et al., 2003).

Differential pulse anodic stripping voltammetry (DPASV) is a technique that is useful for identifying relatively low concentrations of  $Pb^{2+}$  and has found many applications in adsorption

and partitioning experiments. This technique has been particularly useful for quantifying the  $K_d$ , or partitioning ratio of Pb in the solid-to-liquid phase ( $K_d = [\text{total solid-phase metal in mg kg}^{-1}] / [\text{dissolved metal in mg L}^{-1}]$ ). While the exact  $K_d$  value is a function of pH, organic matter content, substrate type, total metal burden, and concentrations of competing ligands, such studies typically show that Pb has very strong solid-phase partitioning. Partitioning ratios determined by DPASV generally range from  $10^3$  to  $10^6$  in soils in the typical pH range (Sauvé et al., 2000b). Aualiitia and Pickering (1987) used thin film ASV to compare the relative affinity of Pb for different inorganic particulates. They reported that Mn(IV) oxides completely adsorbed the Pb, regardless of pH in the range of 3 to 9, and had the highest affinity for Pb in their study. The adsorption of Pb to pedogenic Fe-oxides, Al-hydroxides, clay minerals, and Fe ores was reported to be pH-dependent. Sauvé et al. (1998) used DPASV to study the effects of organic matter and pH on Pb adsorption in an orchard soil. They demonstrated that Pb complexation to dissolved organic matter (DOM) increased Pb solubility, and that 30 to 50% of the dissolved Pb was bound to DOM at pH 3 to 4, while >80% of the dissolved Pb was bound to DOM at neutral pH. They concluded that in most soils, Pb in solution would not be found as  $\text{Pb}^{2+}$  but as bound to DOM. Sauvé et al. (2000a) compared the relative affinity of  $\text{Pb}^{2+}$  for synthetic ferrihydrite, leaf compost, and secondary oxide minerals collected from soils. They reported that the inorganic mineral phases were more efficient at lowering the amount of  $\text{Pb}^{2+}$  that was available in solution than the leaf compost. Glover et al. (2002) used DPASV in studying the effects of time and organic acids on Pb adsorption to goethite. They found that Pb adsorption to goethite was very rapid, and remained unchanged after a period of about 4 h. Lead desorption was found to be much slower. The presence of salicylate appeared to increase the amount of Pb that desorbed from goethite more so than oxalate.

#### **AX7.1.2.2 Tracing the Fate of Atmospherically-delivered Lead in Terrestrial Ecosystems**

Radiogenic Pb isotopes offer a powerful tool for separating anthropogenic Pb from natural Pb derived from mineral weathering (Erel and Patterson, 1994; Erel et al., 1997; Semlali et al., 2001). This has been particularly useful for studying Pb in mineral soil, where geogenic Pb often dominates. The three radiogenic stable Pb isotopes ( $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ , and  $^{208}\text{Pb}$ ) have a heterogeneous distribution in the earth's crust primarily because of the differences in the half-lives of their respective parents ( $^{238}\text{U}$ ,  $T_{1/2} = 4.7 \times 10^9$  year;  $^{235}\text{U}$ ,  $T_{1/2} = 0.7 \times 10^9$  year;  $^{232}\text{Th}$ ,

$T_{1/2} = 14 \times 10^9$  year). The result is that the ore bodies from which anthropogenic Pb are typically derived are usually enriched in  $^{207}\text{Pb}$  relative to  $^{206}\text{Pb}$  and  $^{208}\text{Pb}$  when compared with Pb found in granitic rocks. Graney et al. (1995) analyzed a dated core from Lake Erie, and found that the  $^{206}\text{Pb}/^{207}\text{Pb}$  value in sediment deposited in the late 1700s was 1.224, but in 20th-century sediment, the ratio ranged from 1.223 to 1.197. This shift in the Pb isotopic composition represents the introduction of a significant amount of anthropogenic Pb into the environment. Bindler et al. (1999) and Emmanuel and Erel (2002) analyzed the isotopic composition of Pb in soil profiles in Sweden and the Czech Republic, respectively, and determined that mineral soils immediately below the organic horizon had a mixture of both anthropogenic and geogenic Pb. Anthropogenic Pb has been detected relatively deep into the soil profile (>25 cm) in Europe, presumably this represents very “old” (i.e., pre-1900) Pb (Steinnes and Friedland, 2005).

Erel and Patterson (1994) used radiogenic Pb isotopes to trace the movement of industrial Pb from topsoils to groundwaters to streams in a remote mountainous region of Yosemite National Park in California. They calculated that total 20th-century industrial Pb input to their study site was approximately  $0.4 \text{ g Pb m}^{-2}$ . Lead concentrations in organic material were highest in the upper soil horizons, and decreased with depth. During snowmelt, Pb in the snowpack was mixed with the anthropogenic and geogenic Pb already in the topsoil, and spring melts contained a mixture of anthropogenic and geogenic particulate Pb. During base flows, however, 80% of the Pb export from groundwater and streams was from natural granite weathering (Erel and Patterson, 1994).

Uranium-238 series  $^{210}\text{Pb}$  also provides a tool for tracing atmospherically delivered Pb in soils. After  $^{222}\text{Rn}$  ( $T_{1/2} = 3.8$  days) is produced from the decay of  $^{226}\text{Ra}$  ( $T_{1/2} = 1600$  years), some fraction of the  $^{222}\text{Rn}$  escapes from rocks and soils to the atmosphere. It then decays relatively rapidly to  $^{210}\text{Pb}$  ( $T_{1/2} = 22.3$  years), which has a tropospheric residence time of a few weeks (Koch et al., 1996). Fallout  $^{210}\text{Pb}$  is deposited onto forests via wet and dry deposition, similar to anthropogenic Pb deposition in forests, and is thus useful as a tracer for non-native Pb in soils. Lead-210 is convenient to use for calculating the residence time of Pb in soil layers, because its atmospheric and soil fluxes can be assumed to be in steady state at undisturbed sites (Dörr and Münnich, 1989; Dörr, 1995; Kaste et al., 2003). Atmospheric  $^{210}\text{Pb}$  ( $^{210}\text{Pb}_{\text{ex}}$  hereafter,  $^{210}\text{Pb}$  in “excess” of that supported by  $^{222}\text{Rn}$  in the soil) must be calculated by subtracting the amount of

$^{210}\text{Pb}$  formed in soils by the in-situ decay of  $^{222}\text{Rn}$  from the total  $^{210}\text{Pb}$  (Moore and Poet, 1976; Nozaki et al., 1978).

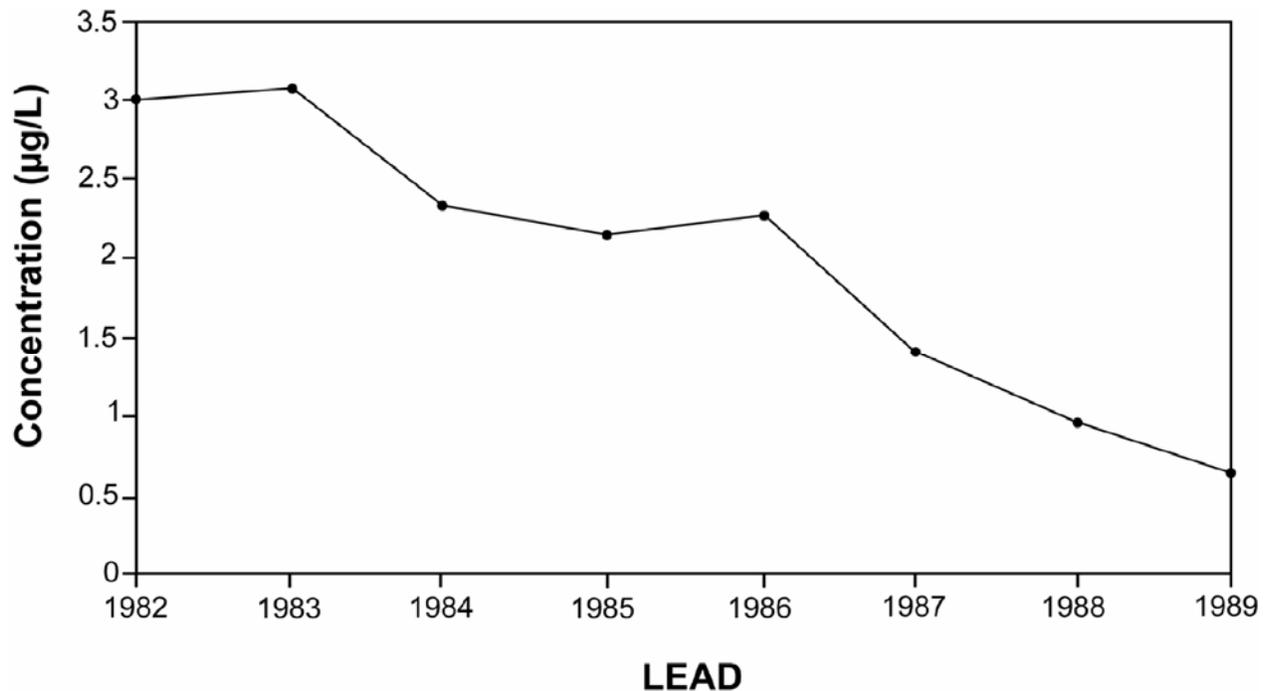
Benninger et al. (1975) measured fallout  $^{210}\text{Pb}$  in soils and streamwater at Hubbard Brook and at an undisturbed forest in Pennsylvania. They estimated atmospheric  $^{210}\text{Pb}$  export in streamwaters to be <0.02% of the standing  $^{210}\text{Pb}$  crop in the organic horizons. They used a simple steady-state model to calculate the residence time of Pb in the organic horizons to be 5,000 years. This overestimate of the Pb residence time in the organic horizons was likely a result of the low resolution of their sampling. Since they only sampled the upper 6 cm of soil and the drainage waters, they did not accurately evaluate the distribution of  $^{210}\text{Pb}$  in the soil column in between. Dörr and Münnich (1989, 1991) used  $^{210}\text{Pb}$  profiles in soils of southern Germany to evaluate the behavior of atmospherically delivered Pb. They calculated the vertical velocity of Pb by dividing the relaxation depth (i.e., the depth at which  $^{210}\text{Pb}$  activity decreases to  $1/e$ , or approximately 37% of its surface value) by the  $^{210}\text{Pb}$  mean life of 32 years. They reported downward transit velocities of atmospherically deposited Pb at  $0.89 \pm 0.33 \text{ mm year}^{-1}$ . The downward transport of atmospheric Pb was not affected by pH or soil type. However, since Pb velocities in the soil profile were identical to carbon velocities calculated with  $^{14}\text{C}$ , they concluded that Pb movement in forest soils is probably controlled by carbon transport. Kaste et al. (2003) used  $^{210}\text{Pb}$  to model the response time of atmospherically delivered Pb in the O horizon at Camel's Hump Mountain in Vermont. They concluded that the forest floor response time was between 60 and 150 years, depending on vegetation zone and elevation. Using  $^{206}\text{Pb}$ : $^{207}\text{Pb}$ , they also demonstrated that some gasoline-derived Pb migrated out of the O horizon and into the mineral soil in the deciduous vegetation zone on the mountain, while all of the atmospheric Pb was retained in the upper 20 cm of the soil profile. Klaminder et al. (2006) used  $^{210}\text{Pb}$  distributions to calculate the residence time of Pb in soils of Northern Sweden. They concluded that atmospheric Pb had a residence time of ~200 years in the mor layer of mature forests. They also provided evidence suggesting that forests in an earlier stage of ecological succession have a much shorter Pb residence time in the mor horizon (<50 years).

Researchers assessing the fate of atmospheric Pb in soils have also relied on repeated sampling of soils and vegetation for total Pb (e.g., Zhang, 2003). This technique works best when anthropogenic Pb accounts for the vast majority of total Pb in a particular reservoir. Johnson et al. (1995a), Yanai et al. (2004), and Friedland et al. (1992) used O horizon (forest

floor) time-series data to evaluate the movement of gasoline-derived Pb in the soil profile. These studies have concluded that the distribution of Pb in the upper soil horizons has changed over the past few decades. Yanai et al. (2004) documented a decline in Pb from the Oie horizon between the late 1970s to the early 1990s in remote forest soils in New Hampshire. Johnson et al. (1995a) and Friedland et al. (1992) demonstrated that some fraction of Pb had moved from the O horizon to the mineral soil during the 1980s at Hubbard Brook and at selected remote sites in the northeastern United States, respectively. Evans et al. (2005) demonstrated that Pb concentrations in the litter layer (fresh litter + Oi horizon) sampled in a transect from Vermont to Quebec decreased significantly between 1979 and 1996, reflecting a decrease in Pb deposition to forests and upper soil horizons during that time period. Miller et al. (1993) and Wang and Benoit (1997) used forest floor time-series data to model the response time ( $e$  folding time, the time it takes a reservoir to decrease to the  $1/e$ , (ca. 37%) of its original amount) of Pb in the forest floor. Miller et al. (1993) calculated O horizon response times of 17 years for the northern hardwood forest and 77 years in the spruce-fir zone on Camel's Hump Mountain in Vermont. Wang and Benoit (1997) determined that the O horizon would reach steady state with respect to Pb ( $1.3 \mu\text{g g}^{-1}$  Pb) by 2100. Both suggested that the vertical movement of organic particles dominated Pb transport in the soil profile.

### **AX7.1.2.3 Inputs/Outputs of Atmospherically-delivered Lead in Terrestrial Ecosystems**

The concentration of Pb in contemporary rainfall in the mid-Atlantic and northeastern United States is on the order of 0.5 to 1  $\mu\text{g/L}$  (Wang et al., 1995; Kim et al., 2000; Scudlark et al., 2005). Long-term trends in nine elemental concentrations (including Pb) in wet deposition were measured on the Delmarva Peninsula near Lewes, Delaware, over the period from 1982 to 1989 (Figure AX7-1.2.1) (Scudlark et al., 1994). Of the nine elements measured, only Pb (and possibly Al and Cd), indicated a decreasing trend over time (3  $\mu\text{g/L}$  in 1982 to <1.0  $\mu\text{g/L}$  in 1989). The authors attributed the dramatic decrease in Pb to the phasing out of Pb-alkyl additives from gasoline as mandated in the 1972 Clean Air Act. For comparison, rainfall measured in Los Angeles (CA) during 2003-2004 averaged 0.15  $\mu\text{g/L}$ , but showed nearly an order-of-magnitude variation, presumably because of the arid environmental (Sabin et al., 2005). See also Table 2-22 for additional rainfall information.



**Figure AX7-1.2.1. Long-term (1982-1989) annual trends in lead concentrations (µg/L) in Lewes, Delaware precipitation.**

Source: Scudlark et al. (1994)

The role of dry deposition in the total deposition of Pb to terrestrial ecosystems is not constrained well. Researchers have estimated that dry deposition accounts for anywhere between 10 to >90% of total Pb deposition (Galloway et al., 1982; Wu et al., 1994; Sabin et al., 2005). Migon et al. (1997) found total, wet, and dry Pb deposition to be 8.6, 1.6 and 7.0 µg/m<sup>2</sup>/d, respectively, over the Ligurian Sea, France. Dry deposition velocities were estimated at approximately 0.2 cm/sec, similar to anthropogenic element deposition velocities measured at remote lakes around Lake Michigan (Yi et al., 2001). Yi et al. (2001) found that dry deposition velocities for elements were correlated with particle concentrations in the following order: coarse > total particle > fine particle. Refer to Table 2-21 for additional information on dry, wet, and total Pb deposition velocities and fluxes.

Arid environments appear to have a much higher fraction of dry deposition: total deposition (Sabin et al., 2005). Furthermore, it is possible that Clean Air Act Legislation enacted in the late 1970s preferentially reduced Pb associated with fine particles, so the relative

contributions of dry deposition may have changed in the last few decades. If the major source of Pb to a terrestrial ecosystem is resuspended particulates from transportation corridors, then the particle size fraction that dominates deposition may be relatively coarse ( $>50 \mu\text{m}$ ) relative to other atmospheric sources (Pirrone et al., 1995; Sansalone et al., 2003).

Total contemporary loadings to terrestrial ecosystems are approximately  $1 \text{ to } 2 \text{ mg m}^{-2} \text{ year}^{-1}$  (Wu et al., 1994; Wang et al., 1995; Simonetti et al., 2000; Sabin et al., 2005). This is a relatively small annual flux of Pb if compared to the reservoir of approximately  $0.5 \text{ to } 4 \text{ g m}^{-2}$  of gasoline-derived Pb that is already in surface soils over much of the United States (Friedland et al., 1992; Miller and Friedland, 1994; Erel and Patterson, 1994; Marsh and Siccama, 1997; Yanai et al., 2004; Johnson et al., 2004; Evans et al., 2005). While vegetation can play an important role in sequestering Pb from rain and dry deposition (Russell et al., 1981), direct uptake of Pb from soils by plants appears to be low (Klaminder et al., 2005). High elevation areas, particularly those near the base level of clouds often have higher burdens of atmospheric contaminants (Siccama, 1974). A Pb deposition model by Miller and Friedland (1994) predicted  $2.2 \text{ and } 3.5 \text{ g Pb m}^{-2}$  deposition for the 20th century in the deciduous zone (600 m) and the coniferous zone (1000 m), respectively. More recently, Kaste et al. (2003) used radiogenic isotope measurements on the same mountain to confirm higher loadings at higher elevation. They measured  $1.3 \text{ and } 3.4 \text{ g gasoline-derived Pb m}^{-2}$  in the deciduous zone and coniferous zones, respectively. Higher atmospheric Pb loadings to higher elevations are attributed to (1) the higher leaf area of coniferous species, which are generally more prevalent at high elevation; (2) higher rainfall at higher elevation; and (3) increased cloudwater impaction at high elevation (Miller et al., 1993).

Although inputs of Pb to ecosystems are currently low, Pb export from watersheds via groundwater and streams is substantially lower than inputs. Therefore, even at current input levels, watersheds are accumulating industrial Pb. However, burial/movement of lead over time down into lower soil/sediment layers also tends to sequester it away from more biologically active parts of the watershed (unless later disturbed or redistributed, e.g., by flooding, dredging, etc.). Seeps and streams at the HBEF have Pb concentrations on the order of  $10 \text{ to } 30 \text{ pg Pb g}^{-1}$  (Wang et al., 1995). At a remote valley in the Sierra Nevada, Pb concentrations in streamwaters were on the order of  $15 \text{ pg Pb g}^{-1}$  (Erel and Patterson, 1994). Losses of Pb from soil horizons are assumed to be via particulates (Dörr and Münnich, 1989; Wang and Benoit, 1996, 1997). Tyler

(1981) noted that Pb losses from a spruce forest A-horizon soil in Sweden were influenced by season; with highest Pb fluxes being observed during warm, wet months. He suggested that DOC production and Pb movement were tightly linked.

Surface soils across the United States are enriched in Pb relative to levels expected from solely natural geogenic inputs (Friedland et al., 1984; Herrick and Friedland, 1990; Francek, 1992; Erel and Patterson, 1994; Marsh and Siccama, 1997; Yanai et al., 2004; Murray et al., 2004). While some of this contaminant Pb is attributed to paint, salvage yards, shooting ranges, and the use of Pb arsenate as a pesticide in localized areas (Francek, 1997), Pb contamination of surface soils is essentially ubiquitous because of atmospheric pollution associated with metal smelting and production, the combustion of fossil fuels, and waste incineration (Newhook et al., 2003; Polissar et al., 2001). Surface soils in Michigan, for example, typically range from 8 to several hundred ppm Pb (Francek, 1992; Murray et al., 2004). Soils collected and analyzed beneath 50 cm in Michigan, however, range only from 4 to 60 ppm Pb (Murray et al., 2004). In remote surface soils from the Sierra Nevada Mountains, litter and upper soil horizons are 20 to 40 ppm Pb, and approximately 75% of this Pb has been attributed to atmospheric deposition during the 20th century (Erel and Patterson, 1994). Repeated sampling of the forest floor (O horizon) in the northeastern United States demonstrates that the organic layer has retained much of the Pb load deposited on ecosystems during the 20th century. Total Pb deposition during the 20th century has been estimated at 1 to 3 g Pb m<sup>-2</sup>, depending on elevation and proximity to urban areas (Miller and Friedland, 1994; Johnson et al., 1995a). Forest floors sampled during the 1980s and 1990s, and in early 2000 had between 0.7 and 2 g Pb m<sup>-2</sup> (Friedland et al., 1992; Miller and Friedland, 1994; Johnson et al., 1995a; Kaste et al., 2003; Yanai et al., 2004; Evans et al., 2005). The pool of Pb in above- and below-ground biomass at the HBEF is on the order of 0.13 g Pb m<sup>-2</sup> (Johnson et al., 1995a).

The amount of Pb that has leached into mineral soil appears to be on the order of 20 to 50% of the total anthropogenic Pb deposition. Kaste et al. (2003) and Miller and Friedland (1994) demonstrated that Pb loss from the forest floor at Camel's Hump Mountain in Vermont depended on elevation. While the mineral soil in the deciduous forest had between 0.4 and 0.5 g Pb m<sup>-2</sup> (out of 1 to 2 g Pb m<sup>-2</sup> in the total soil profile), at higher elevations the thicker coniferous forest floor retained more than 90% of the total Pb deposition (Kaste et al., 2003). Johnson et al. (1995a) determined that the forest floor at HBEF in the mid-1980s had about 0.75 g Pb m<sup>-2</sup>.

Compared to their estimated 20th-century atmospheric Pb deposition of  $0.9 \text{ g Pb m}^{-2}$ , the forest floor has retained 83% of the atmospheric Pb loadings (Johnson et al., 1995a). Johnson et al. (2004) noted that gasoline-derived Pb was a significant component of the labile Pb at the HBEF. They calculated that Pb fluxes to the HBEF by atmospheric pollution were essentially equivalent to the Pb released by mineral weathering over the past 12,000 years. Marsh and Siccama (1997) used the relatively homogenous mineral soils underneath formerly plowed land in Rhode Island, New Hampshire and Connecticut to assess the depth-distribution of atmospheric Pb. They reported that 65% of the atmospheric Pb deposited during the 20th century is in the mineral soil and 35% is in the forest floor. At their remote study site in the Sierra Nevada Mountains, Erel and Patterson (1994) reported that most of the anthropogenic Pb was associated with the humus fraction of the litter layer and soils sampled in the upper few cm.

Atmospherically delivered Pb is probably present in ecosystems in a variety of different biogeochemical phases. A combination of Pb adsorption processes and the precipitation of Pb minerals will typically keep dissolved Pb species low in soil solution, surface waters, and streams (Sauvé et al., 2000a; Jackson et al., 2005). While experimental and theoretical evidence suggest that the precipitation of inorganic Pb phases and the adsorption of Pb on inorganic phases can control the biogeochemistry of contaminant Pb (Nriagu, 1974; Ruby et al., 1994; Jackson et al., 2005), the influence of organic matter on the biogeochemistry of Pb in terrestrial ecosystems cannot be ignored in many systems. Organic matter can bind to Pb, preventing Pb migration and the precipitation of inorganic phases (Manceau et al., 1996; Xia et al., 1997; Lang and Kaupenjohann, 2003). As the abundance of organic matter declines in soil, Pb adsorption to inorganic soil minerals and the direct precipitation of Pb phases may dominate the biogeochemistry of Pb in terrestrial ecosystems (Ostergren et al., 2000a,b; Sauvé et al., 2000a).

## **Conclusions**

Advances in technology since the 1986 Lead AQCD have allowed for a quantitative determination of the mobility, distribution, uptake, and fluxes of atmospherically delivered Pb in ecosystems. Among other things, these studies have shown that industrial Pb represents a significant fraction of total labile Pb in watersheds. Selective chemical extractions and synchrotron-based X-ray studies have shown that industrial Pb can be strongly sequestered by organic matter and by secondary minerals such as clays and oxides of Al, Fe, and Mn. Some of

these studies have provided compelling evidence that the biomineralization of Pb phosphates by soil organisms can play an important role in the biogeochemistry of Pb. Surface soils sampled relatively recently demonstrate that the upper soil horizons (O + A horizons) are retaining most of the industrial Pb burden introduced to the systems during the 20th century. The migration and biological uptake of Pb in ecosystems is relatively low. The different biogeochemical behaviors of Pb reported by various studies may be a result of the many different analytical techniques employed, or they may be a result of natural variability in the behavior of Pb in different systems.

### ***Lead Uptake into Plants***

Plants take up Pb via their foliage and through their root systems (U.S. Environmental Protection Agency, 1986a; Pålsson, 1989). Surface deposition of Pb onto plants may represent a significant contribution to the total Pb in and on the plant, as has been observed for plants near smelters and along roadsides (U.S. Environmental Protection Agency, 1986a). The importance of atmospheric deposition on above-ground plant Pb uptake is well-documented (Dalenberg and Van Driel, 1990; Jones and Johnston, 1991; Angelova et al., 2004). Data examined from experimental grassland plots in southeast England demonstrated that atmospheric Pb is a greater contributor than soil-derived Pb in crop plants and grasses (Jones and Johnston, 1991). A study by Dalenberg and Van Driel (1990) showed that 75 to 95% of the Pb found in field-grown test plants (i.e., the leafy material of grass, spinach, and carrot; wheat grain; and straw) was from atmospheric deposition. Angelova et al. (2004) found that tobacco grown in an industrial area accumulated significant amounts of Pb from the atmosphere, although uptake from soil was also observed. The concentration of Pb in tobacco seeds was linearly related to the concentration of Pb in the exchangeable and carbonate-bound fractions of soil, as measured using sequential extraction (Angelova et al., 2004). Lead in soil is more significant when considering uptake into root vegetables (e.g., carrot, potato), since, as was noted in the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a), most Pb remains in the roots of plants.

There are two possible mechanisms (symplastic or apoplastic) by which Pb may enter the root of a plant. The symplastic route is through the cell membranes of root hairs; this is the mechanism of uptake for water and nutrients. The apoplastic route is an extracellular route between epidermal cells into the intercellular spaces of the root cortex. Previously, Pb was

thought to enter the plant via the symplastic route, probably by transport mechanisms similar to those involved in the uptake of calcium or other divalent cations (i.e., transpirational mass flow, diffusion, or active transport). However, it also had been speculated that Pb may enter the plant via the apoplastic route (U.S. Environmental Protection Agency, 1986a). Sieghardt (1990) determined that the mechanism of Pb uptake was via the symplastic route only and that the apoplastic pathway of transport was stopped in the primary roots by the endodermis. He studied the uptake of Pb into two plants, *Minuartia verna* (moss sandwort) and *Silene vulgaris* (bladder campion) that colonize metal-contaminated sites. In the roots of both plants, Pb was found mainly in the root cortex. Active ion uptake was required to transport the Pb into the stele and then into the shoots of the plant (Sieghardt, 1990).

Although some plants translocate more Pb to the shoots than others, most Pb remains in the roots of plants. Two mechanisms have been proposed to account for this relative lack of translocation to the shoots: (1) Pb may be deposited within root cell wall material, or (2) Pb may be sequestered within root cell organelles (U.S. Environmental Protection Agency, 1986a). Pålsson (1989) noted that plants can accumulate large quantities of Pb from the soil but that translocation to shoots and leaves is limited by the binding of Pb ions at root surfaces and cell walls. In a study by Wierzbicka (1999), 21 different plant species were exposed to Pb<sup>2+</sup> in the form of Pb-chloride. The plant species included cucumber (*Cucumis sativus*), soy bean (*Soja hispida*), bean (*Phaseolus vulgaris*), rapeseed (*Brassica napus*), rye (*Secale cereale*), barley (*Hordeum vulgare*), wheat (*Triticum vulgare*), radish (*Raphanus sativus*), pea (*Pisum sativum*), maize (*Zea mays*), onion (*Allium cepa*), lupine (*Lupinus luteus*), bladder campion (*Silene vulgaris*), Buckler mustard (*Biscutella laevigata*), and rough hawkbit (*Leontodon hispidus*). Although, the amount of Pb taken up by the plant varied with species, over 90% of absorbed Pb was retained in the roots. Only a small amount of Pb was translocated (~2 to 4%) to the shoots of the plants. Lead in roots was present in the deeper layers of root tissues (in particular, the root cortex) and not only on the root surface. There was no correlation between Pb tolerance (measured as root mass increase expressed as a percentage of controls) and either root or shoot tissue concentrations (Wierzbicka, 1999). The study by Wierzbicka (1999) was the first to report that plants developing from bulbs, in this case the onion, were more tolerant to Pb than plants developing from seeds. This tolerance was assumed to be related to the large amounts of Pb that were transported from the roots and stored in the bulb of the plant (Wierzbicka, 1999).

Uptake of Pb from soil into plants was modeled as part of Eco-SSL development (U.S. Environmental Protection Agency, 2005a). The relationship derived between Pb in the soil and Pb in a plant was taken from Bechtel Jacobs Company (BJC) (1998) and is as follows:

$$\ln(C_p) = 0.561 * \ln(C_{soil}) - 1.328 \quad (AX7-1)$$

where  $C_p$  is the concentration of Pb in the plant (dry weight) and  $C_{soil}$  is the concentration of Pb in the soil. This equation recognizes that the ratio of Pb concentration in plant to Pb concentration in soil is not constant.

### ***Invertebrates***

There was no clear evidence suggesting a differential uptake of Pb into different species of earthworm (*Lumbricus terrestris*, *Aporrectodea rosea*, and *A. caliginosa*) collected around a smelter site near Avonmouth, England (Spurgeon and Hopkin, 1996a). This is in contrast to Pižl and Josens (1995) and Terhivuo et al. (1994) who found *Aporrectodea* spp. accumulated more Pb than *Lumbricus*. The authors suggested that these differences could be due to different feeding behaviors, as *Lumbricus* feeds on organic material and *Aporrectodea* species are geophagous, ingesting large amounts of soil during feeding. The differences between species also may be related to differing efficiencies in excretory mechanisms (Pižl and Josens, 1995). However, the interpretation of species difference is complicated by a number of potentially confounding variables, such as soil characteristics (e.g., calcium or other nutrient levels) (Pižl and Josens, 1995).

The bioaccumulation of Pb from contaminated soil was tested using the earthworm *Eisenia fetida*, and the amount of Pb accumulated did not change significantly until the concentration within soil reached 5000 mg/kg (Davies et al., 2003). This coincided with the lowest soil concentrations at which earthworm mortality was observed. The ratio of the concentration of Pb in worms to the concentration in soil decreased from 0.03 at 100 mg/kg to 0.001 at 3000 mg/kg, but then increased quickly to 0.02 at 5000 mg/kg. The authors concluded that earthworms exhibit regulated uptake of Pb at levels of low contamination (<3000 mg/kg) until a critical concentration is reached, at which point this mechanism breaks down, resulting in unregulated accumulation and mortality. This study was conducted using test methods where

soil was not allowed to equilibrate following the addition of Pb and prior to the addition of the test organisms. This may have resulted in an increased bioavailability and overestimated Pb toxicity relative to actual environmental conditions (Davies et al., 2003). See the discussion in Section AX7.1.2 on the effects of aging on Pb sorption processes.

Lock and Janssen (2002) and Bongers et al. (2004) found that Pb-nitrate was more toxic than Pb-chloride to survival and reproduction of the springtail *Folsomia candida*. However, percolation (removal of the chloride or nitrate counterion) caused a significant decrease in Pb-nitrate toxicity such that there was no difference in toxicity once the counterion was removed (Bongers et al., 2004). No change in toxicity was observed for Pb-chloride once the chloride was removed from the soil. Bongers et al. (2004) suggested that the nitrate ion was more toxic than the chloride ion to springtails.

Uptake of Pb from soil into earthworms was also modeled as part of Eco-SSL development (U.S. Environmental Protection Agency, 2005a). The relationship derived between Pb in the soil and Pb in an earthworm was taken from Sample et al. (1999) and is as follows:

$$\ln(C_{\text{worm}}) = 0.807 * \ln(C_{\text{soil}}) - 0.218 \quad (\text{AX7-2})$$

where  $C_{\text{worm}}$  is the concentration of Pb in the earthworm (dry weight) and  $C_{\text{soil}}$  is the concentration of Pb in the soil. This equation recognizes that the ratio of Pb concentration in worm to Pb concentration in soil is not constant.

### **Wildlife**

Research has been conducted to determine what Pb concentrations in various organs would be indicative of various levels of effects. For example, Franson (1996) compiled data to determine what residue levels were consistent with three levels of effects in Falconiformes (e.g., falcons, hawks, eagles, kestrels, ospreys), Columbiformes (e.g., doves, pigeons), and Galliformes (e.g., turkey, pheasant, partridge, quail, chickens). The three levels of effect were (1) subclinical, which are physiological effects only, such as the inhibition of  $\delta$ -aminolevulinic acid dehydratase (ALAD; see Section AX7.1.3.3); (2) toxic, a threshold level marking the initiation of clinical signs, such as anemia, lesions in tissues, weight loss, muscular incoordination, green diarrhea, and anorexia; and (3) compatible with death, an approximate threshold value associated with

death in field, captive, and/or experimental cases of Pb poisoning. The tissue Pb levels associated with these levels of effects are presented in Table AX7-1.2.1.

**Table AX7-1.2.1. Tissue Lead Levels in Birds Causing Effects  
(taken from Franson, 1996)**

<b>Order</b>	<b>Blood (µg/dL)</b>	<b>Liver (ppm wet wt.)</b>	<b>Kidney (ppm wet wt.)</b>
<i>Falconiformes</i>			
Subclinical	0.2 – 1.5	2 – 4	2 – 5
Toxic	>1	>3	>3
Compatible with death	>5	>5	>5
<i>Columbiformes</i>			
Subclinical	0.2 – 2.5	2 – 6	2 – 20
Toxic	>2	>6	>15
Compatible with death	>10	>20	>40
<i>Galliformes</i>			
Subclinical	0.2 – 3	2 – 6	2 – 20
Toxic	>5	>6	>15
Compatible with death	>10	>15	>50

Tissue residue levels below the subclinical levels in Table AX7-1.2.1 should be considered “background” (Franson, 1996). Levels in the subclinical range are indicative of potential injury from which the bird would probably recover if Pb exposure was terminated. Toxic residues could lead to death. Residues above the compatible-with-death threshold are consistent with Pb-poisoning mortality (Franson, 1996). Additional information on residue levels for Passeriformes (e.g., sparrows, starlings, robins, cowbirds), Charadriiformes (e.g., gulls, terns), Gruiformes (e.g., cranes), Ciconiformes (e.g., egrets), Gaviformes (e.g., loons), and Strigiformes (e.g., owls) is available in Franson (1996). Scheuhammer (1989) found blood Pb concentrations of between 0.18 and 0.65 µg/mL in mallards corresponded to conditions associated with greater than normal exposure to Pb but that should not be considered Pb poisoning.

Lead concentrations in various tissues of mammals also have been correlated with toxicity (Ma, 1996). The tissues commonly analysed for Pb are blood, liver, and kidney. Typical baseline levels of blood Pb are approximately 4 to 8 µg/dL for small mammals, and 2 to 6 µg/dL for mature cattle. Typical baseline levels of Pb in liver are 1 to 2 mg/kg dw for small mammals. Typical baseline levels of Pb in kidney are 0.2 to 1.5 mg/kg dw for mice and voles, but shrews typically have higher baseline levels of 3 to 19 mg/kg dw. Ma (1996) concluded that Pb levels less than 5 mg/kg dw in liver and 10 mg/kg dw in kidney were not associated with toxicity, but that levels greater than 5 mg/kg dw in liver and greater than 15 mg/kg dw in kidney could be taken as a chemical biomarker of toxic exposure to Pb in mammals. Humphreys (1991) noted that the concentrations of Pb in liver and kidney can be elevated in animals with normal blood Pb concentrations (and without exhibiting clinical signs of Pb toxicity), because Pb persists in these organs longer than in blood.

Uptake of Pb from soil into small mammals was also modeled as part of Eco-SSL development (U.S. Environmental Protection Agency, 2005a). The relationship derived between Pb in the soil and Pb in the whole-body of a small mammal was taken from Sample et al. (1998) and is as follows:

$$\text{Ln}(C_{\text{mammal}}) = 0.4422 * \text{Ln}(C_{\text{soil}}) + 0.0761 \quad (\text{AX7-3})$$

Where  $C_{\text{mammal}}$  is the concentration of Pb in small mammals (dry weight) and  $C_{\text{soil}}$  is the concentration of Pb in the soil. Similar to the uptake equations for plants (Eq. 8-1) and earthworms (Eq 8-2), the equation for mammalian uptake recognizes that the ratio of Pb concentration in small mammals to Pb concentration in soil is not constant.

#### **AX7.1.2.4 Resistance Mechanisms**

Many mechanisms related to heavy metal tolerance in plants and invertebrates have been described, including avoidance (i.e., root redistribution, food rejection), exclusion (i.e., selective uptake and translocation), immobilization at the plant cell wall, and excretion (i.e., foliar leakage, moulting) (Tyler et al., 1989; Patra et al., 2004). The following section reviews the recent literature on the resistance mechanisms of plants and invertebrates through mitigation of Pb (1) toxicity or (2) exposure.

### ***Detoxification Mechanisms***

Lead sequestration in cell walls may be the most important detoxification mechanism in plants. Calcium may play a role in this detoxification by regulating internal Pb concentrations through the formation of Pb-containing precipitates in the cell wall (Antosiewicz, 2005). Yang et al. (2000) screened 229 varieties of rice (*Oryza sativa*) for tolerance or sensitivity to Pb and found that the oxalate content in the root and root exudates was increased in Pb-tolerant varieties. The authors suggested that the oxalate reduced Pb bioavailability, and that this was an important tolerance mechanism (Yang et al., 2000). Sharma et al. (2004) found Pb-sulfur and Pb-sulfate in the leaves, and Pb-sulfur in the roots of *Sesbania drummondii* (Rattlebox Drummond), a Pb hyperaccumulator plant grown in Pb-nitrate solution. They hypothesized that these sulfur ligands were indicative of glutathione and phytochelatin, which play a role in heavy metal homeostasis and detoxification (Sharma et al., 2004).

Sea pinks (*Armeria maritima*) grown on a metal-contaminated site (calamine spoils more than 100 years old) accumulated 6H the concentrations of Pb in brown (dead and withering) leaves than green leaves (Szarek-Lukaszewska et al., 2004). The concentration of Pb in brown leaves was similar to that in roots. This greater accumulation of Pb into older leaves was not observed in plants grown hydroponically in the laboratory. The authors hypothesized that this sequestering of Pb into the oldest leaves was a detoxification mechanism (Szarek-Lukaszewska et al., 2004).

Terrestrial invertebrates also mitigate Pb toxicity. Wilczek et al. (2004) studied two species of spider, the web-building *Agelena labyrinthica*, and the active hunter wolf spider *Pardosa lugubris*. The activity of metal detoxifying enzymes (via the glutathione metabolism pathways) was greater in *A. labyrinthica* and in females of both species (Wilczek et al., 2004).

Marinussen et al. (1997) found that earthworms can excrete 60% of accumulated Pb very quickly once exposure to Pb-contaminated soils has ended. However, the remainder of the body burden is not excreted, possibly due to the storage of Pb in waste nodules that are too large to be excreted (Hopkin, 1989). Gintenreiter et al. (1993) found that Lepidoptera larvae (in this case, the gypsy moth *Lymantria dispar*) eliminated Pb, to some extent, in the meconium (the fluid excreted shortly after emergence from the chrysalis).

Lead, in the form of pyromorphite ( $\text{Pb}_5(\text{PO}_4)_3\text{Cl}$ ), was localized in the anterior pharynx region of the nematode *Ceanorhabditis elegans* (Jackson et al., 2005). The authors hypothesized

that the nematode may detoxify Pb via its precipitation into pyromorphite, which is relatively insoluble (Jackson et al., 2005).

### ***Avoidance Response***

Studies with soil invertebrates hypothesize that these organisms may avoid soil with high Pb concentrations. For example, Bengtsson et al. (1986) suggested that the lower Pb concentrations in earthworm tissues may be a result of lowered feeding activity of worms at higher Pb concentrations in soil.

### **AX7.1.2.5 Physiological Effects of Lead**

Several studies have measured decreased blood ALAD activity in birds and mammals exposed to Pb (U.S. Environmental Protection Agency, 1986a). Recent studies on the physiological effects of Pb to consumers have focused on heme synthesis (as measured by ALAD activity and protoporphyrin concentration), lipid peroxidation, and production of fatty acids. Effects on growth are covered in Section AX7.1.4.

Biochemically, Pb adversely affects hemoglobin synthesis in birds and mammals. Early indicators of Pb exposure in birds and mammals include decreased blood ALAD concentrations and increased protoporphyrin IX activity. The effects of Pb on blood parameters and the use of these parameters as sensitive biomarkers of exposure has been well documented (Eisler, 1988; U.S. Environmental Protection Agency, 2005b). However, the linkage between these biochemical indicators and ecologically relevant effects is less well understood. Low-level inhibition of ALAD is not generally considered a toxic response, because this enzyme is thought to be present in excess concentrations; rather, it may simply indicate that the organism has recently been exposed to Pb (Henny et al., 1991).

Schlick et al. (1983) studied ALAD inhibition in mouse bone marrow and erythrocytes. They estimated that an absorbed dose of between 50 and 100 µg Pb-acetate/kg body weight per day would result in long-term inhibition of ALAD.

Beyer et al. (2000) related blood Pb to sublethal effects in waterfowl along the Coeur d'Alene River near a mining site in Idaho. The sublethal effects measured included, among others, red blood cell ALAD activity and protoporphyrin levels in the blood. As found in other studies, ALAD activity was the most sensitive indicator of Pb exposure, decreasing to 3%

of the reference value at a blood Pb concentration of 0.68 mg/kg ww (wet weight).

Protoporphyrin concentrations showed a 4.2-fold increase at this same concentration.

Henny et al. (1991) studied osprey along the Coeur d'Alene River. There were no observations of death, behavioral abnormalities, or reduced productivity related to Pb exposure, although inhibition of blood ALAD and increased protoporphyrin concentrations were measured in ospreys. Henny et al. (1991) hypothesized that no impacts to osprey were observed, even though swan mortality was documented in the area because swans feed at a lower trophic level (i.e., Pb does not biomagnify, and thus is found at higher concentrations in lower trophic level organisms).

Hoffman et al. (2000a) also studied the effects of Coeur d'Alene River sediment on waterfowl, focusing on mallard ducklings for 6 weeks after hatching. The study revealed that a 90% reduction in ALAD activity and a greater than 3-fold increase in protoporphyrin concentration occurred when blood Pb reached a concentration of 1.41 mg/kg ww as a result of the ducklings being fed a diet composed of 12% sediment (3449 mg/kg Pb). Those ducklings fed a diet composed of 24% sediment were found to have a mean blood Pb concentration of 2.56 mg/kg ww and a greater than 6-fold increase in protoporphyrin concentration. Hoffman et al. (2000b) also studied Canada Geese (*Branta canadensis*) goslings in a similar fashion. The results revealed that, while blood Pb concentrations in goslings were approximately half (0.68 mg/kg ww) of those found in ducklings under the same conditions (12% diet of 3449 mg/kg sediment Pb), goslings showed an increased sensitivity to Pb exposure. Goslings experienced a 90% reduction in ALAD activity and a 4-fold increase in protoporphyrin concentration, similar to conditions found in the ducklings, although blood Pb concentrations were half those found in the ducklings. More serious effects were seen in the goslings when blood Pb reached 2.52 mg/kg, including decreased growth and mortality.

Redig et al. (1991) reported a hawk LOAEL (lowest observed adverse effect level) of 0.82 mg/kg-day for effects on heme biosynthetic pathways. Lead dosages as high as 1.64 to 6.55 mg/kg-day caused neither mortality nor clinical signs of toxicity. A dose of 6.55 mg/kg-day resulted in blood Pb levels of 1.58 µg/mL. There were minimal changes in immune function (Redig et al., 1991).

Repeated oral administration of Pb resulted in biochemical alterations in broiler chickens (Brar et al., 1997a,b). At a dose of 200 mg/kg-day Pb-acetate, there were significant increases in

plasma levels of uric acid and creatinine and significant declines in the levels of total proteins, albumin, glucose, and cholesterol. Brar et al. (1997a) suggested that increased uric acid and creatinine levels could be due to an accelerated rate of protein catabolism and/or kidney damage. They also suggested that the decline in plasma proteins and albumin levels may be caused by diarrhea and liver dysfunction due to the Pb exposure. Brar et al. (1997b) also found that significant changes in plasma enzymes may be causing damage to other organs.

Lead can cause an increase in tissue lipid peroxides and changes in glutathione concentrations, which may be related to peroxidative damage of cell membranes (Mateo and Hoffman, 2001). There are species-specific differences in resistance to oxidative stress (lipid peroxidation), which may explain why Canada geese are more sensitive to Pb poisoning than mallards (Mateo and Hoffman, 2001). Lead also caused an increase in the production of the fatty acid arachidonic acid, which has been associated with changes in bone formation and immune response (Mateo et al., 2003a). The effects observed by Mateo et al. (2003a,b) were associated with very high concentrations of Pb in the diet (1840 mg Pb/kg diet), much higher than would be found generally in the environment, and high enough that birds decreased their food intake.

Lead also induces lipid peroxidation in plants. Rice plants exposed to a highly toxic level of Pb (1000  $\mu$ M in nutrient solution) showed elevated levels of lipid peroxides, increased activity of superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase (Verma and Dubey, 2003). The elevated levels of these enzymes suggest the plants may have an antioxidative defense mechanism against oxidative injury caused by Pb (Verma and Dubey, 2003).

#### **AX7.1.2.6 Factors that Modify Organism Response**

Research has demonstrated that Pb may affect survival, reproduction, growth, metabolism, and development in a wide range of species. These effects may be modified by chemical, biological, and physical factors. The factors that modify responses of organisms to Pb are described in the following sections.

##### ***Genetics***

Uptake and toxicity of Pb to plants are influenced strongly by the type of plant. Liu et al. (2003) found that Pb uptake and translocation by rice plants differed by cultivar (a cultivated

variety of plant produced by selective breeding) but was not related to genotype. Twenty cultivars were tested from three genotypes. The differences in Pb concentrations among cultivars were smallest when comparing concentrations in the grains at the ripening stage. This study also found that toxicity varied by cultivar; at 800 mg Pb/kg soil, some cultivars were greatly inhibited, some were significantly improved, and others showed no change.

Dearth et al. (2004) compared the response of Fisher 344 (F344) rats and Sprague-Dawley (SD) rats to exposure via gavage to 12 mg Pb/mL as Pb-acetate. Blood Pb levels in the F344 dams were higher than those of the SD dams. Lead delayed the timing of puberty and suppressed hormone levels in F344 offspring. These effects were not observed in the offspring of SD rats, even when the dose was doubled. The authors conclude that F344 rats are more sensitive to Pb (Dearth et al., 2004).

### ***Biological Factors***

Several biological factors may influence Pb uptake and organism response, including organism age, sex, species, feeding guild, and, for plants, the presence of mycorrhizal fungi. Monogastric animals are more sensitive to Pb than ruminants (Humphreys, 1991).

Younger organisms may be more susceptible to Pb toxicity (Eisler, 1988; Humphreys, 1991). Nestlings are more sensitive to the effects of Pb than older birds, and young altricial birds (species unable to self-regulate body heat at birth, such as songbirds), are considered more sensitive than precocial birds (species that have a high degree of independence at birth, such as quail, ducks, and poultry) (Scheuhammer, 1991).

Gender can also have an effect on the accumulation of Pb by wildlife (Eisler, 1988). Female birds accumulate more Pb than males (Scheuhammer, 1987; Tejedor and Gonzalez, 1992). These and other authors have related this to the increased requirement for calcium in laying females.

Different types of invertebrates accumulate different amounts of Pb from the environment (U.S. Environmental Protection Agency, 1986a). There may be species- and sex-specific differences in accumulation of Pb into invertebrates, specifically arthropods. This has been shown by Wilczek et al. (2004) who studied two species of spider, the web-building *A. labyrinthica* and the active hunter wolf spider *P. lugubris*. The body burdens of Pb in the wolf spider were higher than in the web-building spider, and this may be due to the more

effective use of glutathione metabolism pathways in *A. labyrinthica*. Body burdens of females were lower than those of males in both species. This was also observed in spiders by Rabitsch (1995a). Females are thought to be able to detoxify and excrete excess metals more effectively than males (Wilczek et al., 2004). Lead accumulation has been measured in numerous species of arthropods with different feeding strategies. Differences were observed between species (Janssen and Hogervorst, 1993; Rabitsch, 1995a) and depending upon sex (Rabitsch, 1995a), developmental stage (Gintenreiter et al., 1993; Rabitsch, 1995a), and season (Rabitsch, 1995a).

Uptake of Pb may be enhanced by symbiotic associations between plant roots and mycorrhizal fungi. Similar to the mechanism associated with increased uptake of nutrients, mycorrhizal fungi also may cause an increase in the uptake of Pb by increasing the surface area of the roots, the ability of the root to absorb particular ions, and the transfer of ions through the soil (U.S. Environmental Protection Agency, 1986a). There have been contradictory results published in the literature regarding the influence of mycorrhizal organisms on the uptake and toxicity of Pb to plants (see review in Pålsson, 1989). Lin et al. (2004) found that the bioavailability of Pb increased in the rhizosphere of rice plants, although the availability varied with Pb concentration in soil. Bioavailability was measured as the soluble plus exchangeable Pb fraction from sequential extraction analysis. The authors hypothesized that the enhanced solubility of Pb may be due to a reduced pH in the rhizosphere or, more likely, the greater availability of organic ligands, which further stimulates microbial growth (Lin et al., 2004). Increased bioavailability of Pb in soil may increase the uptake of Pb into plants, although this was not assessed by Lin et al. (2004). However, Dixon (1988) found that red oak (*Quercus rubra*) seedlings with abundant ectomycorrhizae had lower Pb concentrations in their roots than those seedlings without this fungus, although only at the 100 mg Pb/kg sandy loam soil concentration (no differences were found at lower Pb concentrations). Lead in soil also was found to be toxic to the ectomycorrhizal fungi after 16 weeks of exposure to 50 mg Pb/kg or more (Dixon, 1988). Malcova and Gryndler (2003) showed that maize root exudates from mycorrhizal fungi can ameliorate heavy metal toxicity until a threshold metal concentration was surpassed. This may explain the conflicting results in the past regarding the uptake and toxicity of Pb to plants with mycorrhizal fungi.

The type of food eaten is a major determinant of Pb body burdens in small mammals, with insectivorous animals accumulating more Pb than herbivores or granivores (U.S. Environmental

Protection Agency, 1986a). In fact, the main issue identified by the EPA (U.S. Environmental Protection Agency, 1986a) related to invertebrate uptake of Pb was not toxicity to the invertebrates, but accumulation of Pb to levels that may be toxic to their consumers. Several authors suggest that shrews are a good indicator of metal contamination, because they tend to accumulate higher levels of metals than herbivorous small mammals (see data summary in Sample et al. (1998)). Shrews accumulate higher levels of metals in contaminated habitats, because their diet mainly consists of detritivores (i.e., earthworms) and other soil invertebrates in direct contact with the soil (Beyer et al., 1985).

### ***Physical/Environmental Factors***

#### ***Plants***

The uptake and distribution of Pb into higher plants from the soil is affected by various chemical and physical factors including the chemical form of Pb, the presence of other metal ions, soil type, soil pH, cation exchange capacity (CEC), the amount of Fe/Mn-oxide films present, organic matter content, temperature, light, and nutrient availability. A small fraction of Pb in soil may be released into the soil water, which is then available to be taken up by plants (U.S. Environmental Protection Agency, 1986a).

The form of Pb has an influence on its toxicity to plants. For example, Pb-oxide is less toxic than more bioavailable forms such as Pb-chloride or Pb-acetate. In a study by Khan and Frankland (1983), radish plants were exposed to Pb-oxide and Pb-chloride in a loamy sand at pH 5.4, in a 42-day study. In a tested concentration range of 0 to 5000 mg/kg, root growth was inhibited by 24% at 500 mg/kg for Pb-chloride and an EC<sub>50</sub> of 2400 mg/kg was calculated from a dose-response curve. Plant growth ceased at 5000 mg/kg and shoots exhibited an EC<sub>50</sub> of 2800 mg/kg. For Pb-oxide exposure (concentration range of 0 to 10,000 mg/kg), reported results indicate an EC<sub>50</sub> of 12,000 mg/kg for shoot growth and an EC<sub>50</sub> of 10,000 mg/kg for root growth. There was no effect on root growth at 500 mg/kg and a 26% reduction at 1000 mg/kg Pb oxide.

