EPA/600/R-23/375
January 2024
www.epa.gov/isa

Integrated Science Assessment for Lead

Appendix 11: Effects of Lead in Terrestrial and Aquatic Ecosystems

January 2024

Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

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DOCUMENT GUIDE

This Document Guide is intended to orient readers to the organization of the Lead (Pb) Integrated Science Assessment (ISA) in its entirety and to the sub-section of the ISA at hand (indicated in bold). The ISA consists of the Front Matter (list of authors, contributors, reviewers, and acronyms), Executive Summary, Integrated Synthesis, and 12 appendices, which can all be found at https://assessments.epa.gov/isa/document/&deid=359536.

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ACRONYMS AND ABBREVIATIONS

ACE	abundance-based coverage estimator
AChE	acetylcholinesterase
Ag	silver
ALAD	aminolevulinic acid dehydratase
AMF	arbuscular mycorrhizal fungi
AQCD	Air Quality Criteria Document
As	arsenic
ASTM	American Society for Testing and Materials
AVS	acid volatile sulfide
AWCD	average cell wall color development
AWQC	ambient water quality criteria
BAF	bioaccumulation factor
BCF	bioconcentration factor
BEST	Biomonitoring of Environmental Status and Trends
BLL	blood lead level
BLM	biotic ligand model
BMF	biomagnification factors
BRT	boosted regression tree
BSAF	biota-sediment accumulation factor
Ca	calcium
CAT	catalase
CCA	canonical correspondence analysis
CCC	criterion continuous concentration
Cd	cadmium
CEC	cation exchange capacity
CF	conversion factor
CMC	criteria maximum concentration
CORT	corticosterone
CRADA	Cooperative Research and Development Agreement
CSMW	California State Mussel Watch
Cu	copper
d	day(s)
DOC	dissolved organic carbon
dpf	days postfertilization
dph	days posthatch
DOM	dissolved organic matter
DT	diatom + tetramin
EC_{50}	half maximal effect concentration
eCEC	effective cation exchange capacity
Eco-SSL	ecological soil screening level
EDTA	ethylenediaminetetraacetic acid
FCORT	fecal corticosterone
FCV	final chronic value
GABA	gamma-aminobutyric acid
GPx	glutathione peroxidase
GSH	glutathione
GST	glutathione-S-transferase
HAB	harmful algal bloom
hpf	hours postfertilization
IC	inhibitory concentration
ISA	Integrated Science Assessment
K_d	partition coefficient
LECES	Level of Biological Organization, Exposure, Comparison, Endpoint, and Study Design
LH	luteinizing hormone
LOEC	lowest observed effect concentration
LOAEL	lowest observed adverse effect level
LRMN	Large River Monitoring Network
LUFA	Landwirtschaftliche Untersuchungs- und Forschungsanstalt
MATC	maximum acceptable toxicant concentration
MBC	microbial biomass carbon
MDA	malondialdehyde
ME	mining ecotype
Mg	magnesium
MIC	minimum inhibitory concentration
MLR	multiple linear regression
mo	month(s)
MRG	metal-rich granules
MTC	maximum tolerable concentration
MW	molecular weight
NAAQS	National Ambient Air Quality Standards
NASGLP	North American Soil Geochemical Landscapes Project
NAWQA	National Water Quality Assessment
NEC	no-effect concentration
NME	nonmining ecotype
NOAA	National Oceanic and Atmospheric Administration
NOEC	no-observed-effect concentration
NOM	natural organic matter
n.s.	nonsignificant
OC	organic carbon
OM	organic matter
OP	omnivores-predator

OTU	operational taxonomic unit	TBMF	trophic biomagnification factor
Pb	lead	TEC	threshold effect concentration
PEC	probable effects concentration	TRF	terminal restriction fragment
PECOS	Population, Exposure, Comparison, Outcome and Study Design	TTF	trophic transfer factor
PMF	Picher mine field	U.S. EPA	United States Environmental Protection Agency
PNEC	predicted no-effect concentration	USGS	United States Geological Survey
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals	WACAP	Western Airborne Contaminants Assessment Project
ROS	reactive oxygen species	WEOC	water-extractable organic carbon
SEM	simultaneously extracted metal	wk	week(s)
SOD	superoxide dismutase	WQC	water quality criteria
SSD	species sensitivity distribution	YCT	yeast, cereal leaves, and trout pellet
T3	triiodothyronine	yr	year(s)
T4	thyroxine	Zn	zinc