Soil pH is the most influential soil property with respect to uptake and accumulation of Pb into plant species. This is most likely due to increased bioavailability of Pb created by low soil pH. At low soil pH conditions, markedly elevated Pb toxicity was reported for red spruce (*P. rubens*) (Seiler and Paganelli, 1987). At a soil pH of 4.5, ryegrass (*Lolium hybridum*)

and oats (*Avena sativa*) had significantly higher Pb concentrations after 3 months of growth compared to plants grown at pH 6.4 (Allinson and Dzialo, 1981).

### *Invertebrates*

The uptake of Pb into invertebrates depends on the physical environment and parameters such as pH, calcium concentration, organic matter content, and CEC. Greater accumulation is found generally when the soil pH or organic content is lower (U.S. Environmental Protection Agency, 1986a).

Soil pH has a significant influence on uptake of Pb into invertebrates. Perämäki et al. (1992) studied the influence of soil pH on uptake into the earthworm *Aporrectodea caliginosa*. Lead accumulation was lowest at the highest pH values, but there was no statistical difference due to variability in the data. Variability in the response also was found by Bengtsson et al. (1986), who reared earthworms (*Dendrobaena rubida*) in acidified soils at pH 4.5, 5.5, or 6.5. Lead uptake into worms was pH-dependent, although the highest concentrations were not always found at the lowest pH. There was no clear relationship between Pb concentration in cocoons and soil pH, and Pb concentrations were higher in the hatchlings than in the cocoons. As has been reported in many other studies (Neuhauser et al., 1995), concentration factors (ratio of Pb in worm to Pb in soil) were lower at higher Pb concentrations in soil. The authors attribute some of this to a lowered feeding activity in worms at higher Pb concentrations (Bengtsson et al., 1986).

Beyer et al. (1987) and Morgan and Morgan (1988) recognized that other factors beyond soil pH could influence the uptake of Pb into earthworms, which may be the cause of the inconsistencies reported by several authors. Both studies evaluated worm uptake of Pb relative to pH, soil calcium concentration, and organic matter content. Morgan and Morgan (1988) also considered CEC, and Beyer et al. (1987) considered concentrations of phosphorus, potassium, or magnesium in soil. Both studies found that calcium concentrations in soil were correlated with soil pH. Morgan and Morgan (1988) also found that CEC was correlated with percentage organic matter. Soil pH (coupled with CEC) and soil calcium were found to play significant roles in the uptake of Pb into worms (Beyer et al., 1987; Morgan and Morgan, 1988). Beyer et al. (1987) noted that concentrations of phosphorus in soil had no effect.

### ***Nutritional Factors***

Diet is a significant modifier of Pb absorption and of toxic effects in many species of birds and mammals (Eisler, 1988). Dietary deficiencies in calcium, zinc, iron, vitamin E, copper, thiamin, phosphorus, magnesium, fat, protein, minerals, and ascorbic acid increased Pb absorption and its toxic effects (Eisler, 1988).

Mateo et al. (2003b) studied intraspecies sensitivity to Pb-induced oxidative stress, by varying the vitamin E content of mallard diets. Vitamin E can protect against peroxidative damage and was found to decrease the lipid peroxidation in nerves of birds; however, it did not alleviate any sign of the Pb poisoning. The authors hypothesize that inhibition of antioxidant enzymes and interaction with sulfhydryl groups of proteins may have a greater influence on Pb toxicity than lipid peroxidation (Mateo et al., 2003b). The effects observed by Mateo et al. (2003b) were associated with very high concentrations of Pb in diet (1840 mg Pb/kg diet), much higher than would be found generally in the environment, and high enough that the birds decreased their food intake.

Mallard ducklings were exposed to Pb-contaminated sediment and either a low nutrition or optimal nutrition diet (Douglas-Stroebel et al., 2005). Lead exposure combined with a nutritionally inferior diet caused more changes in behavior (as measured by time bathing, resting, and feeding) than Pb exposure or low-nutrition diet alone. These effects may be due to the low-nutrition diet being deficient in levels of protein, amino acids, calcium, zinc, and other nutrients.

Zebra finches (*Taeniopygia guttata*) were exposed to Pb-acetate via drinking water at 20 mg/L for 38 days, along with either a low- or high-calcium diet (Snoeijs et al., 2005). Lead uptake into tissues was enhanced by a low-calcium diet. Lead did not affect body weight, hematocrit, or adrenal stress response. Lead suppressed the humoral immune response only in females on a low-calcium diet, suggesting increased susceptibility of females to Pb (Snoeijs et al., 2005).

### ***Interactions with Other Pollutants***

Lead can interact with other pollutants to exert toxicity in an antagonistic (less than additive), independent, additive, or synergistic (more than additive) manner. Concurrent exposure to Pb and additional pollutant(s) can affect the ability of plants to uptake Pb or the other pollutant. However, the uptake and toxic response of plants exposed to Pb combined with

other metals is inconsistent (Påhlsson, 1989). Therefore, no generalizations can be made about the relative toxicity of metal mixtures. For example, An et al. (2004) conducted acute, 5-day bioassays on cucumber exposed to Pb, Pb + copper, Pb + cadmium, or Pb + copper + cadmium in a sandy loam soil of pH 4.3. Shoot and root growth were measured. Depending on the tissue and metal combination, additivity, synergism, or antagonism was observed in the responses to these metals. In fact, the response in roots was not consistent with the response in shoots for the binary mixtures. However, the combined effects were greater in the roots than the shoots, which may be explained by the tendency for Pb and other heavy metals to be retained in the roots of plants. In addition, the pattern of metal bioaccumulation into plant tissue did not always correlate with the toxic response. However, antagonism was observed in the response of roots and shoots exposed to all three metals, and this was reflected in the decreased accumulation of metals into plant tissues. The authors hypothesized that this may be due to the formation of less bioavailable metal complexes (An et al., 2004).

He et al. (2004) found that selenium and zinc both inhibited the uptake of Pb into Chinese cabbage (*Brassica rapa*) and lettuce (*Lactuca sativa*). Zinc applied at 100 mg/kg or selenium applied at 1 mg/kg decreased the uptake of Pb (present in soil at 10 mg/kg as Pb-nitrate) into lettuce by 15% and 20%, respectively, and into Chinese cabbage by 23 and 20%, respectively.

Selenium compounds were evaluated to determine whether they could change the inhibition of ALAD in liver, kidney, or brain of mice exposed to Pb-acetate (Perottoni et al., 2005). Selenium did not affect the inhibition of ALAD in the kidney or liver, but it did reverse the ALAD inhibition in mouse brain.

Co-occurrence of cadmium with Pb resulted in reduced blood Pb concentrations in rats (Garcia and Corredor, 2004). The authors hypothesized that cadmium may block or antagonize the intestinal absorption of Pb, or the metallothionein induced by cadmium may sequester Pb. However, this was not observed in pigs, where blood Pb concentrations were greater when cadmium was also administered (Phillips et al., 2003). The effect on growth rate also was additive when both metals were given to young pigs (Phillips et al., 2003).

### **AX7.1.2.7 Summary**

The current document expands upon and updates knowledge related to the uptake, detoxification, physiological effects, and modifying factors of Pb toxicity to terrestrial organisms.

#### ***Surface Deposition onto Plants***

Recent work (Dalenberg and Van Driel, 1990; Jones and Johnston, 1991; Angelova et al., 2004) has supported previous results and conclusions that surface deposition of Pb onto above-ground vegetation from airborne sources may be significant (U.S. Environmental Protection Agency, 1986a). Similarly, it has been well documented previously that Pb in soil also is taken up by plants, although most remains in the roots, there is little translocation to shoots, leaves, or other plant parts (U.S. Environmental Protection Agency, 1986a). More recent work continues to support this finding (Sieghardt, 1990), and one study found increased tolerance in species with bulbs, possibly due to the storage of Pb in the bulb (Wierzbicka, 1999).

#### ***Uptake Mechanism into Plants***

Lead was thought previously to be taken up by plants via the symplastic route (through cell membranes), although it was unknown whether some Pb also may be taken up via the apoplastic route (between cells) (U.S. Environmental Protection Agency, 1986a). Recent work has shown that the apoplastic route of transport is stopped in the primary roots by the endodermis (Sieghardt, 1990), supporting the previous conclusion that the symplastic route is the most significant route of transport into plant cells.

#### ***Species Differences in Uptake into Earthworms***

Different species of earthworm accumulated different amounts of Pb, and this was not related to feeding strategy (U.S. Environmental Protection Agency, 1986a). This is supported by recent work, which has shown *Aporrectodea* accumulated more than *Lumbricus* (Terhivuo et al., 1994; Pižl and Josens, 1995), although this is not consistently observed (Spurgeon and Hopkin, 1996a).

### ***Speciation and Form of Lead***

Recent work supports previous conclusions that the form of metal tested, and its speciation in soil, influence uptake and toxicity to plants and invertebrates (U.S. Environmental Protection Agency, 1986a). The oxide form is less toxic than the chloride or acetate forms, which are less toxic than the nitrate form of Pb (Khan and Frankland, 1983; Lock and Janssen, 2002; Bongers et al., 2004). However, these results must be interpreted with caution, as the counterion (e.g., the nitrate ion) may be contributing to the observed toxicity (Bongers et al., 2004).

### ***Detoxification in Plants***

Lead may be deposited in root cell walls as a detoxification mechanism (U.S. Environmental Protection Agency, 1986a), and this may be influenced by calcium concentrations (Antosiewicz, 2005). Yang et al. (2000) suggested that the oxalate content in root and root exudates reduced the bioavailability of Pb in soil, and that this was an important tolerance mechanism. Other hypotheses put forward recently include the presence of sulfur ligands (Sharma et al., 2004) and the sequestration of Pb in old leaves (Szarek-Lukaszewska et al., 2004) as detoxification mechanisms.

### ***Detoxification in Invertebrates***

Lead detoxification has not been studied extensively in invertebrates. Glutathione detoxification enzymes were measured in two species of spider (Wilczek et al., 2004). Lead may be stored in waste nodules in earthworms (Hopkin, 1989) or as pyromorphite in the nematode (Jackson et al., 2005).

### ***Physiological Effects***

The effects on heme synthesis (as measured by ALAD activity and protoporphyrin concentration, primarily) have been well-documented (U.S. Environmental Protection Agency, 1986a) and continue to be studied (Schlick et al., 1983; Scheuhammer, 1989; Henny et al., 1991; Redig et al., 1991; Beyer et al., 2000; Hoffman et al., 2000a,b). However, Henny et al. (1991) caution that changes in ALAD and other enzyme parameters are not always related to adverse effects, but simply indicate exposure. Other effects on plasma enzymes, which may damage

other organs, have been reported (Brar et al., 1997a,b). Lead also may cause lipid peroxidation (Mateo and Hoffman, 2001), which may be alleviated by vitamin E, although Pb poisoning may still result (Mateo et al., 2003b). Changes in fatty acid production have been reported, which may influence immune response and bone formation (Mateo et al., 2003a).

### ***Response Modification***

Genetics, biological factors, physical/environmental factors, nutritional factors, and other pollutants can modify terrestrial organism response to Pb. Fisher 344 rats were found to be more sensitive to Pb than Sprague-Dawley rats (Dearth et al., 2004). Younger animals are more sensitive than older animals (Eisler, 1988; Scheuhammer, 1991), and females generally are more sensitive than males (Scheuhammer, 1987; Tejedor and Gonzalez, 1992; Snoeijs et al., 2005). Monogastric animals are more sensitive than ruminants (Humphreys, 1991). Insectivorous mammals may be more exposed to Pb than herbivores (Beyer et al., 1985; Sample et al., 1998), and higher trophic-level consumers may be less exposed than lower trophic-level organisms (Henny et al., 1991). Diets deficient in nutrients (including calcium) result in increased uptake of Pb (Snoeijs et al., 2005) and greater toxicity (Douglas-Stroebel et al., 2005) in birds, relative to diets containing adequate nutrient levels.

Mycorrhizal fungi may ameliorate Pb toxicity until a threshold is surpassed (Malcova and Gryndler, 2003), which may explain why some studies show increased uptake into plants (Lin et al., 2004) while others show no difference or less uptake (Dixon, 1988). Uptake of Pb into plants and soil invertebrates increases with a decrease in soil pH. However, calcium content, organic matter content, and cation exchange capacity of soils also have had a significant influence on uptake of Pb into plants and invertebrates (Beyer et al., 1987; Morgan and Morgan, 1988).

Interactions of Pb with other metals are inconsistent, depending on the endpoint measured, the tissue analyzed, the animal species, and the metal combination (Phillips et al., 2003; An et al., 2004; He et al., 2004; Garcia and Corredor, 2004; Perottoni et al., 2005).

### **AX7.1.3 Exposure-Response of Terrestrial Species**

Section AX7.1.3 summarized the most important factors related to uptake of Pb by terrestrial organisms, the physiological effects of Pb, and the factors that modify terrestrial

organism responses to Pb. Section AX7.1.4 outlines and highlights the critical recent advancements in the understanding of the toxicity of Pb to terrestrial organisms. This section begins with a summary of the conclusions from the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) and then summarizes the more recent critical research conducted on effects of Pb on primary producers, consumers, and decomposers. All concentrations are expressed as mg Pb/kg soil dw, unless otherwise indicated.

Lead exposure may adversely affect organisms at different levels of organization, i.e., individual organisms, populations, communities, or ecosystems. Generally, however, 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). Potential effects at the population level or higher are, of necessity, extrapolated from individual level studies. Available population, community, or ecosystem level studies are typically conducted at sites that have been contaminated or adversely affected by multiple stressors (several chemicals alone or combined with physical or biological stressors). Therefore, the best documented links between lead and effects on the environment are with effects on individual organisms. Impacts on terrestrial ecosystems are discussed in Section 7.1.5 and Annex AX7.1.5.

The summary of recent critical advancements in understanding toxicity relies heavily on the work completed by a multi-stakeholder group, consisting of federal, state, consulting, industry, and academic participants, led by the EPA to develop Ecological Soil Screening Levels (Eco-SSLs). Eco-SSLs describe the concentrations of contaminants in soils that would result in little or no measurable effect on ecological receptors (U.S. Environmental Protection Agency, 2005a). They were developed by the U.S. EPA for use in screening-level assessments at Superfund sites to identify contaminants requiring further evaluation in an ecological risk assessment and were not designed to be used as cleanup target levels. The Eco-SSLs are intentionally conservative in order to provide confidence that contaminants, which could present an unacceptable risk, are not screened out early in the evaluation process. That is, at or below these levels, adverse effects are considered unlikely. Eco-SSLs were derived for terrestrial plants, soil invertebrates, birds, and mammals. Detailed procedures using an extensive list of acceptability and exclusion criteria (U.S. Environmental Protection Agency, 2005a) were used in screening the toxicity studies to ensure that only those that met minimum quality standards were used to develop the Eco-SSLs. In addition, two peer reviews were completed during the

Eco-SSL development process. The first was a consultation with the EPA Science Advisory Board (SAB) in April 1999, and the second was a peer review workshop in July 2000, which was open to the public.

Several conservative factors were incorporated into the development of the Eco-SSLs. For the plant and invertebrate Eco-SSLs, studies were scored to favor relatively high bioavailability. For wildlife Eco-SSLs, only species with a clear exposure link to soil were considered (generalist species, species with a link to the aquatic environment, or species which consume aerial insects were excluded), simple diet classifications were used (100% plants, 100% earthworms or 100% animal prey) when in reality wildlife consume a varied diet, species were assumed to forage exclusively at the contaminated site, relative bioavailability or Pb in soil and diet was assumed to be 1, and the TRV was selected as the geometric mean of NOAELs unless this value was higher than the lowest bounded LOAEL for mortality, growth or reproduction (U.S. Environmental Protection Agency, 2005a,b).

Areas of research that were not addressed are effects from irrelevant exposure conditions relative to airborne emissions of Pb (e.g., Pb shot, Pb paint, injection studies, studies conducted on mine tailings, and studies conducted with hydroponic solutions); mixture toxicity (addressed in Section AX7.1.3); issues related to indirect effects (e.g., effects on predator/prey interactions, habitat alteration, etc.); and human health-related research (e.g., hypertension), which is addressed in other sections of this document.

The toxicity data presented herein should be reviewed with a note of caution regarding their relevance to field conditions. Laboratory studies, particularly those using Pb-spiked soil, generally do not allow the soil to equilibrate following the addition of Pb and prior to the addition of test organisms. This may result in increased bioavailability and overestimation of Pb toxicity relative to actual environmental conditions (Davies et al., 2003).

#### **AX7.1.3.1 Summary of Conclusions from the 1986 Lead Criteria Document**

The 1996 Lead AQCD, Volume II (U.S. Environmental Protection Agency, 1986a) reviewed the literature on the toxicity of Pb to plants, soil organisms, birds, and mammals. The main conclusions from that document are provided below.

### ***Primary Producers***

Commonly reported effects of Pb on vascular plants include the inhibition of photosynthesis, respiration, and/or cell elongation, all of which reduce plant growth. However, it was noted that studies of other effects on plant processes such as maintenance, flowering, and hormone development had not been conducted; therefore, no conclusion could be reached concerning effects of Pb on these processes.

The EPA (U.S. Environmental Protection Agency, 1986a) concluded that most plants experience reduced growth when Pb concentrations in soil moisture (the film of moisture surrounding soil particles in the root zone of soil) exceed 2 to 10 mg/kg. It also was concluded that most plants would experience reduced growth (inhibition of photosynthesis, respiration, or cell elongation) in soils of  $\geq 10,000$  mg/kg when soil composition and pH are such that bioavailability of Pb in the soil is low (see Section AX7.1.3 for details on factors affecting bioavailability of Pb in soil). Acid soils or soils with low organic matter tend to increase Pb bioavailability and would inhibit plants at much lower Pb concentrations (e.g., as low as <100 mg/kg).

Many effect levels have been reported at Pb concentrations much lower than 10,000 mg/kg soil. For example, effects on rye grass (*Lolium rigidum*) exposed to Pb in soil included inhibition of germinating root elongation (at <2.5 mg/kg), absence of root growth (at 5 mg/kg), or 55% inhibition of seed germination (at 20 to 40 mg/kg). Stunted growth in radish (*Raphanus sativus*) was observed at 1000 mg/kg soil, with complete growth inhibition at 5000 mg/kg, when Pb was added as Pb-chloride; effects were less severe when the Pb was added as Pb-oxide.

### ***Consumers***

The EPA (U.S. Environmental Protection Agency, 1986a) concluded that food is the largest contributor of Pb to animals, with inhalation rarely accounting for more than 10 to 15% of daily intake of Pb and drinking water exposures being quite low. It also was concluded that a regular dose of 2 to 8 mg/kg-day causes death in most animals. Grazing animals may consume more than 1 mg/kg-day in habitats near smelters and roadsides, but no toxic effects were documented in these animals.

### ***Decomposers***

Lack of decomposition has been observed as a particular problem around smelter sites. Lead concentrations between 10,000 and 40,000 mg/kg soil can eliminate populations of decomposer bacteria and fungi (U.S. Environmental Protection Agency, 1986a). Lead may affect decomposition processes by direct toxicity to specific groups of decomposers, by deactivating enzymes excreted by decomposers to break down organic matter, or by binding with the organic matter and rendering it resistant to the action of decomposers.

Microorganisms are more sensitive than plants to Pb in soil. Delayed decomposition may occur at between 750 and 7500 mg/kg soil (depending on soil type and other conditions). Nitrification is inhibited by 14% at 1000 mg/kg soil.

### ***U.S. Environmental Protection Agency Staff Review of 1986 Criteria Document***

The EPA reviewed the 1986 Lead AQCD and presented an overall summary of conclusions and recommendations (U.S. Environmental Protection Agency, 1990). The major conclusion was that available laboratory and field data indicated that high concentrations of Pb can affect certain plants and alter the composition of soil microbial communities. It was noted that few field studies were available in which Pb exposures and associated effects in wildlife were reported.

#### **AX7.1.3.2 Recent Studies on the Effects of Lead on Primary Producers**

Several studies published since 1986 have reported terrestrial plant exposure to Pb in soil, many of which were reviewed during the development of the Eco-SSLs (U.S. Environmental Protection Agency, 2005b). The relevant information from the Eco-SSL document (U.S. Environmental Protection Agency, 2005b) is summarized below. A literature search and review also was conducted to identify critical papers published since 2002, which is when the literature search was completed for Eco-SSL development, and no new papers were identified as critical to the understanding of Pb toxicity to terrestrial primary producers.

Effects observed in studies conducted since the 1986 Lead AQCD are similar to those reported previously and include decreased photosynthetic and transpiration rates and decreased growth and yield (U.S. Environmental Protection Agency, 2005b). The phytotoxicity of Pb is considered relatively low, due to the limited availability and uptake of Pb from soil and soil

solution and minimal translocation of Pb from roots to shoots (Påhlsson, 1989). Although many laboratory toxicity studies have reported effects on plants, there are few reports of phytotoxicity from Pb exposure under field conditions. For example, Leita et al. (1989) and Sieghardt (1990) reported high concentrations of Pb and other metals in soil and vegetation collected around mining areas in Europe, with no toxicity symptoms observed in plants or fruit.

The literature search completed for the terrestrial plant Eco-SSL development identified 439 papers for detailed review, of which 28 met the minimum criteria (U.S. Environmental Protection Agency, 2005a). Thirty ecotoxicological endpoints were gleaned from these 28 papers and were further evaluated; most of those evaluated growth (biomass), which was considered the most sensitive and ecologically relevant endpoint (U.S. Environmental Protection Agency, 2005b). Five of the endpoints, representing four species tested under three different combinations of pH and organic matter content, were used to develop the Eco-SSL of 120 mg/kg (115 mg/kg rounded to two significant digits) (Table AX7-1.3.1).

**Table AX7-1.3.1. Plant Toxicity Data Used to Develop the Eco-SSL**

<b>Plant Species</b>	<b>Soil pH</b>	<b>% Organic Matter</b>	<b>Toxicity Parameter</b>	<b>Pb in Soil (mg/kg dw)</b>
Loblolly pine ( <i>Pinus taeda</i> )	4	2.5	MATC* (growth)	144
Red maple ( <i>Acer rubrum</i> )	4	2.5	MATC (growth)	144
Berseem clover ( <i>Trifolium alexandrium</i> )	6.3	0.94	MATC (growth)	316
Berseem clover	6.7	3.11	MATC (growth)	141
Rye grass ( <i>Lolium rigidum</i> )	5.6	0.1	MATC (growth)	22
			Geometric Mean	115

\*MATC = Maximum Acceptable Threshold Concentration, or the geometric mean of the NOAEC (no-observed-adverse-effect concentration) and LOAEC (lowest-observed-adverse-effect concentration).

Source: U.S. Environmental Protection Agency (2005b).

The 25 ecotoxicological endpoints that were not used to develop the Eco-SSL for plants are presented in Table AX7-1.3.2. The first six endpoints were considered eligible for Eco-SSL derivation but were not used; the remainder did not meet all of the requirements to be considered for inclusion in the Eco-SSL derivation process.

**Table AX7-1.3.2. Plant Toxicity Data Not Used to Develop the Eco-SSL**

<b>Plant Species</b>	<b>Soil pH</b>	<b>% Organic Matter</b>	<b>Toxicity Parameter</b>	<b>Pb in Soil (mg/kg dw)</b>
<b>Studies eligible for Eco-SSL derivation, but not used</b>				
Berseem clover ( <i>Trifolium alexandrinum</i> )	6.7	3.11	MATC	141
Tomato ( <i>Lycopersicon esculentum</i> )	7.73	1.70	MATC	71
Tomato	8.20	0.86	MATC	71
Fenugreek ( <i>Trigonella foenum-graecum</i> )	8.3	0.5	MATC	283
Spinach ( <i>Spinacea oleracea</i> )	6.7	3.0	MATC	424
Corn ( <i>Zea mays</i> )	6.5	2.1	MATC	158
Sow thistle ( <i>Sonchus oleraceus</i> )	7.23	1.6	MATC	2,263
<b>Studies not eligible for Eco-SSL derivation</b>				
Loblolly pine ( <i>Pinus taeda</i> )	5.5	3.4	NOAEC	480
Red oak ( <i>Quercus rubra</i> )	6	1.5	LOAEC	100
Spinach	6.7	0.0	NOAEC	600
Alfalfa ( <i>Medicago sativa</i> )	6.4	1.0	NOAEC	250
Alfalfa	6.9	1.7	NOAEC	250
Alfalfa	6.9	1.7	NOAEC	250
Radish ( <i>Raphanus sativus</i> )	6.9	1.0	LOAEC	500
Radish	6.9	1.0	LOAEC	100
Radish	6.9	1.0	LOAEC	100
Onion ( <i>Allium cepa</i> )	8.3	0.5	LOAEC	50
Radish	5.1	8.0	NOAEC	600
Carrot ( <i>Daucus carota</i> )	7.0	0.6	NOAEC	85
Peas ( <i>Pisum sativum</i> )	7.0	0.6	NOAEC	85
Barley ( <i>Hordeum vulgare</i> )	6.0	2.5	NOAEC	1,000
Alfalfa	6.9	4.8	NOAEC	250
Tomato	7.45	2.06	MATC	35
Spinach	6.7	8.0	NOAEC	600
Radish	6.2	8.0	NOAEC	600
Radish	7.1	8.0	NOAEC	600

\*MATC = Maximum Acceptable Threshold Concentration, or the geometric mean of the NOAEC (no-observed-adverse-effect concentration) and LOAEC (lowest-observed-adverse-effect concentration).

Source: U.S. Environmental Protection Agency (2005b).

### AX7.1.3.3 Recent Studies on the Effects of Lead on Consumers

Since the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a), there have been several studies in which birds and mammals were exposed to Pb via ingestion (primarily through dietary Pb). Many of these were reviewed during development of the Eco-SSLs (U.S. Environmental Protection Agency, 2005b). The relevant information from the Eco-SSL document (U.S. Environmental Protection Agency, 2005b) is described below. A literature search and review was conducted to identify critical papers published since 2002. These recent critical papers are described briefly below. No studies were found that used inhalation exposures to evaluate endpoints such as survival, growth, and reproduction in birds or mammals. All studies described below exposed organisms via ingestion (drinking water or diet) or gavage.

The Eco-SSLs for avian and mammalian consumers are presented as Pb concentrations in soil. These concentrations were calculated by assuming exposure to Pb via incidental soil ingestion and ingestion of Pb-contaminated food, and using a NOAEL as the TRV (U.S. Environmental Protection Agency, 2005a). A simplified version of the equation used to calculate the Eco-SSL is:

$$HQ = \frac{[(C_{soil} \times IR_{soil}) + (C_{food} \times IR_{food})] \div BW}{TRV} \quad (AX7-4)$$

where:

- HQ = hazard quotient (1 mg Pb/kg bw/day)
- $C_{soil}$  = concentration of Pb in soil (mg Pb/kg soil)
- $IR_{soil}$  = incidental soil ingestion rate (kg soil/day)
- $C_{food}$  = concentration of Pb in food (mg Pb/kg food)
- $IR_{food}$  = food ingestion rate (kg food/day)
- BW = body weight (kg)
- TRV = toxicity reference value (mg Pb/kg bw/day)

Food ingestion was estimated by modeling the uptake of Pb from soil into each diet component (e.g., vegetation, invertebrates, etc.). Bioavailability of Pb in soil and food was assumed to be 100%. The Eco-SSL is equivalent to the concentration of Pb in soil that results in an HQ = 1. The two factors that may have the most significant influence on the resulting Eco-SSL are the assumption of 100% bioavailability of Pb in soil and diet and the selection of the

TRV. The toxicity data that were reviewed to develop the TRV are presented in the following subsections.

Representative avian and mammalian wildlife species were selected for modeling Pb exposures to wildlife with different diets and calculating the Eco-SSL. The avian species selected were dove (herbivore), woodcock (insectivore), and hawk (carnivore). The mammalian species selected were vole (herbivore), shrew (insectivore), and weasel (carnivore). The lowest of the three back-calculated soil concentrations, which resulted in an HQ = 1, was selected as the Eco-SSL. For Pb, the lowest values were for the insectivorous species of bird and mammal.

### ***Avian Consumers***

Effects on birds observed in studies conducted since the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) are similar to those reported previously: mortality, changes in juvenile growth rate and weight gain, effects on various reproductive measures, and changes in behavior (U.S. Environmental Protection Agency, 2005b). Reproductive effects following Pb exposure included declines in clutch size, number of young hatched, and number of young fledged as well as decreased fertility or eggshell thickness. Few significant reproductive effects have been reported in birds at Pb concentrations below 100 mg/kg in the diet (Scheuhammer, 1987).

The literature search completed for Eco-SSL development identified 2,429 papers for detailed review for either avian or mammalian species, of which 54 met the minimum criteria for further consideration for avian Eco-SSL development (U.S. Environmental Protection Agency, 2005b). The 106 toxicological data points for birds that were further evaluated included biochemical, behavioral, physiological, pathological, reproductive, growth, and survival effects. Growth and reproduction data were used to derive the Eco-SSL (Table AX7-1.3.3; Figure AX7-1.3.1). The geometric mean of the NOAELs was calculated as 10.9 mg/kg-day, which was higher than the lowest bounded LOAEL (the term “bounded” means that both a NOAEL and LOAEL were obtained from the same study). Therefore, the highest bounded NOAEL that was lower than the lowest bounded LOAEL for survival, growth, or reproduction (1.63 mg Pb/kg bw-day) was used as the TRV (U.S. Environmental Protection Agency, 2005b). The TRV was used to back-calculate the Eco-SSL of 11 mg/kg soil for avian species (U.S.

**Table AX7-1.3.3. Avian Toxicity Data Used to Develop the Eco-SSL**

Avian Species	No. of Doses	Route of Exposure	Exposure Duration	Duration Units	Age	Age Units	Lifestage	Sex	Effect Type	Effect Measure	Response Site	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
<b>Reproduction</b>													
Japanese quail	4	FD	5	w	6	w	LB	F	REP	PROG	WO	0.194	1.94
Chicken	3	FD	4	w	NR	NR	LB	F	REP	PROG	WO	1.63	3.26
Chicken	4	FD	30	d	22	w	LB	F	EGG	ALWT	EG	2.69	4.04
Mallard	2	FD	76	d	NR	NR	SM	F	EGG	ESTH	EG	5.63	
American kestrel	3	FD	6	mo	1-6	yr	AD	F	REP	RSUC	WO	12.0	
Japanese quail	5	FD	5	w	6	d	JV	M	REP	TEWT	TE	12.6	126
Japanese quail	5	FD	5	w	1	d	JV	M	REP	TEWT	TE	67.4	135
Japanese quail	3	FD	32	d	NR	NR	AD	F	REP	PROG	WO	125	
Japanese quail	5	FD	12	w	0	d	LB	B	REP	EGPN	EG		0.110
Japanese quail	4	FD	12	w	NR	NR	LB	F	REP	PROG	WO		0.194
Chicken	5	FD	10	w	NR	NR	LB	F	REP	PROG	WO		3.26
Ringed turtle dove	2	DR	11	w	NR	NR	AD	M	REP	TEWT	TE		11.8
Japanese quail	2	FD	1	w	14	w	JV	F	REP	TPRD	WO		93.1
Japanese quail	2	FD	27	d	NR	NR	AD	F	REP	PROG	WO		377

AX7-66

**Table AX7-1.3.3 (cont'd). Avian Toxicity Data Used to Develop the Eco-SSL**

Avian Species	No. of Doses	Route of Exposure	Exposure Duration	Duration Units	Age	Age Units	Lifestage	Sex	Effect Type	Effect Measure	Response Site	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
<b>Growth</b>													
Japanese quail	3	FD	5	w	1	d	JV	F	GRO	BDWT	WO	1.56	15.6
Japanese quail	3	FD	2	w	1	d	JV	B	GRO	BDWT	WO	2.77	
Japanese quail	2	FD	2	w	1	d	JV	NR	GRO	BDWT	WO	4.64	
Japanese quail	3	FD	4	w	0	d	JV	F	GRO	BDWT	WO	5.93	59.3
Chicken	4	FD	4	w	4	w	JV	NR	GRO	BDWT	WO	6.14	61.4
Chicken	4	FD	4	w	4	w	JV	NR	GRO	BDWT	WO	7.10	71.0
Japanese quail	5	FD	12	w	0	d	JV	F	GRO	BDWT	WO	11.1	111
Japanese quail	5	FD	12	w	1	w	JV	F	GRO	BDWT	WO	11.2	112
Japanese quail	5	FD	2	w	6	d	JV	NR	GRO	BDWT	WO	12.6	126
Japanese quail	5	FD	1	w	1	d	JV	NR	GRO	BDWT	WO	13.5	67.4
Chicken	2	FD	21	d	1	d	JV	B	GRO	BDWT	WO	14.2	
Duck	3	GV	3	mo	24	w	MA	F	GRO	BDWT	WO	20.0	
American kestrel	4	GV	10	d	1	d	JV	NR	GRO	BDWT	WO	25.0	125
Chicken	2	FD	20	d	1	d	JV	B	GRO	BDWT	WO	28.4	
Japanese quail	5	FD	14	d	1	d	JV	B	GRO	BDWT	WO	34.5	

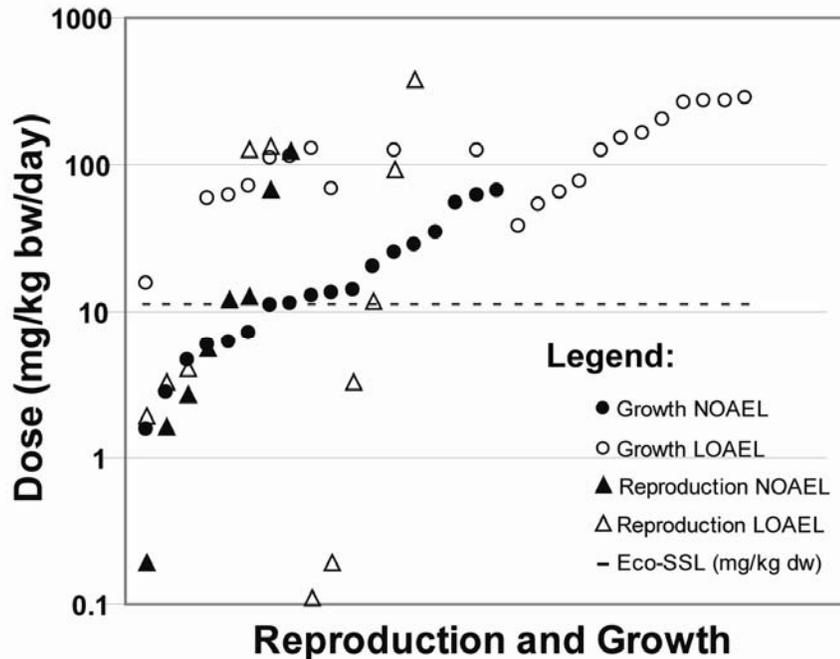
AX7-67

**Table AX7-1.3.3 (cont'd). Avian Toxicity Data Used to Develop the Eco-SSL**

Avian Species	No. of Doses	Route of Exposure	Exposure Duration	Duration Units	Age	Age Units	Lifestage	Sex	Effect Type	Effect Measure	Response Site	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
American kestrel	4	FD	60	d	1-2	yr	AD	B	GRO	BDWT	WO	54.3	
Chicken	5	FD	2	w	1	d	JV	M	GRO	BDWT	WO	61.3	123
Mallard	4	FD	8	d	9	d	JV	NR	GRO	BDWT	WO	66.9	
Chicken	5	FD	20	d	1	d	JV	M	GRO	BDWT	WO		38.2
Chicken	2	FD	3	w	1	d	JV	M	GRO	BDWT	WO		53.1
Japanese quail	3	FD	32	d	NR	NR	AD	F	GRO	BDWT	WO		64.3
Chicken	2	FD	19	d	1	d	JV	M	GRO	BDWT	WO		76.3
Chicken	3	FD	2	w	1	d	JV	M	GRO	BDWT	WO		124
Chicken	4	FD	14	d	8	d	JV	M	GRO	BDWT	WO		152
Chicken	2	FD	20	d	1	d	JV	M	GRO	BDWT	WO		163
Chicken	2	OR	4	w	NR	NR	JV	B	GRO	BDWT	WO		200
Chicken	2	FD	7	d	1	d	JV	M	GRO	BDWT	WO		262
Chicken	2	FD	2	w	1	d	JV	M	GRO	BDWT	WO		270
Chicken	2	FD	7	d	1	d	IM	NR	GRO	BDWT	WO		273
Chicken	2	FD	14	d	8	d	JV	M	GRO	BDWT	WO		282

AD = adult; ALWT = albumin weight; B = both; BDWT = body weight changes; d = days; DR = drinking water; EG = egg; EGG = effects on eggs; EGP = egg production; ESTH = eggshell thinning; F = female; FD = food; GRO = growth; GV = gavage; JV = juvenile; LB = laying bird; MA = mature; M = male; mo = months; NR = not reported; OR = other oral; PROG = progeny counts or numbers; REP = reproduction; RSUC = reproductive success; SM = sexually mature; TE = testes; TEWT = testes weight; TPRD = total production; w = weeks; WO = whole organism; yr = years.

Source: U.S. Environmental Protection Agency (2005b)



**Figure AX7-1.3.1. Avian reproduction and growth toxicity data considered in development of the Eco-SSL.**

Source: U.S. Environmental Protection Agency (2005b).

Environmental Protection Agency, 2005b). For more information on the rationale for selecting TRVs, please refer to U.S. Environmental Protection Agency (2003).

Many of the toxicity data presented in the Eco-SSL document (U.S. Environmental Protection Agency, 2005b) are lower than those discussed in the 1986 Lead AQCD. The TRV and resulting Eco-SSL were derived using many conservative assumptions. For example, the EPA (U.S. Environmental Protection Agency, 2005b) recognizes that toxicity is observed over a wide range of doses (<1 to >100 mg Pb/kg bw/day), even when considering only reproductive effects in the same species. In addition, the TRV of 1.63 mg/kg-day is lower than most of the reported doses that have been associated with measured effects. This is true for not only survival, growth, and reproductive effects but also biochemical, behavioral, physiological, and pathological effects, which generally are observed at lower concentrations than effects on growth or reproduction. In addition, the Eco-SSL was back-calculated using conservative modeling assumptions. Therefore, the Eco-SSL of 11 mg/kg may be considered a conservative value.

Very little research has been done to expand the knowledge of the toxicity of Pb to birds since the Eco-SSL work was done. However, several studies have been conducted on waterfowl. Toxicity data for waterfowl (in particular, mallards) were included in the soil Eco-SSL development process (Table AX7-1.3.3), although mallards may be more exposed to contaminants in sediment than soil. Effects on waterfowl may vary depending on the form of Pb, characteristics of the sediment, the foraging strategy of the species (which may vary during reproduction), and the nutritional status of the animal. Sediment is recognized as an important route of exposure for waterfowl, particularly those species that dabble (i.e., forage on invertebrates in the sediment) (Beyer et al., 2000; Douglas-Stroebel et al., 2005). Douglas-Stroebel et al. (2005) found that mallard ducklings exposed to Pb-contaminated sediment and a low nutrition diet exhibited more changes in behavior (as measured by time bathing, resting, and feeding) than Pb exposure or low nutrition exposure alone. These effects may be due to the low nutrition diet being deficient in levels of protein, amino acids, calcium, zinc, and other nutrients.

Beyer et al. (2000) related blood Pb to sublethal effects in waterfowl along the Coeur d'Alene River near a mining site in Idaho. The authors suggested that 0.20 mg/kg ww blood Pb represents the no-effect level. This no-effect blood concentration corresponds to a sediment Pb concentration of 24 mg/kg. A sediment concentration of 530 mg/kg, associated with a blood Pb concentration of 0.68 mg/kg ww, is suggested to be the lowest-effect concentration. These results are consistent with those of Scheuhammer (1989) who found blood Pb concentrations of 0.18 µg/mL to 0.65 µg/mL in mallards corresponded to conditions associated with greater than normal exposure to Pb, but that that should not be considered Pb poisoning. The study by Beyer et al. (2000) related blood Pb to waterfowl mortality and concluded that some swan mortality may occur at blood Pb levels of 1.9 mg/kg ww, corresponding to a sediment Pb concentration of 1800 mg/kg. Using the mean blood level of 3.6 mg/kg ww from all moribund swans in the study, it was predicted that half of the swans consuming sediment at the 90th percentile rate would die with chronic exposure to sediment concentrations of 3600 mg/kg.

### ***Mammalian Consumers***

Effects on mammals observed in studies conducted since the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) are similar to those reported previously: mortality, effects on reproduction, developmental effects, and changes in growth (U.S. Environmental

Protection Agency, 2005b). Very little research has been done to expand the knowledge of the toxicity of Pb to mammalian wildlife, since the Eco-SSL work was done. Most studies conducted on mammals use laboratory animals to study potential adverse effects of concern for humans, and such studies are summarized in other sections of this document.

Of the 2,429 papers identified in the literature search for Eco-SSL development, 219 met the minimum criteria for further consideration for mammalian Eco-SSL development (U.S. Environmental Protection Agency, 2005b). The 343 ecotoxicological endpoints for mammals that were further evaluated included biochemical, behavioral, physiological, pathological, reproductive, growth, and survival effects. Growth and reproduction data were used to derive the Eco-SSL (Table AX7-1.3.4, Figure AX7-1.3.2). The geometric mean of the NOAELs was calculated as 40.7 mg/kg-day, which was higher than the lowest bounded LOAEL for survival, growth, or reproduction. Therefore, the highest bounded NOAEL that was lower than the lowest bounded LOAEL for survival, growth, or reproduction (4.7 mg Pb/kg bw-day) was used as the TRV (U.S. Environmental Protection Agency, 2005b). The TRV was used to back-calculate the Eco-SSL of 56 mg/kg soil (U.S. Environmental Protection Agency, 2005b). For more information on the rationale for selecting TRVs, please refer to U.S. Environmental Protection Agency (2003).

A review of the data presented in the Eco-SSL document (U.S. Environmental Protection Agency, 2005b) reveals that effects on survival generally are observed at Pb doses much greater than those reported in the 1986 Lead AQCD, where it was concluded that most animals would die when consuming a regular dose of 2 to 8 mg Pb/kg bw-day (U.S. Environmental Protection Agency, 1986a). However, the data presented in the Eco-SSL document (U.S. Environmental Protection Agency, 2005b) generally do not support this. While five studies reported decreased survival at these levels, 34 other studies reported no mortality or a LOAEL for mortality at significantly higher doses (U.S. Environmental Protection Agency, 2005b). The five studies that supported this low toxic level were conducted on three species (mouse, rat, and cow) and used either gavage or drinking water as the exposure method. The 34 other studies included data on these three species as well as five other species (rabbit, dog, pig, hamster, and shrew) and included gavage and drinking water as well as food ingestion exposure methods. The NOAELs for survival ranged from 3.5 to 3200 mg/kg-day (U.S. Environmental Protection Agency, 2005b). Therefore, the review of data in the Eco-SSL document suggests effects on survival of

**Table AX7-1.3.4. Mammalian Toxicity Data Used to Develop the Eco-SSL**

<b>Mammalian Species</b>	<b>No. of Doses</b>	<b>Route of Exposure</b>	<b>Exposure Duration</b>	<b>Duration Units</b>	<b>Age</b>	<b>Age Units</b>	<b>Lifestage</b>	<b>Sex</b>	<b>Effect Type</b>	<b>Effect Measure</b>	<b>Response Site</b>	<b>NOAEL (mg/kg bw/day)</b>	<b>LOAEL (mg/kg bw/day)</b>
<b>Reproduction</b>													
Rat	5	DR	62	d	21	d	GE	F	REP	PRWT	WO	0.71	7.00
Rat	6	DR	21	d	NR	NR	GE	F	REP	PRWT	WO	1.00	5.00
Rat	3	DR	35	d	NR	NR	AD	M	REP	RSUC	WO	2.60	26.0
Rat	4	DR	62	d	21	d	GE	B	REP	PRWT	WO	3.00	6.0
Sheep	3	FD	27	w	NR	NR	GE	F	REP	RSUC	WO	4.50	—
Rat	6	DR	21	d	NR	NR	GE	F	REP	PRWT	WO	5.00	10.0
Guinea pig	3	DR	40	d	NR	NR	GE	F	REP	PRWT	WO	5.50	—
Rat	5	FD	92	w	21	d	JV	M	REP	TEWT	TE	7.50	74.9
Rat	4	DR	23.8	d	21	d	LC	F	REP	Other	WO	8.90	
Rat	5	DR	23.8	d	21	d	GE	F	REP	Other	WO	9.10	45.0
Cotton rat	3	DR	7	w	NR	NR	AD	M	REP	RHIS	RT	12.4	170
Rat	4	GV	9	w	10	w	JV	M	REP	SPCV	TE	18.0	180
Rat	3	DR	100	d	21	d	GE	F	REP	PRWT	WO	25.4	—
Rat	2	FD	35	d	70	d	LC	F	REP	PRWT	WO	27.5	—
Rat	4	DR	60	d	NR	NR	SM	M	REP	TEWT	TE	31.6	63.2
Rat	4	DR	56	d	70	d	LC	F	REP	PROG	WO	32.5	—
Rat	3	DR	31	d	NR	NR	LC	F	REP	PRWT	WO	33.3	111
Rat	4	GV	41	d	NR	NR	GE	F	REP	PRWT	WO	41.0	54.6
Rat	5	DR	1	w	94	d	JV	M	REP	SPCL	SM	47.3	82.0
Rat	4	DR	30	d	NR	NR	SM	M	REP	Other	SV	56.0	285

AX7-72

**Table AX7-1.3.4 (cont'd). Mammalian Toxicity Data Used to Develop the Eco-SSL**

<b>Mammalian Species</b>	<b>No. of Doses</b>	<b>Route of Exposure</b>	<b>Exposure Duration</b>	<b>Duration Units</b>	<b>Age</b>	<b>Age Units</b>	<b>Lifestage</b>	<b>Sex</b>	<b>Effect Type</b>	<b>Effect Measure</b>	<b>Response Site</b>	<b>NOAEL (mg/kg bw/day)</b>	<b>LOAEL (mg/kg bw/day)</b>
Hamster	2	DR	51	d	15	w	GE	F	REP	PROG	WO	64.8	—
Hamster	2	DR	14	d	11	w	GE	F	REP	PROG	WO	64.9	—
Rat	4	DR	37	d	NR	NR	GE	F	REP	PRWT	WO	90.1	270
Rat	5	GV	12	d	NR	NR	GE	F	REP	RSEM	EM	100	150
Rat	3	DR	68	d	25	d	GE	F	REP	PRWT	WO	115	—
Rat	4	DR	77	d	25	d	GE	F	REP	PRWT	WO	116	—
Rat	2	DR	21	d	NR	NR	LC	F	REP	PRWT	WO	120	—
Mouse	3	FD	8	w	2	mo	GE	M	REP	SPCV	TE	144	1,440
Mouse	7	FD	30	d	NR	NR	LC	F	REP	PRWT	WO	202	506
Mouse	7	FD	30	d	NR	NR	LC	F	REP	PRWT	WO	202	506
Rat	4	DR	21	d	NR	NR	GE	F	REP	DEYO	WO	276	552
Rat	5	DR	10	w	NR	NR	AD	M	REP	TEWT	MT	294	587
Rat	2	GV	102	d	30	d	GE	F	REP	PRWT	WO	441	—
Rat	2	DR	9	mo	NR	NR	SM	M	REP	RHIS	TE	600	—
Rat	4	FD	4	d	NR	NR	LC	F	REP	PRWT	WO	601	1,500
Rat	4	DR	13	w	NR	NR	JV	M	REP	FERT	WO	639	—
Mouse	4	GV	60	d	NR	NR	AD	F	REP	RPRD	OV	—	2.00
Rat	3	FD	339	d	26-27	d	JV	B	REP	PRWT	WO	—	2.49
Rat	2	DR	9	mo	21	d	JV	F	REP	DEYO	WO	—	2.94
Mouse	2	DR	6	mo	21	d	JV	F	REP	DEYO	WO	—	3.62
Mouse	4	GV	52	d	2	mo	GE	F	REP	PROG	EM	—	5.50
Rat	2	DR	120	d	1	d	GE	M	REP	SPCL	TE	—	6.76

AX7-73

Table AX7-1.3.4 (cont'd). Mammalian Toxicity Data Used to Develop the Eco-SSL

Mammalian Species	No. of Doses	Route of Exposure	Exposure Duration	Duration Units	Age	Age Units	Lifestage	Sex	Effect Type	Effect Measure	Response Site	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Mouse	2	DR	5	w	NR	NR	AD	M	REP	TEDG	TE	—	16.6
Mouse	2	GV	2	w	NR	NR	JV	M	REP	SPCL	SM	—	46.4
Rat	2	FD	102	d	NR	NR	GE	F	REP	PROG	WO	—	49.6
Rat	2	GV	3	mo	8	w	SM	M	REP	TEDG	TE	—	50.0
Rat	2	DR	18	d	NR	NR	GE	F	REP	PRWT	WO	—	55.5
Rat	3	DR	90	d	NR	NR	AD	M	REP	SPCL	SM	—	61.2
Mouse	2	DR	23	d	NR	NR	GE	F	REP	PRWT	WO	—	78.6
Mouse	2	DR	62	d	NR	NR	GE	F	REP	PRWT	WO	—	99.8
Mouse	2	DR	18	w	6-8	w	LC	F	REP	PRWT	WO	—	137
Mouse	2	DR	12	w	9	w	SM	M	REP	PRFM	WO	—	139
Mouse	4	FD	18	d	NR	NR	GE	F	REP	PRWT	WO	—	154
Rat	2	DR	4	w	99	d	JV	M	REP	SPCL	SM	—	171
Rat	5	DR	6	w	4	mo	GE	F	REP	RHIS	WO	—	175
Rat	2	DR	22	d	NR	NR	GE	F	REP	PRWT	WO	—	178
Rat	3	DR	30	d	52	d	JV	M	REP	GREP	PG	—	198
Rat	2	DR	13	w	NR	NR	GE	F	REP	PRWT	WO	—	200
Rat	2	DR	21	d	80	d	JV	F	REP	PRWT	WO	—	218
Rat	4	FD	3	w	NR	NR	LC	F	REP	PRWT	WO	—	221
Rat	2	FD	1	w	19	w	LC	F	REP	PRWT	WO	—	222
Rat	4	FD	3	w	NR	NR	LC	F	REP	PRWT	WO	—	230
Rat	3	FD	25	d	NR	NR	LC	F	REP	PRWT	WO	—	258
Rat	2	DR	21	d	NR	NR	LC	F	REP	PRWT	WO	—	330

AX7-74

**Table AX7-1.3.4 (cont'd). Mammalian Toxicity Data Used to Develop the Eco-SSL**

Mammalian Species	No. of Doses	Route of Exposure	Exposure Duration	Duration Units	Age	Age Units	Lifestage	Sex	Effect Type	Effect Measure	Response Site	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Rat	2	DR	30	d	52	d	JV	M	REP	SPCL	SM	—	354
Rat	2	DR	17	d	NR	NR	GE	F	REP	PRWT	WO	—	360
Rat	2	DR	24	d	NR	NR	LC	F	REP	PRWT	WO	—	360
Rat	2	DR	12	d	NR	NR	GE	F	REP	PRWT	WO	—	362
Rat	2	DR	30	d	27	d	JV	M	REP	SPCL	SM	—	364
Mouse	2	DR	44	d	NR	NR	GE	F	REP	PRWT	WO	—	381
Mouse	2	DR	14	d	NR	NR	LC	F	REP	PRWT	WO	—	381
Rat	2	DR	50	d	24	d	JV	F	REP	RBEH	WO	—	381
Mouse	2	DR	45	d	50-100	d	GE	F	REP	ODVP	WO	—	404
Rat	2	DR	22	d	NR	NR	GE	F	REP	PRWT	WO	—	420
Mouse	2	DR	48	d	NR	NR	GE	F	REP	PRWT	WO	—	437
Rat	2	DR	9	mo	3	mo	SM	M	REP	SPCL	TE	—	579
Rat	2	DR	9	mo	NR	NR	SM	M	REP	TEDG	TE	—	600
Rat	2	DR	3	w	14	w	LC	F	REP	PRWT	WO	—	635
Mouse	2	FD	7	d	NR	NR	GE	F	REP	RSUC	EM	—	646
Rat	2	DR	126	d	1	d	GE	F	REP	PROG	WO	—	651
Rat	2	DR	20	w	10	w	GE	F	REP	PRWT	WO	—	750
Mouse	2	DR	4	d	NR	NR	LC	F	REP	PRWT	WO	—	762
Rat	2	FD	2	w	NR	NR	LC	F	REP	PRWT	WO	—	828
Rat	2	FD	7	d	NR	NR	LC	F	REP	PRWT	WO	—	833
Rat	2	FD	21	d	NR	NR	LC	F	REP	PRWT	WO	—	991
Mouse	4	DR	18	w	11	w	JV	F	REP	TEWT	WO	—	1,370

AX7-75

Table AX7-1.3.4 (cont'd). Mammalian Toxicity Data Used to Develop the Eco-SSL

Mammalian Species	No. of Doses	Route of Exposure	Exposure Duration	Duration Units	Age	Age Units	Lifestage	Sex	Effect Type	Effect Measure	Response Site	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Rat	2	FD	30	d	NR	NR	LC	F	REP	PRWT	WO	—	1,770
Mouse	2	DR	14	w	NR	NR	GE	B	REP	PROG	WO	—	1,990
Rat	2	FD	16	d	NR	NR	LC	F	REP	PROG	WO	—	2,570
Rat	2	FD	7	d	NR	NR	LC	F	REP	PRWT	WO	—	2,570
Rat	2	FD	25	d	NR	NR	LC	F	REP	PRWT	WO	—	2,570
Rat	M	FD	27	d	NR	NR	LC	C	REP	PROG	WO	—	2,840
Mouse	2	DR	14	w	21	d	JV	B	REP	PROG	WO	—	3,630
Rat	2	FD	17	d	NR	NR	LC	F	REP	PRWT	WO	—	6,170
<b>Growth</b>													
Horse	2	FD	15	w	20-21	w	JV	M	GRO	BDWT	WO	0.15	—
Rat	2	FD	21	d	0	d	JV	F	GRO	BDWT	WO	0.5	—
Rat	6	DR	21	d	NR	NR	GE	F	GRO	BDWT	WO	1.00	5.00
Rat	5	DR	7	d	50	d	AD	F	GRO	BDWT	WO	1.27	13.0
Cattle	4	OR	7	w	1	w	JV	M	GRO	BDWT	WO	1.99	—
Rat	3	DR	14	d	21	d	JV	F	GRO	BDWT	WO	2.40	—
Rat	2	DR	332	d	28	d	JV	B	GRO	BDWT	WO	2.98	—
Rat	4	DR	7	w	21	d	GE	F	GRO	BDWT	WO	4.70	8.90
Dog	3	FD	7	mo	NR	NR	JV	NR	GRO	BDWT	WO	4.71	—
Rat	3	DR	30	d	22-24	d	JV	M	GRO	BDWT	WO	5.64	28.2
Rat	4	DR	23	d	22	d	JV	F	GRO	BDWT	WO	5.80	29.0
Cattle	3	OR	84	d	NR	NR	JV	M	GRO	BDWT	WO	7.79	—
Rat	2	OR	6	w	NR	NR	AD	M	GRO	BDWT	WO	9.10	—

AX7-76

**Table AX7-1.3.4 (cont'd). Mammalian Toxicity Data Used to Develop the Eco-SSL**

Mammalian Species	No. of Doses	Route of Exposure	Exposure Duration	Duration Units	Age	Age Units	Lifestage	Sex	Effect Type	Effect Measure	Response Site	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Rat	2	GV	8	w	NR	NR	JV	F	GRO	BDWT	WO	10.0	—
Rat	3	DR	6	mo	NR	NR	AD	M	GRO	BDWT	WO	10.6	532
Rabbit	3	GV	10	d	1	d	JV	F	GRO	BDWT	WO	10.7	50.4
Rat	2	DR	140	d	21	d	JV	M	GRO	BDWT	WO	10.7	—
Rat	2	DR	6	w	NR	NR	JV	M	GRO	BDWT	WO	15.1	—
Rat	2	FD	10	w	NR	NR	JV	M	GRO	BDWT	WO	15.4	—
Rat	2	OR	6	w	NR	NR	AD	M	GRO	BDWT	WO	15.5	—
Rat	2	DR	7	w	NR	NR	JV	M	GRO	BDWT	WO	16.1	—
Mouse	3	DR	14	d	0	d	JV	NR	GRO	BDWT	WO	16.3	163
Rat	4	GV	9	w	10	w	JV	M	GRO	BDWT	WO	18.0	180
Rat	3	FD	339	d	26-27	d	JV	B	GRO	BDWT	WO	18.3	—
Rat	4	GV	29	d	NR	NR	SM	F	GRO	BDWT	WO	18.9	—
Rat	7	DR	10	w	NR	NR	JV	M	GRO	BDWT	WO	24.3	—
Rat	4	DR	56	d	70	d	LC	F	GRO	BDWT	WO	32.5	—
Sheep	5	FD	84	d	NR	NR	JV	M	GRO	BDWT	WO	32.7	—
Rat	2	DR	10	w	NR	NR	JV	M	GRO	BDWT	WO	38.5	—
Cattle	4	FD	7	w	16	w	JV	M	GRO	BDWT	WO	43.0	—
Rat	2	GV	28	d	2	d	JV	B	GRO	BDWT	WO	50.0	—
Rat	5	DR	4	w	94	d	JV	M	GRO	BDWT	WO	71.5	178
Rat	4	GV	12	d	2	d	JV	B	GRO	BDWT	WO	75.0	225
Rat	2	FD	4	w	NR	NR	JV	M	GRO	BDWT	WO	100	—
Rat	6	DR	10	w	NR	NR	JV	M	GRO	BDWT	WO	120	383

AX7-77

**Table AX7-1.3.4 (cont'd). Mammalian Toxicity Data Used to Develop the Eco-SSL**

<b>Mammalian Species</b>	<b>No. of Doses</b>	<b>Route of Exposure</b>	<b>Exposure Duration</b>	<b>Duration Units</b>	<b>Age</b>	<b>Age Units</b>	<b>Lifestage</b>	<b>Sex</b>	<b>Effect Type</b>	<b>Effect Measure</b>	<b>Response Site</b>	<b>NOAEL (mg/kg bw/day)</b>	<b>LOAEL (mg/kg bw/day)</b>
Mouse	3	FD	4	w	3	mo	JV	B	GRO	BDWT	WO	136	1360
Mouse	2	DR	18	w	6-8	w	LC	F	GRO	BDWT	WO	137	—
Mouse	2	DR	12	w	NR	NR	GE	M	GRO	BDWT	WO	139	—
Rat	3	DR	30	d	52	d	JV	M	GRO	BDWT	WO	169	508
Rat	2	DR	4	w	99	d	JV	B	GRO	BDWT	WO	171	—
Rat	4	GV	18	d	3	d	JV	M	GRO	BDWT	WO	180	—
Mouse	3	DR	6	w	7	w	SM	M	GRO	BDWT	WO	187	373
Rat	4	GV	18	d	2	d	JV	B	GRO	BDWT	WO	200	—
Rat	2	GV	91	d	NR	NR	JV	M	GRO	BDWT	WO	200	—
Rat	2	DR	21	d	80	d	JV	F	GRO	BDWT	WO	218	—
Rat	4	FD	1	w	NR	NR	LC	F	GRO	BDWT	WO	230	460
Rat	4	DR	30	d	NR	NR	JV	M	GRO	BDWT	WO	285	—
Mouse	5	DR	10	w	NR	NR	JV	M	GRO	BDWT	WO	362	—
Rat	2	DR	30	d	52	d	JV	M	GRO	BDWT	WO	364	—
Rat	4	GV	14	d	14	d	JV	NR	GRO	BDWT	WO	400	800
Rat	5	GV	14	d	20	d	JV	NR	GRO	BDWT	WO	400	800
Rat	2	FD	14	mo	0	d	JV	NR	GRO	BDWT	WO	431	—
Rat	2	GV	102	d	30	d	LC	F	GRO	BDWT	WO	441	—
Mouse	4	GV	12	d	6	d	JV	M	GRO	BDWT	WO	534	—
Mouse	7	FD	30	d	NR	NR	LC	F	GRO	BDWT	WO	632	1264
Rat	2	DR	126	d	1	d	GE	F	GRO	BDWT	WO	651	—
Rat	2	DR	20	w	10	w	GE	F	GRO	BDWT	WO	750	—

AX7-78

**Table AX7-1.3.4 (cont'd). Mammalian Toxicity Data Used to Develop the Eco-SSL**

<b>Mammalian Species</b>	<b>No. of Doses</b>	<b>Route of Exposure</b>	<b>Exposure Duration</b>	<b>Duration Units</b>	<b>Age</b>	<b>Age Units</b>	<b>Lifestage</b>	<b>Sex</b>	<b>Effect Type</b>	<b>Effect Measure</b>	<b>Response Site</b>	<b>NOAEL (mg/kg bw/day)</b>	<b>LOAEL (mg/kg bw/day)</b>
Mouse	7	FD	28	d	NR	NR	LC	F	GRO	BDWT	WO	1260	2530
Rat	4	FD	18	d	NR	NR	LC	F	GRO	BDWT	WO	1500	
Rat	2	DR	9	d	21	d	JV	M	GRO	BDWT	WO	—	3.30
Cattle	2	FD	283	d	7	mo	JV	M	GRO	BDWT	WO	—	15.0
Rat	3	DR	92	d	25	d	GE	F	MPH	GMPH	TB	—	28.7
Rat	4	DR	7	d	25	d	GE	F	GRO	BDWT	WO	—	29.0
Rat	2	DR	5	d	26	d	JV	F	GRO	BDWT	WO	—	29.0
Rat	2	DR	26	d	22	d	JV	F	GRO	BDWT	WO	—	29.5
Rat	2	DR	14	d	26	d	JV	F	MPH	Other	TA	—	29.9
Rat	2	DR	10	d	26	d	JV	F	GRO	BDWT	WO	—	30.4
Mouse	2	GV	3	w	NR	NR	JV	M	GRO	BDWT	WO	—	46.4
Dog	2	OR	5	w	<1	yr	JV	NR	GRO	BDWT	WO	—	50.0
Shrew	4	FD	31	d	NR	NR	JV	B	GRO	BDWT	WO	—	61.5
Rat	3	GV	58	d	2	d	JV	B	GRO	BDWT	WO	—	100
Pig	2	FD	13	w	4	w	JV	NR	GRO	BDWT	WO	—	173
Rat	2	GV	29	d	2	d	JV	F	GRO	BDWT	WO	—	200
Rat	2	FD	5	w	NR	NR	MA	NR	GRO	BDWT	WO	—	272
Rat	2	GV	6	d	1	d	JV	B	GRO	BDWT	WO	—	328
Rat	2	DR	30	d	27	d	JV	M	GRO	BDWT	WO	—	354
Rat	2	DR	50	d	24	d	JV	M	GRO	BDWT	WO	—	371
Rat	2	GV	28	d	2	d	JV	M	GRO	BDWT	WO	—	400
Rat	4	GV	14	d	18	d	JV	NR	GRO	BDWT	WO	—	400

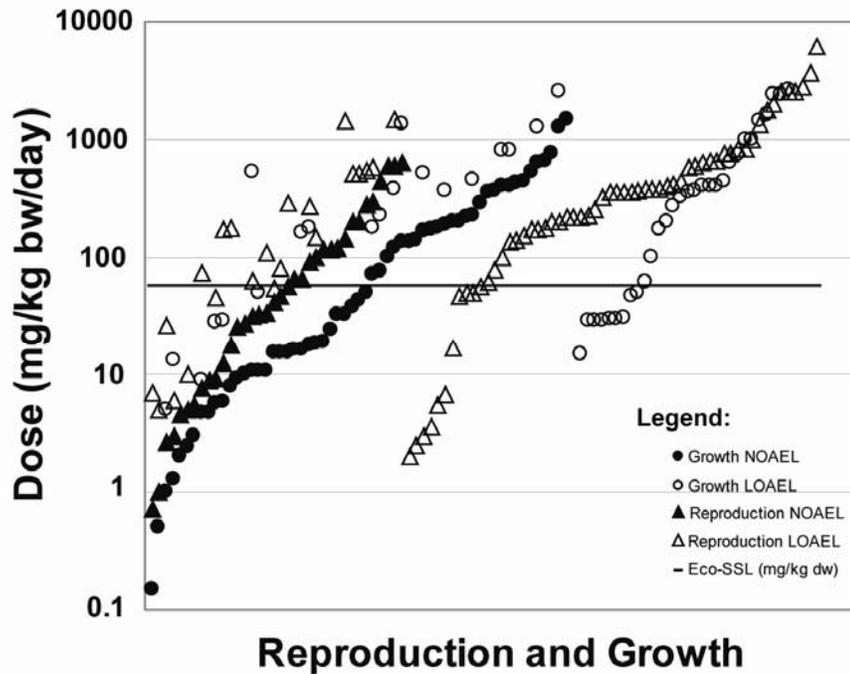
AX7-79

**Table AX7-1.3.4 (cont'd). Mammalian Toxicity Data Used to Develop the Eco-SSL**

Mammalian Species	No. of Doses	Route of Exposure	Exposure Duration	Duration Units	Age	Age Units	Lifestage	Sex	Effect Type	Effect Measure	Response Site	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Mouse	2	DR	45	d	50-100	d	GE	F	GRO	BDWT	WO	—	404
Rat	4	FD	1	w	NR	NR	LC	F	GRO	BDWT	WO	—	442
Rat	2	DR	6	w	14	w	LC	F	GRO	BDWT	WO	—	638
Mouse	4	DR	10	w	11	w	JV	F	GRO	BDWT	WO	—	748
Rat	2	FD	21	d	NR	NR	LC	F	GRO	BDWT	WO	—	991
Rat	2	GV	18	d	2	d	JV	B	GRO	BDWT	WO	—	1000
Rat	2	FD	2	w	0	d	JV	NR	GRO	BDWT	WO	—	1430
Rat	4	GV	14	d	24	d	JV	NR	GRO	BDWT	WO	—	1600
Rat	2	FD	2	w	60-80	d	JV	M	GRO	BDWT	WO	—	2390
Rat	3	GV	14	d	16	d	JV	NR	GRO	BDWT	WO	—	2400
Rat	2	FD	14	d	60	d	JV	M	GRO	BDWT	WO	—	2650

AD = adult; B = both; BDWT = body weight changes; d = days; DEYO = death of young; DR = drinking water; F = female; FD = food; FERT = fertility; GMPH = general morphology; GRO = growth; GV = gavage; JV = juvenile; LC = lactation; M = male; MA = mature; mo = months; MPH = morphology; NR = not reported; ODVP = offspring development; OR = other oral; PG = prostate gland; PROG = progeny counts or numbers; PRWT = progeny weight; RBPH = reproductive behavior; REP = reproduction; RHIS = reproductive organ histology; RSEM = resorbed embryos; RSUC = reproductive success (general); RT = reproductive tissue; SM = sexually mature; SPCL = sperm cell counts; SPCV = sperm cell viability; TA = tail; TB = tibia; TE = testes; TEDG = testes degeneration; TEWT = testes weight; w = weeks; WO = whole organism; yr = years.

Source: U.S. Environmental Protection Agency (2005b).



**Figure AX7-1.3.2. Mammalian reproduction and growth toxicity data considered in development of the Eco-SSL.**

Source: U.S. Environmental Protection Agency (2005b).

wildlife generally would occur at doses greater than the 2 to 8 mg/kg-day reported to be toxic to most animals in the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a).

#### **AX7.1.3.4 Recent Studies on the Effects of Lead on Decomposers**

Recent studies on effects of Pb to two groups of decomposers are summarized in this subsection. Effects on terrestrial invertebrates, such as earthworms and springtails, are described first, followed by effects on microorganisms.

##### ***Effects on Invertebrates***

Since the 1986 Lead AQCD, there have been several studies in which terrestrial invertebrates were exposed to Pb in soil. Many of these were reviewed during the development of the Eco-SSLs (U.S. Environmental Protection Agency, 2005b). The relevant information from the Eco-SSL document is described below.

A literature search and review was conducted to identify critical papers published since 2002. Effects on earthworms and other invertebrates observed in studies conducted since the 1986 Lead AQCD are similar to those reported previously: mortality and decreased growth and reproduction (Lock and Janssen, 2002; Davies et al., 2002; Rao et al., 2003; Bongers et al., 2004; Nursita et al., 2005; U.S. Environmental Protection Agency, 2005b).

The literature search completed for terrestrial invertebrate Eco-SSL development identified 179 papers for detailed review, of which 13 met the minimum criteria for further consideration (U.S. Environmental Protection Agency, 2005b). Most of the 18 ecotoxicological endpoints that were further evaluated measured reproduction or survival as the ecologically relevant endpoint. Four of these, representing one species under three different pH test conditions were used to develop the Eco-SSL of 1700 mg/kg soil (Table AX7-1.3.5).

**Table AX7-1.3.5. Invertebrate Toxicity Data Used to Develop the Eco-SSL**

Invertebrate Species	Soil pH	% Organic Matter	Toxicity Parameter	Pb in Soil (mg/kg dw)
Collembola ( <i>Folsomia candida</i> )	6.0	10	MATC <sup>1</sup> (reproduction)	3162
Collembola	4.5	10	MATC (reproduction)	3162
Collembola	5.0	10	MATC (reproduction)	894
Collembola	6.0	10	MATC (reproduction)	894
			Geometric Mean	1682

\* MATC = Maximum Acceptable Threshold Concentration, or the geometric mean of the NOEC (no-observed-effect concentration) and LOEC (lowest-observed-effect concentration).

Source: U.S. Environmental Protection Agency (2005b).

In a study designed to test the toxicity of Pb to the earthworm *Eisenia fetida*, Davies et al. (2002) found that the 28-day LC<sub>50</sub> (± 95% confidence intervals) for Pb in soils contaminated with Pb(NO<sub>3</sub>)<sub>2</sub> was 4379 ∇ 356 mg/kg. Twenty-eight day EC<sub>50</sub> values (±95% confidence intervals) for weight change and cocoon production were 1408 ∇ 198 and

971 ∇ 633 mg/kg, respectively. Significant mortalities were noted at concentrations of 2000 mg/kg. These data are consistent with those reported in the Eco-SSL document (U.S. Environmental Protection Agency, 2005b) for the same species of earthworm.

Nursita et al. (2005) found no mortality and no adverse effects on reproduction (i.e., number of juveniles) of the collembolan *Proisotoma minuta* exposed for 42 days to 300, 750, 1500, or 3000 mg Pb/kg as Pb-nitrate in an acidic (pH = 4.88) sandy loam soil. It was noted that the soils were allowed to equilibrate for 4 weeks after adding the Pb-nitrate before the organisms were added. The observation of no effect at 3000 mg/kg is consistent with that of Sandifer and Hopkin (1996). Sandifer and Hopkin (1996) determined a NOEC (no-observed-effect concentration) and LOEC (lowest-observed-effect concentration) for collembolan reproduction of 2000 and 5000 mg/kg, respectively. (A MATC [maximum-acceptable-threshold concentration] of 3162 mg/kg was used to develop the Eco-SSL).

The remaining 14 toxicity endpoints that were not used to develop the Eco-SSL for invertebrates are presented in Table AX7-1.3.6. None of these endpoints was considered eligible for Eco-SSL derivation.

Lock and Janssen (2002) exposed the potworm *Enchytraeus albidus* to Pb, as Pb-nitrate. The 21-day LC<sub>50</sub> was 4530 mg/kg, and the 42-day EC<sub>50</sub> for juvenile reproduction was 320 mg/kg. The F1 generation was then grown to maturity in the same concentration soil and subsequently used in a reproduction test. The EC<sub>50</sub> for the F1 generation (394 mg/kg) was similar to that of the P generation. The authors concluded that the two-generation assay did not increase the sensitivity of the test (Lock and Janssen, 2002). None of the 18 toxicity endpoints evaluated in detail during development of the Eco-SSLs used this species. The LC<sub>50</sub> reported for the potworm was higher than reported for nematodes and similar to that reported for the earthworm. The EC<sub>50</sub> for reproduction was lower than reported for the earthworm or collembola.

Recent work by Bongers et al. (2004) cautioned against attributing all toxicity observed in a spiked-soil toxicity test to Pb. They found that the counterion may also contribute to the toxicity of Pb in the springtail *Folsomia candida*. This may have implications on the interpretation of the Eco-SSL data, because the toxicity of the counterion (nitrate) was not taken into account during Eco-SSL development. Percolation (removal of the counterion) had no statistically significant effect on Pb-chloride toxicity (LC<sub>50</sub> = 2900 mg/kg for both non-percolated and percolated soil; EC<sub>50</sub> for reproduction = 1900 mg/kg or 2400 mg/kg for

**Table AX7-1.3.6. Invertebrate Toxicity Data Not Used to Develop the Eco-SSL**

<b>Invertebrate Species</b>	<b>Soil pH</b>	<b>% Organic Matter</b>	<b>Toxicity Parameter</b>	<b>Pb in Soil (mg/kg dw)</b>
Nematode	4	1.14	LC <sub>50</sub>	285
Nematode	4	1.14	LC <sub>50</sub>	297
Nematode	4	4.2	LC <sub>50</sub>	847
Nematode	4	4.2	LC <sub>50</sub>	1341
Nematode	6.2	1.7	LC <sub>50</sub>	1554
Nematode	5.1	3.0	LC <sub>50</sub>	891
Earthworm	6.3	10.0	EC <sub>50</sub>	1940
Earthworm	6.1	10.0	EC <sub>50</sub>	1629
Earthworm	6.0	10.0	LC <sub>50</sub>	3716
Earthworm	6.5	10.0	ILL	1.16
Nematode	4	10	LC <sub>50</sub>	1434
Nematode	4	10	NOAEC	2235
Nematode	6.1	3.4	LC <sub>50</sub>	13.9
Nematode	6.2	2.2	LC <sub>50</sub>	11.6

\*NOAEC (no-observed-adverse-effect concentration); LC<sub>50</sub> (concentration lethal to 50% of test population); EC<sub>50</sub> (effect concentration for 50% of test population); ILL (incipient lethal level).

Source: U.S. Environmental Protection Agency (2005b).

non-percolated or percolated soil, respectively). However, percolation did have a significant effect on Pb-nitrate toxicity (LC<sub>50</sub> = 980 mg/kg or 2200 mg/kg for non-percolated or percolated soil, respectively; EC<sub>50</sub> for reproduction = 580 mg/kg or 1700 mg/kg for non-percolated or percolated soil, respectively). Lead nitrate was more toxic than Pb-chloride for survival and reproduction. However, the toxicity of Pb, from chloride or nitrate, was not significantly different after the counterion was percolated out of the test soil. It is noted that the soil was left for 3 weeks to equilibrate before testing. Lock and Janssen (2002) also found that Pb-nitrate was more toxic than Pb-chloride, and they used Pb-nitrate in their experiments because 1000 mg/kg Pb-chloride did not produce any mortality in their range-finding tests. This difference in

chloride and nitrate toxicity has not been found for earthworms (Neuhauser et al., 1985; Bongers et al., 2004).

Rao et al. (2003) exposed the earthworm *Eisenia fetida* to Pb-oxide in an artificial soil with a pH of 6 at the LC<sub>50</sub> concentration of 11 mg/kg. Exposure for 14 days resulted in a number of effects including body fragmentation, protrusions, rupture of the cuticle, etc. Many of these effects may trigger defensive mechanisms. For example, fragmentation of the affected posterior region was followed by regeneration and a new ectoderm layer was formed to cover affected areas, both of which processes may serve to prevent soil bacteria from further affecting the earthworm (Rao et al., 2003).

### ***Effects on Microorganisms and Microbial Processes***

Microorganisms and microbial processes were not included in the Eco-SSL development process (see Attachment 1-2 of OSWER Directive 92857-55 dated November 2003 in U.S. Environmental Protection Agency [2005a]). Many reasons were given, including that it is unlikely that site conditions would only pose unacceptable risk to microbes and not be reflected as unacceptable risks to higher organisms; that the significance of laboratory-derived effects data to the ecosystem is uncertain; and that the spatial (across millimeter distances) and temporal (within minutes to hours) variation makes understanding ecological consequences challenging. Microbial endpoints often vary dramatically based on moisture, temperature, oxygen, and many non-contaminant factors. Therefore, the recommendation arising from the Eco-SSL development process was that risks to microbes or microbial processes not be addressed through the chemical screening process but that they should be addressed within a site-specific risk assessment (U.S. Environmental Protection Agency, 2005b).

Few studies on the effects of Pb to microbial processes have been published since 1986. As the direct toxicity to fungi and bacterial populations are difficult to determine and interpret, indicators for soil communities are often measured as proxies for toxicity (e.g., urease activity in soil). Recent studies of this nature (Doelman and Haanstra, 1986; Wilke, 1989; Haanstra and Doelman, 1991) are summarized in this subsection. The Pb concentrations in these recent studies (1000 to 5000 mg/kg) are consistent with those reported in the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) as associated with effects on microbial processes (750 to 7500 mg/kg).

The effects of Pb-chloride on the processes of nitrification and nitrogen mineralization were studied in a 28-day experiment by Wilke (1989). The authors reported that nitrification was increased by 12 and 16% at levels of 1000 and 4000 mg/kg, respectively, and that nitrogen mineralization was reduced by 32 and 44% at concentrations of 1000 and 4000 mg/kg, respectively.

The effects of Pb on arylsulfatase (Haanstra and Doelman, 1991) and urease activity (Doelman and Haanstra, 1986) in soil were investigated. LC<sub>50</sub>s for decreases in arylsulfatase activity were reported at Pb concentrations of 3004 and 4538 mg/kg in a silty loam soil, at pH 6 and 8, respectively. The LC<sub>50</sub> for a decrease in urease activity was 5060 mg Pb/kg in a sandy loam soil.

In laboratory microcosm studies Cotrufo et al. (1995) found that decomposition of oak (*Quercus ilex*) leaf litter was reduced at elevated Pb (~20 mg Pb g<sup>-1</sup> C) levels after 8 months compared to controls (~2 mg Pb g<sup>-1</sup> C). The researchers found soil respiration and amount of soil mycelia correlated negatively with soil Pb, Zn and Cr concentration.

#### **AX7.1.3.5 Summary**

The current document expands upon and updates knowledge related to the effects of Pb on terrestrial primary producers, consumers, and decomposers.

#### ***Primary Producers***

The effects of Pb on terrestrial plants include decreased photosynthetic and transpiration rates in addition to decreased growth and yield. The phytotoxicity of Pb is considered to be relatively low, and there are few reports of phytotoxicity from Pb exposure under field conditions. Recently, phytotoxicity data were reviewed for the development of the Eco-SSL (U.S. Environmental Protection Agency, 2005b). Many of the toxicity data presented in the Eco-SSL document (U.S. Environmental Protection Agency, 2005b) are lower than those discussed in the 1986 Lead AQCD, although both documents acknowledged that toxicity is observed over a wide range of concentrations of Pb in soil (tens to thousands of mg/kg soil). This may be due to many factors, such as soil conditions (e.g., pH, organic matter) and differences in bioavailability of the Pb in spiked soils perhaps due to lack of equilibration of the Pb solution with the soil after spiking. Most phytotoxicity data continue to be developed for agricultural plant species (i.e.,

vegetable and grain crops). Few data are available for trees or native herbaceous plants, although two of the five toxicity endpoints used to develop the Eco-SSL were for trees and two were for clover.

### ***Consumers***

Effects of Pb on avian and mammalian consumers include decreased survival, reproduction, and growth as well as effects on development and behavior. There remain few field effects data for consumers, except from sites with multiple contaminants, for which it is difficult to attribute toxicity specifically to Pb. Avian and mammalian toxicity data recently were reviewed for the development of Eco-SSLs (U.S. Environmental Protection Agency, 2005b). Many of the toxicity data presented in the Eco-SSL document (U.S. Environmental Protection Agency, 2005b) are lower than those discussed in the 1986 Lead AQCD, although the EPA (U.S. Environmental Protection Agency, 2005b) recognizes that toxicity is observed over a wide range of doses (<1 to >1000 mg Pb/kg bw-day). Most toxicity data for birds have been derived from chicken and quail studies, and most data for mammals have been derived from laboratory rat and mouse studies. Data derived for other species would contribute to the understanding of Pb toxicity, particularly for wildlife species with different gut physiologies. In addition, data derived using environmentally realistic exposures, such as from Pb-contaminated soil and food, may be recommended. Finally, data derived from inhalation exposures, which evaluate endpoints such as survival, growth, and reproduction, would contribute to understanding the implications of airborne releases of Pb.

### ***Decomposers***

Effects of Pb on soil invertebrates include decreased survival, growth, and reproduction. Effects on microorganisms include changes in nitrogen mineralization and enzyme activities. Recent data on 1986 Lead toxicity to soil invertebrates and microorganisms are consistent with those reported in the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a), with toxicity generally observed at concentrations of hundreds to thousands of mg/kg soil. Studies on microbial processes may be influenced significantly by soil parameters, and the significance of the test results is not clear.

### ***Ecological Soil Screening Levels (Eco-SSLs)***

Eco-SSLs are concentrations of contaminants in soils that would result in little or no measurable effect on ecological receptors (U.S. Environmental Protection Agency, 2005a). They were developed following rigorous scientific protocols and were subjected to two rounds of peer review. Due to conservative modeling assumptions (e.g., metal exists in most toxic form or highly bioavailable form, high food ingestion rate, high soil ingestion rate) which are common to screening processes, several Eco-SSLs are derived below the average background soil concentration for a particular contaminant.

The Eco-SSLs for terrestrial plants, birds, mammals, and soil invertebrates are 120, 11, 56, and 1700 mg Pb/kg soil, respectively.

### **AX7.1.4 Effects of Lead on Natural Terrestrial Ecosystems**

The concept that organisms are part of larger systems that include both biotic and abiotic components of the environment dates back to the naturalists of the Victorian era. However, the breakthrough in what we now consider the ecosystem approach to ecology occurred in the 1950s and 1960s when E.P. and H.T. Odum pioneered the quantitative analysis of ecosystems (Odum, 1971). This approach encouraged the calculation of energy flows into, out of, and within explicitly defined ecosystems. The rapid development of computer technology aided in the growth of ecosystem ecology by allowing the development and use of increasingly complex models for estimating fluxes that could not be directly measured.

It was not long before the quantitative analysis of ecosystems was extended to examine the flows of nutrients and other chemical compounds. In temperate terrestrial systems, the watershed was identified as a convenient and informative experimental unit (Bormann and Likens, 1967). A major conceptual breakthrough in the watershed approach was that drainage water chemistry could be used as an indicator of the “health” of the ecosystem. In a system limited by nitrogen, for example, elevated concentrations of  $\text{NO}_3^-$  in drainage waters indicate that the ecosystem is no longer making optimal use of available nutrients.

The ecosystem approach can also be used effectively in the study of trace element biogeochemistry. Input-output budgets can be used to determine whether an ecosystem is a net source or sink of a trace element. Changes to the input-output balance over time can be used to assess the effects of natural or experimental changes in deposition, land use, climate, or other

factors. In addition, examination of fluxes within the ecosystem (in plant uptake, soil solutions, etc.) can be used to understand the processes that are most influential in determining the fate and transport of the trace element.

Many published ecosystem studies include data for 1 to 3 years, the typical duration of research grant funding or doctoral dissertation research. While these studies enrich our understanding of terrestrial ecosystems, the most valuable studies are those that are maintained over many years. Natural variations in climate, pests, animal migrations, and other factors can make inferences from short-term studies misleading (Likens, 1989). To nurture long-term research, the National Science Foundation supports a network of Long-Term Ecological Research (LTER) sites that represent various biomes.

This section describes terrestrial ecosystem research on Pb, focusing on work done since the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) and highlighting key long-term studies. Unfortunately, there are few studies that feature long-term data on trace metal behavior at multiple levels of organization. Therefore, this examination of the effects of Pb on terrestrial ecosystems combines insights from long- and short-term investigations as well as studies at scales including whole ecosystems, communities, populations, and individual species.

#### **AX7.1.4.1 Effects of Terrestrial Ecosystem Stresses on Lead Cycling**

Terrestrial ecosystems may respond to stressors in a variety of ways, including reductions in the vigor and/or growth of vegetation, reductions in biodiversity, and effects on microbial processes. Each of these effects may lead to the “leakage” of nutrients, especially nitrogen, in drainage waters. The reduced vigor or growth of vegetation results in a lower uptake of nitrogen and other nutrients from soils. Reduced biodiversity accompanied by lower total net primary productivity for the ecosystem would also result in a lower nutrient uptake. Effects of stress in microbial populations are less obvious. If the stress reduces microbial activity rates, then nutrients bound in soil organic matter (e.g., organic nitrogen compounds) will likely be mineralized at a lower rate and retained in the system. On the other hand, disturbances such as clear-cutting, ice-storm damage, and soil freezing can result in substantial nutrient losses from soils (Bormann et al., 1968; Likens et al., 1969; Mitchell et al., 1996; Groffman et al., 2001; Houlton et al., 2003).

Since the movement and fate of Pb in terrestrial ecosystems is strongly related to the organic matter cycle (Section AX7.1.2), stressors that could lead to disruption or alteration of the soil organic matter pool are of particular concern in assessing effects of ecosystem stress on Pb cycling. By binding soluble Pb, soil organic matter acts as a barrier to the release of Pb to drainage waters (Wang et al., 1995; Kaste et al., 2003; Watmough and Hutchinson, 2004). As a result, concentrations of Pb in soil solutions and drainage waters tend to be low (Driscoll et al., 1988; Wang et al., 1995; Bacon and Bain, 1995; Johnson et al., 1995a). Through decomposition and leaching, soluble organic matter is released to solution, and with it, some Pb is also mobilized. Wang and Benoit (1996) found that essentially all of the Pb in soil solutions in a hardwood forest in New Hampshire was bound to dissolve organic matter (DOM). This release of soluble Pb does not typically result in elevated surface water Pb concentrations, because (1) organic matter has a relatively long residence time in most temperate soils (Gosz et al., 1976; Schlesinger, 1997), so only a small fraction of the organic matter pool is dissolved at any time; (2) DOM-Pb complexes solubilized in upper soil horizons may be precipitated or adsorbed lower in the soil profile; (3) the DOM to which Pb is bound may be utilized by microbes, allowing the associated Pb to bind anew to soil organic matter. Together, these factors tend to moderate the release of Pb to surface waters in temperate terrestrial ecosystems. However, stressors or disturbances that result in increased release of DOM from soils could result in the unanticipated release of Pb to groundwater and/or surface waters.

### ***Acidification***

The effect of acidification on ecosystem cycling of Pb is difficult to predict. Like most metals, the solubility of Pb increases as pH decreases (Stumm and Morgan, 1995), suggesting that enhanced mobility of Pb should be found in ecosystems under acidification stress. However, Pb is also strongly bound to organic matter in soils and sediments. Reductions in pH may cause a decrease in the solubility of DOM, due to the protonation of carboxylic functional groups (Tipping and Woof, 1990). Because of the importance of Pb complexation with organic matter, lower DOM concentrations in soil solution resulting from acidification may offset the increased solubility of Pb and hence decrease the mobility of the organically bound metal.

In a study of grassland and forest soils at the Rothamsted Experiment Station in England, long-term (i.e., >100 years) soil acidification significantly increased the mobility of Pb in the soil

(Blake and Goulding, 2002). However, the increased mobility was only observed in very acid soils, those with pH of <4.5. The fraction of exchangeable Pb (extracted with 0.1 M CaCl<sub>2</sub>) increased from about 3% to 15% of the total Pb in the most acidified soils. Similarly, the fraction of organically bound Pb increased from about 2% of total Pb in neutral soils to 12% of total Pb in the most acidified soils. Similarly, Nouri and Reddy (1995) observed higher levels of diethylenetriaminepentaacetic-acid-[DTPA]-extractable Pb in soils in a loblolly pine forest treated with simulated acid rain, but only in the most acidic treatment, with simulated rain with a pH of 3.5.

Although acidification may increase the mobility of Pb in some soils, it is not clear that this Pb is actually moving through or out of the soil profile. In an examination of running waters in Sweden, Johansson et al. (1995) found no relationship between acidification and Pb concentrations in drainage waters and concluded that Pb concentrations were governed by the DOM concentration, which masked any association with acidification. In an in situ lysimeter study, Bergkvist (1986) measured lower concentrations of Pb in soil solutions draining experimentally acidified plots than in unacidified plots. In a laboratory study using large soil columns, Merino and García-Rodeja (1997) observed no effect of experimental acidification on the release of Pb to soil solution. Thus, while acidification may increase the potential mobility of Pb in soils, as indicated by increases in labile soil fractions such as exchangeable and DTPA-extractable Pb, the actual movement of Pb in the soil is limited by DOM solubilization and transport. It is worth noting that in all of these studies, significant effects of acidification were observed for other trace metals (Bergkvist, 1986; Johansson et al., 1995; Merino and García-Rodeja, 1997).

Acidification may enhance Pb export to drainage water in very sandy soils, soils with limited ability to retain organic matter. Studies in the McDonald's Branch watershed in the New Jersey pine barrens, where soil texture is similar to beach sands, suggested little Pb retention in the mineral soil (Swanson and Johnson, 1980; Turner et al., 1985). If acidification results in the mobilization of Pb and organic matter into these mineral soils, then increased streamwater Pb concentrations would likely follow.

### ***Land Use and Industry***

Changes in land use also represent potentially significant changes in the cycling of organic matter in terrestrial ecosystems. Conversion of pasture and croplands to woodlands changes the nature and quantity of organic matter inputs to the soil. In temperate climates, forest ecosystems tend to accumulate organic matter in an O horizon on the forest floor, whereas organic matter in grasslands and agricultural fields is concentrated in an A horizon at the soil surface. Andersen et al. (2002) compared the trace metal concentrations in arable fields in Denmark to nearby sites that had been converted to forest land. After 34 years of afforestation, the soils showed no significant difference in Pb concentration or fractionation, despite significant acidification of the soils. Afforestation had no effect on the soil carbon concentration, suggesting that land use change may have little effect on Pb cycling unless soil carbon pools are affected.

Similarly, the introduction of industrial activity may have consequences for organic matter cycling, and subsequently, Pb mobilization. In a rare long-term study of polluted soils, Egli et al. (1999) studied the changes in trace metal concentrations in forest soils at a site in western Switzerland between 1969 and 1993. The site is 3 to 6 km downwind from an aluminum industrial plant that operated between the 1950s and 1991. In the 24-year period of study, the site experienced significant declines in organic carbon in surface (0 to 5 cm depth) and subsurface (30 to 35 cm) soils. In the 30 to 35 cm layer, the organic carbon concentration declined by more than 75%. Extractable Pb (using an ammonium acetate and EDTA mixture) declined by 35% in the same layer. The authors suggested that the Pb lost from the soil had been organically bound. While this study indicates that loss of soil carbon can induce the mobilization and loss of Pb from terrestrial ecosystems, it is also worth noting that the decline in soil Pb was considerably smaller than the decline in organic carbon. This suggests that Pb mobilized during organic matter decomposition can resorb to remaining organic matter or perhaps to alternate binding sites (e.g., Fe and Mn oxides).

The effects of industries that emit Pb to the atmosphere are discussed in Sections AX7.1.5.2 and AX7.1.5.3 below.

Forest harvesting represents a severe disruption of the organic matter cycle in forest ecosystems. Litter inputs are severely reduced for several years after cutting (e.g., Hughes and Fahey, 1994). The removal of the forest canopy results in reduced interception of precipitation,

and, therefore, increased water flux to the soil surface. Also, until a new canopy closes, the soil surface is exposed to increased solar radiation and higher temperatures. Together, the higher moisture and temperature in surface soils tend to increase the rate of organic matter decomposition. Several studies have estimated decreases of up to 40% in the organic matter content of forest floor soils after clear-cutting (Covington, 1981; Federer, 1984; Johnson et al., 1995b). This loss of organic matter from the forest floor could result in the mobilization of organically complexed Pb. However, observations from clear-cut sites in the United States and Europe indicate that forest harvesting causes little or no mobilization of Pb from forest soils.

At the Hubbard Brook Experimental Forest in New Hampshire, whole-tree harvesting, the most intensive form of clear-cutting, resulted in very small increases in Pb concentrations in soil solutions draining the Oa soil horizon despite substantial reductions in the organic matter mass of that horizon (Fuller et al., 1988; Johnson et al., 1995b). These increases were associated with similarly small increases in dissolved organic carbon (DOC) concentrations in the Oa horizon soil water. Output of Pb from the watershed stream was unaffected by clear-cutting. Similarly, Berthelsen and Steinnes (1995) observed small decreases in the Pb content of the Oa horizon (“H” in the European system of soil classification) in clear-cut sites in Norway, compared to uncut reference sites. This mobilization of Pb from the Oa horizon was accompanied by an increase in the Pb content of the upper mineral soil horizons. The Pb decline in the Oa horizon was accompanied by a decrease in the organic matter content, leading the authors to attribute the Pb dynamics to leaching with DOM. In a study conducted in Wales, Durand et al. (1994) observed lower Pb outputs from a stream draining a clear-cut watershed than from where the stream drained the upper reaches of the watershed, which were uncut. The DOC and H<sup>+</sup> outputs were also lower in the clear-cut area. These patterns persisted in all 5 years of the study.

Forest harvesting is a severe form of ecosystem disturbance, and, thus, it is somewhat surprising that studies of clear-cutting have shown little or no effect on Pb mobility or loss from forest ecosystems. Perhaps the strong complexation behavior of Pb with natural organic matter results in the retention of Pb in forest soils. Even in cases where Pb is mobilized in forest floor soils (Fuller et al., 1988; Berthelsen and Steinnes, 1995), there is no evidence of loss of Pb from the ecosystem, indicating that mineral soils are efficient in capturing and retaining any Pb that is mobilized in the forest floor. Therefore, the principal risk associated with forest harvesting is the loss of Pb in particulate form to drainage waters through erosion. In some relatively remote

lakes in the United Kingdom the distribution of sediment-bound trace elements (including Pb) have been affected by forestry activities and catchment erosion (Yang and Rose, 2005). Yang and Rose (2005) believe that more contaminated soil in-wash could increase sediment heavy metal concentrations while less contaminated soil in-wash could dilute sediment heavy metal levels.

### *Climate Change*

Atmospheric Pb is not likely to contribute significantly to global climate change. Lead compounds have relatively short residence times in the atmosphere, making it unlikely that they will reach the stratosphere. Also, Pb compounds are not known to absorb infrared radiation and, therefore, are unlikely to contribute to stratospheric ozone depletion or global warming.

Climate change does, however, represent a disturbance to terrestrial ecosystems. Unfortunately, the potential linkages between climate-related stress and Pb cycling are poorly understood. As in the previous examples, effects related to alterations in organic matter cycling may influence Pb migration. For example, an increase in temperature leading to increased rates of organic matter decomposition could lead to temporary increases in DOM concentrations and smaller steady-state pools of soil organic matter. Either of these factors could result in increased concentrations of Pb in waters draining terrestrial ecosystems.

Climate change may also affect the fluctuations of temperature and/or precipitation in terrestrial ecosystems. For example, there is some evidence for recent increases in the frequency of soil freezing events in the northeastern United States (Mitchell et al., 1996). Soil freezing occurs when soils have little or no snow cover to insulate them from cold temperatures and results in an increased release of nitrate and DOC from the O horizons of forest soils (Mitchell et al., 1996; Fitzhugh et al., 2001). Increased DOC losses from O horizons subjected to freezing may also increase Pb mobilization.

Increased fluctuations in precipitation may induce more frequent flooding, with potentially significant consequences for Pb contamination of floodplain ecosystems. Soils collected from the floodplain of the Elbe River, in Germany, contained elevated concentrations of Pb and other trace metals (Krüger and Grongroft, 2003). Tissues of plants from floodplain sites did not, however, contain higher Pb concentrations than control sites. More frequent

or more severe flooding would likely result in increased inputs of Pb and other metals to floodplain soils.

#### **AX7.1.4.2 Effects of Lead Exposure on Natural Ecosystem Structure and Function**

The effects of Pb exposure on natural ecosystems are confounded by the fact that Pb exposure cannot be decoupled from other factors that may also affect the ecosystem under consideration. Principal among these factors are other trace metals and acidic deposition. Emissions of Pb from smelting and other industrial activities are accompanied by other trace metals (e.g., Zn, Cu, and Cd) and sulfur dioxide (SO<sub>2</sub>) that may cause toxic effects independently or in concert with Pb. Reductions in the use of alkyl-Pb additives in gasoline have resulted in significant decreases in Pb deposition to natural ecosystems in the northeastern United States (Johnson et al., 1995a). However, the period in which Pb deposition has declined (ca. 1975 to the present) has also seen significant reductions in the acidity (i.e., increased pH) of precipitation in the region (Likens et al., 1996; Driscoll et al., 1998). Therefore, changes in ecosystem Pb fluxes may be the result of reduced Pb inputs and/or reduced acidity.

Experimental manipulation studies do not suffer from these confounding effects, because Pb can be added in specific amounts, with or without other compounds. Unfortunately, ecosystem-level manipulations involving Pb additions have not been undertaken. Therefore, we must use observations from field studies of Pb behavior in sites exposed to various forms of Pb pollution to assess the effects of Pb on terrestrial ecosystems. This section includes a discussion of effects of Pb in the structure and function of terrestrial ecosystems. Effects on energy flows (food chain effects) and biogeochemical cycling are discussed in Section AX7.1.5.3.

#### ***Sites Affected by Nearby Point Sources of Lead***

Natural terrestrial ecosystems near smelters, mines, and other industrial plants have exhibited a variety of effects related to ecosystem structure and function. These effects include decreases in species diversity, changes in floral and faunal community composition, and decreasing vigor of terrestrial vegetation.

All of these effects were observed in ecosystems surrounding the Anaconda smelter in southwestern Montana, which operated between 1884 and 1980 (Galbraith et al., 1995). Soils in affected areas around the Anaconda smelter were enriched in Pb, arsenic, copper, cadmium, and

zinc; had very low pH; and were determined to be phytotoxic to native vegetation (Kapustka et al., 1995). The elevated soil arsenic and metal concentrations occurred despite significantly lower organic matter concentrations in affected soils relative to reference sites (Galbraith et al., 1995). Line-transect measurements indicated that affected sites had an average of 6.9 species per 10-m of transect, compared to 20.3 species per 10-m in the reference areas. More than 60% of the reference sites supported coniferous (58%) or deciduous (3%) forest communities, whereas less than 1% of the affected sites retained functioning forest stands. Abundant dead timber and stumps confirmed that the affected sites were once as forested as the reference sites. Affected grassland sites were also less diverse and had higher abundances of invasive species than reference grasslands. More than 50% of the affected sites were classified as bare ground. The occurrence of bare ground was significantly correlated with the phytotoxicity scores derived by Kapustka et al. (1995), indicating a link between phytotoxicity and the loss of vegetation in the affected area.

Because of the plant community changes near the Anaconda smelter, the vertical diversity of habitats in the affected ecosystems decreased, with only shrubs and soil remaining as viable habitats. Galbraith et al. (1995) also used the Bureau of Land Management's habitat evaluation procedure (HEP) to estimate habitat suitability indices (HSI) for two indicator species, marten (*Martes americana*) and elk (*Cervus elaphus*). The HSI value ranges from 0 (poor habitat) to 1 (ideal habitat). In sites affected by the Anaconda smelter, HSI values for marten averaged 0.0, compared to 0.5 to 0.8 for the reference sites. For elk, affected sites had an average HSI of 0.10, compared to 0.31 at reference sites.

Similar observations were made in the area surrounding Palmerton, Pennsylvania, where two zinc smelters operated between 1898 and 1980. Soils in the area were enriched in Cd, Zn, Pb, and Cu, with concentrations decreasing with distance from the smelter sites (Beyer et al., 1985; Storm et al., 1994). Smelting was determined to be the principal source of Pb in soils in residential and undeveloped areas around Palmerton (Ketterer et al., 2001), which lies on the north side of a gap in Blue Mountain, a ridge running roughly east-west in east-central Pennsylvania. Much of the north-facing side of Blue Mountain within 3 km of the town is bare ground or sparsely vegetated, whereas the surrounding natural landscape is predominantly oak forest (Sopper, 1989; Storm et al., 1994). Biodiversity in affected areas is considerably lower than at reference sites, a pattern attributed to emissions from the smelters (Beyer et al., 1985;

Sopper, 1989). The history is complicated, however, by the land use history of the area. Logging and fire in the early 20th century may also have played a role in the changes in the terrestrial ecosystems (Jordan, 1975). Extensive logging occurred after the smelters began operation, suggesting that some of the logging may have been salvage logging in affected areas. Regardless, the smelter emissions appear to have inhibited the regrowth of ecosystems compared to those in nearby unaffected areas. As in Anaconda, MT, the changes in the structure and function of the Palmerton ecosystem changed its suitability as a habitat for fauna that would normally inhabit the area. Storm et al. (1994) did not find amphibians or common invertebrates in two study sites nearest to the smelters. In the larger study area, they documented elevated concentrations of Pb, Cd, Cu, and Zn in tissues of species ranging in size from red-backed salamanders (*Pletheron cenereus*) to white-tailed deer (*Odocoilius virginianus*).

Metal pollution around a Pb-Zn smelter near Bristol, England has not resulted in the loss of oak woodlands within 3 km of the smelter, despite significant accumulation of Pb, Cd, Cu, and Zn in soils and vegetation (Martin and Bullock, 1994). However, the high metal concentrations have favored the growth of metal-tolerant species in the woodland.

The effects of Pb and other chemical emissions on terrestrial ecosystems near smelters and other industrial sites decrease downwind from the source. Several studies using the soil burden as an indicator have shown that much of the contamination occurs within a radius of 20 to 50 km around the emission source (Miller and McFee, 1983; Martin and Bullock, 1994; Galbraith et al., 1995; Spurgeon and Hopkin, 1996a; see also Section AX7.1.2). For example, the concentration of Pb in forest litter declined downwind from a Pb-Zn smelter near Bristol, UK, from 2330 to 3050 ppm in a stand 2.9 km from the smelter to 45 to 110 ppm in a stand 23 km from the smelter (Martin and Bullock, 1994). Thus, while sites near point sources of Pb may experience profound effects on ecosystem structure and function, the extent of those effects is limited spatially. Elevated metal concentrations around smelters have been found to persist despite significant reductions in emissions (Hrsak et al., 2000). Most terrestrial ecosystems are far enough from point sources that long-range Pb transport is the primary mechanism for Pb inputs.

### *Sites Affected by Long-Range Lead Transport*

Because the effects of anthropogenic Pb emissions tend to be restricted in geographic extent, most natural terrestrial ecosystems in the U.S. sites have Pb burdens derived primarily from long-range atmospheric transport. Pollutant Pb represents a large fraction of the Pb in many of these ecosystems. In particular, many of these sites have accumulated large amounts of Pb in soils. For example, at the Hubbard Brook Experimental Forest in New Hampshire, the amount of Pb in the forest floor was estimated to have increased from about 1.35 kg ha<sup>-1</sup> in 1926 (before the introduction of alkyl-Pb additives in gasoline) to 10.5 kg ha<sup>-1</sup> in 1977 (Johnson et al., 1995a). They also estimated the atmospheric Pb deposition from 1926 to 1987 to be 8.7 kg ha<sup>-1</sup>, an amount that could account for nearly all of the increase in Pb in the forest floor during the period. The input of precipitation Pb to the Hubbard Brook ecosystem in the six decades spanning 1926 to 1987 was more than half of the total Pb estimated to have been released by mineral weathering in the entire 12,000- to 14,000-year post-glacial period (14.1 kg ha<sup>-1</sup>: [Johnson et al., 2004]). Other studies employing Pb budgets (Miller and Friedland, 1994; Watmough et al., 2004), and Pb isotopes (Bacon et al., 1995, 1996; Watmough et al., 1998; Bindler et al., 1999; Hansmann and Köppel, 2000; Kaste et al., 2003), have also shown that pollutant Pb, primarily from gasoline combustion, represents a quantitatively significant fraction of labile Pb in temperate soils, especially in the upper, organic-rich horizons.

Despite years of elevated atmospheric Pb inputs and elevated concentrations in soils, there is little evidence that sites affected primarily by long-range Pb transport have experienced significant effects on ecosystem structure or function. Low concentrations of Pb in soil solutions, the result of strong complexation of Pb by soil organic matter, may explain why few ecological effects have been observed. At Hubbard Brook, for example, the concentration of Pb in soil solutions draining the Oa horizon is <0.1 μM and is even lower in solutions draining mineral-soil horizons (Driscoll et al., 1988; Wang et al., 1995). Friedland and Johnson (1985) measured similar concentrations in soil solutions collected from deciduous and spruce-fir stands on Camel's Hump Mountain in Vermont. In an undeveloped, forested watershed in Maryland, Scudlark et al. (2005) found that atmospheric input of some elements (Al, Cd, Ni, Zn) is effectively transmitted through the watershed, whereas other elements (Pb, As, Se, Fe, Cr, Cu) are strongly sequestered, in the respective order noted.