APPENDIX 11 EFFECTS OF LEAD IN TERRESTRIAL AND AQUATIC ECOSYSTEMS

Summary of Causality Determinations for Welfare Effects of Lead

This appendix characterizes the scientific evidence that supports causality determinations for lead (Pb) exposure and the effects of Pb in terrestrial and aquatic ecosystems and biota. In assessing the overall evidence, the strengths and limitations of individual studies were evaluated. More details on the causal framework used to reach these conclusions are included in the Preamble to the Integrated Science Assessments (U.S. EPA, 2015). The evidence presented throughout this appendix supports the following causality determinations (bolded text indicates a change since the 2013 Integrated Science Assessment for Pb).

Level		Effect	Terrestrial[a]	Freshwater[a]	Saltwater[a]
Community- and Ecosystem		Community and Ecosystem Effects	Likely Causal	Likely Causal	**Suggestive**
Population–Level Endpoints	Organism–Level Responses	Reproductive and Developmental Effects - Plants	Inadequate	Inadequate	Inadequate
		Reproductive and Developmental Effects - Invertebrates	Causal	Causal	**Likely Causal**
		Reproductive and Developmental Effects - Vertebrates	Causal	Causal	Inadequate
		Growth - Plants	Causal	Likely Causal	Inadequate
		Growth - Invertebrates	Likely Causal	Causal	Inadequate
		Growth - Vertebrates	Inadequate	Inadequate	Inadequate
		Survival - Plants	Inadequate	Inadequate	Inadequate
		Survival - Invertebrates	Causal	Causal	Inadequate
		Survival - Vertebrates	Likely Causal	Causal	**Suggestive**
		Neurobehavioral Effects - Invertebrates	Likely Causal	Likely Causal	Inadequate
		Neurobehavioral Effects - Vertebrates	Likely Causal	Likely Causal	Inadequate
	Suborganismal Responses	Hematological Effects - Invertebrates	Inadequate	Likely Causal	Suggestive
		Hematological Effects - Vertebrates	Causal	Causal	Inadequate
		Physiological Stress - Plants	Causal	Likely Causal	Inadequate
		Physiological Stress - Invertebrates	Likely Causal	Likely Causal	Suggestive
		Physiological Stress - Vertebrates	Likely Causal	Likely Causal	Inadequate

[a]Based on the weight of evidence for causal determination in Table II of the ISA Preamble (U.S. EPA, 2015).

The Executive Summary, Integrated Synthesis, and all other appendices of this Pb ISA can be found at https://assessments.epa.gov/isa/document/&deid=359536.

11.1 Introduction, Scope, Concepts, and Tools

This appendix synthesizes and evaluates the most policy-relevant scientific information on Pb welfare effects to help form the foundation for the review of the secondary (welfare[1]-based) National Ambient Air Quality Standards (NAAQS) for lead (Pb). The focus of this appendix is on studies published since the 2013 Integrated Science Assessment (ISA) for Pb (2013 Pb ISA) U.S. EPA (2013) that examine Pb interactions with the biotic components of terrestrial and aquatic ecosystems, including effects on vegetation and wildlife. Pb transport through abiotic compartments (air, soil, water, and sediment) is covered in Appendix 1: Lead Source to Concentration: https://assessments.epa.gov/isa/document/&deid=359536. Section 11.1 of this appendix includes key concepts and tools useful for characterizing the effects of Pb on biota. Section 11.2 examines the bioavailability, bioaccumulation, and effects of Pb in terrestrial ecosystems. The effects of Pb in terrestrial environments are followed by information on the bioavailability, bioaccumulation and effects of Pb in freshwater (Section 11.3) and saltwater (Section 11.4) ecosystems.