In ecosystems where Pb concentrations in soil solutions are low, toxicity levels for vegetation are not likely to be reached regardless of the soil Pb concentration. Furthermore, mycorrhizal infection of tree roots appears to reduce the translocation of Pb from roots to shoots (Marschner et al., 1996; Jentschke et al., 1998). In a study of mycorrhizal and non-mycorrhizal Norway spruce (*Picea abies* (L.) Karst.), mycorrhizal infection of roots was not affected by Pb dose. Some, but not all, species of mycorrhizae showed reductions in the amount of extrametrical mycelium with Pb exposure but only at solution concentrations of 5  $\mu\text{M}$ , a level at least 50 times greater than typical concentrations in forest soils. In a related study, the growth rate of mycorrhizal fungi was unaffected at solution Pb concentrations of 1 and 10  $\mu\text{M}$ , but decreased at 500  $\mu\text{M}$  (Marschner et al., 1999).

Low soil solution Pb concentrations and the influence of mycorrhizal symbionts also result in low uptake of Pb by terrestrial vegetation. The net flux of Pb into vegetation in the northern hardwood forest at Hubbard Brook in the 1980s was estimated as only 1  $\text{g ha}^{-1} \text{ year}^{-1}$  (Johnson et al., 1995a), representing 3% of the precipitation input. Klaminder et al. (2005) also measured a Pb uptake of 1  $\text{g ha}^{-1} \text{ year}^{-1}$  in a spruce-pine forest in northern Sweden. Despite plant uptake fluxes being very low, they are sensitive to differences and changes in Pb deposition. Berthelsen et al. (1995) observed decreases in the Pb content of stem, twig, leaf, and needle tissues of a variety of tree species in Norway between 1982 and 1992, when atmospheric Pb deposition declined by approximately 70%. They also observed significantly lower Pb concentrations in tree tissues collected in northern Norway versus southern Norway, where atmospheric Pb deposition is greater.

Even at subtoxic concentrations, Pb and other metals may influence species diversity in terrestrial ecosystems. However, little work has been done on the effect of low-level metal concentrations on species diversity. In one study, plant species diversity was positively correlated to the concentration of available Pb in natural and artificial urban meadows in Britain (McCrea et al., 2004). The authors hypothesized that Pb may inhibit phosphorous uptake by dominant species, allowing less abundant (but more Pb-tolerant) ones to succeed.

#### **AX7.1.4.3 Effects of Lead on Energy Flows and Biogeochemical Cycling**

In terrestrial ecosystems, energy flow is closely linked to the carbon cycle. The principal input of energy to terrestrial ecosystems is through photosynthesis, in which  $\text{CO}_2$  is converted to

biomass carbon. Because of this link between photosynthesis and energy flow, any effect that Pb has on the structure and function of terrestrial ecosystems (as discussed in Section AX7.1.5.3.) influences the flow of energy into the ecosystem. This section focuses on how Pb influences energy transfer within terrestrial ecosystems, which begin with the decomposition of litter and other detrital material by soil bacteria and fungi, and cascade through the various components of the detrital food web. Because the mobility of Pb in soils is closely tied to organic matter cycling, decomposition processes are central to the biogeochemical cycle of Pb. This section concludes with a discussion of how biogeochemical cycling of Pb has changed in response to the changing Pb inputs to terrestrial ecosystems.

### *Effects of Lead on Detrital Energy Flows*

Lead can have a significant effect on energy flows in terrestrial ecosystems. At some sites severely affected by metal pollution, death of vegetation can occur, dramatically reducing the input of carbon to the ecosystem (Jordan, 1975; Galbraith et al., 1995). Subsequently, wind and erosion may remove litter and humus, leaving bare mineral soil, a nearly sterile environment in which very little energy transfer can take place (Little and Martin, 1972; Galbraith et al., 1995).

At Pb-affected sites that can retain a functioning forest stand, the rate of decomposition of litter may be reduced, resulting in greater accumulation of litter on the forest floor than in unpolluted stands. Numerous investigators have documented significant declines in litter decomposition rates (Cotrufo et al., 1995; Johnson and Hale, 2004) and/or the rate of carbon respiration (Laskowski et al., 1994; Cotrufo et al., 1995; Saviozzi et al., 1997; Niklinska et al., 1998; Palmborg et al., 1998; Aka and Darici, 2004) in acid- and metal-contaminated soils or soils treated with Pb. The resulting accumulation of organic matter on the soil surface can be dramatic. For example, an oak woodland 3 km from a smelter in Bristol, England had a litter layer mass 10 times greater than the mass in a similar stand 23 km from the smelter (Martin and Bullock, 1994).

Reduced decomposition rates in polluted ecosystems are the result of the inhibition of soil bacteria and fungi and its effects on microbial community structure (Bååth, 1989). Kuperman and Carreiro (1997) observed 60% lower substrate-induced respiration in heavily polluted grassland soils near the U.S. Army's Aberdeen Proving Ground in Maryland. This decline in carbon respiration was associated with 81% lower bacterial biomass and 93% lower fungal

biomass. Similar declines in the activities of carbon-, nitrogen-, and phosphorus-acquiring enzymes were also observed. Such dramatic effects have only been observed in highly contaminated ecosystems. In a less contaminated grassland site near a Pb factory in Germany, Chander et al. (2001) observed a lower ratio of microbial biomass carbon to soil organic carbon in polluted soils. The ratio of basal respiration to microbial biomass (the “metabolic quotient,”  $qCO_2$ ) declined with increasing metal concentration, though this observation depended on the procedure for measuring microbial biomass (substrate-induced respiration versus fumigation-extraction). The combined effect of lower microbial biomass per unit soil carbon and similar or lower  $qCO_2$  on polluted sites indicates that the ability of soil microorganisms to process carbon inputs is compromised by metal pollution.

The type of ecosystem also plays a role in determining the effects of Pb and other metals on the microbial processing of litter. Forest soils in temperate zones accumulate organic matter at the soil surface to a greater degree than in grasslands. This organic-rich O horizon can support a large microbial biomass; but it is also an effective trap for Pb inputs, because of the association between Pb and soil organic matter. At highly contaminated forest sites, microbial biomass and enzyme activities may be depressed (Fritze et al., 1989; Bååth et al., 1991), causing slower decomposition of the litter.

In addition to effects on decomposition and carbon transformations, Pb and other trace metals can also influence key nitrogen cycling processes. Studies in the 1970s demonstrated that Pb and other metals inhibit the mineralization of nitrogen from soil organic matter and nitrification (Liang and Tabatabai, 1977, 1978), resulting in lower nitrogen availability to plants. More recent research has documented significant inhibitory effects of Pb and other metals on the activities of several enzymes believed to be crucial to nitrogen mineralization in soils (Senwo and Tabatabai, 1999; Acosta-Martinez and Tabatabai, 2000; Ekenler and Tabatabai, 2002). This suggests that the inhibitory effect of Pb and other metals is broad-based, and not specific to any particular metabolic pathway. In reducing environments, the rate of denitrification is also depressed by trace metals. Fu and Tabatabai (1989) found that  $2.5 \mu\text{mol g}^{-1}$  of Pb (ca.  $500 \text{ mg/kg}^{-1}$ ) was sufficient to cause 0, 27, and 52% decreases in nitrogen reductase activity in three different soils.

Metal pollution can also affect soil invertebrate populations. Martin and Bullock (1994) observed lower abundances of a variety of woodlice, millipedes, spiders, insects, and earthworms

in an oak woodland site 3 km from a Pb-Zn smelter in Bristol, England, compared to a reference site 23 km from the smelter. The differences were most dramatic when expressed per unit mass of litter. Several species that were abundant in the reference site were not found in the contaminated woodland. For example, the abundance of the woodlice *Trichoniscus pusillus* was 151 individuals per m<sup>2</sup> in the reference woodland, but none were found in the contaminated soils. This was also true of 2 of the 3 millipede species, and 4 of the 5 earthworm species studied. At six sites within 1 km from the smelters, no earthworms were present at all (Spurgeon and Hopkin, 1996a). Contamination at this site has apparently reduced both the population and biodiversity of the soil invertebrate community.

The effect of metal pollution on soil invertebrates may be a threshold-type response. In a study conducted in woodlands near two zinc smelters in Noyelles-Godault, in northern France, soils at the most polluted site were devoid of mites and millipedes, while the remaining sites had diversity measures similar to control sites (Grelle et al., 2000).

While Pb pollution affects the population and diversity of soil fauna, there is little evidence of significant bioaccumulation of Pb in the soil food web (see also Section AX7.1.3). In the Bristol, England study, Pb concentrations in earthworms were lower than soil Pb concentrations and much lower than litter Pb concentrations (Martin and Bullock, 1994). Litter-dwelling mites had Pb concentrations that were 10% of the average litter concentration. The predator centipedes *Lithobius forficatus* and *L. variegatus* had mean Pb concentrations of 18.6 and 44.0 mg kg<sup>-1</sup>, respectively, two orders of magnitude lower than the Pb concentration of litter (2193 mg kg<sup>-1</sup>) and lower than the concentrations of their known prey species. In a study conducted in a Norway spruce forest affected primarily by automobile exhaust from a nearby highway, earthworms had Pb concentrations similar to the soil (Roth, 1993). Almost all of the litter decomposers, however, had Pb concentrations that were less than 20% of the litter. All but 3 of the zoophagous arthropods had Pb concentrations that were less than 40% of their prey; the remaining 3 had Pb concentrations similar to their prey. Because of the absence of significant bioaccumulation in the soil food web, predator species will be affected by Pb pollution primarily through effects on the abundance of their prey (Spurgeon and Hopkin, 1996b).

Taken as a whole, ecosystem-level studies of the soil food web indicate that Pb can affect energy flows in terrestrial ecosystems through two principal mechanisms. In the most severely polluted sites, the death of primary producers directly decreases the flow of energy into the

ecosystems. More commonly, the accumulation of toxic levels of Pb or other metals in litter and soil decreases the rate of litter decomposition through decreases in microbial biomass and/or respiration. These reductions can subsequently affect higher trophic levels that depend on these organisms. It is important to note that sites that have exhibited significant disruption to energy flows and the terrestrial food web are sites that have experienced severe metal contamination and adverse effects from SO<sub>2</sub> from smelters or other metals-related activities.

### *Lead Dynamics in Terrestrial Ecosystems*

Lead inputs to terrestrial ecosystems in the United States have declined dramatically in the past 30 years, primarily because of the almost complete elimination of alkyl-Pb additives in gasoline in North America. Also, Pb emissions from smelters have declined as older plants have been shut down or fitted with improved emissions controls. Unfortunately, there are few long-term data sets of precipitation inputs to terrestrial ecosystems. At the Hubbard Brook Experimental Forest, in New Hampshire, Pb input in bulk deposition declined by more than 97% between 1976 and 1989 (Johnson et al., 1995a). Studies of freshwater sediments also indicate a dramatic decline in Pb inputs since the mid-1970s (Graney et al., 1995; Johnson et al., 1995a; Farmer et al., 1997; Brännvall et al., 2001a,b).

Reported concentrations of Pb in waters draining natural terrestrial ecosystems have always been low (Wang et al., 1995; Bacon and Bain, 1995; Johnson et al., 1995a; Vinogradoff et al., 2005), generally less than 1 ng L<sup>-1</sup>, even at moderately polluted sites (Laskowski et al., 1995). Consequently, most terrestrial ecosystems in North America and Europe remain sinks for Pb despite reductions in atmospheric Pb deposition of more than 95%. At Hubbard Brook, for example, the input of Pb in bulk precipitation declined from 325 g ha<sup>-1</sup> year<sup>-1</sup> between 1975 and 1977 compared to 29 g ha<sup>-1</sup> year<sup>-1</sup> between 1985 and 1987 (Johnson et al., 1995a). During the same period, the output of Pb in stream water declined from 6 g ha<sup>-1</sup> year<sup>-1</sup> to 4 g ha<sup>-1</sup> year<sup>-1</sup>. Thus, despite the decline in Pb input, 85% of the incoming Pb was still retained in the terrestrial ecosystem in the later time period. Similar observations have been made in Europe, where the use of leaded gasoline has also declined in the last few decades. At the Glensaugh Research Station in Scotland, the input of Pb to the forest ecosystem was estimated as 42.6 g ha<sup>-1</sup> year<sup>-1</sup> between 2001 and 2003, about six times the stream export of 7.2 g ha<sup>-1</sup> year<sup>-1</sup> (Vinogradoff et al., 2005). Similarly, Huang and Matzner (2004) reported a throughfall flux of 16.5 g ha<sup>-1</sup> year<sup>-1</sup> at

the forested Lehstenbach catchment in Bavaria, about six times the efflux in runoff of 2.82 g ha<sup>-1</sup> year<sup>-1</sup>.

Lead pollution has resulted in the accumulation of large Pb burdens in terrestrial ecosystems (see also Section AX7.1.2). Despite reductions in emissions, this accumulation of Pb continues, though at markedly lower rates. The large pool of Pb bound in soils may potentially be a threat to aquatic ecosystems (see Section AX7.2), depending on its rate of release from the soil. Early estimates of the residence time of Pb in the forest floor ranged from 220 to 5,000 years (Benninger et al., 1975; Friedland and Johnson, 1985; Turner et al., 1985). However, more recent literature suggests that Pb is transported more rapidly within soil profiles than previously believed. The pool of Pb in forest floor soils of the northeastern United States declined significantly in the late 20th century. Friedland et al. (1992) reported a 12% decline in the amount of Pb in forest floor soils at 30 sites in the region between 1980 and 1990, a much greater decline than would be expected for a pool with a residence time of 220 to 5,000 years. At Hubbard Brook, the pool of Pb in the forest floor declined by 29% between 1977 and 1987, an even more rapid rate of loss than reported by Friedland et al. (1992). More recently, Evans et al. (2005) reported significant declines in the Pb content of forest floor soils in the northeastern United States and eastern Canada between 1979 and 1996. The magnitude of the decrease in Pb content was greatest at their sites in southern Vermont, and smallest at sites on the Gaspé Peninsula in Quebec, reflecting the historic gradient in Pb deposition in the region. At the Vermont site, the Pb concentration in the litter layer (Oi horizon) was 85% lower in 1996 than in 1979. In the Gaspé peninsula of Quebec, the decrease was only 50%.

Since drainage water Pb concentrations remain low, the Pb released from forest floor soils in the past has been largely immobilized in mineral soils (Miller and Friedland, 1994; Johnson et al., 1995a; Johnson and Petras, 1998; Watmough and Hutchinson, 2004; Johnson et al., 2004). This is supported by evidence from Pb-isotope analyses. Gasoline-derived Pb has a <sup>206</sup>Pb:<sup>207</sup>Pb ratio that can be easily discriminated from Pb in the rocks from which soils are derived. Using isotopic mixing models with gasoline-Pb and Pb in soil parent materials as end members, a number of researchers have documented the accumulation of pollutant Pb in mineral soils (Bindler et al., 1999; Kaste et al., 2003; Watmough and Hutchinson, 2004; Bacon and Hewitt, 2005; Steinnes and Friedland, 2005). In a hardwood stand on Camel's Hump Mountain in Vermont, as much as 65% of the pollutant Pb deposited to the stand had moved into mineral

horizons by 2001 (Kaste et al., 2003). In a spruce-fir stand, containing a thicker organic forest floor layer, penetration of pollutant Pb into the mineral soil was much lower.

This recent research has resulted in a reevaluation of the turnover time of Pb in forest floor soils. The Camel's Hump data suggest that Pb resides in the forest floor of deciduous stands for about 60 years and about 150 years in coniferous stands (Kaste et al., 2003). These values are somewhat greater than those published previously by Miller and Friedland (1994), who used a Pb budget approach. Extremely rapid turnover of Pb was observed in some hardwood forest floor soils in south-central Ontario (Watmough et al., 2004). Their estimated turnover times of 1.8 to 3.1 years are much lower than any other published values, which they attribute to the mull-type forest floor at their sites. Mull-type forest floors are normally underlain by organic-rich A horizons, capable of immobilizing Pb released from the forest floor. Indeed, at the same site in Ontario, Watmough and Hutchinson (2004) found that 90% of the pollutant Pb could be found in this A horizon.

The time period over which the accumulated Pb in soils may be released to drainage waters remains unclear. If Pb moves as a pulse through the soil, there may be a point in the future at which problematic Pb concentrations occur. However, several authors have argued against this hypothesis (Wang and Benoit, 1997; Kaste et al., 2003; Watmough et al., 2004), contending that the strong linkage between Pb and DOM will result in a temporally dispersed release of Pb in the form of Pb-DOM complexes. Thus, the greatest threat is likely to be in the most highly contaminated areas surrounding point sources of Pb, where the amount of Pb accumulated in the soil is high, and the death of vegetation has resulted in reduced soil organic matter levels.

#### **AX7.1.4.4 Summary**

Atmospheric Pb pollution has resulted in the accumulation of Pb in terrestrial ecosystems throughout the world. In the United States, pollutant Pb represents a significant fraction of the total Pb burden in soils, even in sites remote from smelters and other industrial plants. However, few significant effects of Pb pollution have been observed at sites that are not near point sources of Pb. Evidence from precipitation collection and sediment analyses indicates that atmospheric deposition of Pb has declined dramatically (>95%) at sites unaffected by point sources of Pb, and

there is little evidence that Pb accumulated in soils at these sites represents a threat to groundwaters or surface water supplies.

The highest environmental risk for Pb in terrestrial ecosystems exists at sites within about 50 km of smelters and other Pb-emitting industrial sites. Assessing the risks specifically associated with Pb is difficult, because these sites also experience elevated concentrations of other metals and because of effects related to SO<sub>2</sub> emissions. The concentrations of Pb in soils, vegetation, and fauna at these sites can be two to three orders of magnitude higher than in reference areas (see Sections AX7.1.2 and AX7.1.4.2). In the most extreme cases, near smelter sites, the death of vegetation causes a near-complete collapse of the detrital food web, creating a terrestrial ecosystem in which energy and nutrient flows are minimal. More commonly, stress in soil microorganisms and detritivores can cause reductions in the rate of decomposition of detrital organic matter. Although there is little evidence of significant bioaccumulation of Pb in natural terrestrial ecosystems, reductions in microbial and detritivorous populations can affect the success of their predators. Thus, at present, industrial point sources represent the greatest Pb-related threat to the maintenance of sustainable, healthy, diverse, and high-functioning terrestrial ecosystems in the United States.

## **AX7.2 AQUATIC ECOSYSTEMS**

### **AX7.2.1 Methodologies Used in Aquatic Ecosystem Research**

As discussed in previous sections, aerial deposition is one source of Pb deposition to aquatic systems. Consequently, to develop air quality criteria for Pb, consideration must be given to not only the environmental fate of Pb, but also to the environmental effects of Pb in the aquatic environment through consideration of laboratory toxicity studies and field evaluations. Perhaps the most straightforward approach for evaluating the effects of Pb is to consider extant criteria for Pb in aquatic ecosystems, i.e., water and sediment quality criteria. A key issue in developing Pb water and sediment criteria that are broadly applicable to a range of water bodies is properly accounting for Pb bioavailability and the range in species sensitivities. This section summarizes how these criteria are derived, the types of toxicity studies considered, and key factors that influence the bioavailability of Pb in surface water and sediment to aquatic life. Because Pb in the aquatic environment is often associated with other metals (e.g., cadmium,

copper, zinc), the importance of considering the toxicity of metal mixtures is also discussed. Finally, some issues related to background Pb concentrations are briefly addressed. It is beyond the scope of this section to review all methodologies in aquatic system research, but good reviews can be found in summary books, such as Rand et al. (1995).

### AX7.2.1.1 Analytical Methods

Common analytical methods for measuring Pb in the aquatic environment are summarized in Table AX7-2.1.1. For relevance to the ambient water quality criteria (AWQC) and sediment quality criteria for Pb discussed below, minimum detection limits should be in the low parts per billion (ppb) range for surface water and the low parts per million (ppm) range for sediment.

**Table AX7-2.1.1. Common Analytical Methods for Measuring Lead in Water, Sediment, and Tissue**

Analysis Type	Analytical Method
Direct-Aspiration (Flame) Atomic Absorption Spectroscopy (AAS)	EPA SW-846 Method 7420 <sup>a</sup> , EPA Method 239.1 <sup>b</sup> , Standard Method 3111 <sup>c</sup>
Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)	EPA SW-846 Method 7421 <sup>a</sup> , EPA Method 239.2 <sup>b</sup> , Standard Method 3113 <sup>c</sup>
Inductively Coupled Plasma (ICP)	EPA SW-846 Method 6010B <sup>a</sup> , EPA Method 200.7 <sup>b</sup> , Standard Method 3120 <sup>c</sup>
Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)	EPA SW-846 Method 6020 <sup>a</sup> , EPA Method 200.8 <sup>b</sup>

<sup>a</sup> U.S. Environmental Protection Agency (1986c) Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW-846). Third Edition, September 1986; Final Updates I (7/92), IIA (8/93), II (9/94), IIB (1/95), III (12/96), IIIA (4/98), IIIB (11/04).

<sup>b</sup> U.S. Environmental Protection Agency (1991) Methods for the Determination of Metals in Environmental Samples. EPA/600/4-91-010. June 1991 (Supplement I, EPA/600/R-94-111, May 1994).

<sup>c</sup> American Public Health Association (1995) Standard Methods for the Examination of Water and Wastewater, 19th Edition. American Public Health Association, American Water Works Association, Water Pollution Control Federation.

In addition to the methods presented in Table AX7-2.1.1, many of the methods discussed in Section AX7.1.1 can be applied to suspended solids and sediments collected from aquatic

ecosystems. Just as in the terrestrial environment, the speciation of Pb and other trace metals in natural freshwaters and seawater plays a crucial role in determining their reactivity, mobility, bioavailability, and toxicity. Many of the same speciation techniques employed for the speciation of Pb in terrestrial ecosystems (see Section AX7.1.1 and AX7.1.2) are applicable in aquatic ecosystems.

There is now a better understanding of the potential effects of sampling, sample handling, and sample preparation on aqueous-phase metal speciation. Thus, a need has arisen for dynamic analytical techniques that are able to capture a metal's speciation, in-situ and in real time. Some of these recently developed dynamic trace metal speciation techniques include:

- Diffusion gradients in thin-film gels (DGT)
- Gel integrated microelectrodes combined with voltammetric in situ profiling (GIME-VIP)
- Stripping chronopotentiometry (SCP)
- Flow-through and hollow fiber permeation liquid membranes (FTPLM and HFPLM)
- Donnan membrane technique (DMT)
- Competitive ligand-exchange/stripping voltammetry (CLE-SV)

Various dynamic speciation techniques were compared in a study by Sigg et al. (2006) using freshwaters collected in Switzerland. They found that techniques involving in-situ measurement (GIME-VIP) or in-situ exposure (DGT, DMT, and HFPLM) appeared to be the most appropriate for avoiding Pb and other trace metal speciation artifacts associated with sampling and sample handling.

#### **AX7.2.1.2 Ambient Water Quality Criteria: Development**

The EPA's procedures for deriving AWQC are described in Stephan et al. (1985) and are summarized here. With few exceptions, AWQC are derived based on data from aquatic toxicity studies conducted in the laboratory. In general, both acute (short term) and chronic (long term) AWQC are developed. Depending on the species, the toxicity studies considered for developing acute criteria range in length from 48 to 96 hours. Acceptable endpoints for acute AWQC development are mortality and/or immobilization, expressed as the median lethal concentration (LC<sub>50</sub>) or median effect concentration (EC<sub>50</sub>). For each species, the geometric mean of the acceptable LC<sub>50</sub>/EC<sub>50</sub> data is calculated to determine the species mean acute value (SMAV).

For each genera, the geometric mean of the relevant SMAVs is then calculated to determine the genus mean acute value (GMAV). The GMAVs are then ranked from high to low, and the final acute value (FAV; the 5th percentile of the GMAVs, based on the four GMAVs surrounding the 5th percentile) is determined. Because the FAV is based on LC<sub>50</sub>/EC<sub>50</sub> values (which represent unacceptably high levels of effect), the FAV is divided by two to estimate a low-effect level. This value is then termed the acute criterion, or criterion maximum concentration (CMC). Based on the most recent AWQC document for Pb (U.S. Environmental Protection Agency, 1985), Table AX7-2.1.2 shows the freshwater SMAVs and GMAVs for Pb, and the resulting freshwater CMC. Note that the freshwater AWQC are normalized for the hardness of the site water, as discussed further below in Section AX7.2.1.3.

**Table AX7-2.1.2. Development of Current Acute Freshwater Criteria for Lead (U.S. Environmental Protection Agency, 1985)<sup>1</sup>**

<b>Rank</b>	<b>Species</b>	<b>GMAV (µg/L)</b>	<b>SMAV (µg/L)</b>
10	Midge ( <i>Tanytarsus dissimilis</i> )	235,900	235,900
9	Goldfish ( <i>Carassius auratus</i> )	101,100	101,100
8	Guppy ( <i>Poecilia reticulata</i> )	66,140	66,140
7	Bluegill ( <i>Lepomis macrochirus</i> )	52,310	52,310
6	Fathead minnow ( <i>Pimephales promelas</i> )	25,440	25,440
5	Brook trout ( <i>Salvelinus fontinalis</i> )	4,820	4,820
4	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	2,448	2,448
3	Snail ( <i>Aplexa hypnorum</i> )	1,040	1,040
2	Cladoceran ( <i>Daphnia magna</i> )	447.8	447.8
1	Amphipod ( <i>Gammarus pseudolimnaeus</i> )	142.6	142.6
		<b>FAV = 67.54 µg/L</b>	
		<b>CMC = 33.77 µg/L</b>	

<sup>1</sup> All values are normalized to a hardness of 50 mg/L (see Section AX7.2.1.3).

To develop chronic AWQC, acceptable chronic toxicity studies should encompass the full life cycle of the test organism, although for fish, early life stage or partial life cycle toxicity studies are considered acceptable. Acceptable endpoints include reproduction, growth and development, and survival, with the effect levels expressed as the chronic value, which is the geometric mean of the no-observed-effect concentration (NOEC)<sup>1</sup> and the lowest-observed-effect concentration (LOEC)<sup>2</sup>. Although a chronic criterion could be calculated as the 5th percentile of genus mean chronic values (GMCVs), sufficient chronic toxicity data are generally lacking, as is the case for Pb. Consequently, an acute-chronic ratio (ACR) is typically applied to the FAV to derive the chronic criterion. As the name implies, the ACR is the ratio of the acute LC<sub>50</sub> to the chronic value, based on studies with the same species and in the same dilution water. For Pb, the final ACR is 51.29, which results in a final chronic value (FCV) of 1.317 µg/L (at a hardness of 50 mg/L). The U.S. EPA guidelines for developing AWQC (Stephan et al., 1985) are now more than 20 years old and thus are not reflective of scientific advances in aquatic toxicology and risk assessment that have developed since the 1980s. For example, the toxicological importance of dietary metals has been increasingly recognized and approaches for incorporating dietary metals into regulatory criteria are being evaluated (Meyer et al., 2005). Other issues include consideration of certain sublethal endpoints that are currently not directly incorporated into AWQC development (e.g., endocrine toxicity, behavioral responses) and protection of threatened and endangered (T&E) species (U.S. Environmental Protection Agency, 2003). In deriving appropriate and scientifically defensible air quality criteria for Pb, it will be important that the state-of-the-science for metals toxicity in aquatic systems be incorporated into the development process.

Subsequent sections summarize some of the toxicity studies that meet the AWQC development guidelines, with an emphasis on key studies published since the last Pb AWQC were derived in 1984.

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<sup>1</sup> The NOEC is the highest concentration tested that did not result in statistically significant effects relative to the control.

<sup>2</sup> The LOEC is the lowest concentration tested that resulted in statistically significant effects relative to the control.

### **AX7.2.1.3 Ambient Water Quality Criteria: Bioavailability Issues**

In surface waters, the environmental fate of metal contaminants is mitigated through adsorption, complexation, chelation, and other processes that affect bioavailability. The toxicity of divalent cations tends to be highest in soft waters with low concentrations of dissolved organic matter and suspended particles. In an acidic environment (pH <4), the ionic form of most metals generally predominates and is considered to be the more toxic form. As the pH increases, carbonate, oxide, hydroxide, and sulfide complexes of the metals tend to predominate, and tend to be less toxic (Florence, 1977; Miller and Mackay, 1980). The portion of dissolved metal available for uptake or bioaccumulation is influenced by modifying factors that “sequester” the metal in an environmental matrix, thereby reducing the bioavailability of the metal at the sites of action. Metals can become complexed (bound) to a ligand that can make metals either more toxic (via transport mechanisms) or less toxic (by changing the metal’s biological activity). Metals that complex tightly to ligands generally are not readily bioavailable and, thus, are less toxic to aquatic biota than their free-metal ion counterparts (Carlson et al., 1986; McCarthy, 1989). There are many kinds of ligands, organic and inorganic, as well as natural and man-made. Ligands found in natural surface waters and municipal and industrial effluent discharges include glycine, ammonia, oxalate, humic or fulvic acids, hydroxide, carbonate, bicarbonate, chloride, and hydrogen sulfide (Stumm and Morgan, 1970; Martin, 1986; Pagenkopf, 1986).

Recognizing the importance of calcium and magnesium ions (hardness) in modifying Pb toxicity, the current freshwater AWQC for Pb are normalized based on the hardness of the site water. The acute freshwater criteria, for example, are 30, 65, and 136 µg/L at hardness levels of 50, 100, and 200 mg/L (as CaCO<sub>3</sub>). Although it has been known for some time that other water quality parameters such as pH, dissolved organic carbon (DOC), and alkalinity affect the bioavailability of metals to aquatic biota, it was the relatively recent development of the biotic ligand model (BLM) that allows for AWQC to potentially consider all of these factors. Paquin et al. (2002) provided a thorough review of the factors influencing metal bioavailability and how research over the last few decades has culminated in the development of the BLM.

By understanding the binding affinities of various natural ligands in surface waters and how the freshwater fish gill interacts with free cations in the water, one can predict how metals exert their toxic effects (Schwartz et al., 2004). Models developed prior to the BLM are the free-ion activity model (FIAM) and the gill surface interaction model (GSIM). The FIAM accounts

for the binding of free-metal ion and other metal complexes to the site of toxic action in an organism; and it also considers competition between metal species and other cations (Paquin et al., 2002). The GSIM is fundamentally similar to the FIAM in that it accounts for competition between metal ions and hardness cations at the physiological active gill sites, but whereas the FIAM is largely conceptual, the GSIM was used in interpreting toxicity test results for individual metals and metal mixtures (Pagenkopf, 1983). The BLM was adapted from the GSIM and uses the biotic ligand, rather than the fish gill as the site of toxic action (Di Toro et al., 2001; Paquin et al., 2002). This approach, therefore, considers that the external fish gill surface contains receptor sites for metal binding (Schwartz et al., 2004) and that acute toxicity is associated with the binding of metals to defined sites (biotic ligands) on or within the organism (Paquin et al., 2002). The model is predicated on the theory that mortality (or other toxic effects) occurs when the concentration of metal bound to biotic ligand exceeds a threshold concentration (Di Toro et al., 2001; Paquin et al., 2002). Free metal cations “out compete” other cations and bind to the limited number of active receptor sites on the gill surface, which may ultimately result in suffocation and/or disruption of ionoregulatory mechanisms in the fish, leading to death (Di Toro et al., 2001; Paquin et al., 2002). Because the BLM uses the biotic ligand (not the fish gill) as the site of action, the model can be applied to other aquatic organisms, such as crustaceans, where the site of action is directly exposed to the aqueous environment (Di Toro et al., 2001).

Although the BLM is currently being considered as a tool for regulating metals on a site-specific basis, there are potential limitations in using the BLM to regulate metals in surface waters that should be understood in developing air quality criteria for lead. For example, BLMs developed to-date have focused on acute mortality/immobilization endpoints for fish and invertebrates. Chronic exposures are typically of greatest regulatory concern, but chronic BLMs to date have received limited attention (De Schamphelaere and Janssen, 2004). In addition, BLMs account for uptake of dissolved metal, but dietary metals have also been shown to contribute to uptake by aquatic biota and, in some cases, increased toxicity. Besser et al. (2005) observed that chronic (42-day) Pb toxicity to the amphipod *Hyaella azteca* was greater from a combined aqueous and dietary exposure than from a water-only exposure and the authors conclude that estimates of chronic toxicity thresholds for Pb should consider both aqueous and dietary exposure routes. The feasibility of incorporating dietary metals into BLMs is under investigation. Another important issue that must be addressed in developing and applying a

BLM in AWQC development is the type of dissolved organic matter used in model development relative to the types of dissolved organic matter at the site of interest. Richards et al. (2001) demonstrated that natural organic matter (NOM) of different types differentially influenced Pb and Cu accumulation by gills of rainbow trout. There are also other ligands not accounted for in the BLM that require more research. Bianchini and Bowles (2002) emphasized the importance of reduced sulfur as a metal ligand, limitations in scientific knowledge on reduced sulfur, and provided recommendations for studies necessary to incorporate sulfide ligands into the BLM.

The U.S. EPA is currently revising the aquatic life AWQC for lead, which will include toxicity data published after the 1985 AWQC were released and incorporation of the BLM is being evaluated. If an acute BLM is incorporated into the revised AWQC for lead, the chronic criteria would likely be estimated using an ACR (the same approach used when the acute criterion is based on empirical toxicity data; see above).

#### **AX7.2.1.4 Sediment Quality Criteria: Development and Bioavailability Issues**

As with metals in surface waters, the environmental fate of metal contaminants in sediments is moderated through various binding processes that reduce the concentration of free, bioavailable metal. Sediments function as a sink for Pb, as with most metals. Lead compounds such as Pb-carbonates, Pb-sulfates, and Pb-sulfides predominate in sediments (Prosi, 1989). Total Pb has a higher retention time and a higher percentage is retained in sediments compared to copper and zinc (Prosi, 1989). Lead is primarily accumulated in sediments as insoluble Pb complexes adsorbed to suspended particulate matter. Naturally occurring Pb is bound in sediments and has a low geochemical mobility (Prosi, 1989). Organic-sulfide and moderately reducible fractions are less mobile, whereas cation-exchangeable fractions and easily-reducible fractions are more mobile and more readily bioavailable to biota (Prosi, 1989). Most Pb transported in surface waters is in a particulate form, originating from the erosion of sediments in rivers or produced in the water column (Prosi, 1989).

Sediment quality criteria have yet to be adopted by the EPA, but an equilibrium partitioning procedure has recently been published (U.S. Environmental Protection Agency, 2005c). The EPA has selected an equilibrium partitioning approach because it explicitly accounts for the bioavailability of metals. This approach is based on mixtures of cadmium, copper, Pb, nickel, silver, and zinc. Equilibrium partitioning (EqP) theory predicts that metals

partition in sediment between acid-volatile sulfide, pore water, benthic organisms, and other sediment phases such as organic carbon. When the sum of the molar concentrations of simultaneously extracted metal ( $\Sigma\text{SEM}$ ) minus the molar concentration of AVS is less than zero, it can accurately be predicted that sediments are not toxic because of these metals. Note that this approach can be used to predict the lack of toxicity, but not the presence of toxicity. It is important to emphasize that metals must be evaluated as a mixture using this approach. If individual metals, or just two or three metals, are measured in sediment,  $\Sigma\text{SEM}$  would be misleadingly small and it may inaccurately appear that  $\Sigma\text{SEM} / \text{AVS}$  is less than 1.0.

If  $\Sigma\text{SEM} / \text{AVS}$  is normalized to the organic carbon fraction (i.e.,  $(\Sigma\text{SEM} / \text{AVS})/f_{\text{OC}}$ ), mortality can be more reliably predicted by accounting for both the site-specific organic carbon and AVS concentrations. When evaluating a metal mixture containing cadmium, copper, Pb, nickel, silver, and zinc, the following predictions can be made (U.S. Environmental Protection Agency, 2005c):

- A sediment with  $(\text{SEM} / \text{AVS})/f_{\text{OC}} < 130 \mu\text{mol/g}_{\text{OC}}$  should pose low risk of adverse biological effects due to these metals.
- A sediment with  $130 \mu\text{mol/g}_{\text{OC}} < (\text{SEM} / \text{AVS})/f_{\text{OC}} < 3000 \mu\text{mol/g}_{\text{OC}}$  may have adverse biological effects due to these metals.
- In a sediment with  $(\text{SEM} / \text{AVS})/f_{\text{OC}} > 3000 \mu\text{mol/g}_{\text{OC}}$ , adverse biological effects may be expected.

A third approach is to measure pore water concentrations of cadmium, copper, Pb, nickel, and zinc and then divide the concentrations by their respective FCVs. If the sum of these quotients is  $< 1.0$ , these metals are not expected to be toxic to benthic organisms.

It should be noted that although EPA has endorsed the AVS-SEM approach for developing sediment guidelines, other investigators do not necessarily agree, as the AVS-SEM approach may not be relevant to benthic organisms that ingest sediment particles. For example, Griscom et al. (2002) found that metals associated with either reduced or oxidized sediment particles can be assimilated by deposit and suspension feeding bivalve species due to the low pH and moderate reducing conditions in bivalve guts. Although Lee et al. (2000) agreed that the AVS-based approach may be appropriate for protecting some benthic organisms from acute toxicity associated with exposure to very high pore water concentrations of metals, they questioned whether the AVS-based approach is relevant to less contaminated sediments under

more natural conditions with vertical stratification of oxygen concentrations and, hence, varying levels of AVS. The authors concluded that important uncertainties remain in the application of the AVS-SEM approach as a regulatory tool. Similarly, some studies suggest that AVS-SEM measurements in the natural environment must be interpreted cautiously as AVS can be quite variable with sediment depth and season (Van den Berg et al., 1998). Finally, Long et al. (1998) critically compared the ability of AVS-SEM and dry weight-normalized concentrations to predict toxicity of sediment-associated trace metals. Based on an analysis of 77 field-collected sediment samples, Long et al. (1998) found that dry weight-normalized concentrations were equally or slightly more accurate than AVS-SEM in predicting non-toxic and toxic results in laboratory bioassays. Long et al. suggests that a limitation of the AVS-SEM approach is that the criteria were not derived from field studies with mixture of toxic chemicals and thus may be less relevant to sites comprising complex chemical mixtures. Thus, although the AVS-SEM approach for developing sediment quality criteria is being pursued by the U.S. EPA, there is clearly not scientific consensus on this approach, at least not for all circumstances.

Many alternative approaches for developing sediment quality guidelines are based on empirical correlations between metal concentrations in sediment to associated biological effects, based on sediment toxicity tests (Long et al., 1995; Ingersoll et al., 1996; MacDonald et al., 2000). However, these guidelines are based on total metal concentrations in sediment and do not account for the bioavailability of metals between sediments. Sediment quality guidelines proposed for Pb from these other sources are shown in Table AX7-2.1.3.

**Table AX7-2.1.3. Recommended Sediment Quality Guidelines for Lead**

Source	Water Type	Guideline Type	Conc. (mg/kg dw)
MacDonald et al. (2000)	Freshwater	TEC	35.8
		PEC	128
Ingersoll et al. (1996)	Freshwater	ERL	55
		ERM	99
Long et al. (1995)	Saltwater	ERL	46.7
		ERM	218

TEC = Threshold effect concentration; PEC = Probable effect concentration; ERL = Effects range – low;  
ERM = Effects range – median

#### AX7.2.1.5 Metal Mixtures

As discussed above, the EPA's current approach for developing sediment criteria for Pb and other metals is to consider the molar sum of the metal concentrations ( $\Sigma$ SEM). Although a similar approach has not been applied to AWQC, metal mixtures have been shown to be more toxic than individual metals (Spehar and Fiandt, 1986; Enserink et al., 1991). Spehar and Fiandt (1986) evaluated the acute and chronic toxicity of a metal mixture (arsenic, cadmium, chromium, copper, mercury, and Pb) to fathead minnows (*Pimephales promelas*) and a daphnid (*Ceriodaphnia dubia*). In acute tests, the joint toxicity of these metals was observed to be more than additive for fathead minnows and nearly strictly additive for daphnids. In chronic tests, the joint toxicity of the metals was less than additive for fathead minnows and nearly strictly additive for daphnids. One approach for considering the additive toxicity of Pb with other metals is to use the concept of toxic units (TUs). Toxic units for each component of a metal mixture are derived by dividing metal concentrations by their respective acute or chronic criterion. The TUs for all the metals in the mixture are then summed. A  $\Sigma$ TU > 1.0 suggests the metal mixture is toxic (note that this is the same approach as discussed above for developing metal sediment criteria based on pore water concentrations). According to Norwood et al. (2003), the TU approach is presently the most appropriate model for predicting effects of metal mixtures based on the currently available toxicity data. However, it should also be emphasized that the TU approach is most appropriate at a screening level, because the true toxicity of the mixture is dependent on the relative amounts of each metal. The TU approach is also recommended with mixtures containing less than six metals.

Lead and other metals often co-occur in sediments with other toxicants, such as organic contaminants. Effects-based sediment quality guidelines (SQGs) have been developed over the past 20 years to aid in the interpretation of the relationships between complex chemical contamination and adverse biological effects (Long et al., 2006). Mean sediment quality guideline quotients (mSQGQs) can be calculated by dividing the concentrations of chemicals in sediments by their respective SQGs and then calculating the mean of the quotients for the individual chemicals. Long et al. (2006) performed a critical review of this approach and found that it reasonably predicts the incidence and magnitude of toxicity in laboratory tests and the incidence of impairment to benthic communities increases incrementally with increasing mSQGQs. However, the authors pointed out some of the limitations of this approach, such as a

lack of agreement on the level of mSQGQs, masking of an individual chemical's effect due to data aggregation, lack of SQGs for all chemicals of concern, and mSQGQs were not initially derived as a regulatory standard or criterion, thus there is a reluctance to use them in enforcement or remediation (Long et al., 2006).

For assessing Pb effects on aquatic ecosystems, it is not truly feasible to account for metal mixtures, because these will obviously vary highly from site to site. However, the toxicity of metal mixtures in surface water should be considered on a site-specific basis.

#### **AX7.2.1.6 Background Lead**

Because Pb is naturally occurring, it is found in all environmental compartments including surface water, sediment, and aquatic biota. Background Pb concentrations are spatially variable depending on geological features and local characteristics that influence Pb speciation and mobility. In the European Union risk assessments for metals, an “added risk” approach has been considered that assumes only the amount of metal added above background is relevant in a toxicological evaluation. However, this approach ignores the possible contribution of background metal levels to toxic effects, and background metal levels are regionally variable, precluding the approach from being easily transferable between sites. In terms of deriving environmental criteria for Pb, background levels should be considered on a site-specific basis if there is sufficient information that Pb concentrations are naturally elevated. As discussed previously, the use of radiogenic Pb isotopes is useful for source apportionment.

#### **AX7.2.2 Distribution of Lead in Aquatic Ecosystems**

Atmospheric Pb is delivered to aquatic ecosystems primarily through deposition (wet and/or dry) or through erosional transport of soil particles (Baier and Healy, 1977; Dolske and Sievering, 1979; and Yang and Rose, 2005). A number of physical and chemical factors govern the fate and behavior of Pb in aquatic systems. The EPA summarized some of these controlling factors in the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a). For example, the predominant form of Pb in the environment is in the divalent ( $\text{Pb}^{2+}$ ) form and complexation with inorganic and organic ligands is dependent on pH (Lovering, 1976; Rickard and Nriagu, 1978). A significant portion of Pb in the aquatic environment exists in the undissolved form (i.e., bound to suspended particulate matter). The ratio of Pb in suspended solids to Pb in filtrate

varies from 4:1 in rural streams to 27:1 in urban streams (Getz et al., 1977). In still waters, Pb is removed through sedimentation at a rate determined by temperature, pH, oxidation-reduction (redox) potential, organic content, grain size, and chemical form of Pb in the water and biological activities (Jenne and Luoma, 1977). Since the publication of the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a), knowledge of the properties of Pb in aquatic ecosystems has expanded. This section will provide further detail on the chemical species and the environmental factors affecting speciation of Pb in the aquatic environment. In addition, quantitative distributions of Pb in water, sediment, and biological tissues will be presented for aquatic ecosystems throughout the United States. Finally, recent studies discussing the tracing of Pb in aquatic systems will be summarized.

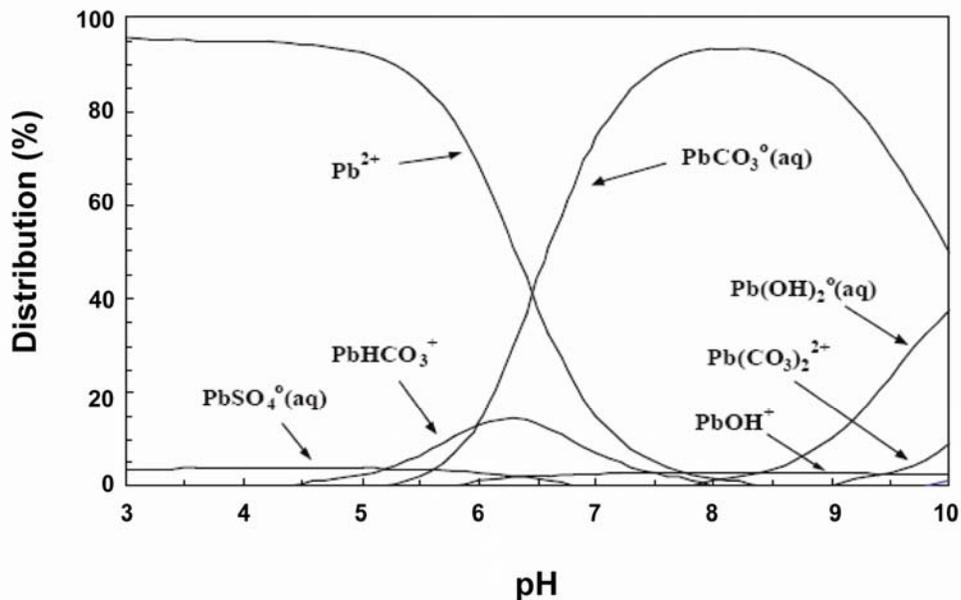
#### **AX7.2.2.1 Speciation of Lead in Aquatic Ecosystems**

The speciation of Pb in the aquatic environment is controlled by many factors. The primary form of Pb in aquatic environments is divalent ( $\text{Pb}^{2+}$ ), while  $\text{Pb}^{4+}$  exists only under extreme oxidizing conditions (Rickard and Nriagu, 1978). Labile forms of Pb (e.g.,  $\text{Pb}^{2+}$ ,  $\text{PbOH}^+$ ,  $\text{PbCO}_3$ ) are a significant portion of the Pb inputs to aquatic systems from atmospheric washout. Lead is typically present in acidic aquatic environments as  $\text{PbSO}_4$ ,  $\text{PbCl}_4$ , ionic Pb, cationic forms of Pb-hydroxide, and ordinary Pb-hydroxide ( $\text{Pb(OH)}_2$ ). In alkaline waters, common species of Pb include anionic forms of Pb-carbonate ( $\text{Pb(CO}_3\text{)}^-$ ) and  $\text{Pb(OH)}_2$ . Speciation models have been developed based on the chemical equilibrium model developed by Tipping (1994) to assist in examining metal speciation. The EPA MINTEQA2 computer model (<http://www.epa.gov/ceampubl/mmedia/minteq/>) is one such equilibrium speciation model that can be used to calculate the equilibrium composition of dilute aqueous solutions in the laboratory or in natural aqueous systems. The model is useful for calculating the equilibrium mass distribution among dissolved species, adsorbed species, and multiple solid phases under a variety of conditions, including a gas phase with constant partial pressures. In addition to chemical equilibrium models, the speciation of metals is important from a toxicological perspective. The BLM was developed to study the toxicity of metal ions in aquatic biota and was previously described in Section AX7.2.1.3. Further detail on speciation models is not provided herein, rather a general overview of major speciation principles are characterized in the following sections.

## Acidity (pH)

### Freshwater

Most of the Pb in aquatic environments is in the inorganic form (Sadiq, 1992). The speciation of inorganic Pb in freshwater aquatic ecosystems is dependent upon pH and the available complexing ligands. Solubility varies according to pH, temperature, and water hardness (Weber, 1993). Lead rapidly loses solubility above pH 6.5 (Rickard and Nriagu, 1978) and as water hardness increases. In freshwaters, Pb typically forms strong complexes with inorganic  $\text{OH}^-$  and  $\text{CO}_3^{2-}$  and weak complexes with  $\text{Cl}^-$  (Long and Angino, 1977; Bodek et al., 1988). The primary form of Pb at low pH (<6.5) is predominantly  $\text{Pb}^{2+}$  and less abundant inorganic forms include  $\text{Pb}(\text{HCO}_3)_2$ ,  $\text{Pb}(\text{SO}_4)_2^{2-}$ ,  $\text{PbCl}_2$ ,  $\text{PbCO}_3$ , and  $\text{Pb}_2(\text{OH})_2\text{CO}_3$  (Figure AX7-2.2.1). At higher pH (>7.5), Pb forms hydroxide complexes ( $\text{PbOH}^+$ ,  $\text{Pb}(\text{OH})_2$ ,  $\text{Pb}(\text{OH})_3^-$ ,  $\text{Pb}(\text{OH})_4^{2-}$ ).



**Figure AX7-2.2.1. Distribution of aqueous lead species as a function of pH based on a concentration of 1  $\mu\text{g Pb/L}$  (U.S. Environmental Protection Agency, 1999).**

Organic compounds in surface waters may originate from natural (e.g., humic or fulvic acids) or anthropogenic sources (e.g., nitrilotriacetone and ethylenediaminetetraacetic acid [EDTA]) (U.S. Environmental Protection Agency, 1986b). The presence of organic complexes has been shown to increase the rate of solution of Pb bound as Pb-sulfide (Lovering, 1976). Soluble organic Pb compounds are present at pH values near 7 and may remain bound at pH values as low as 3 (Lovering, 1976; Guy and Chakrabarti, 1976). At higher pH (7.4 to 9), Pb-organic complexes are partially decomposed. Water hardness and pH were found to be important in Pb-humic acid interactions (O'Shea and Mancy, 1978). An increase in pH increased the concentration of exchangeable Pb complexes, while an increase in hardness tended to decrease the humic acid-Pb interactions. Thus, the metals involved in water hardness apparently inhibit the exchangeable interactions between metals and humic acids.

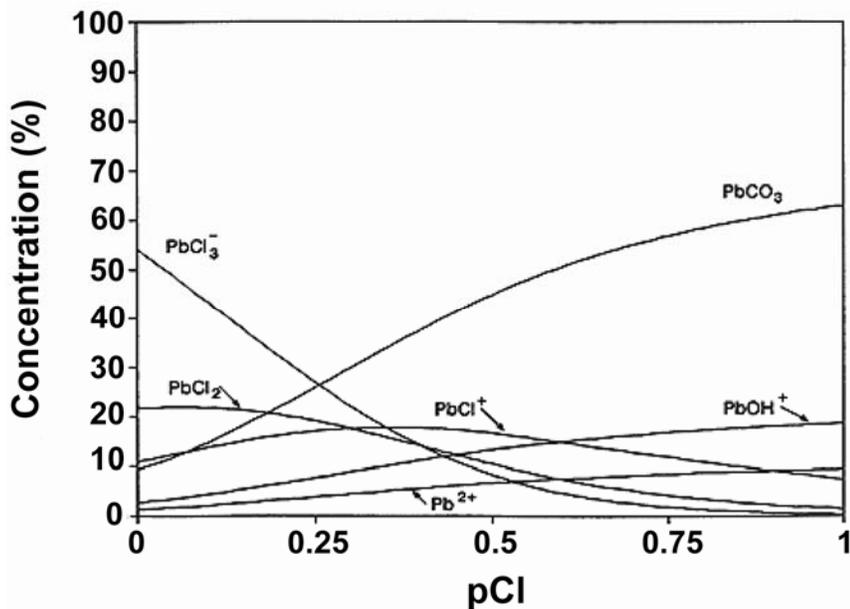
### Marine Water

In marine systems, an increase in salinity increases complexing with chloride and carbonate ions and reduces the amount of free  $Pb^{2+}$ . In seawaters and estuaries at low pH, Pb is primarily bound to chlorides ( $PbCl$ ,  $PbCl_2$ ,  $PbCl_3^{\ominus}$ ,  $PbCl_4^{2\ominus}$ ) and may also form inorganic  $Pb(HCO_3)_3$ ,  $Pb(SO_4)_2^{2\ominus}$ , or  $PbCO_3$ . Elevated pH in saltwater environments results in the formation of Pb hydroxides ( $PbOH^+$ ,  $Pb(OH)_2$ ,  $Pb(OH)_3^{\ominus}$ ,  $Pb(OH)_4^{2\ominus}$ ) (Figure AX7-2.2.2). A recent examination of Pb species in seawater as a function of chloride concentration suggested that the primary species were  $PbCl_3^{\ominus} > PbCO_3 > PbCl_2 > PbCl^+$  and  $Pb(OH)^+$  (Fernando, 1995). Lead in freshwater and seawater systems is highly complexed with carbonate ligands suggesting that Pb is likely to be highly available for sorption to suspended materials (Long and Angino, 1977).

Current information suggests that inorganic Pb is the dominant form in seawater; however, it has been shown that organically bound Pb complexes make up a large portion of the total Pb (Capodaglio et al., 1990).

### **Sorption**

Sorption processes (i.e., partitioning of dissolved Pb to suspended particulate matter or sediments) appear to exert a dominant effect on the distribution of Pb in the environment (U.S. Environmental Protection Agency, 1979). Sorption of Pb results in the enrichment of



**Figure AX7-2.2.2. Lead speciation versus chloride content (Fernando, 1995).**

bed sediments, particularly in environments with elevated organic matter content from anthropogenic sources. Lead adsorption to aquatic sediments is correlated with pollution in sites containing high levels of anthropogenic organic content, even under acidic conditions (Tada and Suzuki, 1982; Brook and Moore, 1988; Davis and Galloway, 1993; Botelho et al., 1994; Davis et al., 1996). Particulate-bound forms are more often linked to urban runoff and mining effluents (Eisler, 2000).

Solid Pb complexes form when Pb precipitates or adsorbs to suspended particulate matter and sediments. Inorganic Pb adsorption to suspended organic matter or sediments is dependent on parameters such as, pH, salinity, water hardness, and the composition of the organic matter (U.S. Environmental Protection Agency, 1979). In addition to suspended organic matter, Pb can adsorb to biofilms (i.e., bacteria) (Nelson et al., 1995; Wilson et al., 2001). Adsorption typically increases with increasing pH, increasing amounts of iron or manganese; and with a higher degree of polarity of the particulate matter (e.g., clays). Adsorption decreases with water hardness (Syracuse Research Corporation, 1999). At higher pH, Pb precipitates as Pb(OH)<sup>+</sup> and PbHCO<sub>3</sub><sup>+</sup> into bed sediments (Weber, 1993). Conversely, at low pH, Pb is negatively sorbed (repelled from the adsorbent surface) (U.S. Environmental Protection Agency, 1979; Gao et al., 2003). In addition, Pb may be remobilized from sediment with a decrease in metal concentration in the

solution phase, complexation with chelating agents (e.g., EDTA), and changing redox conditions (Gao et al., 2003). Changes in water chemistry (e.g., reduced pH or ionic composition) can cause sediment Pb to become remobilized and potentially bioavailable to aquatic organisms (Weber, 1993).

### ***Biotransformation***

Methylation may result in Pb remobilization and reintroduction into the aqueous environment compartment and its subsequent release into the atmosphere (Syracuse Research Corporation., 1999). However, methylation is not a significant environmental pathway controlling the fate of Pb in the aquatic environment. The microbial methylation of Pb in aquatic systems has been demonstrated experimentally, but evidence for natural occurrence is limited (Beijer and Jernelov, 1984; DeJonghe and Adams, 1986). Reisinger et al. (1981) examined the methylation of Pb in the presence of numerous bacteria known to alkylate metals and did not find evidence of Pb methylation under any test condition. Tetramethyl-Pb may be formed by the methylation of Pb-nitrate or Pb-chloride in sediments (Bodek et al., 1988). However, tetramethyl-Pb is unstable and may degrade in aerobic environments after being released from sediments (U.S. Environmental Protection Agency, 1986b). Methylated species of Pb may also be formed by the decomposition of tetraalkyl-Pb compounds (Radojevic and Harrison, 1987; Rhue et al., 1992). Sadiq (1992) reviewed the methylation of Pb compounds and suggested that chemical methylation of Pb is the dominant process and that biomethylation is of secondary importance.

### **AX7.2.2.2 Spatial Distribution of Lead in Aquatic Ecosystems**

#### ***National Water Quality Assessment (NAWQA)***

The 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986b) did not describe the distribution and concentration of Pb throughout aquatic ecosystems of the United States. Consequently, an analysis of readily available data on Pb concentrations was conducted to determine the distribution of Pb in the aquatic environment. Data from the United States Geological Survey (USGS) National Water-Quality Assessment (NAWQA) program were queried and retrieved. NAWQA contains data on Pb concentrations in surface water, bed sediment, and animal tissue for more than 50 river basins and aquifers throughout the country,

and it has been used by the EPA for describing national environmental concentrations for use in developing AWQC. The authors recognize that the NAWQA program encountered analytical challenges with the chemical analysis of Pb in surface waters. The analytical methods available during the NAWQA program were not as sensitive as methods currently applied today. Therefore, analytical detection limits are elevated and a large portion of the data set contains non-detected values. Nevertheless, this data provides a comprehensive overview of Pb concentrations in U.S. surface waters that is supplemented with data from other relevant studies. The following sections describe the estimated concentrations of Pb from NAWQA and other research programs.

NAWQA data are collected during long-term, cyclical investigations wherein study units undergo intensive sampling for 3 to 4 years, followed by low-intensity monitoring and assessment of trends every 10 years. The NAWQA program's first cycle was initiated in 1991; therefore, all available data are less than 15 years old. The second cycle began in 2001 and is ongoing; data are currently available through 30 September 2003. The NAWQA program study units were selected to represent a wide variety of environmental conditions and contaminant sources; therefore, agricultural, urban, and natural areas were all included. Attention was also given to selecting sites covering a wide variety of hydrologic and ecological resources.

NAWQA sampling protocols are designed to promote data consistency within and among study units while minimizing local-scale spatial variability. Water-column sampling is conducted via continuous monitoring, fixed-interval sampling, extreme-flow sampling, as well as seasonal, high-frequency sampling in order to characterize spatial, temporal, and seasonal variability as a function of hydrologic conditions and contaminant sources. Sediment and tissue samples are collected during low-flow periods during the summer or fall to reduce seasonal variability. Where possible, sediment grab samples are collected along a 100-m stream reach, upstream of the location of the water-column sampling. Five to ten depositional zones at various depths, covering left bank, right bank, and center channel, are sampled to ensure a robust representation of each site. Fine-grained samples from the surficial 2 to 3 cm of bed sediment at each depositional zone are sampled and composited. Tissue samples are collected following a National Target Taxa list and decision trees that help guide selection from that list to accommodate local variability.

The NAWQA dataset was chosen over other readily available national databases (i.e. the USEPA-maintained database for the STorage and RETrieval [STORET] of chemical, physical, and biological data), because the study design and methods used to assess the water quality of each study unit are rigorous and consistent, and, as such, these data may be presented with a high level of confidence. This is in stark contrast to the STORET database, which essentially serves as a depot for any organization wishing to share data they have generated. This lack of a consistent methodology or QA/QC protocol has led to the STORET data being highly qualified and offered with only a mild level of confidence. Furthermore, because there is no standard for site selection within STORET, the database may be biased toward contaminated sites. Finally, and, perhaps most importantly, the majority of the available Pb data in STORET predate the use of clean techniques for Pb quantification.

The authors recognize the existence of several local and regional datasets that may be of quality equal to NAWQA; however, due to the national scope of this assessment, these datasets were not included in the following statistical analyses. However, because the NAWQA database does not cover lakes and the marine/estuarine environment, and we were unable to identify any monitoring data of similar quality, local and regional datasets were used in these cases to provide general information on environmental Pb concentrations.

### Data Acquisition and Analysis

The following data were downloaded for the entire United States (all states) from the NAWQA website (<http://water.usgs.gov/nawqa/index.html>): site information, dissolved Pb concentration in surface water ( $\mu\text{g/L}$ ), total Pb concentration ( $\mu\text{g/g}$ ) in bed sediment ( $<63 \mu\text{m}$ )<sup>3</sup>, and Pb concentration in animal tissue ( $\mu\text{g/g dw}$ ). Using the land use classification given for each site, the data were divided into two groups: “natural” and “ambient” (Table AX7-2.2.1). All samples were considered to fall within the ambient group (the combined contribution of natural and anthropogenic sources), whereas the natural group comprised “forest,” “rangeland,” or “reference” samples only<sup>4</sup>. These groups follow those defined and recommended for use by the EPA’s Framework for Inorganic Metals Risk Assessment (U.S. Environmental Protection

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<sup>3</sup> NAWQA sediment samples are sieved to  $<63 \mu\text{m}$  to promote the collection of fine-grained surficial sediments, which are natural accumulators of trace elements.

<sup>4</sup> The authors acknowledge that while Pb samples collected from sites classified under these three land use categories will most closely reflect natural background concentrations, atmospheric input of lead may be present.

**Table AX7-2.2.1. NAWQA Land Use Categories and Natural/Ambient Classification**

<b>NAWQA Land Use Categories</b>	<b>Classification</b>
Agricultural	Ambient
Commercial/Industrial	Ambient
Cropland	Ambient
Forest	Ambient/Natural
Mining	Ambient
Mixed	Ambient
NA	Ambient
Orchard/Vineyard	Ambient
Other/Mixed	Ambient
Pasture	Ambient
Rangeland	Ambient/Natural
Reference	Ambient/Natural
Residential	Ambient
Urban	Ambient

Agency, 2004b). Finally, in addition to the natural/ambient classification, tissue samples were further divided into “whole organism” and “liver” groups.

All data were compiled in spreadsheets wherein non-detect values were converted to one-half the detection limit and the total number of samples, percentage of non-detect values (percent censorship), minimum, maximum, median, mean, standard deviation, and cumulative density functions were calculated for each endpoint for both the natural and ambient groups.

As discussed below, some datasets were highly censored; however, deletion of non-detect data has been shown to increase the relative error in the mean to a greater extent than inclusion of non-detects as ½ of the detection limit (Newman et al., 1989); therefore means and other statistics were calculated using the latter method for this analysis. Finally, since all data were geo-referenced, a geographic information system (GIS; ArcGIS) was used to generate maps, conduct spatial queries and analyses, and calculate statistics.

## *Lead Distributions Generated from the NAWQA Database*

### Natural versus Ambient Groups

There were four to eight times more ambient surface water (Table AX7-2.2.2) and bulk sediment (Table AX7-2.2.3) samples in the compiled dataset than natural samples. This is most likely a function of both the NAWQA program site selection process and the fact that sites unaffected by human activities are extremely limited. The spatial distributions of natural and ambient surface water/sediment sites were fairly comparable, with natural samples located in almost all of the same areas as ambient samples except in the Midwest (Ohio, Illinois, Iowa, and Michigan), where natural sites were not present (Figure AX7-2.2.3). This exception may be because these areas are dominated by agricultural and urban areas. The same spatial distributions were observed for the natural and ambient liver and whole organism tissue samples (Figure AX7-2.2.4 and Figure AX7-2.2.5).

**Table AX7-2.2.2. Summary Statistics of Ambient and Natural Levels of Dissolved Lead in Surface Water**

<b>Statistic</b>	<b>Surface Water Dissolved Pb (<math>\mu\text{g/L}</math>)</b>	
	<b>Natural</b>	<b>Ambient</b>
% Censorship	87.91	85.66
N	430	3445
Minimum	0.04	0.04
Maximum	8.40	29.78
Mean	0.52	0.66
Standard Deviation	0.59	1.20
Median	0.50	0.50
90th Percentile	0.50	0.50
95th Percentile	0.50	1.10
96th Percentile	0.67	2.00
97th Percentile	1.00	2.34
98th Percentile	1.79	3.58
99th Percentile	2.48	5.44

**Table AX7-2.2.3. Summary Statistics of Ambient and Natural Levels of Total Lead in <63  $\mu\text{m}$  Bulk Sediment**

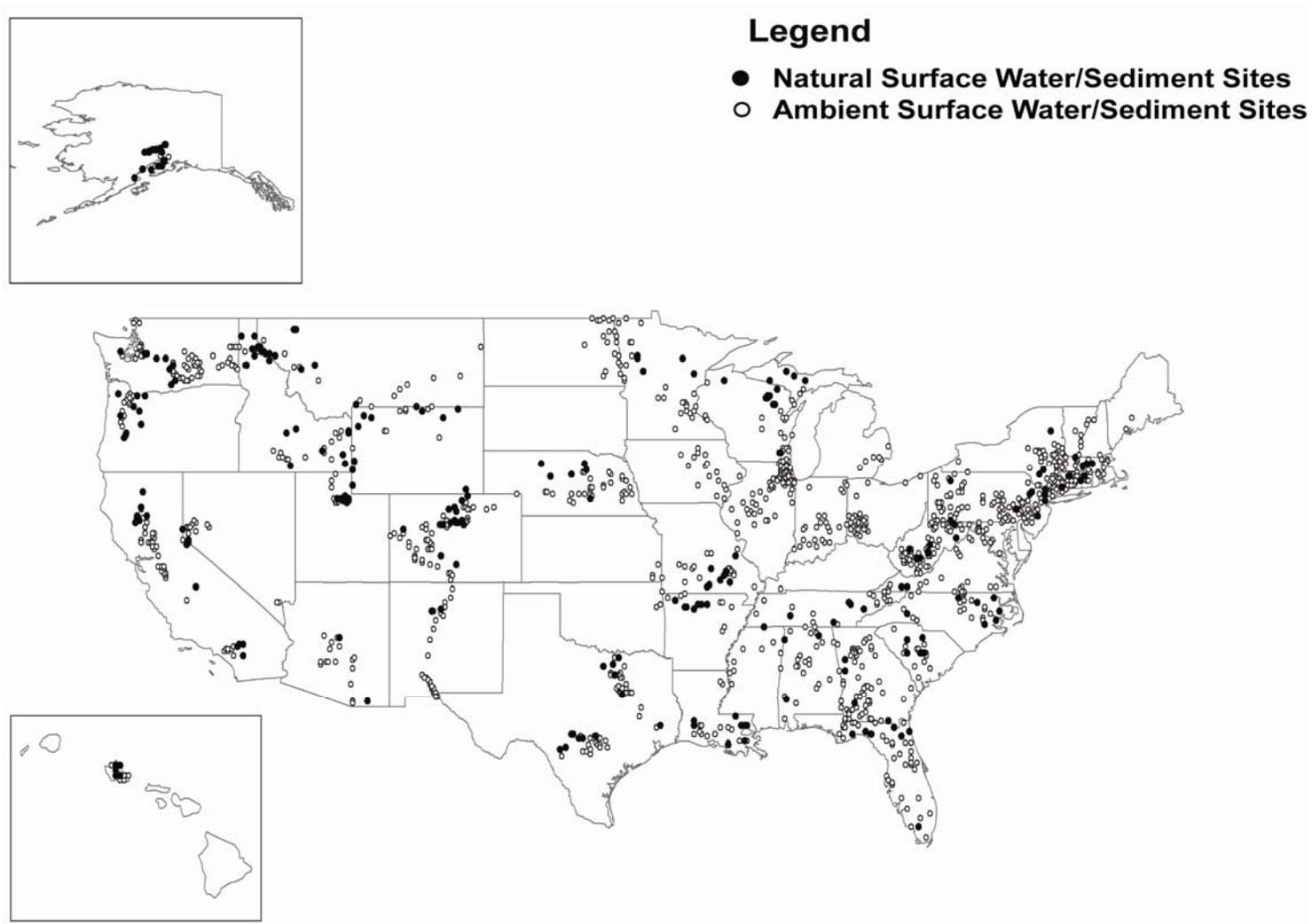
Statistic	Bulk Sediment <63 $\mu\text{m}$ Total Lead ( $\mu\text{g/g}$ )	
	Natural	Ambient
% Censorship	1.16	0.48
N	258	1466
Minimum	0.50	0.50
Maximum	12000	12000
Mean	109.07	120.11
Standard Deviation	786.74	672.41
Median	22.00	28.00
90th percentile	66.30	120.00
95th percentile	161.50	200.00

### Surface Water

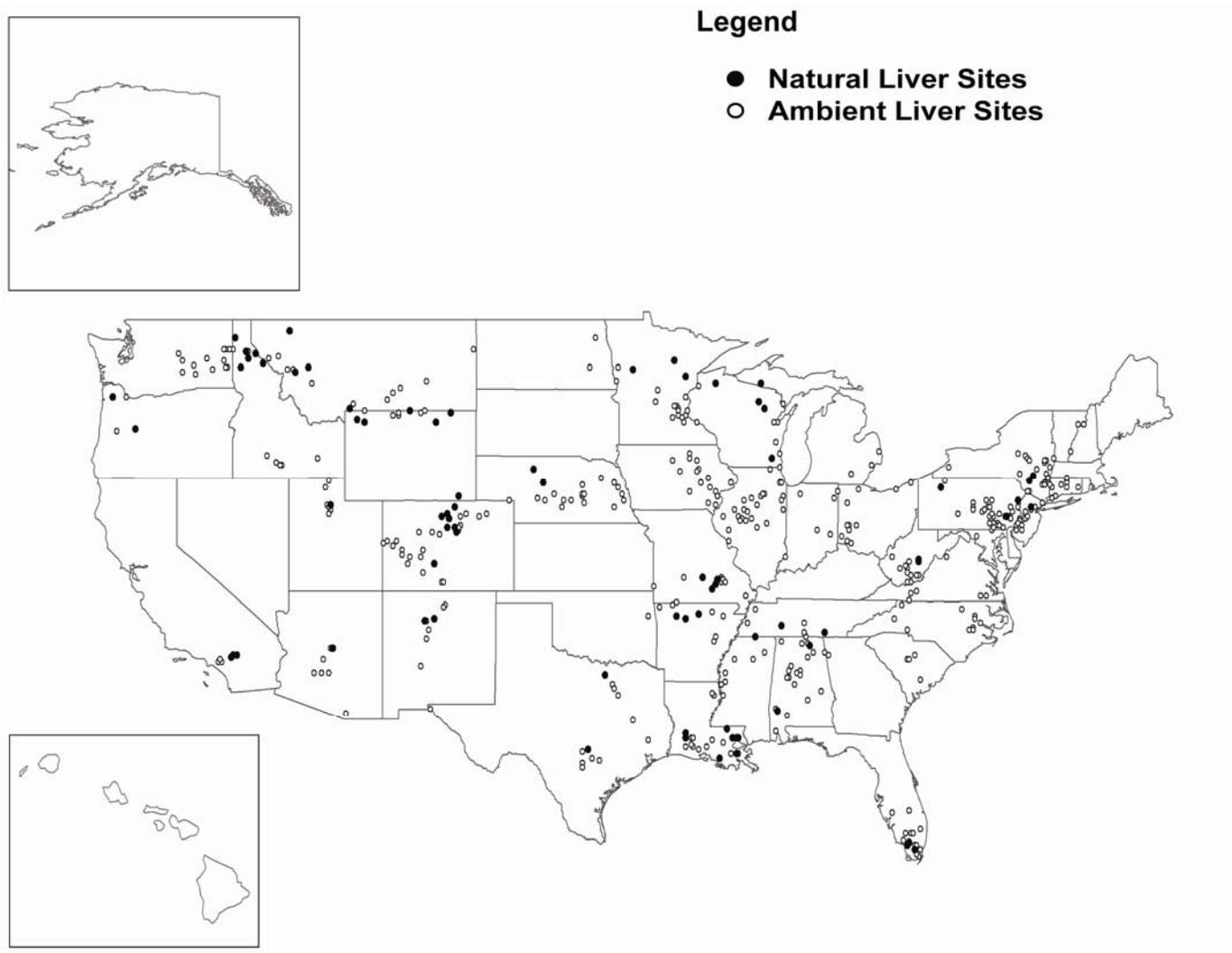
The total number of surface water Pb samples was 3,445; however these data were highly censored with 85.66% of the ambient samples (2951/3445) and 87.91% of the natural samples (378/430) below the detection limit<sup>5</sup> (Table AX7-2.2.2). Consequently, the majority of the variability between these two datasets fell between the 95th and 100th (maximum) percentiles, as was shown by the frequency distributions of the two groups deviating only at the upper and lower tails with most of the overlapping data falling at 0.50  $\mu\text{g/L}$  (one-half of the most common detection limit, 1.0  $\mu\text{g/L}$ ; Figure AX7-2.2.6). As expected, due to the definitions of the natural and ambient groups, the 95th and 100th percentiles were consistently higher for the ambient samples than the natural samples. Similarly, the mean ambient Pb concentration (0.66  $\mu\text{g/L}$ ) was higher than the mean natural Pb concentration (0.52  $\mu\text{g/L}$ ).<sup>6</sup>

<sup>5</sup> The NAWQA dataset contains multiple detection limits for Pb in surface water that have decreased over time. While the majority of data were analyzed with a detection limit of 1.0  $\mu\text{g/L}$  (before 2000/2001), the most recent samples were analyzed with either a 0.5, 0.2, 0.16, or 0.08  $\mu\text{g/L}$  detection limit (after 2000/2001), and some older samples (N = 20) were analyzed with a detection limit of 2.0  $\mu\text{g/L}$ .

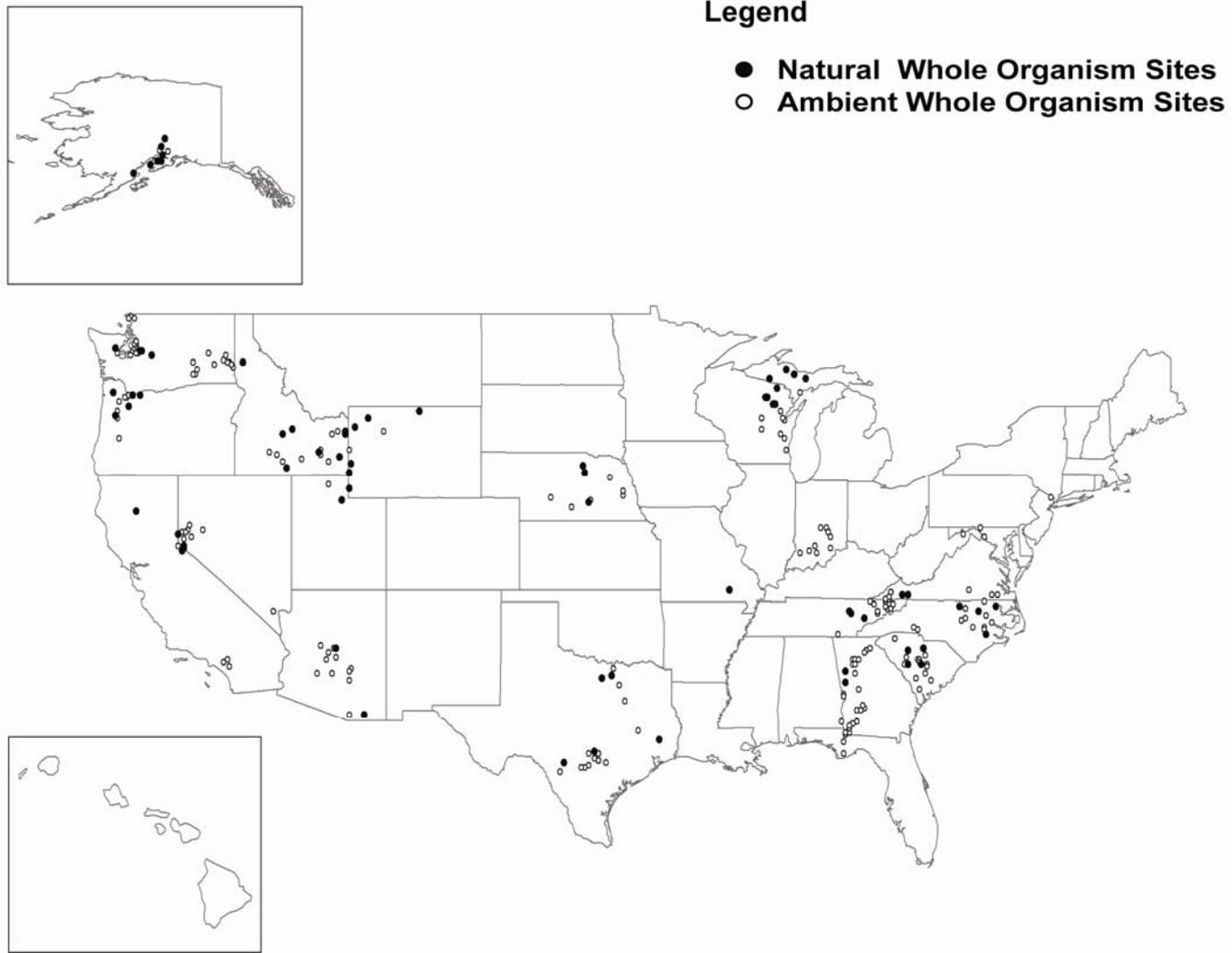
<sup>6</sup> The same pattern was observed upon calculating the mean Pb concentrations based on detect data only (ambient mean = 1.66  $\mu\text{g/L}$ , natural mean = 0.87  $\mu\text{g/L}$ ); however, as previously discussed, calculations included non-detect data as  $\frac{1}{2}$  of the detection limit to reduce the relative error in the mean.



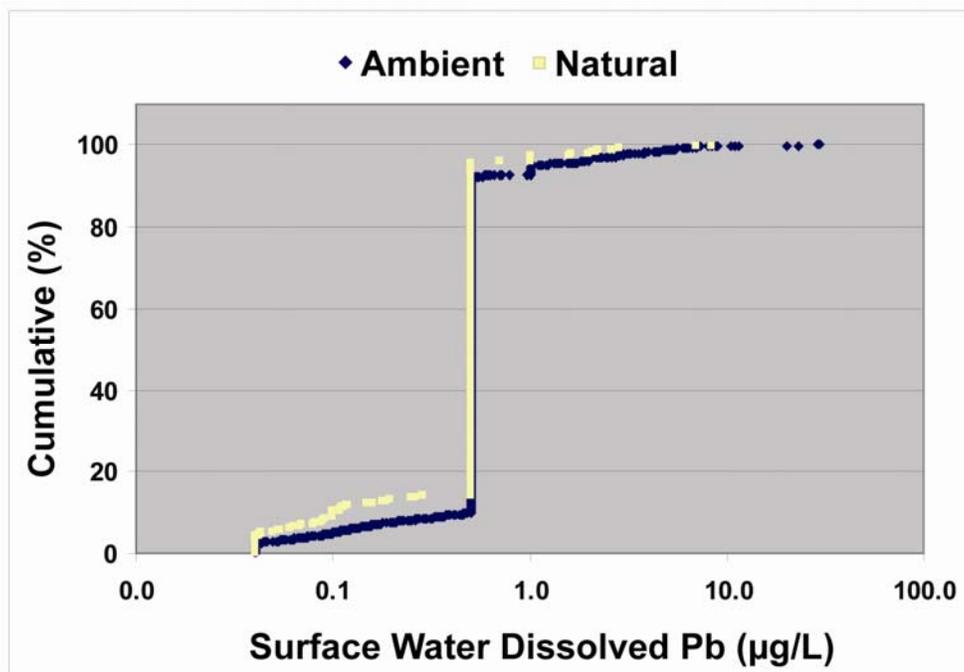
**Figure AX7-2.2.3. Spatial distribution of natural and ambient surface water/sediment sites (Surface water: natural N = 430, ambient N = 3445; Sediment: natural N = 258, ambient N = 1466).**



**Figure AX7-2.2.4. Spatial distribution of natural and ambient liver tissue sample sites (Natural N = 83, Ambient N = 559).**



**Figure AX7-2.2.5. Spatial distribution of natural and ambient whole organism tissue sample sites (Natural N = 93, Ambient N = 332).**

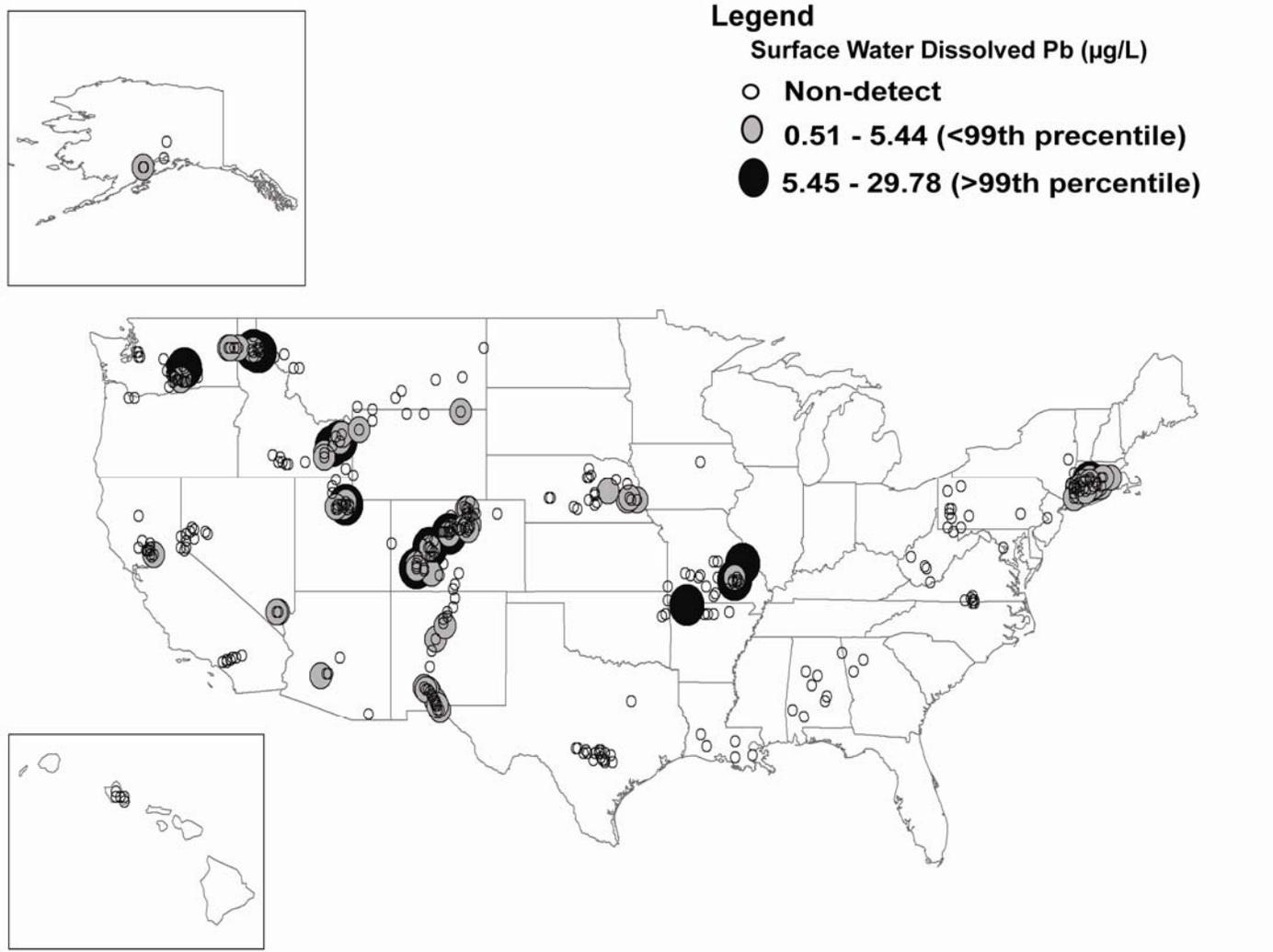


**Figure AX7-2.2.6. Frequency distribution of ambient and natural levels of surface water dissolved lead (µg/L).**

Due to the preponderance of non-detectable (ND) measurements, assessing national trends in surface water-dissolved Pb concentrations was not possible. However, areas with elevated Pb concentrations were identified by classifying the data with detectable Pb concentrations above and below the 99th percentile. The 99th percentile (versus the 95th percentile) was chosen in this instance to represent extreme conditions given the small window of variability in the dataset. By convention, the 95th percentile was used in subsequent analyses of this type. Areas with high surface water Pb concentrations were observed in Washington, Idaho, Utah, Colorado, Arkansas, and Missouri (Figure AX7-2.2.7). The maximum measured Pb concentration was located in Canyon Creek at Woodland Park, ID, a site classified as mining land use.

### Sediment

There were approximately one-half of the number of surface water data available for sediments (N = 1466). In contrast to the surface water data, however, very few sediment data were below the detection limit (7/1466 ambient ND, 3/258 natural ND; Table AX7-2.2.3). As expected, the mean ambient Pb concentration was higher than the mean natural Pb



**Figure AX7-2.2.7. Spatial distribution of dissolved lead in surface water (N = 3445).**

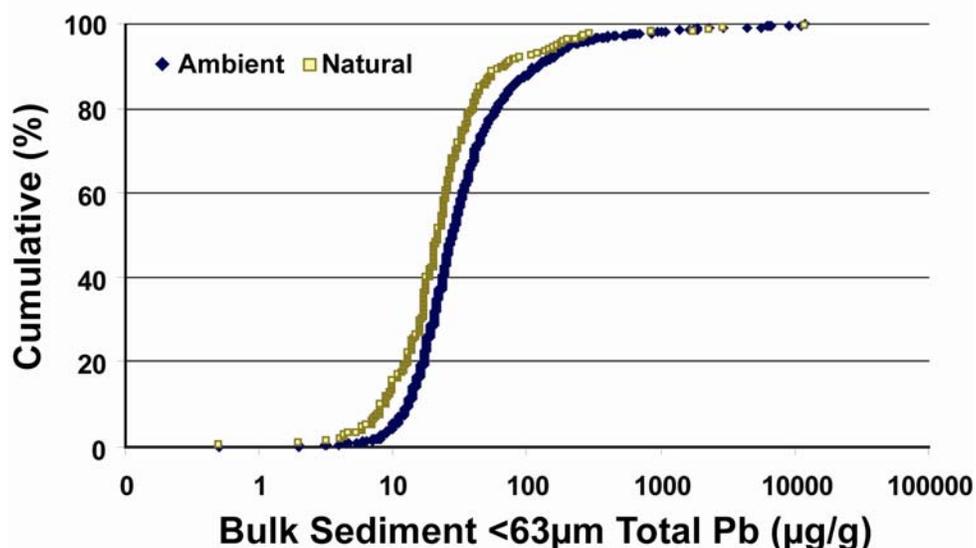
concentration (120.11 and 109.07  $\mu\text{g/g}$ , respectively). Similarly, the median ambient Pb concentration was higher than the median natural Pb concentration (28.00 and 22.00  $\mu\text{g/g}$ , respectively) and the ambient 95th percentile was higher than the natural 95th percentile (200.00 and 161.50  $\mu\text{g/g}$ , respectively). While the natural and ambient surface water Pb distributions differed only at the extremes, the natural sediment Pb percentiles were consistently lower than the ambient percentiles throughout the distributions (Figure AX7-2.2.8). Unlike the surface water dataset, because the sediment dataset was not heavily censored, assessing national trends in sediment Pb concentrations was possible. The data were mapped and categorized into the four quartiles of the frequency distribution (Figure AX7-2.2.9). The following observations were made:

- Sediment Pb concentrations generally increased from west to east (the majority of sites along East Coast had Pb concentrations in the fourth quartile of the sediment Pb concentration frequency distribution).
- Several “hot spots” of concentrated sites with elevated sediment Pb concentrations were apparent in various western states.
- Sediment Pb concentrations were generally lowest in the midwestern states (the majority of sites in North Dakota, Nebraska, Minnesota, and Iowa had Pb concentrations in the first or second quartile of sediment Pb concentration frequency distribution).

As was seen with surface water Pb concentrations, the highest measured sediment Pb concentrations were found in Idaho, Utah, and Colorado. Not surprisingly, of the top 10 sediment Pb concentrations recorded, 7 were measured at sites classified as mining land use.

### *Tissue*

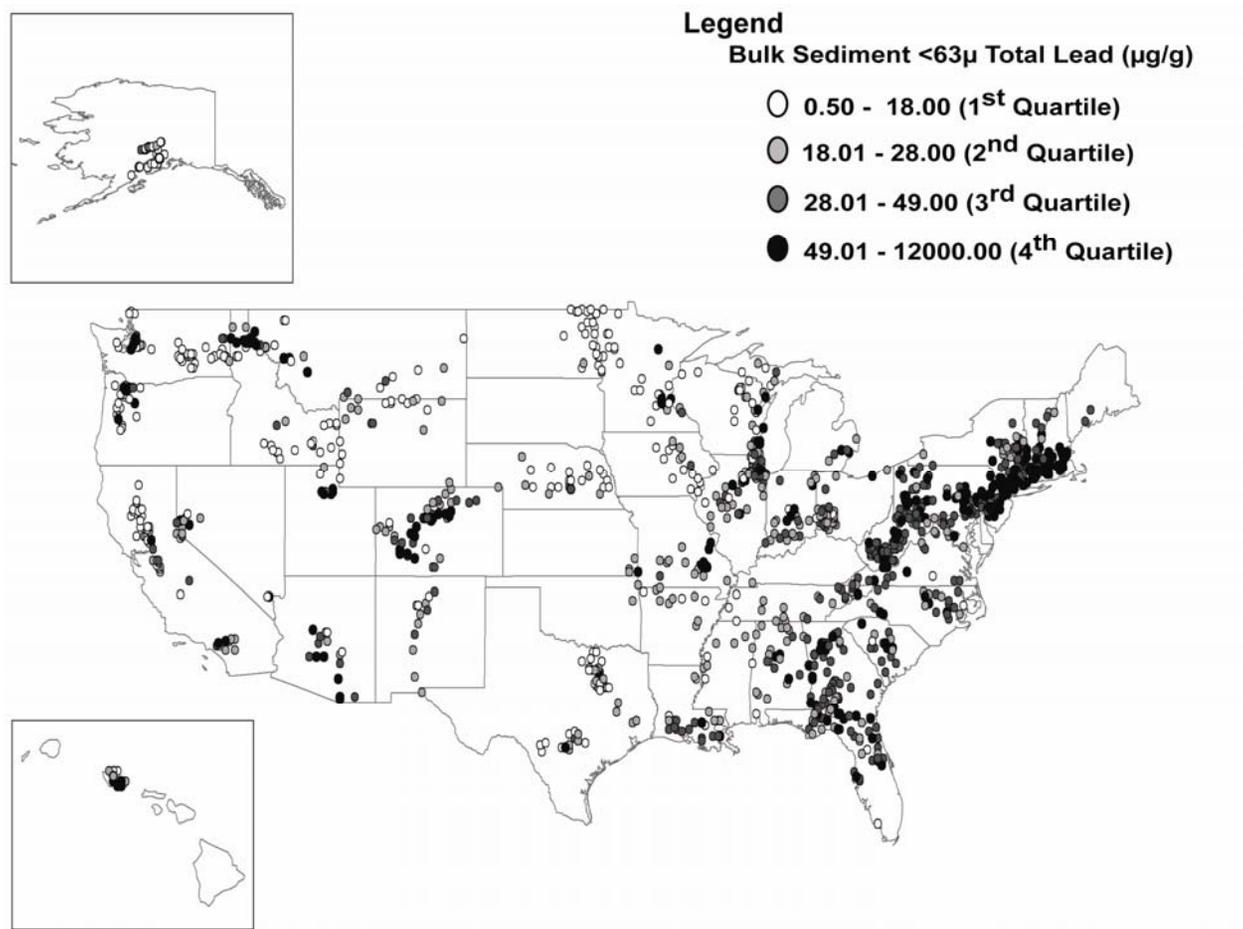
As was true for the surface water data, there were a high number of tissue samples below the detection limit (47/93 natural whole organism ND, 130/332 ambient whole organism ND, 74/83 natural liver ND, 398/559 ambient liver ND; Table AX7-2.2.4). In general, more non-censored data were available for whole organism samples than liver samples, and for ambient sites than natural sites. As expected, for whole organism samples, the 95th percentile Pb concentration measured at ambient sites was higher than that measured at natural sites (3.24 and 2.50  $\mu\text{g/g}$ , respectively); however, Pb liver concentration 95th percentiles for ambient and natural samples were very similar, with the natural 95th percentile actually higher than the



**Figure AX7-2.2.8. Frequency distribution of ambient and natural levels of bulk sediment <63  $\mu\text{m}$  total Pb ( $\mu\text{g/g}$ ).**

ambient 95th percentile (1.26 and 1.06  $\mu\text{g/g}$ , respectively). In addition, as expected, the median and mean Pb liver concentrations of ambient samples (0.15 and 0.36  $\mu\text{g/g}$ , respectively) were higher than the median and mean Pb liver concentrations of natural samples (0.11 and 0.28  $\mu\text{g/g}$ , respectively). The same pattern was observed in the whole organism median and mean Pb concentrations (ambient: median = 0.59, mean = 1.03; natural: median = 0.35, mean = 0.95  $\mu\text{g/g}$ ). In addition, the frequency distributions of the liver and whole organism Pb concentrations followed the same trends, with the natural percentiles consistently lower than the ambient percentiles throughout the distributions (Figure AX7-2.2.10 and Figure AX7-2.2.11).

These whole organism results were compared with findings from the 1984 U.S. Fish and Wildlife Service (USFWS) National Contaminant Biomonitoring Program (NCBP) (Schmitt and Brumbaugh, 1990). As part of this program, 321 composite samples of 3 to 5 whole, adult fish of a single species were collected from 109 river and Great Lake stations throughout the country. Samples were analyzed for Pb concentrations ( $\mu\text{g/g}$  ww) and the geometric mean, maximum, and 85th percentile were calculated. Upon comparing these summary statistics with the equivalent NAWQA ambient group value (NCBP stations were representative of both natural and anthropogenically influenced conditions), a very strong agreement between the two analyses was



**Figure AX7-2.2.9. Spatial distribution of total lead in bulk sediment <63 μm (N = 1466).**

**Table AX7-2.2.4. Summary Statistics of Ambient and Natural Levels of Lead in Whole Organism and Liver Tissues**

Statistic	Tissue Pb ( $\mu\text{g/g}$ dry weight)			
	Whole Organism		Liver	
	Natural	Ambient	Natural	Ambient
% Censorship	50.54	39.16	89.16	71.20
N	93	332	83	559
Minimum	0.08	0.08	0.01	0.01
Maximum	22.60	22.60	3.37	12.69
Mean	0.95	1.03	0.28	0.36
Standard Deviation	2.53	1.74	0.54	0.96
Median	0.35	0.59	0.11	0.15
90th percentile	1.40	2.27	0.37	0.59
95th percentile	2.50	3.24	1.26	1.06

observed for each endpoint (Table AX7-2.2.5). For example, NCBP and NAWQA geometric mean Pb concentrations were nearly identical (0.55 and 0.54  $\mu\text{g/g}$  dw, respectively) and the 85th percentiles only differed by 0.5  $\mu\text{g Pb/g dw}$  (NCBP, 1.10 and NAWQA, 1.60). The authors acknowledge that a high degree of censorship is present in both of these datasets and no firm conclusions can be drawn by comparing these means. The objective of this exercise was limited to showing how the NAWQA data compare to other national datasets.

As was the case with surface water data, the high amount of non-detectable measurements did not allow for a national assessment of spatial trends in Pb tissue concentrations. Instead, areas with high Pb tissue concentrations were identified by classifying the data above and below the 95th percentile. Similar to surface water and sediments, tissue concentrations were found to be elevated in Washington, Idaho, Utah, Colorado, Arkansas, and Missouri; however, several of the highest measured Pb concentrations were also found in study units in the southwestern and

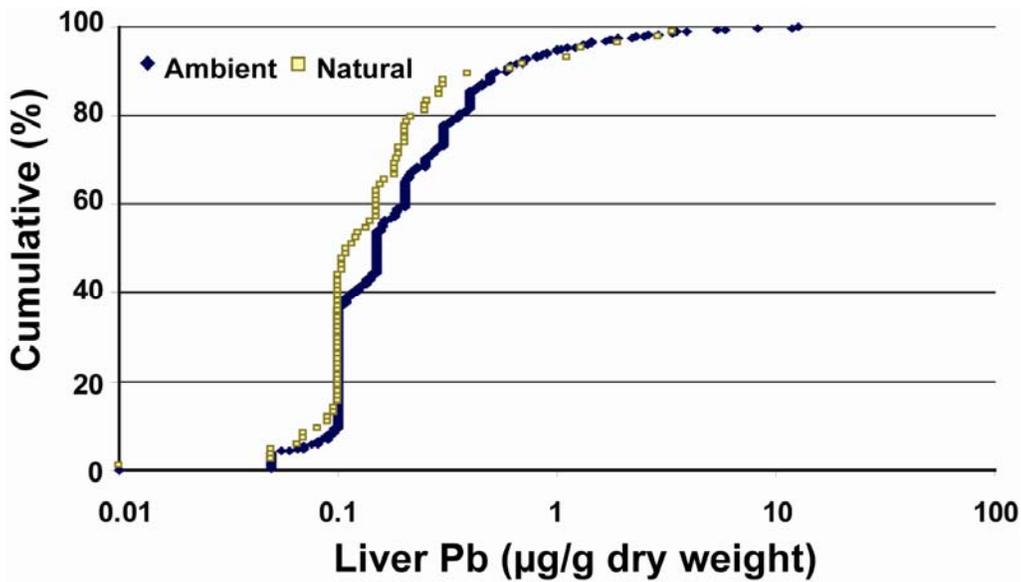


Figure AX7-2.2.10. Frequency distribution of ambient and natural levels of lead in liver tissue (µg/g dry weight).

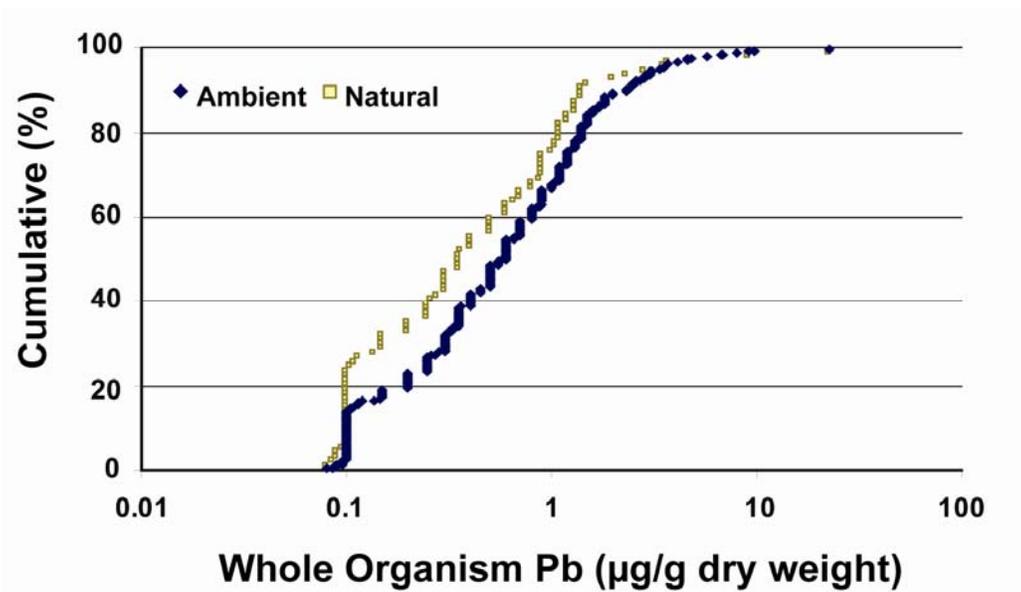


Figure AX7-2.2.11. Frequency distribution of ambient and natural levels of lead in whole organism tissue (µg/g dry weight).

**Table AX7-2.2.5. Comparison of NCBP and NAWQA Ambient Lead Levels in Whole Organism Tissues**

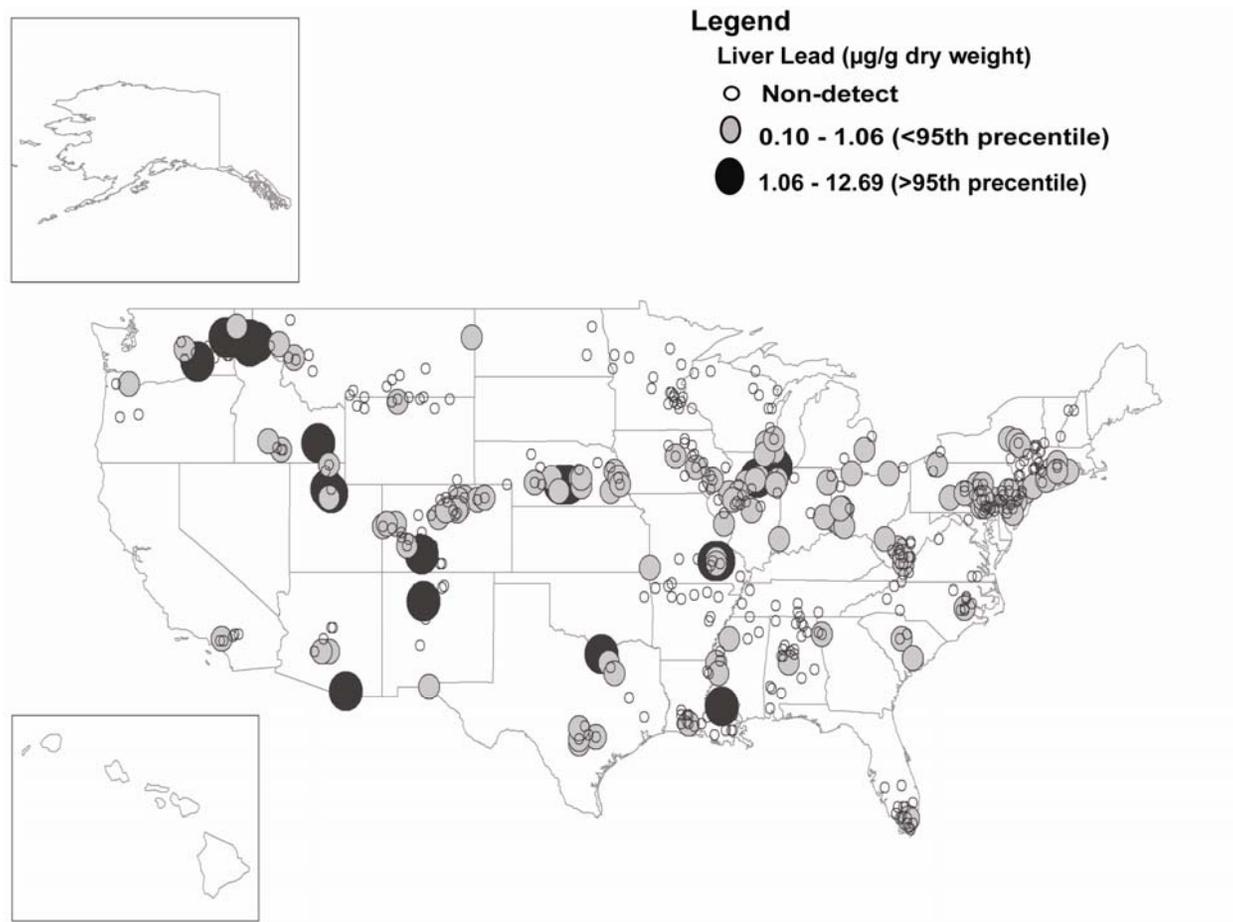
Statistic	Whole Organism Lead Concentration (µg/g dry weight)	
	NCBP <sup>1</sup>	NAWQA
Geometric Mean	0.55	0.54
Maximum	24.40	22.60
85th Percentile	1.10	1.60

<sup>1</sup> To convert between wet and dry weight, wet weight values were multiplied by a factor of five.