11.1.1 Scoping and Criteria for Study Inclusion

This appendix builds upon the assessment of effects of Pb on ecosystems reported in the 2013 Pb ISA (U.S. EPA, 2013) and in prior Air Quality Criteria Documents (AQCDs) from 1977 (U.S. EPA, 1977), 1986 (U.S. EPA, 1986), and 2006 (U.S. EPA, 2006). The framework used to define the scope of the ecological effects portion of the current ISA is modeled after the Population, Exposure, Comparison, Outcome, and Study Design (PECOS) used for human health effects (Appendix 12: https://assessments.epa.gov/isa/document/&deid=359536). For the health appendices, the PECOS statement defines the objectives of the review and establishes study inclusion criteria, thereby facilitating identification of the most relevant literature to inform the ISA for each health discipline. Similarly, the Level of Biological Organization, Exposure, Comparison, Endpoint, and Study Design (LECES) statement aids in identifying the relevant evidence in the literature for the ecological effects of Pb (Table 12-4; Appendix 12). Studies that reported the effects of Pb on biota were evaluated, included, and discussed in this appendix if they satisfied the following LECES criteria:

11.1.1.1 Level of Biological Organization

Studies considered for this appendix included those that reported Pb effects on species, subspecies or populations of vegetation, microbes, invertebrates, or vertebrates at any lifestage on

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

biological communities or on ecosystems in terrestrial, freshwater, or saltwater environments and transition zones present in the United States or similar to those in the United States. In the 2013 Pb ISA, ecological effects were generally organized in order of increasing biological complexity (i.e., from the subcellular and cellular levels through the individual organism and up to ecosystem-level effects) (U.S. EPA, 2013). This appendix follows the same organizing principle. For effects that occur at the suborganism scale such as perturbation of biomarkers of physiological stress or changes in hematological parameters, emphasis was placed on studies that concurrently reported effects experimentally linked to higher levels of biological organization. Organism-level endpoints such as growth, survival, and reproductive output have been definitively linked to effects at the population level and above. Examples of organism-level endpoints with direct links to population-level effects include mortality, gross abnormalities, survival, fecundity, and growth (Suter et al., 2004). Because of the complexity of processes that can affect an ecosystem and considering that Pb rarely occurs as the only contaminant in natural systems, it is difficult to attribute effects observed at higher levels of biological organization solely to Pb.

11.1.1.2 Exposure

The deleterious effects of any given concentration of Pb can vary greatly under different environmental and experimental conditions, as well as the duration and pathway of exposure. Relevant concentrations for this assessment take into consideration the range of Pb concentrations in environmental media from U.S. locations (Table 11-1) and the available evidence for concentrations at which effects are observed in microbes, plants, invertebrates, and vertebrates. Effects observed at or near environmental concentrations of Pb measured in soil, sediment, and water are emphasized. For the studies included in the 2013 Pb ISA, evidence from exposures or doses generally ranged "from current levels to one or two orders of magnitude above current levels" (U.S. EPA, 2013). Statements regarding concentrations considered for literature inclusion were not provided in earlier United States Environmental Protection Agency (U.S. EPA) reviews of this metal. To focus on studies that are the most policy-relevant with regard to current environmental concentrations of Pb in the United States, concentration guidelines were applied when evaluating the literature published since the 2013 Pb ISA (Appendix 12: https://assessments.epa.gov/isa/document/&deid=359536). These guidelines were derived by taking into consideration data that was current at the time of the 2013 Pb ISA on Pb concentrations in soils, water and sediments in the United States. The concentration guideline for literature screening in this ISA is approximately one order of magnitude higher than upper bound values from available environmental surveys (Table 1-1 from 2013 Pb ISA). For soil, the concentration guideline for screening of terrestrial studies of Pb exposure and effects was set at approximately 230 mg Pb/kg of soil, although higher concentrations were considered if the study added new information on a mechanism of action, or if the higher concentration was part of a series that contributed exposure-response information and included other concentrations below 230 mg Pb/kg. The concentration guideline for screening aqueous exposure studies was approximately 10 µg Pb/L, although higher concentrations were considered if the study added new information on a mechanism of action or if the higher concentration was part of a series that contributed exposure-response information. The concentration guideline for screening sediment studies was approximately 300 mg Pb/kg dry weight or lower. Studies at very high concentrations of Pb were excluded unless they were part of a series in an experimental exposure-response study and at least one concentration in the test series was in the ranges stated above. The approach for selection of the Pb concentrations used as guidelines and additional information on scoping for the literature for ecological effects of Pb is provided in Appendix 12. Initial literature search and screening steps for this review identified many studies conducted at higher concentrations of Pb that were ultimately excluded from the draft ISA (https://hero.epa.gov/hero/index.cfm/project/page/project_id/4081).