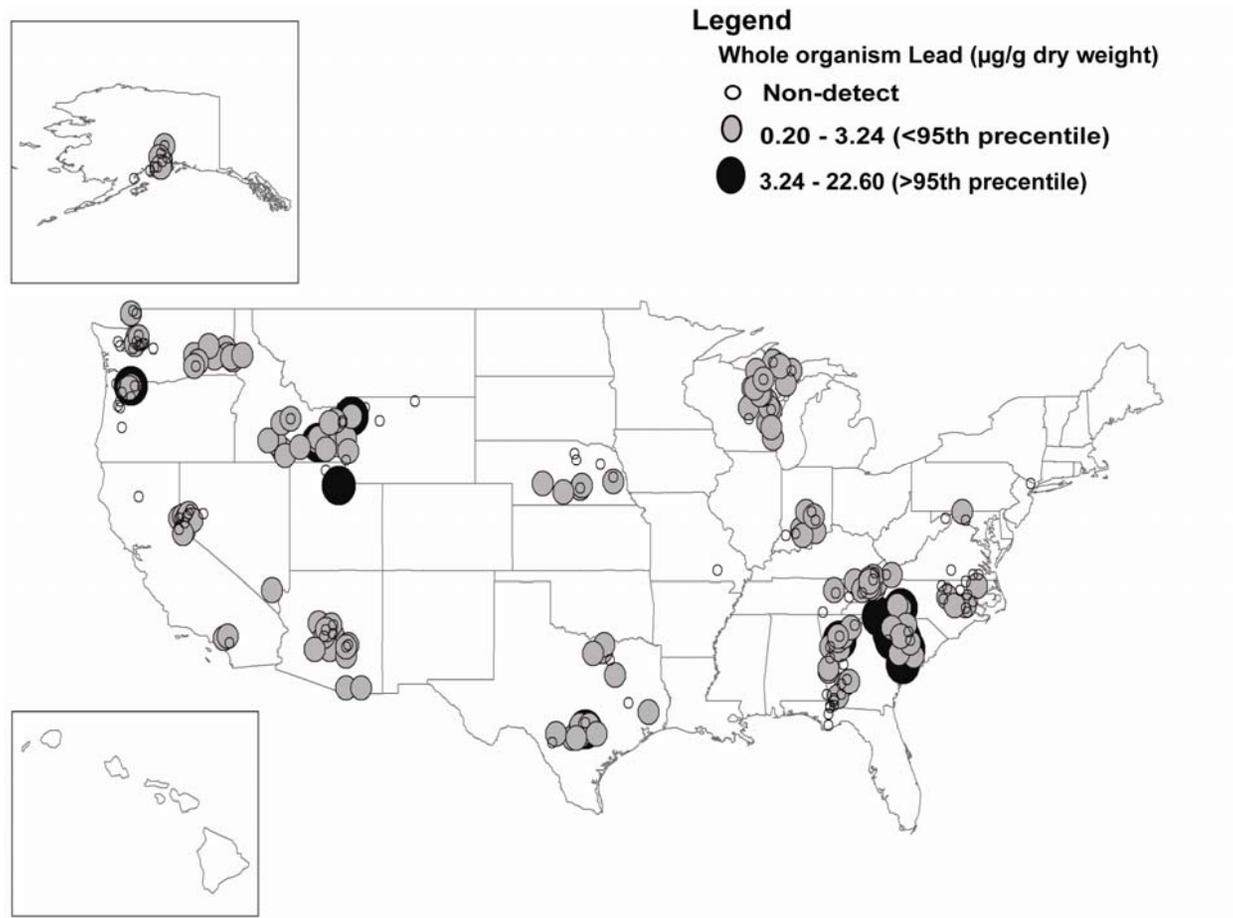
southeastern states (Figure AX7-2.2.12 and Figure AX7-2.2.13). As expected, the majority of the samples with elevated Pb concentrations were taken from sites classified as urban, commercial/industrial, or mining.

### **Input and Distribution of Lead in Other Aquatic Systems**

Because the NAWQA database does not cover lakes or the sea where atmospheric deposition of Pb is highly likely, the primary literature was searched for studies using ultra-clean sampling/analytical techniques to characterize Pb concentrations in these environments. Lead concentrations in lakes and oceans were generally found to be much lower than those measured in the lotic waters assessed by NAWQA. Surface water concentrations of dissolved Pb measured in Hall Lake, Washington in 1990 ranged from 2.1 – 1015.3 ng/L (Balistrieri et al., 1994). Nriagu et al. (1996) found that the average surface water dissolved Pb concentrations measured in the Great Lakes (Superior, Erie, and Ontario) between 1991 and 1993 were 3.2, 6.0, and 9.9 ng/L, respectively. Lead concentrations ranged from 3.2 – 11 ng/L across all three lakes. Similarly, 101 surface water total Pb concentrations measured at the HOT station ALOHA between 1998 and 2002 ranged from 25 – 57 pmol/kg (5 – 11 ng/kg; (Boyle et al., 2005). Based on the fact that Pb is predominately found in the dissolved form in the open ocean (<90%; Schaule and Patterson, 1981), dissolved Pb concentrations measured at these locations would likely have been even lower than the total Pb concentrations reported.



**Figure AX7-2.2.12. Spatial distribution of lead in liver tissues (N = 559).**



**Figure AX7-2.2.13. Spatial distribution of lead in whole organism tissues (N = 332).**

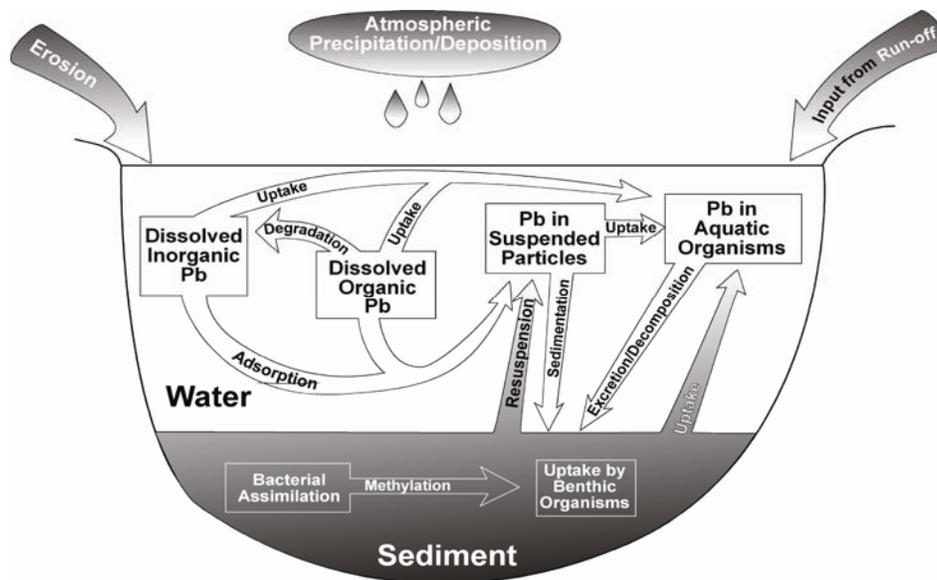
In open waters of the North Atlantic the decline of Pb concentrations has been associated with the phasing out of leaded gasoline in North America and western Europe (Véron et al., 1998). Likewise, Pb restrictions in gasoline appear to have been effective in reducing atmospheric Pb loading to the Okefenokee Swamp in southern Georgia/northern Florida (Jackson et al., 2004). Based on sediment cores from the Okefenokee Swamp, Pb concentrations were approximately 0.5 mg/kg prior to industrial development, reached a maximum of approximately 31 mg/kg from about 1935 to 1965, and following passage of the Clean Air Act in 1970 concentrations have declined to about 18 mg/kg in 1990 (Jackson et al., 2004). Trends in metals concentrations (roughly 1970-2001) in sediment cores from 35 reservoirs and lakes in urban and reference settings were analyzed by Mahler et al. (2006) to determine the effects of three decades of legislation, regulation, and changing demographics and industrial practices in the United States on concentrations of metals in the environment. The researchers found that decreasing trends outnumbered increasing trends for all seven metals analyzed (Cd, Cr, Cu, Pb, Hg, Ni, and Zn). The most consistent trends were for Pb and Cr: For Pb, 83% of the lakes had decreasing trends and 6% had increasing trends; for Cr, 54% of the lakes had decreasing trends and none had increasing trends. Mass accumulation rates of metals in cores, adjusted for background concentrations, decreased from the 1970s to the 1990s, where median changes ranged from 246% (Pb) to 23% (Hg and Zn). The largest decreases were found in lakes located in dense urban watersheds where the overall metals contamination in recently deposited sediments decreased to on-half its 1970s median value. However, Mahler et al. (2006) found that anthropogenic mass accumulation rates in dense urban lakes remained elevated over those in lakes in undeveloped watersheds, in some cases by as much as two orders of magnitude (Cr, Cu, and Zn), indicating that urban fluvial source signals can overwhelm those from regional atmospheric sources. In estuarine systems, however, it appears that similar declines following the phase-out of leaded gasoline are not necessarily as rapid. Steding et al. (2000) used isotopic evidence to demonstrate the continued cycling of Pb in the San Francisco Bay estuary. In the southern arm of San Francisco Bay, which has an average depth of <2 m, Steding et al. (2000) found that isotopic compositions were essentially invariant, with 90% of the Pb derived from 1960s-1970s leaded gasoline. The authors attributed this to the limited hydraulic flushing and remobilization of Pb from bottom sediments. In the northern arm of San Francisco Bay, although seasonal and decadal variations in Pb isotope composition were observed, mass balance

calculations indicate that only a small fraction of leaded gasoline fallout from the late 1980s has been washed out of the San Joaquin and Sacramento rivers' drainage basin by 1995 and consequently freshwater inputs remain a Pb source to the bay (Steding et al., 2000). The authors suggest that the continuous source of Pb from the river systems draining into the bay, coupled with benthic remobilization of Pb, indicates that historic gasoline deposits may remain in the combined riparian/estuarine system for decades.

### **AX7.2.2.3 Tracing the Fate and Transport of Lead in Aquatic Ecosystems**

The following section presents a generalized framework for the fate and transport of Pb in aquatic systems (Figure AX7-2.2.14). The primary source of Pb in natural systems is atmospheric deposition (Rickard and Nriagu, 1978; U.S. Environmental Protection Agency, 1986a). Estimated median global atmospheric emission for anthropogenic and natural sources are  $332 \times 10^6$  kg/year and  $12 \times 10^6$  kg/year, respectively (summarized by Giusti et al., 1993). Inorganic and metallic Pb compounds are nonvolatile and will partition to airborne particulates or water vapors (Syracuse Research Corporation., 1999). Dispersion and deposition of Pb is dependent on the particle size (U.S. Environmental Protection Agency, 1986a; Syracuse Research Corporation., 1999). More soluble forms of Pb will be removed from the atmosphere by washout in rain.

In addition to atmospheric deposition, Pb may enter aquatic ecosystems through industrial or municipal wastewater effluents, storm water runoff, erosion, or direct point source inputs (e.g., Pb shot or accidental spills). Once in the aquatic environment, Pb will partition between the various compartments of the system (e.g., dissolved phase, solid phase, biota). The movement of Pb between dissolved and particulate forms is governed by factors such as pH, sorption, and biotransformation (see Section AX7.2.2.1). Lead bound to organic matter will settle to the bottom sediment layer, be assimilated by aquatic organisms, or be resuspended in the water column. The uptake, accumulation, and toxicity of Pb in aquatic organisms from water and sediments are influenced by various environmental factors (e.g., pH, organic matter, temperature, hardness, bioavailability). These factors are further described in Section AX7.2.3.4). The remainder of this section discusses some methods for describing the distribution of atmospheric Pb in the aquatic environment.



**Figure AX7-2.2.14. Lead cycle in an aquatic ecosystem.**

Sediment Core Dating and Source Tracing

In addition to directly measuring Pb concentrations in various aquatic compartments (see Section AX7.2.3.3), it is useful to study the vertical distribution of Pb. Sediment profiling and core dating is a method used to determine the extent of accumulation of atmospheric Pb and provide information on potential anthropogenic sources. Sediment concentration profiles are typically coupled with Pb isotopic analysis. The isotope fingerprinting method utilizes measurements of the abundance of common Pb isotopes (i.e.,  $^{204}\text{Pb}$ ,  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ ,  $^{208}\text{Pb}$ ) to distinguish between natural Pb over geologic time and potential anthropogenic sources. Details of this method were described in Section AX7.1.2. The concentration of isotope  $^{204}\text{Pb}$  has remained constant throughout time, while the other isotope species can be linked to various anthropogenic Pb sources. Typically, the ratios or signatures of isotopes (e.g.,  $^{207}\text{Pb}:$  $^{206}\text{Pb}$ ) are compared between environmental samples to indicate similarities or differences in the site being investigated and the potential known sources.

Generally, Pb concentrations in sediment vary with depth. For example, Chow et al. (1973) examined sediment Pb profiles in southern California. Lead concentrations were increased in the shallower sediment depths and comparatively decreased at greater depths. These changes in sediment vertical concentration were attributed to higher anthropogenic Pb fluxes

from municipal sewage, storm runoff, and atmospheric deposition. Similar experiments conducted throughout the United States have also suggested an increase in Pb concentrations in the upper sediment layer concomitant with increases in anthropogenic inputs (Bloom and Crecelius, 1987; Case et al., 1989; Ritson et al., 1999; Chillrud et al., 2003).

Sediment Pb concentration profiles and isotope analysis have also been used to identify specific anthropogenic sources. For example, Flegal et al. (1987) used isotopic ratios to trace sources of Pb in mussels from Monterey Bay, CA to a specific slag deposit. Several investigators have examined isotopic tracers to determine potential regional sources of Pb in eastern North America and the Great Lakes (Flegal et al., 1989b; Graney et al., 1995; Blais, 1996). Water samples from Lake Erie and Lake Ontario were collected and analyzed. Lead isotope ratios ( $^{206}\text{Pb}:$  $^{207}\text{Pb}$ ) from the lakes were compared to known ratios for Pb aerosols derived from industrial sources in Canada and the United States and found to correlate positively. This indicated that a majority of Pb in the lakes was derived from those industrial sources (Flegal et al., 1989b). Similarly, Gallon et al. (2006) used  $^{206}\text{Pb}:$  $^{207}\text{Pb}$  ratios in sediment cores collected from a 300 km transect in Canadian Shield headwater lakes to differentiate Pb contributions from smelter emissions relative to Pb contributions from other anthropogenic inputs. The authors were able to estimate the amounts of smelter-derived Pb in sediment collected along the 300 km transect. Lead isotopes in sediment cores from Quebec and Ontario, Canada were also used to distinguish between the amount of Pb deposited from local Canadian sources (28.4 to 61.7%) and U.S. sources (38.3 to 71.6%) (Blais, 1996). Examination of Pb isotopes in sediment and suspended sediment in the St. Lawrence River were used to identify potential anthropogenic Pb sources from Canada (Gobeil et al., 1995, 2005). Graney et al. (1995) used Pb isotope measurements to describe the differing historic sources of Pb in Lake Erie, Ontario and in Michigan. Temporal changes in Pb isotopic ratios were found to correspond to sources such as regional deforestation from 1860 through 1890, coal combustion and or smelting through 1930, and the influence of leaded gasoline consumption from 1930 to 1980.

The historic record of atmospheric Pb pollution has been studied to understand the natural background Pb concentration and the effects of Pb accumulation on ecosystems (Bindler et al., 1999; Renberg et al., 2000, 2002; Brännvall et al., 2001a,b). The most extensive work in this area has been conducted at pristine locations in Sweden (Bindler et al., 1999). In this study, soil, sediment, and tree rings were sampled for Pb concentrations and isotopic analyses were

conducted on the soil samples. From this record, historic Pb concentrations and Pb accumulation rates were estimated. Present day concentrations in the forest soils ranged from 40 to 100 mg/kg, while a natural background concentration was estimated at <1 mg/kg. The authors were able to model Pb concentrations for the past 6,000 years and also to project Pb concentrations for the next 400 years, given an assumed atmospheric deposition rate of 1 mg Pb m<sup>2</sup>/year. Models such as this are useful tools in determining the critical limits of metals in soils or sediments (Bindler et al., 1999; Renberg et al., 2002).

Lead source association may also be assessed through retrospective measurements. Squire et al. (2002) used a time-series approach to evaluate the change in Pb in San Francisco Bay, CA from 1989 to 1999. This approach involved the use of detailed linear regression models and long-term monitoring data to determine changes in Pb concentrations and to identify events corresponding to those changes. Sediment and water samples were collected throughout the bay and combined with data on effluent discharges, urban runoff, atmospheric deposition, and river discharges. The authors identified a 40% decline of Pb in the southern portion of the bay but found no change in the northern reach. The decline was attributed to a reduction in wastewater source loadings over the previous decade.

#### **AX7.2.2.4 Summary**

Lead is widely distributed in aquatic ecosystems, predominantly originating from atmospheric deposition or point source contribution. The fate and behavior of Pb in aquatic systems is regulated by physical and chemical factors such as pH, salinity, sediment sorption, transformation, and uptake by aquatic biota. In the United States, Pb concentrations in surface waters, sediments, and fish tissues range from 0.04 to 30 µg/L, 0.5 to 12,000 mg/kg, and 0.08 to 23 mg/kg, respectively. Atmospheric sources are generally decreasing, as the United States has removed Pb from gasoline and other products. However, elevated Pb concentrations remain at sites associated with mining wastes or wastewater effluents. Since the 1986 Lead AQCD, much has been learned about the processes affecting Pb fate and transport. Detailed analyses are currently available (i.e., Pb isotope dating) to allow for constructing the history of Pb accumulation and identifying specific Pb contaminant sources. Continued source control along with examination of the physical and chemical properties will further allow for the reduction of Pb concentrations throughout the United States.

### **AX7.2.3 Aquatic Species Response/Mode of Action**

Recent advancements in understanding the responses of aquatic biota to Pb exposure are highlighted in this section. A summary of the conclusions on the review of aquatic responses to Pb from the appropriate sections of the 1986 Lead AQCD, Volume II (U.S. Environmental Protection Agency, 1986a) and the subsequent conclusions and recommendations contained in the EPA staff review of that document (U.S. Environmental Protection Agency, 1990) are also provided. In addition, this section summarizes research subsequent to the 1986 Lead AQCD on Pb uptake into aquatic biota, effects of Pb speciation on uptake, resistance mechanisms to Pb toxicity, physiological effects of Pb, factors that affect responses to Pb, and factors associated with global climate change. Areas of research that are not addressed here include literature related to exposure to Pb shot or pellets and studies that examine human health-related endpoints (e.g., hypertension), which are described in other sections of this document.

#### **AX7.2.3.1 Lead Uptake**

Lead is nutritionally nonessential and non-beneficial and is toxic to living organisms in all of its forms (Eisler, 2000). Lead can bioaccumulate in the tissues of aquatic organisms through ingestion of food and water and adsorption from water (Vázquez et al., 1999; Vink, 2002) and subsequently lead to adverse effects if tissue levels are sufficiently high (see Section AX7.2.5). Recent research has suggested that due to the low solubility of Pb in water, dietary Pb (i.e., lead adsorbed to sediment, particulate matter, and food) may contribute substantially to exposure and toxicity in aquatic biota (Besser et al., 2005). Besser et al. (2004) exposed the amphipod *Hyalella azteca* to concentrations of Pb to evaluate the influence of waterborne and dietary Pb exposure on acute and chronic toxicity. The authors found that acute toxicity was unaffected by dietary exposure but that dietary Pb exposure did contribute to chronic toxic effects (i.e., survival, growth, reproduction) in *H. azteca*. Field studies in areas affected by metal contamination (i.e., Clark Fork River, MO; Coeur d'Alene, ID) (Woodward et al., 1994, 1995; Farag et al., 1994) have also demonstrated the effects of dietary metals on rainbow trout. However, there has been a debate on the importance of dietary exposure, as few controlled laboratory studies have been able to replicate the effects observed in the field studies (Hodson et al., 1978; Mount, 1994; Erikson, 2001). This may be due to differences in the availability of Pb from the dietary sources used in laboratory studies, differences in speciation, and/or

nutritional characteristics of the Pb dosed diets. In many field and laboratory studies, dietary exposure is rarely considered, but food provided to biota in these studies adsorb metals from water. Therefore, both dietary and waterborne exposure are occurring and both may be considered to play roles in eliciting the measured effects.

Lead concentrations in the tissues of aquatic organisms are generally higher in algae and benthic organisms and lower in higher trophic-level consumers (Eisler, 2000). Thus, trophic transfer of Pb through food chains is not expected (Eisler, 2000). Metals are not metabolized; therefore, they are good integrative indicators of exposure in aquatic biota (Luoma and Rainbow, 2005). Metal uptake is complex, being influenced by geochemistry, route of exposure (diet and adsorption), depuration, and growth (Luoma and Rainbow, 2005). This section discusses the factors affecting uptake of Pb by aquatic biota and the state of current research in this area.

As described in Section AX7.2.2.1, the solubility of Pb in water varies with pH, temperature, and ion concentration (water hardness) (Weber, 1993). Lead becomes soluble and bioavailable under conditions of low pH, organic carbon content, suspended sediment concentrations, and ionic concentrations (i.e., low Cd, Ca, Fe, Mn, Zn) (Eisler, 2000). Lead rapidly loses solubility above pH 6.5 (Rickard and Nriagu, 1978) and precipitates out as  $\text{Pb(OH)}^+$  and  $\text{PbHCO}_3^+$  into bed sediments. However, at reduced pH levels or ionic concentrations, sediment Pb can remobilize and potentially become bioavailable to aquatic organisms (Weber, 1993).

The most bioavailable inorganic form of Pb is divalent Pb ( $\text{Pb}^{2+}$ ), which tends to be more readily assimilated by organisms than complexed forms (Erten-Unal et al., 1998). On the other hand, the low solubility of Pb salts restricts movement across cell membranes, resulting in less accumulation of Pb in fish in comparison to other metals (e.g., Hg, Cu) (Baatrup, 1991).

The accumulation of Pb in aquatic organisms is, therefore, influenced by water pH, with lower pHs favoring bioavailability and accumulation. For example, fish accumulated Pb at a greater rate in acidic lakes (pH = 4.9 to 5.4) than in more neutral lakes (pH = 5.8 to 6.8) (Stripp et al., 1990). Merlini and Pozzi (1977) found that pumpkinseed sunfish exposed to Pb at pH 6.0 accumulated three-times as much Pb as fish kept at pH 7.5. However, Albers and Camardese (1993a,b) examined the effects of pH on Pb uptake in aquatic plants and invertebrates in acidic (pH ~5.0) and nonacidic (pH ~6.5) constructed wetlands, ponds, and small lakes in Maine and Maryland. Their results suggested that low pH had little effect on the accumulation of metals by

aquatic plants and insects and on the concentration of metals in the waters of these aquatic systems (Albers and Camardese, 1993a,b).

Three geochemical factors that influence metal bioaccumulation in aquatic organisms include speciation, particulate metal form, and metal form in the tissues of prey items (Luoma and Rainbow, 2005). Lead is typically present in acidic aquatic environments as  $\text{PbSO}_4$ ,  $\text{PbCl}_4$ , ionic Pb, cationic forms of Pb-hydroxide, and ordinary hydroxide  $\text{Pb(OH)}_2$ . In alkaline waters, common species of Pb include anionic forms of Pb-carbonate ( $\text{Pb(CO}_3\text{)}$ ) and  $\text{Pb(OH)}_2$ . Labile forms of Pb (e.g.,  $\text{Pb}^{2+}$ ,  $\text{PbOH}^+$ ,  $\text{PbCO}_3$ ) are a significant portion of the Pb inputs to aquatic systems from atmospheric washout. Particulate-bound forms are more often linked to urban runoff and mining effluents (Eisler, 2000). Little research has been done to link the complex concepts of chemical speciation and bioavailability in natural systems (Vink, 2002). The relationship between the geochemistry of the underlying sediment and the impact of temporal changes (e.g., seasonal temperatures) to metal speciation are particularly not well studied (Vink, 2002; Hassler et al., 2004).

Generally speaking, aquatic organisms exhibit three Pb accumulation strategies: (1) accumulation of significant Pb concentrations with a low rate of loss, (2) excretion of Pb roughly in balance with availability of metal in the environment, and (3) weak net accumulation due to very low metal uptake rate and no significant excretion (Rainbow, 1996). Species that accumulate nonessential metals such as Pb and that have low rates of loss must partition it internally in such a way that it is sparingly available metabolically. Otherwise, it may cause adverse toxicological effects (Rainbow, 1996). Aquatic organisms that exhibit this type of physiological response have been recommended for use both as environmental indicators of heavy metal pollution (Borgmann et al., 1993; Castro et al., 1996; Carter and Porter, 1997) and, in the case of macrophytes, as phytoremediators, because they accumulate heavy metals rapidly from surface water and sediment (Gavrilenko and Zolotukhina, 1989; Simões Gonçalves et al., 1991; Carter and Porter, 1997).

Uptake experiments with aquatic plants and invertebrates (e.g., macrophytes, chironomids, crayfish) have shown steady increases in Pb uptake with increasing Pb concentration in solution (Knowlton et al., 1983; Timmermans et al., 1992). In crayfish, the process of molting can cause a reduction in body Pb concentrations, as Pb incorporated into the crayfish shell is eliminated (Knowlton et al., 1983). Vázquez et al. (1999) reported on the uptake

of Pb from solution to the extracellular and intracellular compartments of 3 species of aquatic bryophytes. Relative to the 6 metals tested, Pb was found to accumulate to the largest degree in the extracellular compartments of all 3 bryophytes. The extracellular metals were defined as those that are incorporated into the cell wall or are found on the outer surface of the plasma membrane (i.e., adsorbed) (Vázquez et al., 1999). Intracellular metals were defined as metals introduced into the cell through a metabolically controlled process.

Arai et al. (2002) examined the effect of growth on the uptake and elimination of trace metals in the abalone *Haliotis*. They reported that older abalones had generally lower whole body concentrations of heavy metals than did younger, rapidly growing individuals. During the rapid growth of juveniles, the organism is less able to distinguish between essential (e.g., Zn), and nonessential metals (e.g., Pb). Once they reach maturity, they develop the ability to differentiate these metals. Li et al. (2004) reported a similar response in zebra fish embryo-larvae. Li et al. (2004) suggested that mature physiological systems are not developed in the embryo-larvae to handle elevated concentrations of metals. Therefore, metals are transported into the body by facilitated diffusion. Both the zebra fish and juvenile abalone demonstrate a rapid accumulation strategy followed by a low rate of loss as described above. There are insufficient data available to determine whether this phenomenon is true for other aquatic organisms.

Growth rates are generally thought to be an important consideration in the comparison of Pb levels in individuals of the same species. The larger the individual the more the metal content is diluted by body tissue (Rainbow, 1996).

Once Pb is absorbed, it may sequester into varying parts of the organism. Calcium appears to have an important influence on Pb transfer. For example, Pb uptake and retention in the skin and skeleton of coho salmon was reduced when dietary Ca was increased (Varanasi and Gmur, 1978). Organic Pb compounds tend to accumulate in lipids, and are taken up and accumulated in fish more readily than inorganic Pb compounds (Pattee and Pain, 2003).

Given the complexities of metal uptake in natural systems, a model incorporating some of the factors mentioned above is desirable. The EPA's Environmental Research Laboratory in Duluth, Minnesota developed a thermodynamic equilibrium model, MINTEQ that predicts aqueous speciation, adsorption, precipitation, and/or dissolution of solids for a defined set of environmental conditions (MacDonald et al., 2002; Playle, 2004). Although not specifically

designed to model uptake, MINTEQA2 provides an indication of what forms of the metal are likely to be encountered by aquatic organisms by estimating the formation of metal ions, complexation of metals, and the general bioavailability of metals from environmental parameters. More recently, a mechanistic model centered on biodynamics has been proposed by Luoma and Rainbow (2005) as a method of tying together geochemical influences, biological differences, and differences among metals to model metal bioaccumulation. The biodynamic model would be useful in determining the potential adverse effects on aquatic biota, which species are most useful as indicators of metal effects, and how ecosystems may change when contaminated by metals. Two prominent models examine trace metal bioavailability and its link to effects (Hassler et al., 2004). These include the free ion activity model (FIAM) and the biotic ligand model (BLM). Specific information on these models, including their limitations, is provided in Section AX7.2.1.3. These models are useful for advancing our understanding of how metal uptake occurs in aquatic organisms and how uptake and toxic effects are linked.

#### Bioconcentration Factors (BCF)

BCFs for Pb are reported for various aquatic plants in Table AX7-2.3.1. The green alga *Cladophora glomerata* is reported as having the highest BCF (Keeney et al., 1976). Duckweed (*Lemna minor*) exhibited high BCF values ranging from 840 to 3560 depending on the method of measurement (Rahmani and Sternberg, 1999). Duckweed that was either previously exposed or not exposed to Pb was exposed to a single dose of Pb-nitrate at 5000 µg/L for 21 days. Duckweed that was previously exposed to Pb removed 70 to 80% of the Pb from the water, while the previously unexposed duckweed removed 85 to 90%. Both plant groups were effective at removing Pb from the water at sublethal levels.

BCFs for Pb are reported for various invertebrates in Table AX7-2.3.2. BCFs for freshwater snails were 738 for a 28-day exposure (Spehar et al., 1978) and 1,700 for a 120-day exposure (Borgmann et al., 1978). Other reported values for invertebrates included a BCF of 1930 for the scud during a 4-day exposure (MacLean et al., 1996), and BCFs of 499 and 1120 for the caddis fly and stonefly, respectively, in 28-day exposures (Spehar et al., 1978). In a 28-day exposure, midge larvae were reported with a BCF of 3670 (Timmermans et al., 1992).

**Table AX7-2.3.1. Bioconcentration Factors for Aquatic Plants**

<b>BCF</b>	<b>Species</b>	<b>Test Conditions</b>	<b>Reference</b>
840 to 2700 (measured digestion)	Duckweed ( <i>Lemna minor</i> )	21 days, Pb-nitrate	Rahmani and Sternberg (1999)
1150 to 3560 (measured solution)	Duckweed	21 days, Pb-nitrate	Rahmani and Sternberg (1999)
16,000 to 20,000	Alga ( <i>Cladophora glomerata</i> )	not specified	Keeney et al. (1976)

**Table AX7-2.3.2. Bioconcentration Factors for Aquatic Invertebrates**

<b>BCF</b>	<b>Species</b>	<b>Test Conditions</b>	<b>Reference</b>
738	Snail ( <i>Physa integra</i> )	28 days, Pb-nitrate	Spehar et al. (1978)
1700	Snail ( <i>Lymnaea palustris</i> )	120 days, Pb-nitrate	Borgmann et al. (1978)
499	Caddis fly ( <i>Brachycentrus sp.</i> )	28 days, Pb-nitrate	Spehar et al. (1978)
1120	Stonefly ( <i>Pteronarcys dorsata</i> )	28 days, Pb-nitrate	Spehar et al. (1978)
1930	Scud ( <i>Hyaella azteca</i> )	4 days, Pb-chloride	MacLean et al. (1996)
3670	Midge larvae ( <i>Chironomus riparius</i> )	28 days	Timmermans et al. (1992)

BCFs for freshwater fish were 42 and 45 for brook trout and bluegill, respectively (Holcombe et al., 1976; Atchison et al., 1977). Although no BCFs have been reported for amphibians, Pb-nitrate was reported to accumulate mainly in the ventral skin and in the kidneys of frogs (Vogiatzis and Loumbourdis, 1999).

Bioconcentration factors and bioaccumulation factors (BAFs) are not necessarily the best predictors of tissue concentration levels given exposure concentration levels (Kapustka et al., 2004). The role of homeostatic mechanisms is a major consideration in tissue concentrations found in exposed biota. Similarly, measuring BCFs and BAFs in organisms may not accurately reflect how metals are treated within the organisms (e.g., partitioning to specific organelles,

sequestering to organ tissues). Therefore, they are not recommended for use in conducting metal risk assessments (Kapustka et al., 2004).

### **AX7.2.3.2 Resistance Mechanisms**

#### ***Detoxification Mechanisms***

Detoxification includes the biological processes by which the toxic qualities, or the probability and/or severity of harmful effects, of a poison or toxin are reduced by the organism. In the case of heavy metals, this process frequently involves the sequestration of the metal, rendering it metabolically inactive. Recent research into heavy metal detoxification in aquatic biota has focused on several physiological and biochemical mechanisms for detoxifying Pb. This section examines these mechanisms and the ability of plants, protists, invertebrates, and fish to mitigate Pb toxicity.

#### ***Plants and Protists***

Deng et al. (2004) studied the uptake and translocation of Pb in wetland plant species surviving in contaminated sites. They found that all plants tended to sequester significantly larger amounts of Pb in their roots than in their shoots. Deng et al. (2004) calculated a translocation factor (TF), the amount of Pb found in the shoots divided by the amount of Pb found in the root system, and found that TFs ranged from 0.02 to 0.80. Concentrations of Pb in shoots were maintained at low levels and varied within a narrow range. Deng et al. (2004) observed that plants grown in Pb-contaminated sites usually contained higher concentrations than the 27 mg/kg toxicity threshold established for plants by Beckett and Davis (1977). Some of the wetland plants examined by Deng et al. (2004) also accumulated high concentrations of metals in shoot tissues; however, these metals were assumed to be detoxified (metabolically unavailable), as no toxic response to these elevated concentrations was observed. Deng et al. (2004) suggested that this ability is likely related to discrete internal metal detoxification tolerance mechanisms.

Phytochelatins are thiol-containing intracellular metal-binding polypeptides that are produced by plants and protists in response to excessive uptake of heavy metals (Zenk, 1996). Phytochelatins are synthesized by the enzyme phytochelatin synthase that is activated by the presence of metal ions and uses glutathione as a substrate. When phytochelatins are synthesized

in sufficient amounts to chelate the metal ion, the enzyme is deactivated (Morelli and Scarano, 2001).

Morelli and Scarano (2001) studied phytochelatin synthesis and stability in the marine diatom *Phaeodactylum tricorutum* in the presence of Pb. They found that when metal exposure was alleviated, significant cellular Pb-phytochelatin complex content was lost. Their findings support a hypothesis of vacuolarization proposed for higher plants (Zenk, 1996), in which metal-phytochelatin complexes are actively transported from the cytosol to the vacuole, where they undergo rapid turnover. Zenk (1996) suggested that the complex dissociates, and the metal-free peptide is subsequently degraded. Morelli and Scarano (2001) proposed concomitant occurrence of phytochelatin synthesis and release during metal exposure, as a coincident detoxification mechanism in *P. tricorutum*.

#### *Aquatic Invertebrates*

Like plants and protists, aquatic animals detoxify Pb by preventing it from being metabolically available, though their mechanisms for doing so vary. Invertebrates use lysosomal-vacuolar systems to sequester and process Pb within glandular cells (Giamberini and Pihan, 1996). They also accumulate Pb as deposits on and within skeletal tissue (Knowlton et al., 1983; Anderson et al., 1997; Boisson et al., 2002), and some can efficiently excrete Pb (Vogt and Quintio, 1994; Prasuna et al., 1996).

Boisson et al. (2002) used radiotracers to evaluate the transfer of Pb into the food pathway of the starfish *Asterias rubens* as well as its distribution and retention in various body compartments. Boisson et al. (2002) monitored Pb elimination after a single feeding of Pb-contaminated molluscs and found that Pb was sequestered and retained in the skeleton of the starfish, preventing it from being metabolically available in other tissues. Elimination (as percent retention in the skeleton) was found to follow an exponential time course. Elimination was rapid at first, but slowed after 1 week, and eventually stabilized, implying an infinite biological half-life for firmly bound Pb. Results of radiotracer tracking suggest that Pb migrates within the body wall from the organic matrix to the calcified skeleton. From there, the metal is either absorbed directly or adsorbed on newly produced ossicles (small calcareous skeletal structures), where it is efficiently retained as mineral deposition and is not metabolically active (Boisson et al., 2002).

AbdAllah and Moustafa (2002) studied the Pb storage capability of organs in the marine snail *Nerita saxtilis*. Enlarged electron-dense vesicles and many granules were observed in digestive cells of these snails and are suggested to be the site of storage of detoxified metals. *N. saxtilis* were found to be capable of concentrating Pb up to 50 times that of surrounding marine water without exhibiting signs of histopathologic changes. This ability has been attributed to chelation with various biochemical compounds, such as thionine (forming metallothionine) (Rainbow, 1996), or complexation with carbonate, forming lipofuchsin (AbdAllah and Moustafa, 2002). Granules observed in lysosomal residual bodies were presumed to be the result of Pb accumulation. The presence of large vacuoles and residual bodies were indicative of the fragmentation phase of digestion, suggesting that Pb was also processed chemically in the digestive cells.

The podocyte cells of the pericardial gland of bivalves are involved in the ultrafiltration of the hemolymph (Giamberini and Pihan, 1996). A microanalytical study of the podocytes in *Dreissena polymorpha* exposed to Pb revealed lysosomal-vacuolar storage/processing similar to that in the digestive cells of *Nerita saxtilis*. The lysosome is thought to be the target organelle for trace metal accumulation in various organs of bivalves (Giamberini and Pihan, 1996). Epithelial secretion is the principal detoxification mechanism of the tiger prawn *Penaeus monodon*. Vogt and Qunitio (1994) found that Pb granules tended to accumulate in the epithelial cells of the antennal gland (the organ of excretion) of juveniles exposed for 5 and 10 days to waterborne Pb. The metal is deposited in vacuoles belonging to the lysosomal system. Continued deposition leads to the formation of electron-dense granules. Mature granules are released from the cells by apocrine secretion into the lumen of the gland, and presumably excreted through the nephridopore (i.e., the opening of the antennal gland). Apocrine secretion is predominant, so that as granules form, they are kept at low levels. Excretion was also found to be a primary and efficient detoxification mechanism in the shrimp *Chrissia halyi* (Prasuna et al., 1996).

Crayfish exposed to Pb have been shown to concentrate the metal in their exoskeleton and exuvia through adsorption processes. More than 80% of Pb found in exposed crayfish has been found in exoskeletons (Knowlton et al., 1983; Anderson et al., 1997). Following exposure, clearance is most dramatic from the exoskeleton. The result of a 3-week Pb-clearance study with red swamp crayfish *Procambarus clarkia*, following a 7-week exposure to 150 µg Pb/L, showed

an 87% clearance from the exoskeleton due, in part, to molting. Other organs or tissues that take up significant amounts of Pb include the gills, hepatopancreas, muscle, and hemolymph, in decreasing order. These parts cleared >50% of accumulated Pb over the 3-week clearance period, with the exception of the hepatopancreas. The hepatopancreas is the organ of metal storage and detoxification, although the molecular mechanisms of metal balance in crayfish have yet to be extensively investigated (Anderson et al., 1997).

### Fish

Most fish use mucus as a first line of defense against heavy metals (Coello and Khan, 1996). In fish, some epithelia are covered with extracellular mucus secreted from specialized cells. Mucus contains glycoproteins, and composition varies among species. Mucosal glycoproteins chelate Pb, and settle, removing the metal from the water column. Fish may secrete large amounts of mucus when they come into contact with potential chemical and biochemical threats. Coello and Khan (1996) investigated the role of externally added fish mucus and scales in accumulating Pb from water, and the relationship of these with the toxicity of Pb in fingerlings of green sunfish, goldfish and largemouth bass. The authors compared trials in which fish scales from black sea bass (*Centropristis striata*) and flounder (*Pseudopleuronectes americanus*) and mucus from largemouth bass were added to green sunfish, goldfish, and largemouth bass test systems and to reference test systems. On exposure to Pb, fish immediately started secreting mucus from epidermal cells in various parts of the body. Metallic Pb stimulated filamentous secretion, mostly from the ventrolateral areas of the gills, while Pb-nitrate stimulated diffuse molecular mucus secretion from all over the body. The addition of largemouth bass mucus significantly increased the  $LT_{50}$  (the time to kill 50%) for green sunfish and goldfish exposed to 250 mg/L of Pb-nitrate. In contrast, Tao et al. (2000) found that mucus reduced the overall bioavailability of Pb to fish but that the reduction was insignificant. Coello and Khan (1996) found that scales were more significant in reducing  $LT_{50}$  than mucous. Fish scales can accumulate high concentrations of metals, including Pb, through chelation with keratin. Scales were shown to buffer the pH of Pb-nitrate in solution and remove Pb from water after which they settled out of the water column. Addition of scales to test water made all species (green sunfish, goldfish, and largemouth bass) more tolerant of Pb.

### Summary of Detoxification Processes

Mechanisms of detoxification vary among aquatic biota and include processes such as translocation, excretion, chelation, adsorption, vacuolar storage, and deposition. Lead detoxification has not been studied extensively in aquatic organisms, but existing results indicate the following:

- Protists and plants produce intracellular polypeptides that form complexes with Pb (Zenk, 1996; Morelli and Scarano, 2001).
- Macrophytes and wetland plants that thrive in Pb-contaminated regions have developed translocation strategies for tolerance and detoxification (Knowlton et al., 1983; Deng et al., 2004).
- Some starfish (asteroids) sequester the metal via mineral deposition into the exoskeleton (Boisson et al., 2002).
- Species of mollusc employ lysosomal-vacuolar systems that store and chemically process Pb in the cells of their digestive and pericardial glands (Giamberini and Pihan, 1996; AbdAllah and Moustafa, 2002).
- Decapods can efficiently excrete Pb (Vogt and Qunitio, 1994; Giamberini and Pihan, 1996) and sequester metal through adsorption to the exoskeleton (Knowlton et al., 1983).
- Fish scales and mucous chelate Pb in the water column, and potentially reduce visceral exposure.

### ***Avoidance Response***

Avoidance is the evasion of a perceived threat. Recent research into heavy metal avoidance in aquatic organisms has looked at dose-response relationships as well as the effects of coincident environmental factors. Preference/avoidance response to Pb has not been extensively studied in aquatic organisms. In particular, data for aquatic invertebrates is lacking.

Using recent literature, this section examines preference-avoidance responses of invertebrates and fish to Pb and some other environmental gradients.

### *Aquatic Invertebrates*

Only one study was identified on avoidance response in aquatic invertebrates. Lefcort et al. (2004) studied the avoidance behavior of the aquatic pulmonate snail *Physella columbiana* from a pond that had been polluted with heavy metals for over 120 years. In a Y-maze test, first generation *P. columbiana* from the contaminated site avoided Pb at 9283 µg/L ( $p < 0.05$ ) and

moved toward Pb at 6255 µg/L ( $p < 0.05$ ). It is thought that attraction to Pb at certain elevated concentrations is related to Pb neuron-stimulating properties (Lefcort et al., 2004). These results are consistent with those from similar studies. Control snails from reference sites, and first and second-generation snails from contaminated sites were capable of detecting and avoiding heavy metals, although the first generation was better than the second generation, and the second was better than the controls at doing so. This suggests that detection and avoidance of Pb is both genetic and environmentally based for *P. columbiana*. Lefcort et al. (2004) observed heightened sensitivity to, and avoidance of, heavy metals by the snails when metals were present in combination.

### Aquatic Vertebrates

Steele et al. (1989) studied the preference-avoidance response of bullfrog (*Rana catesbeiana*) to plumes of Pb-contaminated water following 144-h exposure to 0 to 1000 µg Pb/L. In this laboratory experiment, tadpoles were exposed to an influx of 1000 µg Pb/L at five different infusion rates (i.e., volumes per unit time into the test system). Experiments were videotaped and location data from the tank were used to assess response. No significant differences were seen in preference-avoidance responses to Pb in either nonexposed or previously exposed animals. In a similar subsequent study, Steele et al. (1991) studied preference-avoidance response to Pb in American toad (*Bufo americanus*) using the same exposure range (0 to 1000 µg Pb/L). *B. americanus* did not significantly avoid Pb, and behavioral stress responses were not observed. The results do not indicate whether the tadpoles were capable of perceiving the contaminant. Lack of avoidance may indicate insufficient perception or the lack of physiological stress (Steele et al., 1991).

The olfactory system in fish is involved in their forming avoidance response to heavy metals (Brown et al., 1982; Sveciavičius, 1991). It is generally thought that behavioral avoidance of contaminants may be a cause of reduced fish populations in some water bodies, because of disturbances in migration and distribution patterns (Sveciavičius, 2001). Unfortunately, avoidance of Pb by fish has not been studied as extensively as for other heavy metals (Woodward et al., 1995).

Woodward et al. (1995) studied metal mixture avoidance response in brown trout (*Salmo trutta*), as well as the added effects of acidification. A 1-fold (1H) mixture contained

1.1 µg/L Cd, 12 µg/L Cu, 55 µg/L Zn, and 3.2 µg/L Pb (all metals were in the form of chlorides). Avoidance was quantified as time spent in test water, trip time to test water, and number of trips. Brown trout avoided the 1H mixture as well as the 0.5H, 2H, 4H, and 10H mixtures, but not the 0.1H mixture. Reduced avoidance was observed at higher concentrations (4H and 10H). The authors proposed that the reduced avoidance response was due to impaired perception due to injury. These responses are typical of other fish species to individual metals of similar concentrations (Woodward et al., 1995). This study does not conclusively indicate which of the metals in the mixture may be causing the avoidance response. However, given the neurotoxic effects of Pb, impaired perception is a likely response of Pb-exposed fish.

When test water was reduced in pH from 8 to 7, 6 to 5, brown trout avoidance increased, but with no significant difference between metal treatments and controls. However, in the 1H metal mixture treatment, brown trout made fewer trips into the test water chamber at the lower pHs (Woodward et al., 1995). This response may be related to an increased abundance of Pb cations at lower pH values in the test system.

Scherer and McNicol (1998) investigated the preference-avoidance responses of lake whitefish (*Coregonus clupeaformis*) to overlapping gradients of light and Pb. Whitefish were found to prefer shade in untreated water. Lead concentrations under illumination ranged from 0 to 1000 µg/L, and from 0 to 54,000 µg/L in the shade. Under uniform illumination, Pb was avoided at concentrations above 10 µg/L, but avoidance behavior lacked a dose-dependent increase over concentrations ranging from 10 to 1000 µg Pb/L. Avoidance in shaded areas was strongly suppressed, and whitefish only avoided Pb at concentrations at or above 32,000 µg/L.

### Summary of Avoidance Response

In summary, of those aquatic organisms studied, some are quite adept at avoiding Pb in aquatic systems, while others seem incapable of detecting its presence. Snails have been shown to be sensitive to Pb and to avoid it at high concentrations. Conversely, anuran (frog and toad) species lack an avoidance response to the metal. Fish avoidance of many chemical toxicants has been well established, and it is a dominant sublethal response in polluted waters (Svecevičius, 2001). However, no studies have been located specifically examining avoidance behavior for Pb in fish. Environmental gradients, such as light and pH, can alter preference-avoidance responses.

### **AX7.2.3.3 Physiological Effects of Lead**

This section presents a review of the physiological effects and functional growth responses associated with the exposure of aquatic biota to Pb. Physiological effects of Pb on aquatic biota can occur at the biochemical, cellular, and tissue levels of organization and include inhibition of heme formation, adverse effects to blood chemistry, and decreases in enzyme levels. Functional growth responses resulting from Pb exposure include changes in growth patterns, gill binding affinities, and absorption rates.

#### Biochemical Effects

Lead was observed to have a gender-selective effect on brain endocannabinoid (eCB) (e.g., 2-arachidonylglycerol [2-AG] and *N*-arachidonylethanolamine [AEA]) levels in fathead minnow *Pimephales promelas* (Rademacher et al., 2005). Cannabinoids, such as eCB, influence locomotor activity in organisms. Increased levels of cannabinoids have been shown to stimulate locomotor activity and decreased levels slow locomotor activity (Sañudo-Peña et al., 2000). Male and female fathead minnows were exposed to 0 and 1000 µg/L of Pb. Female minnows in the control group contained significantly higher levels of AEA and 2-AG compared to males. At a concentration of 1000 µg Pb/L, this pattern reversed, with males showing significantly higher levels of AEA in the brain than females (Rademacher et al., 2005). After 14-days exposure to the 1000 µg Pb/L treatment, significantly higher levels of 2-AG were found in male fathead minnows, but no effect on 2-AG levels in females was observed (Rademacher et al., 2005).

Lead acetate slightly inhibited 7-ethoxyresorufin-*o*-deethylase (7-EROD) activity in *Gammarus pulex* exposed for up to 96 h to a single toxicant concentration (EC<sub>50</sub>) (Kutlu and Susuz, 2004). The exact concentration used in the study was not reported. The EROD enzyme is required to catalyze the conjugation and detoxification of toxic molecules and has been proposed as a biomarker for contaminant exposure. The authors believe more detailed studies are required to confirm EROD as a biomarker for Pb exposure. The enzyme group alanine transferases (ALT) has been suggested as a bioindicator/biomarker of Pb stress (Blasco and Puppo, 1999). A negative correlation was observed between Pb accumulation and ALT concentrations in the gills and soft body of *Ruditapes philippinarum* exposed to 350 to 700 µg/L of Pb for 7 days (Blasco and Puppo, 1999).

Studies have identified ALAD in fish and amphibians as a useful indicator of Pb exposure (Gill et al., 1991; Nakagawa et al., 1995a,b). ALAD catalyzes the formation of hemoglobin and early steps in the synthesis of protoporphyrin (Gill et al., 1991; Nakagawa et al., 1995b). The absence of an inhibitory effect on this enzyme following exposure to cadmium, copper, zinc, and mercury suggests that this enzyme reacts specifically to Pb (Johansson-Sjöbeck and Larsson, 1979; Gill et al., 1991). A 0% decrease in ALAD activity was reported in common carp (*Cyprinus carpio*) exposed to a Pb concentration of 10 µg/L for 20 days (Nakagawa et al., 1995b). The recovery of ALAD activity after exposure to Pb has also been examined in carp (Nakagawa et al., 1995a). After 2-week exposure to 200 µg Pb/L, ALAD activity decreased to approximately 25% of value reported for controls (Nakagawa et al., 1995a). Fish removed from the test concentration after 2 weeks and placed in a Pb-free environment recovered slightly, but ALAD activity was only 50% of the controls even after 4 weeks (Nakagawa et al., 1995a). Vogiatzis and Loumbourdis (1999) exposed the frog (*Rana ridibunda*) to a Pb concentration of 14,000 µg/L over 30 days and a 90% decrease in ALAD activity was observed in the frogs.

### Blood Chemistry

Numerous studies have examined the effects of Pb exposure on blood chemistry in aquatic biota. These studies have primarily used fish in acute and chronic exposures to Pb concentrations ranging from 100 to 10,000 µg/L. Decreased erythrocyte, hemoglobin, and hemocrit levels were observed in rosy barb (*Barbus punctatus*) during an 8-week exposure to 126 µg/L of Pb-nitrate (Gill et al., 1991).

No difference was found in red blood cell counts and blood hemoglobin in yellow eels (*Anguilla anguilla*) exposed to 0 and 300 µg/L of Pb for 30 days (Santos and Hall, 1990). The number of white blood cells, in the form of lymphocytes, increased in the exposed eels. The authors concluded this demonstrates the lasting action of Pb as a toxicant on the immune system (Santos and Hall, 1990). Significant decreases in red blood cell counts and volume was reported in blue tilapia (*Oreochromis aureus*) exposed to Pb-chloride at a concentration of 10,000 µg/L for 1 week (Allen, 1993).

Blood components, such as plasma glucose, total plasma protein, and total plasma cholesterol, were unaffected in yellow eels exposed to 300 µg/L of Pb for 30 days (Santos and Hall, 1990). Effects on plasma chemistry were observed in *Oreochromis mossambicus* exposed

to 0, 18,000, 24,000, and 33,000  $\mu\text{g/L}$  of Pb (Ruparelia et al., 1989). Significant decreases in plasma glucose (hypoglycemic levels) were reported at concentrations of 24,000 and 33,000  $\mu\text{g Pb/L}$  after 14 and 21 days of exposure, and at 18,000  $\mu\text{g Pb/L}$  after 21 days of exposure (Ruparelia et al., 1989). Plasma cholesterol levels dropped significantly in comparison to controls after 14 days of exposure to 33,000  $\mu\text{g Pb/L}$  and in all test concentrations after 21 days of exposure (Ruparelia et al., 1989). Similarly, concentrations of blood serum protein, albumin, and globulin were identified as bioindicators of Pb stress in carp (*Cyprinus carpio*) exposed to Pb-nitrates at concentrations of 800 and 8000  $\mu\text{g Pb/L}$  (Gopal et al., 1997).

### Tissues

In fish, the gills serve as an active site for ion uptake. Recent studies have examined the competition between cations for binding sites at the fish gill (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{H}^+$ ,  $\text{Pb}^{2+}$ ) (MacDonald et al., 2002; Rogers and Wood, 2003, 2004). Studies suggest that  $\text{Pb}^{2+}$  is an antagonist of  $\text{Ca}^{2+}$  uptake (Rogers and Wood, 2003, 2004). MacDonald et al. (2002) proposed a gill-Pb binding model that assumes  $\text{Pb}^{2+}$  has a  $\approx 100$  times greater affinity for binding sites at the fish gill than other cations. More toxicity studies are required to quantify critical Pb burdens that could be used as indicators of Pb toxicity (Niyogi and Wood, 2003).

### Growth Responses

A negative linear relationship was observed in the marine gastropod abalone (*Haliotis*) between shell length and muscle Pb concentrations (Arai et al., 2002). Abalones were collected from two sites along the Japanese coast. *Haliotis discus hannai* were collected from along the coast at Onagawa; *Haliotis discus* were collected from along the coast at Amatsu Kominato. The authors did not report significant differences between the two sampling sites. From samples collected at Onagawa, Pb concentrations of 0.03 and 0.01  $\mu\text{g/g}$  were associated with abalone shell lengths of 7.7 cm (3 years old) and 12.3 cm (6 years old), respectively. From samples collected at Amatsu Kominato, Pb concentrations of 0.09 and 0.01  $\mu\text{g/g}$  were associated with abalone shell lengths of 3.9 cm (0 years old) and 15.3 cm (8 years old), respectively (Arai et al., 2002). The authors theorized that young abalones, experiencing rapid growth, do not discriminate between the uptake of essential and nonessential metals. However, as abalones grow larger and their rate of growth decreases, they increasingly favor the uptake of essential

metals over nonessential metals. This is demonstrated by the relatively consistent concentrations of Cu, Mn, and Zn that were reported for the abalone samples (Arai et al., 2002).