For references published since the 2013 Pb ISA, values reported in this appendix for biological effects are from exposures in which concentrations of Pb were analytically verified (measured). Some nominal concentrations are cited but the studies where they are used are experimental gradient studies where the response of an organism or system to a series of increasing exposures is informative with respect to causality. In no case are such studies cited to support quantification of effect concentrations or quantification of the exposure-response relationship. In addition, many studies in terrestrial and aquatic environments report a series of nominal additions of Pb to the environmental medium, but measure the concentrations present in the organisms and analyze their effects on organisms, not the relationship between the medium concentration and effects on organisms. Older studies cited in prior AQCDs or in the 2013 Pb ISA and occasionally referenced in this appendix may have used nominal exposures. For consistency, concentrations of Pb in soil and sediment are reported in mg Pb/kg dry weight (unless otherwise specified) and aqueous concentrations of Pb are reported as µg Pb/L. For study concentrations originally in other units such as µM or ppb, the values are converted to mg Pb/kg or µg Pb/L, and original reported units are retained in parentheses. Only a subset of the studies reporting Pb effects on biota analytically verified the concentration of Pb in media and the test organisms investigated.

11.1.1.3 Comparison

Comparisons in the studies considered for inclusion in this appendix were to an unexposed laboratory control, a reference population, or a site with no detectable exposure or with lower Pb exposure. For ecological effects assessment, both laboratory and field studies (including field experiments and observational studies) can provide useful data (U.S. EPA, 2015). As the number of factors that the study holds constant increases, other than Pb exposure, so does the certainty with which observed variation in outcomes can be attributed to exposure, while the size of effects that the study is capable of attributing to exposure becomes smaller. The ability to hold other variables constant is expected to diminish with increasing biological scale from subcellular processes to whole ecosystems and from laboratory to field. In general, effects of Pb on ecological endpoints are reported in the ISA if they are statistically significant.

11.1.1.4 Endpoint

The biological endpoints considered in this appendix are relevant to the levels of biological organization discussed above. The endpoints encompass individual organism-level or population-level effects on a given species including but not limited to effects on growth, reproduction or development, neurobehavioral effects, reduced survival, or fitness, and photosynthesis. At higher levels of biological organization, endpoints include, but are not limited to, changes in community composition (e.g., shifts in genotypes or species, species extirpation, declines in the total number of species, and decreased species richness), declines in biomass, and other altered ecosystem processes and functions.

11.1.1.5 Study Design

Relevant study designs for assessing Pb effects on ecological receptors include laboratory, mesocosm, observational or experimental field or gradient studies wherein observed effects are measured and analyzed quantitatively, or mechanistic modeling studies that estimate the effect of Pb on an organism, biological population, community, or ecosystem (U.S. EPA, 2015). Controlled exposure studies in laboratory or small-to-medium-scale field settings provide the most direct evidence for the effects of Pb exposure, but their scope of inference may be limited (U.S. EPA, 2013). Exposure-response data from acute bioassays typically report effects on mortality, growth, or reproduction. Chronic bioassays are designed to incorporate effects over the lifespan or partial lifespan of the study subjects, including effects on reproduction. In contrast, mesocosms and field studies include potentially confounding factors (e.g., other metals) or factors known to interact with exposure (e.g., pH), thus increasing the uncertainty in associating the effects observed with exposure to Pb (U.S. EPA, 2013).