#### Other Physiological Effects

Increased levels of Pb in water were found to increase fish production of mucus: excess mucus coagulates were observed over the entire body of fishes. Buildup was particularly high around the gills, and in the worst cases, interfered with respiration and resulted in death by anoxia (Aronson, 1971; National Research Council of Canada., 1973).

#### **AX7.2.3.4 Factors That Modify Organism Response to Lead**

A great deal of research has been undertaken recently to better understand the factors that modify aquatic organism response to metals including lead. A discussion of research on the many factors that can modify aquatic organism response to Pb is provided in this section.

#### ***Influence of Organism Age and Size on Lead Uptake and Response***

It is generally accepted that Pb accumulation in living organisms is controlled, in part, by metabolic rates (Farkas et al., 2003). Metabolic rates are, in-turn, controlled by the physiological conditions of an organism, including such factors as size, age, point in reproductive cycle, nutrition, and overall health. Of these physiological conditions, size and age are the most commonly investigated in relation to heavy metal uptake. This section reviews recent research focusing on relationships between body size, age, and Pb accumulation in aquatic invertebrates and fish.

#### Invertebrates

MacLean et al. (1996) investigated bioaccumulation kinetics and toxicity of Pb in the amphipod *Hyalella azteca*. Their results indicated that body size did not greatly influence Pb accumulation in *H. azteca* exposed to 50 or 100 µg/L of PbCl<sub>2</sub> for 4 days. Canli and Furness (1993) found similar results in the Norway lobster *Nephrops norvegicus* exposed to 100 µg/L of Pb(NO<sub>3</sub>)<sub>2</sub> for 30 days. No significant sex- or size-related differences were found in concentrations of Pb in the tissue. The highest tissue burden was found in the carapaces (42%). Several studies have determined that Pb can bind to the exoskeleton of invertebrates and

sometimes dominate the total Pb accumulated (Knowlton et al., 1983). This adsorption of Pb to the outer surface of invertebrates can result in strong negative relationships for whole-body Pb concentration as a function of body mass (i.e., concentrations decrease rapidly with increased body size and then stabilize) (MacLean et al., 1996).

Drava et al. (2004) investigated Pb concentrations in the muscle of red shrimp *Aristeus antennatus* from the northwest Mediterranean. Lead concentrations ranged from 0.04 to 0.31 µg/g dw. No significant relationships between size and Pb concentration in *A. antennatus* were found, and concentrations were not related to reproductive status.

Arai et al. (2002) analyzed abalones (*Haliotis*) at various life stages from coastal regions of Japan. They investigated growth effects on the uptake and elimination of Pb. Results indicated a significant negative linear relationship between age, shell length and Pb concentrations in muscle tissue. The relationship was consistent despite habitat variations in Pb concentrations between the study sites, suggesting that Pb concentrations changed with growth in the muscle tissue of test specimens and implying that abalone can mitigate Pb exposure as they age.

### Fish

Douben (1989) investigated the effects of body size and age on Pb body burden in the stone loach (*Noemacheilus barbatulus* L.). Fish were caught during two consecutive springs from three Derbyshire rivers. Results indicated that Pb burden increased slightly with age. Similarly, Köck et al. (1996) found that concentrations of Pb in the liver and kidneys of Arctic char (*Salvelinus alpinus*) taken from oligotrophic alpine lakes were positively correlated with age. It has been suggested that fish are not able to eliminate Pb completely, and that this leads to a stepwise accumulation from year to year (Köck et al., 1996). In contrast, Farkas et al. (2003) found a negative relationship between Pb concentrations and muscle and gill Pb concentrations in the freshwater fish *Abramis brama*. Fish were taken from a low-contaminated site and contained between 0.44 and 3.24 µg/g Pb dw. Negative correlations between metal concentration and fish size in low-contaminated waters likely results from variations in feeding rates associated with developmental stages. This hypothesis is consistent with the fact that in low-contaminated waters, feeding is the main route of uptake and feeding rates decrease with development in fish (Farkas et al., 2003).

In summary, relationships between age, size, and Pb body burden in aquatic invertebrates and fish are interspecifically variable and depend on many environment-related variables (e.g., exposure) (Farkas et al., 2003).

### ***Genetics***

There are few studies documenting the effects of Pb on organismal and population genetics, although rapid advances in biotechnology have prompted recent research in this area (Beaty et al., 1998). There are two principal effects that sublethal exposure to a contaminant can have on the genetics of an organism and/or population: (1) a contaminant may influence selection by selecting for certain phenotypes that enable populations to better cope with the chemical; or (2) a contaminant can be genotoxic, meaning it can produce alterations in nucleic acids at sublethal exposure concentrations, resulting in changes in hereditary characteristics or DNA inactivation (Shugart, 1995). Laboratory studies have shown that exposure to  $\text{Pb}^{2+}$  at 10 mg/mL in blood produces chromosomal aberrations (i.e., deviations in the normal structure or number of chromosomes) in some organisms (Cestari et al., 2004). Effects of genotoxicity and toxin-induced selection do not preclude one another, and may act together on exposed populations. This section reviews Pb genotoxicity and the effects of Pb-induced selection in aquatic populations.

### **Selection**

Evidence for genetic selection in the natural environment has been observed in some aquatic populations exposed to metals (Rand et al., 1995; Beaty et al., 1998; Duan et al., 2000; Kim et al., 2003). Because tolerant individuals have a selective advantage over vulnerable individuals in polluted environments, the frequency of tolerance genes will increase in exposed populations over time (Beaty et al., 1998). Several studies have shown that heavy metals can alter population gene pools in aquatic invertebrates. These changes have resulted in decreased genetic diversity and are thought to be a potential source of population instability (Duan et al., 2000; Kim et al., 2003).

Kim et al. (2003) investigated genetic differences and population structuring in the gastropod *Littorina brevicula* from heavy-metal polluted and unpolluted environments. Organisms from polluted sites contained a mean of 1.76  $\mu\text{g Pb/g}$ , while organisms from

unpolluted sites contained 0.33 µg Pb/g. They found significant differences in haplotypes between the test groups and allelic diversity was significantly lower among *L. brevicula* from polluted regions. In contrast, Yap et al. (2004) performed a similar experiment with the green-lipped mussel *Perna viridis*; they found that mussels from contaminated sites containing between 4 and 10 µg Pb/g, as well as other heavy metals, exhibited a higher percentage of polymorphic loci and excess heterozygosity compared to those from uncontaminated sites. The higher level of genetic diversity was attributed to greater environmental heterogeneity (i.e., variation due to pollution gradients) in contaminated sites (Yap et al., 2004).

Duan et al. (2000) investigated amphipod (*Hyalella azteca*) selective mortality and genetic structure following acute exposure to Pb (5.47 mg/L Pb(NO<sub>2</sub>)<sub>2</sub>) as well as exposure to other heavy metals. They found that genetic differentiation consistently increased among survivors from the original population, supporting the hypothesis that heavy metals, including Pb, have the potential to alter the gene pools of aquatic organisms.

### Genotoxicity

Lead exposure in water (50 µg/L) over 4 weeks resulted in DNA strand breakage in the freshwater mussel *Anodonta grandis* (Black et al., 1996), although higher concentrations (up to 5000 µg/L) did not result in significant breakage by the end of the study period. These results suggest that a threshold effect for DNA damage and repair exists, where DNA repair only occurs once a certain body exposure level has been reached. More recently, Cestari et al. (2004) observed similar results in neotropical fish (*Hoplias malabaricus*) that were fed Pb-contaminated food over 18, 41, and 64 days. Lead body burdens in *H. malabaricus* were approximately 21 µg Pb<sup>2+</sup>/g. Results indicated that exposure to Pb significantly increased the frequency of chromosomal aberrations and DNA damage in kidney cell cultures, although when assessed at the end of the longer exposure periods, aberrations were less common.

### ***Environmental Biological Factors***

Environmental factors that are biological in origin can alter the availability, uptake and toxicity of Pb to aquatic organisms. These factors can be grouped into living and non-living constituents. For example, living organisms may sequester Pb from the water column, reducing the availability and toxicity of the metal in the water column to other biota, thus reducing

potential toxic effects in other organisms. Non-living organic material (e.g., components of sloughed-off scales, mucus, carcasses, and other decomposing, humic material) can similarly combine with Pb from the water column, rendering it unavailable. This section will review the literature on biological environmental factors and their influence on the bioavailability, uptake, and toxicity of Pb.

Van Hattum et al. (1996) studied the influence of abiotic variables, including DOC on Pb concentrations in freshwater isopods (*Proasellus meridianus* and *Asellus aquaticus*). They found that BCFs were significantly negatively correlated with DOC concentrations. Thus, as DOC concentrations increased, BCFs decreased in *P. meridianus* and *A. aquaticus*, indicating that DOC acts to inhibit the availability of Pb to these isopods.

Kruatrachue et al. (2002) investigated the combined effects of Pb and humic acid on total chlorophyll content, growth rate, multiplication rate, and Pb uptake of common duckweed. When humic acid was added to the Pb-nitrate test solutions (50, 100, and 200 mg Pb(NO<sub>3</sub>)<sub>2</sub>/ L), toxicity of Pb to duckweed was decreased. The addition of humic acid to the Pb-nitrate solution increased the pH. The authors suggested that there was a proton dissociation from the carboxyl group in the humic acid that complexed with Pb, resulting in a decrease in free Pb ions available to the plant.

Schwartz et al. (2004) collected natural organic matter (NOM) from several aquatic sites across Canada and investigated the effects of NOM on Pb toxicity in rainbow trout (*Oncorhynchus mykiss*). They also looked at toxicity effects as they related to the optical properties of the various NOM samples. The results showed that NOM in test water almost always increased LT<sub>50</sub> and that optically dark NOM tended to decrease Pb toxicity more than did optically light NOM in rainbow trout.

In summary, non-living constituents of biological origin in the environment have been shown to reduce Pb availability and, therefore, toxicity in some aquatic organisms. It is generally thought that this occurs through complexation or chelation processes that take place in the water column.

### ***Physicochemical Environmental Factors***

This section reviews the literature on physicochemical environmental factors and their influence on the bioavailability, uptake, and toxicity of Pb in aquatic organisms. These factors are discussed with regard to their influence individually and in combination.

Studies generally agree that as pH increases, the toxicity of Pb decreases (Horne and Dunson, 1995b; MacDonald et al., 2002). As pH decreases, Pb becomes more soluble and more readily bioavailable to aquatic organisms (Weber, 1993). Significantly lower survival, decreased hatching success, slower development, and increased egg mass and larval mortality were observed in Jefferson salamanders (*Ambystoma jeffersonianum*) and wood frogs (*Rana sylvatica*) exposed to Pb at a pH of 4.5 versus a pH of 5.5 (Horne and Dunson, 1995b). Contradictory results have been reported for invertebrates. Over a 96-h exposure period, mortality increased with decreasing pH for the bivalve *Pisidium casertanum*, while pH-independent mortality was reported for gastropods and crustacea under similar exposure conditions (Mackie, 1989). Cladocerans (*C. dubia*) and amphipods (*H. azteca*) were also more sensitive to Pb toxicity at pH 6 to 6.5 than at higher pH levels (Schubauer-Berigan et al., 1993). Lead was 100 times more toxic to the amphipod *Hyaella azteca* at a pH range of 5.0 to 6.0 (Mackie, 1989) than at a pH range of 7.0 to 8.5 (Schubauer-Berigan et al., 1993). Lead was also more toxic to fathead minnows at lower pH levels (Schubauer-Berigan et al., 1993).

The influence of pH on Pb accumulation has also been observed in sediments. Accumulation of Pb by the isopod *Asellus communis* was enhanced at low pH, after a 20-day exposure to Pb-contaminated sediments (Lewis and McIntosh, 1986). In *A. aquaticus*, temperature increases were found to be more important than increased pH in influencing Pb accumulation (Van Hattum et al., 1996). Increased water temperature was also found to reduce Pb uptake fluxes in green microalga (*Chlorella kesslerii*) (Hassler et al., 2004). Lead and zinc body concentrations in *Asellus* sp. were found to vary markedly with seasonal temperature changes, with greater concentrations present in spring and summer (Van Hattum et al., 1996).

Acute and chronic toxicity of Pb increases with decreasing water hardness, as Pb becomes more soluble and bioavailable to aquatic organisms (Horne and Dunson, 1995a; Borgmann et al., 2005). There is some evidence that water hardness and pH work together to increase or decrease the toxicity of Pb. Jefferson salamanders exposed to Pb for 28 days at low pH and low water hardness experienced 50% mortality, while exposure to Pb at high pH and high water hardness

resulted in 91.7% survival (Horne and Dunson, 1995a). Exposure to Pb at high pH and low water hardness or low pH and high water hardness resulted in 75 and 41.7% survival, respectively (Horne and Dunson, 1995a). Similar results were reported for Jefferson salamanders during a 7-day exposure and wood frogs during 7- and 28-day exposures (Horne and Dunson, 1995c). In some cases, water hardness and pH in the absence of Pb have been shown to affect survival adversely. Mean acute survival of wood frogs and Jefferson salamanders exposed to low pH and low water hardness, in the absence of Pb, was 83.3 and 91.7%, respectively. Mean chronic survival of wood frogs and Jefferson salamanders exposed to low pH and low water hardness, in the absence of Pb, was 79.2 and 41.7%, respectively (Horne and Dunson, 1995c).

High  $\text{Ca}^{2+}$  concentrations have been shown to protect against the toxic effects of Pb (Sayer et al., 1989; MacDonald et al., 2002; Hassler et al., 2004; Rogers and Wood, 2004). Calcium affects the permeability and integrity of cell membranes and intracellular contents (Sayer et al., 1989). As  $\text{Ca}^{2+}$  concentrations decrease, the passive flux of ions (e.g., Pb) and water increases. At the lowest waterborne  $\text{Ca}^{2+}$  concentration (150  $\mu\text{mol/L}$ ), Pb accumulation in juvenile rainbow trout (*Oncorhynchus mykiss*) branchials significantly increased as Pb concentration in water increased (Rogers and Wood, 2004). At higher  $\text{Ca}^{2+}$  concentrations, Pb accumulation did not significantly increase with Pb concentration in water. This result demonstrates the protective effects of waterborne  $\text{Ca}^{2+}$  and supports the suggestion that the  $\text{Ca}^{2+}$  component of water hardness determines the toxicity of Pb to fish (Rogers and Wood, 2004). Rogers and Wood (2004) reported that the uptake of  $\text{Ca}^{2+}$  and  $\text{Pb}^{2+}$  involves competitive inhibition of apical entry at lanthanum-sensitive  $\text{Ca}^{2+}$  channels and interference with the function of the ATP-driven baso-lateral  $\text{Ca}^{2+}$  pump. High mortality was reported in brown trout (*Salmo trutta*) fry exposed to Pb at a waterborne  $\text{Ca}^{2+}$  concentration of 20  $\mu\text{mol/L}$ , while negligible mortality was reported at the same Pb concentration but at a waterborne  $\text{Ca}^{2+}$  concentration of 200  $\mu\text{mol/L}$  (Sayer et al., 1989). Adverse effects to mineral uptake and skeletal development were observed in the latter test group (Sayer et al., 1989).

The bioavailability of Pb and other metals that can be simultaneously extracted in sediments may be modified through the role of acid volatile sulfide (AVS) under anoxic conditions (Tessier and Campbell, 1987; Di Toro et al., 1992; Casas and Crecelius, 1994). The term AVS (iron sulfide is an example) refers to the fraction of the sediment that consists of a

reactive pool of solid-phase sulfide. This phase is available to bind divalent metals that then become unavailable for uptake by aquatic biota. The models proposed by Di Toro et al. (1992) and Casas and Crecelius (1994) predict that when the molar ratio of simultaneously extractable metals (SEM) to AVS in sediments is less than one, the metals will not be bioavailable due to complexation with available sulfide.

Salinity is an important modifying factor to metal toxicity. Verslycke et al. (2003) exposed the estuarine mysid *Neomysis integer* to individual metals, including Pb, and metal mixtures under changing salinity. At a salinity of 5‰, the reported LC<sub>50</sub> for Pb was 1140 µg/L (95% CL = 840, 1440 µg/L). At an increased salinity of 25‰, the toxicity of Pb was substantially reduced (LC<sub>50</sub> = 4274 µg/L [95% CL = 3540, 5710 µg/L]) (Verslycke et al., 2003). The reduction in toxicity was attributed to increased complexation of Pb<sup>2+</sup> with Cl<sup>-</sup> ions.

### ***Nutritional Factors***

The relationship between nutrition and Pb toxicity has not been thoroughly investigated in aquatic organisms. In fact, algae species are the only aquatic organisms to have been studied fairly frequently. Although nutrients have been found to have an impact on Pb toxicity, the mechanisms involved are poorly understood. It is unclear whether the relationship between nutrients and toxicity comprises organismal nutrition (the process by which a living organism assimilates food and uses it for growth and for replacement of tissues), or whether nutrients have interacted directly with Pb, inhibiting its metabolic interaction in the organism. This section reviews the little information that has been gathered from studies documenting apparent Pb-nutrition associations in aquatic organisms.

Jampani (1988) looked at the impact of various nutrients (i.e., sodium acetate, citric acid, sodium carbonate, nitrogen, and phosphates) on reducing growth inhibition in blue-green algae (*Synechococcus aeruginosus*) exposed to 200 mg Pb/L. Exposure to this Pb treatment concentration caused 100% mortality in algae. Results indicated that additional nitrogen, phosphates, and some carbon sources, including sodium acetate, citric acid and sodium carbonate, all protected the algae from Pb toxicity. Algae that had been starved prior to the experiment were found to be significantly more sensitive to Pb exposure. Glucose was the only nutrient tested that did not have a significant impact on Pb toxicity in *S. aeruginosus*. In a similar study by Rao and Reddy (1985) on *Scenedesmus incrassatulus*, nitrogen, phosphate and

carbon sources (including glucose), all had protective effects, and reduced Pb toxicity at 300 and 400 mg Pb/L. Both studies proposed similar hypotheses regarding nutrient-Pb mechanisms that led to reduced toxicity. One hypothesis was that the nutrients were able to reverse toxic effects. The second hypothesis was that the nutrients interacted directly with Pb, in some way sequestering the metal so as to inhibit its metabolic interaction with the organism (Rao and Reddy, 1985; Jampani, 1988).

Rai and Raizada (1989) investigated the effects of Pb on nitrate and ammonium uptake as well as carbon dioxide and nitrogen fixation in *Nostoc muscorum* over a 96-h period. Test specimens were exposed to 10, 20, and 30 mg Pb/L. At 20 mg Pb/L, nitrate uptake was inhibited by 64% after 24 h and by 39% after 96 h. Ammonium uptake was inhibited, and similarly, inhibition decreased from 72% inhibition after 24 h to 26% inhibition after 96 h of exposure. Carbon dioxide fixation and nitrogenase activity followed similar patterns, and results indicated that Pb exposure can affect the uptake of some nutrients in *N. muscorum*.

Adam and Abdel-Basset (1990) studied the effect of Pb on metabolic processes of *Scenedesmus obliquus*. They found that nitrogenase activity was inhibited by Pb nitrate, but enhanced by Pb-acetate. As photosynthetic products and respiratory substrates, carbohydrate and lipid levels were altered by Pb. Above 30 mg/L of Pb-nitrate, both macronutrients were reduced. However, Pb-acetate was found to increase carbohydrate levels. Results suggest that Pb can have an effect on macronutrients in *S. obliquus* and that effects may vary depending on the chemical species.

Simões Gonçalves et al. (1991) studied the impact of light, nutrients, air flux, and Pb, in various combinations, on growth inhibition in the green algae *Selenastrum capricornutum*. Results indicated that at lower Pb concentrations (<0.207 mg/L) and increased nutrient concentrations, algae release more exudates that form inert complexes with Pb anions in the water. This suggests that *S. capricornutum* can use exudates as a protection and that this protective mechanism depends on nutrient supply. These results are consistent with those of Capelo et al. (1993), who investigated uptake of nitrogen and phosphorus in the algae *Selenastrum capricornutum* over time in the absence and presence of 0.207 mg Pb/L. They found that the presence of Pb had no significant influence on the assimilation of nitrogen and phosphorus. However, they did find that in the presence of Pb, algae released higher concentrations of organics with Pb-chelating groups.

Amiard et al. (1994) investigated the impact on soft tissue Pb concentrations of various feeding regimes on oysters (*Crassostrea gigas*) during their spat rearing. They fed test groups of *C. gigas* different amounts of *Skeletonema costatum* and additional natural phytoplankton grown in test solutions. Results showed that size and food intake both negatively correlated with metal concentrations in soft tissue. The authors hypothesized that this relationship was due in part to a diluting effect of the food.

In summary, nutrients affect Pb toxicity in those aquatic organisms that have been studied. Some nutrients seem to be capable of reducing toxicity, though the mechanisms have not been well established. Exposure to Pb has not been shown to reduce nutrient uptake ability, though it has been demonstrated that Pb exposure may lead to increased production and loss of organic material (e.g., mucus and other complex organic ligands) (Capelo et al., 1993).

### ***Interactions with Other Pollutants***

Most of the scientific literature reviewed in this section considered how Pb and other elements combine to affect uptake and exert toxicity. Research on the interactions of Pb with complexing ligands and other physical and biological factors was more thoroughly discussed in Section AX7.2.3.4. Predicting the response of organisms to mixtures of chemicals is difficult (Norwood et al., 2003). There are two schools of thought on addressing chemical mixtures in toxicology. The first focuses on the combined mode of action of the individual mixture substances while the other focuses on the organism response to the mixture as being additive, or some deviation from additive (synergistic or antagonistic). The mode of action model assumes that each of the individual substances has similar pharmacokinetics as well as mode of action. Approaches to modeling combined mode of action include: 1) joint independent action for toxicants with different modes of action; and 2) joint similar mode of action. Within the scientific literature the deviations from additivity approach can be confusing as some authors report concentration additivity, while others report effect additivity. The concentration additivity model assumes that the sum of the concentrations will result in a level of effect similar to the simple sum of the effects observed if each chemical were applied separately (e.g., Herkovits and Perez-Coll, 1991). Toxic units (TU) may be used to describe the concentration additivity results for mixtures (e.g., Hagopian-Schlekat et al., 2001). Effects additivity suggests that the level of effect of a mixture will be the sum of the effects of each chemical used separately. Thus, the

separate concentration-effects models for each chemical are used to predict individual effects and the sum of these predictions is then compared to the actual effect of the mixture. Deviations from the mixture effect are then classified as less than additive (antagonistic) or more than additive (synergistic) (see Piegorsch et al., 1988; Finney 1947 for further information).

The complexity of metal mixture interactions as different metal concentrations, environmental conditions (e.g., temperature, pH), and other factors can cause marked changes in the effects observed (Norwood et al., 2003). In describing Pb interactions with other elements, the different approaches to modeling mixture toxicity are considered. Specific reference to known Pb-metal interactions and implications on Pb uptake and toxicity will also be made in each of the studies below.

Less than additive or antagonistic interactions can reduce metal bioavailability when metals are present in combination, and may lead to reduced potential for toxicity (Hassler et al., 2004). A number of elements act in an antagonistic fashion with Pb. For example, Pb is a well-known antagonist to  $\text{Ca}^{2+}$  (Niyogi and Wood, 2004; Hassler et al., 2004), which is an essential element, required for a number of physiological processes in most organisms. Lead ions have an atomic structure similar to  $\text{Ca}^{2+}$  and can be transported either actively or passively across cell membranes in place of  $\text{Ca}^{2+}$ . An example of this interaction was reported by Behra (1993a,b) where Pb was shown to activate calmodulin reactions in rainbow trout (*O. mykiss*) and sea mussel (*Mytilus* sp.) tissues in the absence of calcium. Calmodulin (CaM) is a major intracellular calcium receptor and regulates the activities of numerous enzymes and cellular processes. Allen (1994) reported that Pb can replace calcium in body structures (e.g., bones, shells); replace zinc in ALAD, which is required for heme biosynthesis; and react with sulfhydryl groups, causing conformation protein distortion and scission of nucleic acids (Herkovits and Perez-Coll, 1991). Lead is also a known antagonist to  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ , and  $\text{Cl}^-$  regulation in fish (Ahern and Morris, 1998; Rogers and Wood, 2003, 2004; Niyogi and Wood, 2004). Li et al. (2004) reported on the interaction of  $\text{Pb}^{2+}$  with  $\text{Cd}^{2+}$  in the context of adsorption from solution by *Phanerochaete chrysosporium*, a filamentous fungus. The authors found that cadmium uptake decreased with increasing concentration of Pb ions with  $\text{Pb}^{2+}$  outcompeting  $\text{Cd}^{2+}$  for binding sites.

Hassler et al. (2004) reported that in the presence of copper ( $\text{Cu}^{2+}$ ), there was a significantly higher rate of internalization of Pb in the green algae *Chlorella kesslerii*. It was

suggested that  $\text{Cu}^{2+}$  may have affected organism physiology through the disruption of cell membrane integrity. This would allow increased cation (i.e.,  $\text{Pb}^{2+}$ ) permeability and, therefore, substantially increase internalization of Pb and result in effects that were more than additive (synergistic). Hagopian-Schlekat et al. (2001) examined the impact of individual metals and complex metal mixtures containing Cd, Cu, Ni, Zn, and Pb to the estuarine copepod *Amphiascus tenuiremis*. The copepods were exposed to metal-spiked sediment and pore water. The mixed metal sediment toxicity tests demonstrated greater than additive toxicity to *A. tenuiremis*. It was postulated that the synergism observed was due to two or more metals affecting the same biological function. Herkovits and Perez-Coll (1991) exposed *Bufo arenarum* larvae to various Pb and zinc concentrations in solution. At low zinc concentrations, (2:1 Pb:Zn ratio), a synergistic toxic effect was observed in the frog larvae relative to the effects observed from exposure to the individual metals and at higher zinc concentrations. Enhanced Pb toxicity was attributed to the interference of Pb with cellular activities due to binding with sulfhydryl polypeptides and nucleic acid phosphates (Herkovits and Perez-Coll, 1991). Allen (1994) reported on the accumulation of numerous metals and ions into specific tissues of the tilapia *Oreochromis aureus*. Tilapia exposed to low concentrations of Pb and mercury (both at 0.05 mg/L) had significantly higher concentrations of Pb in internal organs than those fish exposed to Pb alone. Similarly, low concentrations of cadmium with low concentrations of Pb caused increased uptake of Pb in certain organs (e.g., liver, brain, and caudal muscle).

Lead has been shown to complex with  $\text{Cl}^-$  in aquatic systems. For example, Verslycke et al. (2003) exposed the estuarine mysid *Neomysis integer* to six different metals, including Pb, and a combined metal mixture under changing salinity conditions. At a salinity of 5‰, the reported  $\text{LC}_{50}$  for Pb was 1140  $\mu\text{g/L}$  (840, 1440  $\mu\text{g/L}$ ). At an increased salinity of 25‰, the toxicity of Pb was substantially reduced ( $\text{LC}_{50} = 4274 \mu\text{g/L}$  [3540, 5710  $\mu\text{g/L}$ ]) (Verslycke et al., 2003). This reduction in toxicity was attributed to the increased concentration of  $\text{Cl}^-$  ion due to increased salinity, in that it complexed with divalent Pb in the test system. Exposure of *N. integer* to Pb in combination with the other five metals (Hg, Cd, Cu, Zn, Ni) resulted in roughly strictly additive toxicity (Verslycke et al., 2003).

Long et al. (2006) performed a critical review of the uses of mean sediment quality guideline quotients (mSQGs) in assessing the toxic effects of contaminant mixtures (metals and organics) in sediments. This approach has been used in numerous surveys and studies since

1994. mSQGQs are useful to risk assessors but their inherent limitations and underlying assumptions must be fully understood (see Section AX7.2.1.5).

### ***Summary of Interactions With Other Pollutants***

Norwood et al. (2003) reported that in a review and reinterpretation of published data on the interactions of metals in binary mixtures (n = 15 studies), antagonistic (6) and additive interactions (6) were the most common for Pb. The complexity of the interactions and possible modifying factors makes determining the impact of even binary metal mixtures to aquatic biota difficult (Norwood et al., 2003; Playle, 2004). The two most commonly reported Pb-element interactions are between Pb and calcium and between Pb and zinc. Both calcium and zinc are essential elements in organisms and the interaction of Pb with these ions can lead to adverse effects both by increased Pb uptake and by a decrease in Ca and Zn required for normal metabolic functions.

### **AX7.2.3.5 Factors Associated with Global Climate Change**

It is highly unlikely that Pb has any influence on generation of ground-level ozone, depletion of stratospheric ozone, global warming, or other indicators of global climate change. Lead compounds have relatively short residence times in the atmosphere, making it unlikely that they will reach the stratosphere, and they do not absorb infrared radiation, making them unlikely to contribute to stratospheric ozone depletion or global warming. Also, these compounds are unlikely to have a significant interaction with ground-level nitrogen oxides or volatile organic compounds, thus precluding generation of ground-level ozone.

Approached from another viewpoint, climate change can have a major impact on the fate/behavior of Pb in the environment and, therefore, can subsequently alter organism or ecosystem responses. For example, changes in temperature regime (Q10 rule), changes in precipitation quantity and quality (e.g., acidic deposition) may influence fate, transport, uptake, and bioavailability of Pb (Syracuse Research Corporation., 1999).

### **AX7.2.3.6 Summary**

There have been a number of advancements in the understanding of Pb behavior in the environment and its impact on aquatic organisms since 1986. In particular, greater knowledge of

factors that influence Pb accumulation in aquatic organisms, mechanisms of detoxification and avoidance of Pb, and greater understanding of the interactions of Pb in aquatic systems. All of these factors require some consideration when attempting to determine the potential for exposure and subsequent response of aquatic species to lead. The following section provides a review on the current understanding of lead and its impacts on aquatic biota.

#### **AX7.2.4 Exposure/Response of Aquatic Species**

This section outlines and highlights the critical recent advancements in the understanding of the toxicity of Pb to aquatic biota. The section begins with a review of the major findings and conclusions from the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a). The following sections summarize the research conducted since 1986 on determining the concentrations of Pb that cause the effects discussed in Section 7.2.3.

Lead exposure may adversely affect organisms at different levels of organization, i.e., individual organisms, populations, communities, or ecosystems. Generally, however, 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). Potential effects at the population level or higher are, of necessity, extrapolated from individual level studies. Available population, community, or ecosystem level studies are typically conducted at sites that have been contaminated or adversely affected by multiple stressors (several chemicals alone or combined with physical or biological stressors). Therefore, the best documented links between lead and effects on the environment are with effects on individual organisms. Impacts on aquatic ecosystems are discussed in Section 7.2.5 and Annex AX7.2.5.

##### **AX7.2.4.1 Summary of Conclusions From the Previous Criteria Document**

The 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) reviewed data in the context of the sublethal effects of lead exposure. The document focused on describing the types and ranges of lead exposures in ecosystems likely to adversely impact domestic animals. As such, the criteria document did not provide a comprehensive analysis of the effects of lead to most aquatic primary producers, consumers, and decomposers. For the aquatic environment, general reviews of the effects of lead to algae, aquatic vertebrates, and invertebrates were undertaken. A summary of these reviews is provided below.

### ***Algae***

The 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) reported that some algal species (e.g., *Scenedesmus* sp.) were found to exhibit physiological changes when exposed to high lead or organolead concentrations in situ. The observed changes included increased numbers of vacuoles, deformations in cell organelles, and increased autolytic activity. Increased vacuolization was assumed to be a tolerance mechanism by which lead was immobilized within cell vacuoles.

### ***Aquatic Vertebrates***

The 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) reported that hematological and neurological responses were the most commonly reported effects in aquatic vertebrates. These effects include red blood cell destruction and inhibition of the enzyme ALAD, required for hemoglobin synthesis. At high lead concentrations, neurological responses included neuromuscular distortion, anorexia, muscle tremor, and spinal curvature (e.g., lordosis). The lowest reported exposure concentration causing either hematological or neurological effects was 8 µg/L (U.S. Environmental Protection Agency, 1986a).

### ***Aquatic Invertebrates***

Numerous studies were cited on the effects of lead to aquatic invertebrates in the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a). In general, lead concentrations in aquatic invertebrates were found to be correlated closely with concentrations in water rather than food. Freshwater snails were found to accumulate lead in soft tissue, often in granular bodies of precipitated lead. Mortality and 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.

The review of the NAAQS for Pb (U.S. Environmental Protection Agency, 1990) made only one recommendation reported in the sections of the 1986 Lead AQCD dealing with effects to aquatic biota. This was the need to consider the impact of water hardness on Pb bioavailability and toxicity, to be consistent with the recommendations of the AWQC for the protection of aquatic life (U.S. Environmental Protection Agency, 1985).

#### **AX7.2.4.2 Recent Studies on Effects of Lead on Primary Producers**

Using literature published since the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a), this section examines the toxicity of Pb (individually and in metal mixtures) to algal and aquatic plant growth, its effects on metabolic processes (e.g., nutrient uptake), and its impact on primary productivity in natural systems.

##### ***Toxicity of Lead to Algae***

The toxicity of Pb to algal growth has been investigated for a number of species including *Chlorella vulgaris*, *Closterium acerosum*, *Pediastrum simplex*, *Scenedesmus quadricauda*, *Scenedesmu obliquus*, *Syneshococcus aeruginosus*, and *Nostoc muscorum* (Jampani, 1988; Rai and Raizada, 1989; Adam and Abdel-Basset, 1990; Fargašová, 1993; Bilgrami and Kumar, 1997). Study durations ranged from 7 to 20 days and Pb-nitrate was the most commonly used form of Pb. Effects to algal growth (*Chlorella vulgaris*, *Closterium acerosum*, *Pediastrum simplex*, *Scenedesmus quadricauda*), ranging from minimal to complete inhibition, have been reported at Pb concentrations between 100 and 200,000 µg/L (Jampani, 1988; Bilgrami and Kumar, 1997). Most studies report the percent inhibition in test groups compared to controls rather than calculating the LOEC, NOEC, or EC<sub>50</sub> values. Clinical signs of Pb toxicity include the deformation and disintegration of algae cells and a shortened exponential growth phase (Jampani, 1988; Fargašová, 1993). Other effects of Pb block the pathways that lead to pigment synthesis, thus affecting photosynthesis, the cell cycle and division, and ultimately result in cell death (Jampani, 1988).

From the studies reviewed, *Closterium acerosum* is the most sensitive alga species tested (Bilgrami and Kumar, 1997). Exposure of these algae to 1000 and 10,000 µg/L as lead nitrate for 6 days resulted in cell growth that was 52.6 and 17.4%, respectively, of controls (Bilgrami and Kumar, 1997). *Chlorella vulgaris*, *Pediastrum simplex*, and *Scenedesmus quadricauda* were also exposed to Pb-nitrate in this study. Compared to controls, cell growth at 1000 and 10,000 µg Pb-nitrate/L was 65.3 and 48.7%, 64.5 and 42.7%, and 77.6 and 63.2%, respectively (Bilgrami and Kumar, 1997). *Scenedesmus quadricauda* exhibited a similar magnitude of effects when exposed to lead (Pb<sup>2+</sup>) for 20 days at 0, 5500, 11,000, 16,500, 22,000, 27,500, and 33,000 µg/L (Fargašová, 1993). This study reported an EC<sub>50</sub> for growth inhibition at 13,180 µg/L (95% CI: 10,190, 14,620). Decreased cell number, but increased cell size, was

observed in *Selenastrum capricornutum*<sup>7</sup> exposed to lead (Pb<sup>2+</sup>) at 207.2 µg/L and a Q/V (flux of air [Q] divided by volume of the culture [V]) of 4.7 H 10<sup>13</sup> sec<sup>11</sup> for 9 days (Simões Gonçalves et al., 1991). The Q/V is a measure of culture growth where an increase in the Q/V ratio indicates growth. The pigment concentration per cell decreased with exposure to Pb, so while the algae cells were larger, they were less healthy (Simões Gonçalves et al., 1991). Growth rates were not reported, making comparison with other studies difficult.

High Pb concentrations were required to elicit effects in *Nostoc muscorum* and *Scenedesmus aeruginosus* (Jampani, 1988; Rai and Raizada, 1989). Following 15-day exposures, test groups exposed to 10,000, 20,000, and 30,000 µg Pb/L experienced growth rates that were 90.5, 76.9, and 66.7% of the controls (Rai and Raizada, 1989). *Synechococcus aeruginosus* experienced little inhibition of growth from exposure to Pb-nitrate up to a concentration of 82,000 µg/L (Jampani, 1988). At a test concentration of 100,000 µg/L, complete inhibition of growth was observed, and at a concentration of 200,000 µg/L, algae failed to establish a single colony (Jampani, 1988). *Scenedesmus obliquus* are quite tolerant to the effects of Pb-nitrate and Pb-acetate on growth. Algae exposed to Pb-nitrate or Pb-acetate up to 180,000 µg/L had higher cell numbers than controls (Adam and Abdel-Basset, 1990). Exposure to the highest concentration of 300,000 µg/L Pb-nitrate or Pb-acetate resulted in cell numbers that were 81 and 90% of the controls, respectively (Adam and Abdel-Basset, 1990).

Lead in combination with other metals (e.g., Pb and Cd, Pb and Ni, etc.) is generally less toxic than exposure to Pb alone (Rai and Raizada, 1989). *Nostoc muscorum* exposed to chromium and Pb in combination demonstrated better growth than when exposed to either of the metals alone (Rai and Raizada, 1989). Antagonistic interaction was observed in the exposure of *Nostoc muscorum* to Pb and nickel in combination (Rai and Raizada, 1989). When applied separately, these metals demonstrated different levels of toxicity; however, in combination, they exerted similar effects (Rai and Raizada, 1989). More information on toxic interactions of Pb with other metals is provided in Section AX7.2.3.5.

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<sup>7</sup>The species name *Selenastrum capricornutum* has been changed to *Pseudokirchneriella subcapitata*. The former species name is used in this report.

### ***Aquatic Plants***

The toxicity of Pb to aquatic plant growth has been studied using *Spirodela polyrhiza*, *Azolla pinnata*, and *Lemna gibba* (Gaur et al., 1994; Gupta and Chandra, 1994; Miranda and Ilangovan, 1996). Test durations ranged from 4 to 25 days and test concentrations ranged between 49.7 and 500,000  $\mu\text{g Pb/L}$  (Gaur et al., 1994; Miranda and Ilangovan, 1996). Research on aquatic plants has focused on the effects of Pb on aquatic plant growth and chlorophyll and protein content.

Of the species reviewed here, the effects of Pb on aquatic plant growth are most pronounced in *Azolla pinnata* (Gaur et al., 1994). An  $\text{EC}_{50}$  of 1100  $\mu\text{g/L}$  was reported for *A. pinnata* exposed to Pb-nitrate for 4 days. *S. polyrhiza* exposed to Pb-nitrate under the same test conditions had a reported  $\text{EC}_{50}$  for growth of 3730  $\mu\text{g/L}$  (Gaur et al., 1994). *Lemna gibba* was shown to be the least sensitive plant species to Pb: significant growth inhibition was reported at concentrations of 200,000  $\mu\text{g/L}$  or greater after 25 days of exposure to concentrations of 30,000, 50,000, 100,000, 200,000, 300,000, or 500,000  $\mu\text{g/L}$  (Miranda and Ilangovan, 1996). The maximum growth rate for *L. gibba* was observed at 10 days of exposure. After this point, the growth rate declined in controls and test concentrations (Miranda and Ilangovan, 1996). Clinical signs of Pb toxicity include yellowing and disintegration of fronds, reduced frond size, and chlorosis (Gaur et al., 1994; Miranda and Ilangovan, 1996). Toxicity results suggest that effects to growth from Pb exposure occur in a dose-dependent manner (Gaur et al., 1994).

### ***Effects of Lead on Metabolic Processes***

Algal and aquatic plant metabolic processes are variously affected by exposure to Pb, both singularly and in combination with other metals. Lead adversely affects the metabolic processes of nitrate uptake, nitrogen fixation, ammonium uptake, and carbon fixation at concentrations of 20,000  $\mu\text{g Pb/L}$  or greater (Rai and Raizada, 1989). Lead in combination with nickel has an antagonistic effect on nitrogen fixation and ammonium uptake, but a synergistic effect on nitrate uptake and carbon fixation (Rai and Raizada, 1989). Lead in combination with chromium has an antagonistic effect on nitrate uptake, but it has a synergistic effect on nitrogen fixation, ammonium uptake, and carbon fixation (Rai and Raizada, 1989).

Lead effects on nitrate uptake in *Nostoc muscorum* ( $\mu\text{g NO}_3/\mu\text{g Chl } a$ ) were greatest after 24 h, when exposure to 20,000  $\mu\text{g/L}$  reduced nitrate uptake by 64.3% compared to controls.

Nitrate uptake reported after 48, 72, and 96 h was reduced by 30.0, 37.5, and 38.9%, respectively, compared to controls (Rai and Raizada, 1989). Lead in combination with chromium, both at a test concentration of 20,000  $\mu\text{g/L}$ , demonstrated antagonistic effects on nitrate uptake. Compared to controls, nitrate uptake was reduced by 52.4, 30, 25, and 22.2% at 24, 48, 72 and 96 h, respectively (Rai and Raizada, 1989). The greatest effect on uptake occurred at 24 h when, compared to controls, a 52.4% reduction was reported in the test concentration. Lead and nickel in combination at test concentrations of 20,000 and 1000  $\mu\text{g/L}$ , respectively, resulted in a greater reduction of nitrate uptake than Pb alone at 48, 72, and 96 h (Rai and Raizada, 1989).

After 24, 48, and 72 h of Pb exposure at 20,000  $\mu\text{g/L}$ , nitrogenase activity (nmol  $\text{C}_2\text{H}_4/\mu\text{g}$  protein/hr) in *Nostoc muscorum* was reduced by 39.3, 61.8, and 14.1%, respectively, compared to controls (Rai and Raizada, 1989). A concentration of 207.2  $\mu\text{g Pb/L}$  had little effect on nitrogen or phosphorus assimilation in *Selenastrum capricornutum* over 7 days (Capelo et al., 1993). An antagonistic effect on nitrogenase activity was generally reported for *Nostoc muscorum* exposed to Pb in combination with nickel at 20,000 and 1,000  $\mu\text{g/L}$ , respectively (Rai and Raizada, 1989). Compared to controls, nitrogenase activity was reduced by 42.9, 32.7, and 13.6% at 24, 48, and 72 h, respectively (Rai and Raizada, 1989). Lead and chromium, both administered at a concentration of 20,000  $\mu\text{g/L}$ , had a synergistic impact on nitrogenase activity in *Nostoc muscorum*. Nitrogenase activity in the test group was reduced by 60.7, 60, and 50% compared to the controls at 24, 48, and 72 h, respectively (Rai and Raizada, 1989).

Lead-induced inhibition of ammonium uptake ( $\mu\text{g NH}_4$  uptake/ $\mu\text{g Chl } a$ ) was greatest in *Nostoc muscorum* after 48 h of exposure to 20,000  $\mu\text{g/L}$  of lead. Compared to controls, the Pb test concentration 20,000  $\mu\text{g/L}$  reduced ammonium uptake by 72, 82, 61, and 26 % at 24, 48, 72, and 96 h, respectively (Rai and Raizada, 1989). Lead in combination with nickel at concentrations of 20,000 and 1,000  $\mu\text{g/L}$ , respectively, demonstrated an antagonistic effect on ammonium uptake. Compared to controls, ammonium uptake in the test group was reduced by 44.9, 54.1, 23.3, and 4% at 24, 48, 72, and 96 h, respectively (Rai and Raizada, 1989). Lead in combination with chromium, both at concentrations of 20,000  $\mu\text{g/L}$ , demonstrated a synergistic interaction with 24, 48, 72, and 96 h uptake rates reduced by 87.2, 88.5, 72.5, and 50 %, respectively, compared to controls (Rai and Raizada, 1989).

*Nostoc muscorum* exposed to 20,000 µg Pb/L experienced the greatest reduction in carbon fixation at 0.5 h of exposure: 62% compared to controls. Inhibition of carbon fixation in the test group was less pronounced after 1 and 2 h of exposure: 29 and 13% of controls (Rai and Raizada, 1989). Lead in combination with nickel or chromium had synergistic effects to carbon fixation. Lead and nickel concentrations of 20,000 and 1000 µg/L, respectively, resulted in 0.5, 1, and 2 h carbon fixation rates reduced by 93, 92, and 91%, respectively, compared to controls (Rai and Raizada, 1989). Lead with chromium at concentrations of 20,000 µg/L resulted in 0.5, 1, and 2 h carbon fixation rates reduced by 65, 58, and 50%, respectively, compared to controls.

Nutrients such as nitrogen, phosphate, sodium acetate, sodium carbonate, and citric acid have been shown to protect against the toxic effects of Pb to algae (Jampani, 1988). Nitrogen compounds (ammonium chloride, potassium nitrate, sodium nitrate, sodium nitrite) protected *Synechococcus aeruginosus* from a lethal Pb-nitrate dose of 200,000 µg/L (Jampani, 1988). Two phosphates (K<sub>2</sub>HPO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub>) were found to improve *Synechococcus aeruginosus* survival from 0 to 72% at 200,000 µg/L of Pb-nitrate (Jampani, 1988).

Compared to controls, protein content was reduced by 54.2 and 51.9% in aquatic plants *Vallisneria spiralis* and *Hydrilla verticillata*, respectively, exposed to Pb for 7 days at 20,720 µg/L (Gupta and Chandra, 1994). Decreased soluble protein content has been observed in *Scenedesmus obliquus* exposed to Pb-nitrate or Pb-acetate at concentrations greater than 30,000 µg/L, and in *L. gibba* at concentrations greater than 200,000 µg/L (Adam and Abdel-Basset, 1990; Miranda and Ilangovan, 1996). *Lemna gibba* also showed increased loss of soluble starch at concentrations >200,000 µg/L (Miranda and Ilangovan, 1996). Under the conditions described previously (Gupta and Chandra, 1994), EC<sub>50</sub> values for chlorophyll content were 14,504 and 18,648 µg/L for *Vallisneria spiralis* and *Hydrilla verticillata*, respectively (Gupta and Chandra, 1994). Effects to chlorophyll *a* content have been observed in *Scenedesmus obliquus* at Pb-nitrate and Pb-acetate concentrations >30,000 µg/L (Adam and Abdel-Basset, 1990).

### ***Summary of Toxic Effects Observed in Single-Species Bioassays***

Algae and aquatic plants have a wide range in sensitivity to the effects of Pb in water. Both groups of primary producers experience EC<sub>50</sub> values for growth inhibition between approximately 1000 and >100,000 µg/L (Jampani, 1988; Gaur et al., 1994; Bilgrami and Kumar,

1997). The most sensitive primary producers reported in the literature for effects to growth were *Closterium acersoum* and *Azolla pinnata* (Gaur et al., 1994; Bilgrami and Kumar, 1997). The least sensitive primary producers reported in the literature for effects to growth were *Synechococcus aeruginosus* and *Lemna gibba* (Jampani, 1988; Miranda and Ilangovan, 1996). Exposure to Pb in combination with other metals is generally less toxic to growth than exposure to lead alone. Studies have shown that lead adversely affects the metabolic processes of nitrate uptake, nitrogen fixation, ammonium uptake, and carbon fixation (Rai and Raizada, 1989). Lead in combination with nickel or chromium produced synergistic effects for nitrate uptake, nitrogenase activities, ammonium uptake, and carbon fixation (Rai and Raizada, 1989).

### ***Leads Effects on Primary Productivity***

Lead nitrate and Pb-acetate have been shown to have adverse effects on the primary productivity of aquatic plants in two water bodies in India (Jayaraj et al., 1992). One of the two water bodies was a freshwater tank that receives wastewater and supports a rich population of hyacinths, and the other was a wastewater stabilization pond. Water quality characteristics in the freshwater tank were pH = 7.5, dissolved oxygen = 6 mg/L, and water hardness (CaCO<sub>3</sub>) = 100 mg/L. Water quality characteristics in the wastewater pond were pH = 8.1, dissolved oxygen = 6.2 mg/L, and water hardness (CaCO<sub>3</sub>) = 160 mg/L (Jayaraj et al., 1992). Lead nitrate concentrations of 500, 5000, 10,000, 25,000, and 50,000 µg/L were combined with appropriate water samples in light and dark bottles and suspended in each of the water bodies for 4 h. The concentrations of Pb-acetate (5000, 10,000, 25,000, 50,000, and 100,000 µg/L) were applied in the same manner. The EC<sub>50</sub> values were determined based on the concentration required to inhibit gross productivity (GP) and net productivity (NP) by 50% (Jayaraj et al., 1992). The results demonstrated that Pb-nitrate was more toxic to primary production than Pb-acetate. In the freshwater tank, Pb-nitrate EC<sub>50</sub> values for GP and NP were 25,100 and 6310 µg/L, respectively, compared to Pb-acetate EC<sub>50</sub> values of 50,100 and 28,200 µg/L for GP and NP, respectively (Jayaraj et al., 1992). In the stabilization pond, Pb-nitrate EC<sub>50</sub> values for GP and NP were 31,600 and 28,200 µg/L, respectively, compared to Pb-acetate EC<sub>50</sub> values of 79,400 and 316 µg/L for GP and NP, respectively (Jayaraj et al., 1992). The higher toxicity reported in the freshwater tank was attributed to differences in species composition and diversity. The freshwater tank was dominated by water hyacinths that decreased the photic zone available for

photosynthesis and consumed a great deal of available nutrients. The stabilization pond had a rich nutrient budget, resulting in improved alga growth and species diversity (Jayaraj et al., 1992).

#### **AX7.2.4.3 Recent Studies on Effects of Lead on Consumers**

This section focuses on the effects of Pb to aquatic biota including invertebrates, fish, and other biota with an aquatic life stage (e.g., amphibians). It is not intended to be a comprehensive review of all research conducted. Rather, the intent is to illustrate the concentrations and effects of Pb on freshwater and marine aquatic species. Eisler (2000) provides an overview of much of the recent available literature on the toxicity of Pb to fish and aquatic invertebrates. An extensive literature search was conducted using numerous electronic bibliographic and database services (e.g., DIALOG, EPA ECOTOX) and limited temporally from 1986 to present. This temporal limit was due to the availability of the EPA water quality criteria report for the protection of aquatic life, released in 1986 (U.S. Environmental Protection Agency, 1986b). Based on the results of the literature search and recent reviews of the toxicity of Pb (Eisler, 2000), numerous studies have been published on the toxicity of Pb to aquatic consumers. Hardness, pH, temperature, and other factors are important considerations when characterizing the acute and chronic toxicity of lead (Besser et al., 2005) (Section AX7.2.3.5). However, many of the studies reviewed did not report critical information on control mortality, water quality parameters, or statistical methods, making comparing effects between studies difficult. Studies reporting only physiological responses to Pb exposure (e.g., reduction of ALAD) are not discussed here, as this topic was covered more completely in Section AX7.2.3.4. This section provides a review of toxicity studies conducted with invertebrates, fish, and other aquatic organisms.

##### ***Invertebrates***

Exposure of invertebrates to Pb can lead to adverse effects on reproduction, growth, survival, and metabolism (Eisler, 2000). The following presents information on the toxicity of Pb to invertebrates in fresh and marine waters.

## *Freshwater Invertebrates*

Acute and chronic Pb toxicity data for freshwater invertebrates are summarized in Table AX7-2.4.1. As described in Section AX7.2.3.5, water hardness is a critical factor governing the solubility, bioavailability, and ultimately the toxicity of Pb. The acute and chronic toxicity of Pb increases with decreasing water hardness as Pb becomes more soluble and bioavailable to aquatic organisms. For example, Borgmann et al. (2005) examined the toxicity of 63 metals, including Pb, to *Hyalella azteca* at two levels of water hardness (soft water hardness, 18 mg CaCO<sub>3</sub>/L; hard water, 124 mg CaCO<sub>3</sub>/L). Lead was 23 times more acutely toxic to *H. azteca* in soft water than hard water. Besser et al. (2005) found that acute toxicity to *H. azteca* was also modified by water hardness.

At a mean pH of 7.97 in soft water (hardness (CaCO<sub>3</sub>) = 71 mg/L) mortality was >50% for *H. azteca* at a dissolved Pb concentration of 151 µg/L. The LOEC for survival in hard water (hardness (CaCO<sub>3</sub>) = 275 mg/L) at pH 8.27 was 192 µg/L as dissolved Pb and 466 µg/L as total Pb. Both waterborne and dietary Pb were found to contribute to reduced survival of *H. azteca* (Besser et al., 2005).

Exposure duration may also play an important role in Pb toxicity in some species. For example, Kraak et al. (1994) reported that filtration in the freshwater mussel *Dreissena polymorpha* was adversely affected at significantly lower Pb concentrations over 10 weeks of exposure than was the case after 48 h of exposure.

The influence of pH on lead toxicity in freshwater invertebrates varies between invertebrate species. Over a 96-h exposure period, mortality increased with decreasing pH in the bivalve *Pisidium casertanum*, while pH-independent mortality was reported for gastropod and crustacean species under similar exposure conditions (Mackie, 1989). Cladocerans (*Ceriodaphnia dubia*), amphipods (*H. azteca*), and mayflies (*Leptophlebia marginata*) were also more sensitive to Pb toxicity at lower pH levels (Schubauer-Berigan et al., 1993; Gerhardt, 1994). Lead was 100 times more toxic to the amphipod, *Hyalella azteca*, at a pH range of 5.0 to 6.0 (Mackie, 1989) than at a pH range of 7.0 to 8.5 (Schubauer-Berigan et al., 1993).

The physiology of an aquatic organism at certain life stages may be important when determining the toxicity of metals to test organisms. For example, Bodar et al. (1989) exposed early life stages of *Daphnia magna* to concentrations of Pb(NO<sub>3</sub>)<sub>2</sub>. The test medium had a pH of 8.3 ± 0.2, water hardness (CaCO<sub>3</sub>) of 150 mg/L, and temperature of 20 ± 1 °C. Lead

**Table AX7-2.4.1. Effects of Lead to Freshwater and Marine Invertebrates**

Species	Chemical	Endpoint: Conc. (µg/L)*	Duration of Exposure	Water Chemistry	Test Type - Effect	Reference
<i>Freshwater</i>						
Cladoceran ( <i>Ceriodaphnia dubia</i> )	lead chloride	LC <sub>50</sub> : 280 >2,700 >2,700	48 h	pH: 6-6.5 7-7.5 8-8.5 Hardness: 280-300 mg/L CaCO <sub>3</sub>	static-survival	Schubauer-Berigan et al. (1993)
Worm ( <i>Lumbriculus variegatus</i> )	lead chloride	LC <sub>50</sub> : >8,000 >8,000 >8,000	96 h	pH: 6-6.5 7-7.5 8-8.5 Hardness: 280-300 mg/L CaCO <sub>3</sub>	static-survival	Schubauer-Berigan et al. (1993)
Amphipod ( <i>Hyalella azteca</i> )	lead chloride	LC <sub>50</sub> : <90 >5,400 >5,400	96 h	pH: 6-6.5 7-7.5 8-8.5 Hardness: 280-300 mg/L CaCO <sub>3</sub>	static-survival	Schubauer-Berigan et al. (1993)
Amphipod ( <i>Hyalella azteca</i> )	lead chloride	LC <sub>50</sub> : 27 (20.1-36.4)	8 days	Hardness 130 mg/L pH 7.8-8.6	renewal, 1-week- old amphipods	MacLean et al. (1996)
Amphipod ( <i>Hyalella azteca</i> )	lead chloride	LC <sub>50</sub> : 60 (53.6-67.3)	8 days	Hardness 130 mg/L pH 7.8-8.6	renewal, 10- to 16- week old amphipods	MacLean et al. (1996)
Mayfly ( <i>Leptophlebia marginata</i> )	lead chloride	LC <sub>50</sub> : 1090 (400-133200)	96 h	pH: 4.5	acute - survival	Gerhardt (1994)
Mayfly ( <i>Leptophlebia marginata</i> )	lead chloride	LC <sub>50</sub> : 5000	96 h	pH 7.0	acute - survival	Gerhardt (1994)

**Table AX7-2.4.1 (cont'd). Effects of Lead to Freshwater and Marine Invertebrates**

Species	Chemical	Endpoint: Conc. (µg/L)*	Duration of Exposure	Water Chemistry	Test Type - Effect	Reference
Amphipod ( <i>Hyalella azteca</i> )	lead nitrate	LC <sub>50</sub> : 10 21 18	96 h	pH: 5.0 5.5 6.0	acute-survival	Mackie (1989)
Bivalve ( <i>Pisidium compressum</i> )	lead nitrate	LC <sub>50</sub> : 38,000 21,300 11,400	96 h	pH: 3.5 4.0 4.5	acute- survival	Mackie (1989)
Bivalve ( <i>Pisidium casertanum</i> )	lead nitrate	LC <sub>50</sub> : 23,600 23,500 56,000	96 h	pH: 3.5 4.0 4.5	acute- survival	Mackie (1989)
Gastropod ( <i>Amnicola limosa</i> )	lead nitrate	LC <sub>50</sub> : 10,300 20,600 9,500	96 h	pH: 3.5 4.0 4.5	acute- survival	Mackie (1989)
Mussel ( <i>Dreissena polymorpha</i> )	lead nitrate	EC <sub>50</sub> : 370 91	48 h 10 wks	pH = 7.9; Hardness = 150 mg CaCO <sub>3</sub> /L; Temp = 15 °C	renewal - filtration	Kraak et al. (1994)
Mussel ( <i>Dreissena polymorpha</i> )	lead nitrate	LT <sub>50</sub> : 358	72 days	pH = 7.9; Hardness = 150 mg CaCO <sub>3</sub> /L; Temp = 15 °C	renewal - filtration	Kraak et al. (1994)
Amphipod ( <i>Hyalella azteca</i> )	lead nitrate	LC <sub>50</sub> : 4.8 (3.3 - 7.1)	7 days	pH = 7.37 - 8.27 Hardness = 18 mg CaCO <sub>3</sub> /L DOC = 0.28 mg/L	renewal - survival	Borgmann et al. (2005)
Amphipod ( <i>Hyalella azteca</i> )	lead nitrate	LC <sub>50</sub> : 113 (101 -126)	7 days	pH = 8.21 - 8.46 Hardness = 124 mg CaCO <sub>3</sub> /L; DOC = 1.1 mg/L	renewal - survival	Borgmann et al. (2005)

Table AX7-2.4.1 (cont'd). Effects of Lead to Freshwater and Marine Invertebrates

Species	Chemical	Endpoint: Conc. (Φg/L)*	Duration of Exposure	Water Chemistry	Test Type - Effect	Reference
Mayfly ( <i>Leptophlebia marginata</i> )	lead chloride	LC <sub>50</sub> : 1090 (95% C.I. = 133.2) >5000	96 h	pH = 4.5 - 6.5; DOC - 21.6 mg Cl <sup>11</sup> ; Cond = 7.0 ΦS cm <sup>11</sup>	renewal - survival	Gerhardt (1994)
Cladoceran ( <i>D. magna</i> )	lead nitrate	LC <sub>50</sub> : 0.45	48 h	pH = 8.3 ∇ 0.2 Hardness (CaCO <sub>3</sub> ) = 150 mg/L Temp = 20 °C	static - embryogenesis	Bodar et al. (1989)
Cladoceran ( <i>D. magna</i> )	lead chloride	NOEC: 260	12 to 21 d	Not specified	renewal - reproduction	Enserink et al. (1991)
Cladoceran ( <i>D. magna</i> )	lead chloride	NOEC: 270	10 d	Not specified	renewal - growth	Enserink et al. (1991)
Amphipod ( <i>Hyalella azteca</i> )	lead	LOEC: (Dissolved Pb) 192 (Total Pb) 466	96 h	pH = 8.27 Hardness (CaCO <sub>3</sub> ) = 275 mg/L Temp = 21.1 °C	flow through - survival	Besser et al. (2005)
Tubificid worm ( <i>Tubifex tubifex</i> )	lead nitrate	EC <sub>50</sub> : 237 (183–316) 142 (107–184)	24 h 48 h	pH = 7.5–7.7 Hardness = 245 mg/L Temp = 29.5–31 °C	static - immobilization	Khengarot (1991)
<b>Marine</b>						
Copepod ( <i>Amphiascus tenuiremis</i> )	lead	LC <sub>50</sub> : sediment 2462 μg metal/dry sediment	96 h	pH = 7.7 ∇ 0.1 Dissolved O <sub>2</sub> -6.3 ∇ 0.3 mg/L Salinity – 32 ppt		Hagopian-Schlekat et al. (2001)
Bivalve ( <i>Mytilus galloprovincialis</i> )	lead nitrate	EC <sub>50</sub> : 221 (58.9–346.3) LOEC : 50		artificial seawater	embryogenesis	Beiras and Albentosa (2003)

\* - Brackets after effect concentration are 95% confidence intervals.

concentrations of <100 mg/L had no impact on *Daphnia* egg development. The authors suggested that this might be due to the *Daphnia* egg structure, which consists of two layers: the inner vitelline layer and outer chorion layer. The chorion layer in other species (e.g., rainbow trout) is known to adsorb metals, thereby, preventing ionic injury to the developing embryo.

Exposures to sediment-associated Pb can be toxic to sediment-dwelling organisms. In freshwater sediments, 48-h exposure of water fleas (*Daphnia magna*) to 7000 mg Pb/kg dw significantly reduced mobility, while exposure to 13,400 mg Pb/kg dw for 24 h produced the same effect (Dave, 1992a,b). Longer-term (i.e., 14-day) exposure of midges (*Chironomus tentans*) to sediments containing 31,900 mg Pb/kg dw resulted in 100% mortality.

### *Marine Invertebrates*

In estuarine environments, salinity is an important modifying factor to Pb toxicity. Verslycke et al. (2003) exposed the estuarine mysid *Neomysis integer* to individual metals, including Pb, and metal mixtures under changing salinity. Water temperature (20  $\pm$  1°C) and salinity were reported, although no other water quality parameters were available (e.g., pH, water hardness). At a salinity of 5‰, the reported LC<sub>50</sub> for Pb was 1140  $\mu$ g/L (95% CI: 840, 1440  $\mu$ g/L). At an increased salinity of 25‰, the toxicity of lead was substantially reduced (LC<sub>50</sub> = 4274  $\mu$ g/L [3540, 5710  $\mu$ g/L]) (Verslycke et al., 2003).

Sensitivity to Pb can also vary between genders in some aquatic organisms. For example, Hagopian-Schlekat et al. (2001) examined the toxicity of Pb-chloride in sediment and sediment pore water to female and male estuarine copepods *Amphiascus tenuiremis*. The reported LC<sub>50</sub> for total lead was 2462 mg Pb/kg dw (95% CI: 2097, 2891 mg Pb/kg dw). Gender effects were observed in that male copepods were more sensitive ( $p = 0.038$ ) to Pb than females as determined by generalized linear model analysis.

Beiras and Albentosa (2003) examined the inhibition of embryo development in commercial bivalves *Ruditapes decussatus* and *Mytilus galloprovincialis* after exposure to concentrations of Pb(NO<sub>3</sub>)<sub>2</sub> in seawater. No water chemistry parameters other than temperature were reported (test conducted at 20 °C). An EC<sub>50</sub> range for *R. decussatus* was reported as 156 to 312  $\mu$ g/L, as insufficient data were available to calculate the actual EC<sub>50</sub>. The lowest observable effect concentration (LOEC) was 156  $\mu$ g/L. For *M. galloprovincialis*, the EC<sub>50</sub> was 221  $\mu$ g/L (95% CI: 58.9, 346.3) while the LOEC was reported as 50  $\mu$ g/L.

## ***Fish***

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 (Eisler, 2000). Toxicity in fish has been closely correlated with duration of Pb exposure and uptake (Eisler, 2000). The following presents information on the toxicity of Pb to fish in fresh and marine waters. Table AX7-2.4.2 summarizes the effects of Pb on freshwater and marine fish.

### ***Freshwater Fish***

Many of the toxicity modifying factors described above and in Section AX7.2.3.5 (e.g., pH, DOC) for invertebrates are also important modifying factors for Pb toxicity to fish species. The effects of pH on Pb bioavailability and subsequent toxicity have been well-studied (Sayer et al., 1989; Spry and Wiener, 1991; Schubauer-Berigan et al., 1993; Stouthart et al., 1994; MacDonald et al., 2002; Rogers and Wood, 2003). Schubauer-Berigan et al. (1993) exposed fathead minnow to Pb-chloride over 96 hours. The reported LC<sub>50</sub> ranged from 810 to >5400 µg/L at pH 6 to 6.5 and pH 7 to 8.5, respectively.

Water hardness also has a strong influence on the effects of lead to fish. Chronic exposure of rainbow trout fry to Pb in soft water resulted in spinal deformities at 71 to 146 µg/L after 2 months of exposure (Sauter et al., 1976) or 13.2 to 27 µg/L (Davies and Everhart, 1973; Davies et al., 1976), after 19 months of exposure. When exposed to Pb in hard water, only 0 and 10% of the trout (*Oncorhynchus mykiss*) developed spinal deformities at measured Pb concentrations of 190 and 380 µg/L, respectively. In soft water, 44 and 97% of the trout developed spinal deformities at concentrations of 31 and 62 µg/L, respectively (Davies et al., 1976). The maximum acceptable toxicant concentration (MATC) for rainbow trout fry in soft water was 4.1 to 7.6 µg/L (Davies et al., 1976), while the MATC for brook trout was 58 to 119 µg/L (Holcombe et al., 1976). Histological reproductive abnormalities were noted in mature male rainbow trout at 10 µg/L Pb-nitrate (Ruby et al., 1993).

Schwartz et al. (2004) examined the influence of NOM on Pb toxicity to rainbow trout exposed for 96 h in a static system. The pH of the exposure system ranged between 6.5 and 7.0, temperature was maintained between 9 and 11 EC, and Pb was added as PbCl<sub>2</sub>. NOM from a

**Table AX7-2.4.2. Effects of Pb to Freshwater and Marine Fish**

Species	Chemical	Endpoint: Conc. (Φg/L)	Duration of Exposure	Water Chemistry	Comments	Reference
<i>Freshwater</i>						
Fathead minnow ( <i>Pimephales promelas</i> )	lead chloride	LC <sub>50</sub> : 810 >5,400 >5,400	96 h	pH: 6–6.5 7–7.5 8–8.5 Hardness: 280-300 mg/L CaCO <sub>3</sub>	static, measured	Schubauer-Berigan et al. (1993)
Rainbow trout - mature males ( <i>Oncorhynchus mykiss</i> )	lead nitrate	Reproductive effects: 10	12 days	Hardness 128 mg/L CaCO <sub>3</sub>	Decreased spermatocyte development	Ruby et al. (1993)
Fathead minnow ( <i>Pimephales promelas</i> )	lead acetate	Reproductive Effects: 500	29 days	pH: 7.5–8.5; Hardness 130 mg/L CaCO <sub>3</sub> ; 22–25 °C (Pb 95% soluble)	Fewer viable eggs produced, testicular damage	Weber (1993)
Rainbow trout – Juvenile ( <i>Oncorhynchus mykiss</i> )	lead nitrate	LC <sub>50</sub> : 1000 (800 - 1400)	96 h	pH: 7.9–8.0 DOC = 3 mg/L Hardness (CaCO <sub>3</sub> ) = 140 mg/L	Flow through - Survival	Rogers and Wood (2003)
Common carp ( <i>Cyprinus carpio</i> )	not reported	LC <sub>50</sub> : 6.5 cm fish – 1030 3.5 cm fish – 300	96 h	pH: 7.1 Temperature–15 °C Oxygen sat. 6.4 mg/L	static-renewal - Survival	Alam and Maughan (1995)

AX7-190

number of U.S. rivers and lakes was then added to the test system, and the LT<sub>50</sub> was reported. NOM was found to reduce the toxic effects of Pb to rainbow trout.

Fish size is an important variable in determining the adverse effects of Pb. Alam and Maughan (1995) exposed two different sizes of common carp (*Cyprinus carpio*) to Pb concentrations to observed effects on carp mortality. Water chemistry parameters were reported (pH = 7.1; temperature = 20 EC). Smaller fish (3.5 cm) were found to be more sensitive to Pb than were larger fish (6.5 cm). The reported LC<sub>50</sub>s were 0.44 mg/L and 1.03 mg/L, respectively.

### *Marine Fish*

There were no studies available that examined the toxicity of Pb to marine fish species for the time period examined (1986 to present). However, Eisler (2000) reviewed available research on Pb toxicity to marine species and reported studies done prior to 1986. Acute toxicity values ranged from 50 µg/L to 300,000 µg/L in plaice (*Pleuronectes platessa*) exposed to organic and inorganic forms of Pb (Eisler, 2000). Organolead compounds (e.g., tetramethyl Pb, tetraethyl Pb, triethyl Pb, diethyl Pb) were generally more toxic to plaice than inorganic Pb (Maddock and Taylor, 1980).

### *Other Aquatic Biota*

A paucity of data exist on the effects of Pb to growth, reproduction, and survival of aquatic stages of frogs and turtles. Rice et al. (1999) exposed frog larvae (*Rana catesbeiana*) to 780 µg Pb/L and two oxygen concentrations (3.5 or 7.85 mg/L) for 7 days (Table AX7-2.4.3). Exposure conditions included water hardness of 233 to 244 mg CaCO<sub>3</sub>/L, pH from 7.85 to 7.9, and temperature at 23 EC. Frog larvae were found to display little to no activity in the low oxygen and high Pb treatment. Hypoxia-like behavior was exhibited in larvae exposed to both low and high oxygen concentrations and high Pb. Therefore, larvae of *R. catesbeiana* showed sensitivity to Pb and responded with hypoxia-like behavior. Additionally, the larvae in the Pb treatment were found to have lost body mass relative to controls and the other treatments. Rice et al. (1999) suggested that the decrease in mass likely indicated the beginning of a period of reduced growth rate. Larvae exposed for longer periods (>4 weeks) were smaller and metamorphosed later compared to unexposed individuals.

**Table AX7-2.4.3. Nonlethal Effects in Amphibians**

<b>Species</b>	<b>Chemical</b>	<b>Endpoint: Concentration</b>	<b>Duration of Exposure</b>	<b>Water Chemistry</b>	<b>Comments</b>	<b>Reference</b>
Frogs ( <i>Rana ridibunda</i> )	lead nitrate	Biochemical effects: 14,000 µg/L	30 days	Not specified	Hepatic ALAD decreased by 90%	Vogiatzis and Loumbourdis (1999)
Frogs ( <i>Bufo arenarum</i> )		Mortality: 16 mg Pb <sup>2+</sup> /L	5 days	Not specified	Effects reported include erratic swimming, loss of equilibrium	Herkovits and Perez-Coll (1991)
Frogs ( <i>Rana catesbeiana</i> )		Hypoxia-like behavior: 780 µg/L	7 days	O <sub>2</sub> = 3.5-7.85 mg/L pH = 7.85-7.9 Temp = 23 EC CaCO <sub>3</sub> = 233-244 mg/L	Larvae used	Rice et al. (1999)
Turtle Hatchlings ( <i>Trachemys scripta</i> )	lead acetate	NOEL: 100 µg/g (Survival and behavior)	4 weeks	N/A	Exposure via single injection	Burger et al. (1998)

Herkovits and Pérez-Coll (1991) examined Pb toxicity to amphibian larvae (*Bufo arenarum*). Larvae (n = 50) were exposed for up to 120 h at two Pb concentrations, 8 mg Pb<sup>2+</sup>/L and 16 mg Pb<sup>2+</sup>/L. Relative to controls, the 8 mg Pb<sup>2+</sup>/L treatment group exhibited 40% mortality and the 16 mg Pb<sup>2+</sup>/L group 60% mortality after 120 h (p < 0.05). The authors reported behavioral effects, erratic swimming, and loss of equilibrium during the tests, symptoms that are consistent with the action of Pb on the central and peripheral nervous systems (Rice et al., 1999).

Behavior (i.e., righting, body turnover, seeking cover), growth, and survival of hatchling slider turtles (*Trachemys scripta*) exposed to Pb-acetate were investigated in one study (Burger et al., 1998). In the first part of the study, 6-month-old hatchlings received single Pb-acetate injections at 50 or 100 µg/g body weight (bw). In the second part of the study, 3-week-old turtles were injected once with doses of 250, 1000 or 2500 µg/g bw. There were no differences in survival, growth, or behavior for hatchlings in the first study, however, several effects were reported from the second part of the study at doses in the range of 250 to 2,500 µg/g bw. As the dose increased, so did the plastron length (i.e., ventral section of the shell), carapace length, and weight. The highest dose group had the lowest survival rate with an LD<sub>50</sub> of 500 µg/g bw. Behavioral effects included slower times of righting behavior and seeking cover. The authors suggested a NOEL of 100 µg/g bw for slider turtles for survival and behavior.

#### **AX7.2.4.4 Recent Studies on Effects of Lead on Decomposers**

In this section, decomposers are defined as being bacteria and other microorganisms. Many invertebrates are also potentially considered decomposers, but the effects of Pb to invertebrates have been described in previous sections. There were no toxicity studies located on the effects of Pb to aquatic decomposers in the time period of interest.

#### **AX7.2.4.5 Summary**

Lead in all its forms is known to cause adverse effects in aquatic organisms (Eisler, 2000). Effects to algal growth have been observed at concentrations ranging from 100 to 200,000 µg/L. Clinical signs of Pb toxicity in plants include the deformation and disintegration of algae cells and a shortened exponential growth phase. Other effects of Pb include a blocking of the pathways that lead to pigment synthesis, thus affecting photosynthesis, cell cycle and division, and ultimately resulting in death. The toxicity of Pb to macrophyte growth has been

studied using *Spirodela polyrhiza*, *Azolla pinnata*, and *Lemna gibba*. Test durations ranged from 4 to 25 days and test concentrations ranged between 49.7 and 500,000 µg/L.

Waterborne Pb is highly toxic to aquatic organisms, with toxicity varying with the species and life stage tested, duration of exposure, form of Pb tested, and water quality characteristics. Among the species tested, aquatic invertebrates, such as amphipods and water fleas, were the most sensitive to the effects of Pb, with adverse effects being reported as low as 0.45 µg/L. Effects concentrations for aquatic invertebrates were found to range from 0.45 to 8000 µg/L. Freshwater fish demonstrated adverse effects at concentrations ranging from 10 to >5400 µg/L, depending generally upon water quality parameters. Amphibians tend to be relatively Pb tolerant (Eisler, 2000); however, they may exhibit decreased enzyme activity (e.g., ALAD reduction) and changes in behavior (e.g., hypoxia response behavior). Lead tends to be more toxic with longer-term exposures.

The primary focus of this section was on effects at the individual level of organization. This narrow focus is primarily due to a lack of information on the effects of lead on higher levels of organization (e.g., populations, communities, and ecosystems). The impact of lead at higher levels of biological organization should be considered in future reviews of lead toxicity. In considering these higher levels of organization, cause-effect models and relationships that examine the roles of life history strategy and optimal foraging theory, community processes and associated theory, and ecosystem processes and the influences of redundancy/interactions should be taken into account.

## **AX7.2.5 Effects of Lead on Natural Aquatic Ecosystems**

### **Introduction**

As discussed previously, lead exposure may adversely affect organisms at different levels of organization, i.e., individual organisms, populations, communities, or ecosystems. Generally, however, 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). Potential effects at the population level or higher are, of necessity, extrapolated from individual level studies. Available population, community, or ecosystem level studies are typically conducted at sites that have been contaminated or adversely affected by multiple stressors (several chemicals alone or combined with physical or biological stressors). Therefore,

the best documented links between lead and effects on the environment are with effects on individual organisms.

This section discusses the effects of Pb on natural aquatic ecosystems. Such effects include changes in species composition and richness, ecosystem function, and energy flow due to Pb stress. The format of this section generally follows a conceptual framework for discussing the effects of a stressor such as Pb on an ecosystem. This conceptual framework was developed by the EPA Science Advisory Board (Young and Sanzone, 2002). The essential attributes used to describe ecological condition include landscape condition, biotic condition, chemical and physical characteristics, ecological processes, hydrology and geomorphology and natural disturbance regimes. The majority of the published literature pertaining to Pb and aquatic ecosystems focuses on the biotic condition, one of several essential attributes of an ecosystem as described in Young and Sanzone (2002). For the biotic condition, the SAB framework identifies community extent, community composition, trophic structure, community dynamics, and physical structure as factors for assessing ecosystem health. Other factors for assessing the biotic condition such as effects of Pb on organs, species, populations, and organism conditions (e.g., physiological status) were discussed in Sections AX7.2.3 and AX7.2.4.

For natural aquatic ecosystems, the focus of study in the general literature has been on evaluating ecological stress where the sources of Pb were from urban and mining effluents rather than atmospheric deposition (Poulton et al., 1995; Deacon et al., 2001; Mucha et al., 2003). The atmospheric deposition of Pb in remote lakes has been evaluated; however, the direct effects of Pb on aquatic ecosystems was not evaluated in many cases (Larsen, 1983; Köck et al., 1996; Allen-Gil et al., 1997; Outridge, 1999; Lotter et al., 2002). In other studies, although Pb deposition was studied, the effects of acid deposition on aquatic life were the focus of the study and perceived to be more relevant (Mannio, 2001; Nyberg et al., 2001). Finally, the effects of Pb, other metals, and acidification on phytoplankton have only been inferred based on the paleolimnological record (Tolonen and Jaakkola, 1983; Rybak et al., 1989). The statistical methods used when evaluating the effects of Pb on aquatic ecosystems are important, as more than one variable may be related to the observed effect. Studied variables include water hardness, pH, temperature, and physical factors such as embeddedness, dominant substrate, and velocity. In most cases, single variable statistical techniques were used to evaluate the data.

However, in other cases multivariate techniques were used. Therefore, where appropriate, some detail on the statistical methods used is presented.

Although most of the available studies discussed in this section focus on the biotic condition, one case study examining multiple components of the EPA conceptual framework is also included. The remainder of this section describes the effects of Pb on the biotic condition.

#### **AX7.2.5.1 Case Study: Coeur d'Alene River Watershed**

The Coeur d'Alene River watershed is an area of Idaho impacted by Pb and other metals from historic mining waste releases. Maret et al. (2003) examined several ecological components to determine any negative associations with metals and the watershed communities. The variables examined and associated ecological conditions are presented in Table AX7-2.5.1. In addition to measurements of non-metal variables (e.g., dissolved oxygen levels, water temperature and pH, embeddedness), Cd, Pb, and Zn levels were also compared in affected sites versus reference sites.

Some of the above non-metal variables are important to macroinvertebrate communities. For example, a stream with highly embedded substrate can have a lower number of individuals within a species or a different species composition compared to a stream with less embeddedness (Waters, 1995). Macroinvertebrates from the Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) (EPT) group inhabit the surface of cobble and the interstitial spaces between and underneath cobble. When substrate is embedded, these interstitial spaces are filled, leaving less habitat space for EPT taxa. In another example, water temperature is important; some macroinvertebrates (e.g., stoneflies) are usually only found in cooler water (Harper and Stewart, 1984).

Of the variables examined only metal concentrations, mine density, site elevation, and water temperature were significantly different between reference and mine-affected sites.

A Mann-Whitney t-test was used to evaluate statistical differences between reference and test sites for physical and water quality parameters, while Spearman's rank correlation matrices were used to compare all possible response and explanatory variables. Lead concentrations were significantly correlated with the number of mines in proximity to the watershed. Lead concentrations in sediment and water were strongly correlated to Pb levels in whole caddisflies,  $r^2 = 0.90$  and  $0.63$ , respectively. Furthermore, mine density was significantly correlated to Pb in

**Table AX7-2.5.1. Ecological Attributed Studies by Maret et al. (2003)  
in the Coeur d'Alene Watershed**

Ecological Attribute	Subcategory	Measure
Landscape condition	Areal extent landscape pattern	Basin area (km <sup>2</sup> ) Production mine density/km <sup>2</sup>
Biotic Condition	Organism condition population structure/ dynamics	Caddisfly tissue concentrations (mg/kg) Number of EPT taxa Density of EPT individuals (no./m <sup>2</sup> )
Chemical/physical characteristics	Chemical/physical parameters	Dissolved oxygen (mg/L) Specific conductance (μS/cm) Water temperature (E EC) pH Water hardness (mg/L) Total NO <sub>3</sub> (mg/L) Total P (mg/L) Dissolved NH <sub>3</sub> (mg/L) Sediment Cd, Pb, Zn (mg/kg) Dissolved Cd, Pb, Zn in water (mg/L)
Ecological processes	—	None measured
Hydrology/geomorphology	Channel morphology and distribution	Site elevation (m) Stream gradient (%) Stream discharge (m <sup>3</sup> /s) Stream width (m) Stream depth (m) Open canopy (%) Stream velocity (m/s) Embeddedness (%) Dominant substrate (mm)
Natural disturbance regimes	—	None measured

tissue,  $r^2 = 0.64$ . Although temperature was significantly different between reference and mine-affected sites, temperature conditions were concluded to be non-limiting to aquatic life. For example, reference and mine-affected sites had at least 15 and 13 obligate cold-water taxa, respectively.

A significant negative correlation between Pb in the water column (0.5 to 30 μg/L dissolved) and total taxa richness, EPT taxa richness, and the number of metal-sensitive mayfly

species was observed. Similar, significant negative correlations were found between sediment Pb levels (132 to 6252  $\mu\text{g/g}$ ) and the same macroinvertebrate community metrics and caddisfly tissue levels. Negative correlations were also found between Cd and Zn in the water and sediment and the macroinvertebrate community metrics. In an analysis of cumulative toxicity, Pb was judged to be the most significant metal in sediment related to the cumulative toxicity measured. This study provided multiple lines of evidence (i.e., mine density, metal concentrations, bioaccumulation in caddisfly tissue and benthic invertebrate assemblage structure) of the negative impacts of mining in the Coeur d'Alene River, suggesting that Pb (and other metals) were primary contributors to the effects observed in the Coeur d'Alene River watershed (Maret et al., 2003).

#### **AX7.2.5.2 Biotic Condition**

In an evaluation of the biotic condition, the SAB framework described by Young and Sanzone (2002) identifies community extent, community composition, trophic structure, community dynamics, and physical structure as essential ecological attributes for assessing ecosystem health. The following two sections describe the effects of Pb on community composition, community dynamics, and trophic structure. To date, no available studies were located on the effects of Pb on physical structure (e.g., change in riparian tree canopy height, ecosystem succession).

#### ***Ecosystems and Communities, Community Composition***

To measure community composition, an inventory of the species/taxa found in the ecological system must be conducted. According to the SAB framework, useful measures of composition include the total number of species or taxonomic units, their relative abundance, presence and abundance of native and non-native species, and information on the presence and abundance of focal or special interest species (Young and Sanzone, 2002). Focal or special species of interest can be those that play a critical role in ecosystem processes such as flows of materials or energy within complex food-webs (Young and Sanzone, 2002). Community composition as assessed in Pb studies has included the following measures.

- Changes in energy flow or nutrient cycling:
  - Increased or decreased respiration or biomass
  - Increased or decreased turnover/cycling of nutrients
  
- Changes in community structure:
  - Reduced species abundance (i.e., the total number of individuals of a species within a given area or community)
  - Reduced species richness (i.e., the number of different species present in a community)
  - Reduced species diversity (i.e., a measure of both species abundance and species richness)

Investigators have evaluated the effects of Pb on aquatic communities through microcosm and mesocosm studies in natural aquatic systems. Field studies in the general literature have focused on natural systems that were affected by metal stress from various anthropogenic sources. In most of those natural systems, the sources evaluated were from direct mining waste inputs, rather than atmospheric deposition, of Pb. Studies published since the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) that describe the effects of Pb on natural aquatic ecosystems are presented below and summarized in Table AX7-2.5.2. Studies included here evaluated the effects of Pb on watersheds, landscapes, aquatic ecosystems, aquatic communities, biodiversity, lakes, rivers, streams, estuaries, wetlands, and species interaction.

#### *Aquatic Microcosm Studies*

The examination of simulated aquatic ecosystems (i.e., microcosms) provides limited information on the effects of pollutants on natural systems. Microcosm studies typically focus on only a few aspects of the natural system and do not incorporate all of the ecological, chemical, or biological interactions. Nevertheless, a few microcosm studies have been conducted that indicate potential effects of Pb on the community structure of aquatic ecosystems. Fernandez-Leborans and Antonio-García (1988) evaluated the effect of Pb on a natural community of freshwater protozoans in simulated aquatic ecosystems and found a reduction in the abundance and composition of protozoan species with increasing Pb concentrations (0.05 to 1.0 mg/L) compared to controls. Studies with marine protozoan communities in laboratory microcosms indicated that waterborne Pb exposure reduced protozoan abundance, biomass, and

**Table AX7-2.5.2. Essential Ecological Attributes for Natural Aquatic Ecosystems Affected by Lead**

Category	Species	Condition Measures	Exposure Medium	Location	Exposure Concentrations	Other Metals Present	Reference
<b>Biotic Condition</b>							
Ecosystems and Communities-Community Composition	Protozoan community	Reduced species abundance and diversity	Marine water	Laboratory microcosm	0.02– 0.05 mg/L	N	Fernandez-Leborans and Novillo (1992)
	Protozoan community	Reduced species abundance	Freshwater water	Laboratory microcosm	0.05–1 mg/L	N	Fernandez-Leborans and Antonio-García (1988)
	Protist community	Reduced species abundance and diversity	Marine water	Laboratory microcosm	1 mg/L	N	Fernandez-Leborans and Novillo (1994)
	Meiofauna community	Reduced abundance	Marine sediment	Laboratory microcosm	177 mg/kg dw	Y	Millward et al. (2001)
	Algal community	Increased respiration	Freshwater	Domestic water stabilization pond	25–80 mg/L	?	Jayaraj et al. (1992)
	Algal community	Decreased primary productivity	Freshwater	Sharana Basaveshwara Tank, India	6–32 mg/L	?	Jayaraj et al. (1992)
	Meiobenthic community (primarily nematodes)	Reduced species abundance No effect on abundance	Marine sediment	Laboratory microcosm	1343 mg/kg dw 1580 mg/kg dw	N	Austen and McEvoy (1997)

**Table AX7-2.5.2 (cont'd). Essential Ecological Attributes for Natural Aquatic Ecosystems Affected by Lead**

Category	Species	Condition Measures	Exposure Medium	Location	Exposure Concentrations	Other Metals Present	Reference
	Macroinvertebrate community	Lower total abundance, decreased taxa, and EPT richness, larger percentage of tolerant species of benthic macroinvertebrates.	Freshwater and sediment	Mining sites in the Upper Colorado Basin	<0.001–0.02 mg/L 145-850 mg/kg dw (<63 µM fraction)	Y	Deacon et al. (2001); Mize and Deacon (2002)
	Macroinvertebrate community	Negatively correlated with species richness and diversity indices	Estuary sediment	Douro Estuary, Portugal	0.25–192 mg/kg dw	Y	Mucha et al. (2003)
	Macroinvertebrate community	Reduced species abundance	Freshwater sediment	River Ill and tributaries, France	1–16 mg/kg dw	Y	Rosso et al. (1994)
	Fish, crustacean and macroinvertebrate community	Correlation with changes in species abundance and distribution	Marine Sediment	Spencer Gulf, South Australia	156–5270 mg/kg dw	Y	Ward and Young (1982); Ward and Hutchings (1996)
	Chironomid community	Reduced chironomid richness	Whole organism residue	New Brunswick, Canada	40.3–1,387 mg/kg dw (periphyton) 1.6–131 mg/kg dw (chironomid tissue)	Y	Swansburg et al. (2002)
	Macroinvertebrate community	Lead in tissues negatively correlated with taxa richness, EPT richness, chironomid richness, and species density.	Whole organism residue	Clark Fork River, MT	32.2–67.1 mg/kg dw	Y	Poulton et al. (1995)
	Macroinvertebrate community	Lead in tissues negatively correlated with EPT richness and abundance.	Biofilm residues	Boulder River, MT	32–1540 mg/kg dw	Y	Rhea et al. (2004)

**Table AX7-2.5.2 (cont'd). Essential Ecological Attributes for Natural Aquatic Ecosystems Affected by Lead**

Category	Species	Condition Measures	Exposure Medium	Location	Exposure Concentrations	Other Metals Present	Reference
	Macroinvertebrate Community	Lead in tissues and sediment not correlated to diversity and richness	Sediment and whole organism residue	Aquashicola Creek tributaries, Palmerton, PA	7.5–59.5 mg/kg dw (sediment) 0.25-6.03 mg/kg dw (macroinvertebrates)	Y	Carline and Jobsis (1993)
	Fish Community	Lead in tissues and sediment not correlated to diversity and richness	Sediment and whole organism residue	Aquashicola Creek tributaries, Palmerton, PA	7.5–59.5 mg/kg dw (sediment) 0.1-0.86 mg/kg dw (fish)	Y	Carline and Jobsis (1993)
Ecosystems and Communities-Community Dynamics and Trophic Structure	Snails and tadpoles	Lead affected predator-prey interactions	Sediment	Outdoor mini-ecosystems	Not cited	Y	Lefcort et al. (1999)
	Snails and caddisflies	No avoidance of predator by snail. Caddisfly did respond to predator	Water	Field microcosm for snail; in-stream disturbance for caddisfly	27.7–277.6 mg/kg dw (snail tissue) 223–13,507 mg/kg dw (caddisfly tissue)	Y	Lefcort et al. (2000)
	Fathead minnow	Feeding behavior altered	Water	Laboratory microcosm	0.5–1.0 mg/L	N	Weber (1996)
	American toad	No avoidance of lead	Water	Laboratory microcosm	0.5–1.0 mg/L	N	Steele et al. (1991)
	Mummichog	Feeding behavior altered and predator avoidance affected	Water	Laboratory	0.3–1.0 mg/L	N	Weis and Weis (1998)

diversity at concentrations of 0.02 to 1.0 mg/L Pb (Fernandez-Leborans and Novillo, 1992, 1994).

Austen and McEvoy (1997) studied the effects of Pb on an estuarine meiobenthic community (mainly nematodes) in a microcosm setting using sediment samples collected offshore from England. A multivariate analysis of similarities (ANOSIM) test with square root-transformed data was used to evaluate differences between treatments and controls. Lead was found to significantly affect species abundance at 1343 mg/kg dw relative to a control at 56 mg/kg dw, but no significant adverse effects were observed at the highest dose tested, 1580 mg/kg dw. The authors did not attempt to explain why the 1580 mg/kg dw dose was not significant while the 1343 mg/kg dw dose was. None of the Pb exposures were significantly different than the controls based on separate univariate tests of abundance, richness, and diversity. There were no other confounding metals in the Pb tests, as the experiments were with a single metal dose. In one other mesocosm study, the effects of a mixture of metals (Cu, Cd, Pb, Hg, and Zn) on a salt marsh meiofaunal community were evaluated (Millward et al., 2001).

After 30-days exposure, significant reductions in copepod, gastropod, and bivalve abundances were observed at the highest Pb exposure concentration, 177 mg/kg dw. Ostracods and nematodes were not affected. The authors believed that the response of the meiofauna taxa to metals was in part due to the various feeding strategies in that deposit feeders were most affected.

### *Natural Aquatic Ecosystem Studies*

Lead stress in aquatic ecosystems has also been evaluated in natural communities. Studies examining community-scale endpoints, however, are complex, and interpretation can be confounded by the variability found in natural systems and the presence of multiple stressors. Natural systems frequently contain multiple metals, making it difficult to attribute observed adverse effects to single metals. For example, macroinvertebrate communities have been widely studied with respect to metals contamination and community composition and species richness (Winner et al., 1980; Chadwick et al., 1986; Clements, 1994). In these studies, multiple metals are evaluated and correlations between observed community level effects are ascertained. The results often indicate a correlation between the presence of one or more metals (or total metals) and the negative effects observed. While, correlation may imply a relationship between two

variables, it does not imply causation of effects. The following studies suggest an association between Pb concentration and an alteration of community structure and function (see summary in Table AX7-2.6.2):

#### *Reduced Primary Productivity and Respiration*

Jayaraj et al. (1992) examined the effects of Pb on primary productivity and respiration in an algal community of two water bodies. Concentrations of Pb in water (6 to 80 mg/L) were found to significantly reduce primary productivity and increase respiration. The authors suggested that increased respiration indicated a greater tolerance to or adaptive mechanisms of the resident heterotrophs to cope with lead stress.

#### *Alterations of Community Structure*

Deacon et al. (2001) studied a macroinvertebrate community in mine-affected waters of Colorado. Initially, transplanted bryophytes were used to assess whether metals could bioaccumulate at various mine-affected and unaffected sites (Deacon et al., 2001; Mize and Deacon, 2002). Lead was bioaccumulated by the bryophytes, and median tissue concentrations at mine-affected sites (34 to 299  $\mu\text{g/g dw}$ ) were higher than at reference sites (2.5 to 14.7  $\mu\text{g/g dw}$ ). Lead concentrations in surface water and sediment ranged from  $<0.001$  to 0.02 mg/L and 145 to 850 mg/kg dw ( $<63 \mu\text{m}$  fraction), respectively. The same sites were also evaluated for the effects of various metals on macroinvertebrate communities. Values of total abundance, taxa richness, mayfly, and stonefly abundance were reduced at mining sites. Lead levels along with Cd, Cu, and Zn were correlated with reduced abundance and diversity indices.

Macrobenthic communities studied in a Portuguese estuary were affected by Pb at a range from 0.25 to 192 mg/kg dw (Mucha et al., 2003). Species richness was decreased in areas with increased Pb concentrations in the sediment. Interpretation of Pb effects was complicated by other non-metal stressors, namely sediment particle size and organic matter content. Furthermore, other metals were present (e.g., Al, Cu, Cr, Mn, Zn) and may have affected the community (Mucha et al., 2003).

The effects of Pb on oligochaetes in the Ill River and its tributaries in France were evaluated by Rosso et al. (1994). Lead in sediment (5 to 16  $\mu\text{g/g dw}$  at affected sites) was positively correlated to the abundance of the oligochaete, *Nais* sp., and negatively correlated to

Tubificidae abundance. Lead was the only metal that was positively correlated to *Nais* species, while other metals were negatively correlated to Tubificidae (Rosso et al., 1994).

The effects of metals and particle size on structuring epibenthic sea grass fauna (fish, mollusks, crustaceans, and polychaetes) was evaluated near a Pb smelter in South Australia (Ward and Young, 1982; Ward and Hutchings, 1996). Effluent from the smelter was the primary source of Pb and other metal contamination. Species richness and composition were evaluated near the Pb smelter along with metal concentrations in sediment. Lead levels in sediment (up to 5270 mg/kg dw) correlated with negative effects on species richness and composition, while the other metals evaluated had similar correlations. Therefore, Pb alone could not be identified as the sole metal causing stress.

#### *Tissue Bioaccumulation Associated with Alterations of Community Structure*

Several studies have examined the bioaccumulation of lead in aquatic systems with indices of community structure and function. A focused study on changes in Chironomidae community composition in relation to metal mines (New Brunswick, Canada) identified changes in Chironomidae richness (Swansburg et al., 2002). Lead was not detected (detection limit not given for any matrix) in the water column at any site. However, Pb levels in periphyton were significantly higher at mining sites (40.3 to 1387 mg/kg dw) compared to reference sites (not detected [ND], 33.3 mg/kg dw). Furthermore, Pb in chironomids was significantly higher at mine-affected sites (1.6 to 131 mg/kg dw) compared to reference sites (ND, 10.2 mg/kg dw). The concentrations in biota indicate that Pb is mobile and available to the aquatic community even though water concentrations were undetectable. Chironomidae richness was reduced at the sites receiving mining effluent containing Pb, Cd, Cu, and Zn.

In another study, macroinvertebrate lead tissue concentrations (32.2 to 67.1 mg/kg dw at affected sites) collected from the Clark Fork River, Montana correlated negatively with total richness, EPT richness, and density (Poulton et al., 1995). Mean Pb levels were as high as 67.1 mg/kg dw at sites most affected by lead. However, other metals, including Cd, Cu, and Zn, also were negatively correlated with total richness and EPT richness. Therefore, attribution of the observed effects to Pb is difficult, as other metals may be contributing factors.

In Montana, the potential effects of metals on macroinvertebrate communities in the Boulder River watershed were evaluated (Rhea et al., 2004). Similar to the approach taken by

Poulton et al. (1995), the effects on richness and abundance of EPT taxa were compared to metal concentrations in tissue (i.e., biofilm and macroinvertebrates). Lead levels in biofilm (32 to 1540 mg/kg dw) were significantly correlated with habitat scores and macroinvertebrate indices (e.g., EPT taxa). However, macroinvertebrate tissue Pb levels were not significantly correlated with macroinvertebrate community level metrics. As with most natural systems with potential mine impacts, other metals also correlated with community level effects. However, the authors indicated that Pb concentrations in biofilm appeared to have the most significant impact on macroinvertebrate metrics.

A detailed investigation of sediment, macroinvertebrates, and fish was conducted for tributaries in the Aquashicola Creek watershed near a former zinc smelter in Palmerton, PA (Carline and Jobsis, 1993). The smelter deposited large amounts of Cd, Cu, Pb, and Zn on the surrounding landscape during its operation from 1898 to 1980. The goal of the study was to evaluate if there was a trend in the metal levels in sediment, macroinvertebrate and fish tissue, and community indices going away from the smelter. Sites were chosen, from 7.8 to 24.6 km from the smelter. There were no clear associations between proximity to the smelter and Pb levels in sediment, macroinvertebrate tissue, and fish tissue. Furthermore, there were no associations between proximity to the smelter and macroinvertebrate and fish diversity and richness. The authors suggested that the transport of metals in the watershed has decreased since the smelter ceased operation, and thereby no effects were observed.

### ***Ecosystems and Communities, Community Dynamics, and Trophic Structure***

As described in the SAB framework, community dynamics include interspecies interactions such as competition, predation, and succession (Young and Sanzone, 2002). Measures of biotic interactions (e.g., levels of seed dispersal, prevalence of disease in populations of focal species) provide important information about community condition. If the community dynamics are disrupted, then the trophic structure may also be disrupted. According to the SAB framework, trophic structure refers to the distribution of species/taxa and functional groups across trophic levels. Measures of trophic structure include food web complexity and the presence/absence of top predators or dominant herbivores. Therefore, this section discusses how aquatic species interactions can be affected by Pb. Examples of species interactions can include:

- Predator-prey interactions (e.g., reduced avoidance of predators)
- Prey consumption rate (e.g., increase or decrease in feeding)
- Species competition (e.g., interference with another species, increased aggressive behavior)
- Species tolerance/sensitivity (e.g., the emergence of a dominant species due to contaminant tolerance or sensitivity)

Species interactions are highly relevant to a discussion about the effects of Pb on natural aquatic ecosystems, because effects on species interactions could potentially affect ecosystem function and diversity. Some examples of Pb induced changes in species interactions are presented below (see summary in Table AX7-2.5.2).

#### *Predator-prey Interactions*

Lefcort et al. (1999) examined the competitive and predator avoidance behaviors of snails and tadpoles in outdoor mini-ecosystems with sediment from a metals-contaminated Superfund site (i.e., Pb, Zn, Cd). Previous investigations of aquatic invertebrates and vertebrates yielded Pb tissue concentrations of 9 to 3800 mg/kg dw and 0.3 to 55 mg/kg dw, respectively. Several species interactions were studied in the presence of metal-contaminated sediment:

Snails and tadpoles have similar dietary behaviors. Thus, when placed in the same habitat they will compete for the same food items and negatively affect one another. However, when tadpoles exposed to a predator (i.e., through biweekly additions of 20 mL of water from tanks housing sunfish—10 mL from sunfish-fed snails, 10 mL from sunfish-fed tadpoles) were placed with snails, the tadpoles reduced sediment ingestion, while snails increased ingestion. Thus, snails were exposed to greater quantities of metals in sediment.

In an uncontaminated environment, snail recruitment (i.e., reproduction) was reduced in the presence of tadpoles. The addition of tadpoles increased the competition for food in the form of floating algae and the snails switched to feeding on algae that grew on the sediment. This decrease was due to competition alone. The effects on snail recruitment were even higher when tadpoles, the influence of a predator (i.e., sunfish extract), and metals in the sediment were all present. However, the predator effect was indirect in that the tadpoles hid in the algal mats forcing the snails to feed primarily on the benthic algae that grew on the sediment with high

metal levels. Furthermore, although not significant, Pb levels in snails were higher when tadpoles and sunfish extract were present than when only metals in the sediment were present.

Finally, snail predator avoidance was assessed. Snails (control and lead-exposed) were stimulated with a predator indicator (i.e., crushed snails and an extract of crushed snail). Control snails changed behaviors in the presence of the predator indicator, while exposed snails did not alter their behavior. The authors suggested that metal exposure caused behavioral changes that alter competitive interactions and the perception of predators by the snails. Thus, Pb may affect the predator avoidance response of snails.

In further study, Lefcort et al. (2000) examined the predator avoidance behaviors of snails and caddisflies. In separate experiments, the avoidance behavior of the snail, *Physella columbiana*, and four caddisfly genera (*Agrypnia*, *Hydropsyche*, *Arctopsyche*, *Neothremma*) were evaluated. The snails were collected from reference lakes and lakes downstream of the Bunker Hill Superfund site. The snails from the affected lakes generally had higher cadmium, Pb, and zinc tissue levels implying previous exposure to these metals. Snail predator avoidance behavior was tested by exposure to crushed snail extract. Snails from the affected lakes did not reduce their activity when exposed to the snail extract, implying a reduced predator avoidance. The lack of response may make the snails at the affected lakes more prone to predation.

The caddisflies were evaluated at 36 sites from six different streams. As with the snails, the caddisflies from the affected streams had higher cadmium, Pb and zinc tissue levels. The time for caddisfly larvae to respond (i.e., how long immobile) to disturbance (i.e., lifted from water for 3 seconds and moved to a new location) was evaluated. There was no correlation between tissue metal level and any response variable (Lefcort et al., 2000). Therefore, the authors concluded that preexposure to metals did not reduce predator avoidance for caddisflies.

Weber (1996) examined juvenile fathead minnows exposed to 0, 0.5, or 1.0 ppm Pb in water during a 2-week preexposure and 2-week testing period (4 weeks total exposure). Feeding behavior was evaluated by presenting two prey sizes (2-day-old and 7-day-old *Daphnia magna*). Control fish began switching from larger, more difficult-to-capture 7-day-old daphnids to smaller, easier-to-catch 2-day-old prey by day 3. Lead-exposed fish displayed significant switching at day 3 (at 0.5 ppm) or day 10 (at 1.0 ppm). Thus, exposure to Pb delayed the altering of prey size choices to less energetically costly prey.

Lefcort et al. (1998) exposed spotted frogs (*Rana luteiventris*) to 0.05 to 50 ppm Pb in water for 3 weeks. High levels of Pb reduced the fright response of tadpoles; suggesting a reduced avoidance of predators.

Bullfrog larvae exposed to Pb in water (0.78 mg/L) and high or low dissolved oxygen were monitored for respiratory surfacing behavior (Rice et al., 1999). Larvae had a significantly increased number of trips to the water surface regardless of oxygen content. Thus, the authors suggest that Pb may affect oxygen uptake such that larvae are under greater predation pressure due to increased time spent at the surface.

Weis and Weis (1998) evaluated the effect of Pb exposure on mummichog (*Fundulus heteroclitus*) larvae prey capture rate, swimming behavior, and predator avoidance. Prey capture rates were affected after 4 weeks exposure at 1.0 mg Pb/L. The larvae were also more vulnerable to predation by grass shrimp (*Palaemonetes pugio*) at 1.0 mg Pb/L. Finally, the swimming behavior of mummichog larvae was affected at 0.3 and 1.0 mg Pb/L. Once the larvae were no longer exposed to Pb, they recovered their ability to capture prey and avoid predators.

Clearly, exposure to Pb does affect the predator-prey interactions and the ability of prey to avoid predators. The effect of Pb on these ecological functions may alter community dynamics.

### **AX7.2.5.3 Summary**

The effects of Pb have primarily been studied in instances of point source pollution rather than area-wide atmospheric deposition; thus, the effects of atmospheric Pb on ecological condition remains to be defined. The evaluation of point source Pb within the EPA Ecological Condition Framework has been examined primarily in relation to biotic conditions. The available literature focuses on studies describing the effects of Pb in natural aquatic ecosystems with regard to community composition and species interactions. The effects of Pb on the biotic condition of natural aquatic systems can be summarized as follows: there is a paucity of data in the general literature that explores the effects of Pb in conjunction with all or several of the various components of ecological condition as defined by the EPA. However, numerous studies are available associating the presence of Pb with effects on biotic conditions.

In simulated microcosms or natural systems, environmental exposure to Pb in water and sediment has been shown to affect energy flow and nutrient cycling and benthic community structure. In field studies, Pb contamination has been shown to significantly alter the aquatic

environment through bioaccumulation and alterations of community structure and function. Exposure to Pb in laboratory studies and simulated ecosystems may alter species competitive behaviors, predator-prey interactions, and contaminant avoidance behaviors. Alteration of these interactions may have negative effects on species abundance and community structure. In natural aquatic ecosystems, Pb is often found coexisting with other metals and other stressors. Thus, understanding the effects of Pb in natural systems is challenging given that observed effects may be due to cumulative toxicity from multiple stressors.

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