11.1.1.6 Additional Scoping

Topics within scope also include effects of Pb biogeochemistry on bioavailability in terrestrial, freshwater, and saltwater environments as well as subsequent vulnerability of particular organisms, populations, communities or ecosystems and studies that address key uncertainties and limitations in the evidence identified in the previous review. Topics outside of the scope of this appendix included mixture studies that did not assess Pb effects independently and site-specific studies in non-U.S. locations that do not contribute novel insights on Pb biogeochemistry or effects. As in the 2013 Pb ISA, generally, studies on mine tailings, industrial effluent, sewage, bioremediation of highly contaminated sites and ingestion of Pb shot, pellets or fishing gear are not within the scope of this ISA due to the high concentration of Pb and lack of a connection to air-related sources or processes. This is consistent with the 2006 AQCD, which typically did not include "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)" (Section AX 7.1.3 of (U.S. EPA, 2006)).

11.1.2 Introduction to Ecosystem Connections and Pb Transfers

Metals, including Pb, occur naturally in the geosphere, and anthropogenic enrichment of these elements can lead to elevated concentrations in terrestrial and aquatic ecosystems. Pb is a persistent metal that, once emitted, may cycle through multiple environmental media compartments (e.g., air, soil, water, sediment) prior to exposure to plants and animals, as discussed in Appendix 1: https://assessments.epa.gov/isa/document/&deid=359536 (Section 1.3). In terrestrial ecosystems, non-air media can receive Pb from atmospheric deposition or other sources. The contribution of atmospheric Pb differs by location and there is a lack of source apportionment studies to characterize the amount of Pb from deposition in relation to other Pb sources. Once deposited, Pb can be resuspended into the air or transferred among other environmental media (Section 1.3). Exposure of freshwater and estuarine organisms to Pb, and associated effects, are tied to terrestrial systems via watershed processes. Atmospherically derived Pb can enter aquatic systems through erosional transport of soil particles in runoff from terrestrial systems (Section 1.3.3) or via direct wet or dry deposition over a water surface (Section 1.3.1.2). Once in the aquatic environment, Pb partitions between various compartments (water column, sediment, biota; Section 1.3.3). Saltwater ecosystems include habitats that encompass a range of salinities from just above that of freshwater to that of seawater. These ecosystems may receive Pb contributions from atmospheric deposition (Section 1.3.1.2), riverine transport (Section 1.3.3) and runoff (Section 1.3.3) from terrestrial systems. Ecosystems in more urban areas are also influenced by non-air sources of Pb such as paint, automobiles, wastewater, and industrial activities. Although Pb is present in the natural environment, it has no biological function in plants or animals. Terrestrial, freshwater, and marine/estuarine organisms have developed adaptive physiological responses for living with metals. These adaptations may include intracellular sequestration (e.g., synthesis of metallothioneins or metal-rich granules [MRG]), induction of enzymes involved in oxidative stress response, and modification of metal uptake or elimination rates (Gismondi et al., 2017). Anthropogenic enrichment can result in concentrations that exceed the capacity of organisms to regulate internal concentrations, causing a toxic response and potentially death. Across taxa, effects of Pb exposure are likely mediated through common biological mechanisms. In the case of Pb, ecological receptors and humans are linked via shared pathways of exposure and commonalities in biological response to this metal (Lassiter et al., 2015).

Connections between the atmosphere, the abiotic and biotic compartments of terrestrial and aquatic ecosystems, and humans are acknowledged for Pb. However, for the purposes of this ISA, these topics are divided into different appendices. Within this Ecological Effects appendix, terrestrial, freshwater, and saltwater ecosystems are considered separately because of different environmental and physiological factors that influence Pb toxicity, such as bioavailability of the metal, form of Pb, other water and soil chemistry factors, and organism adaptations.

11.1.3 Concentrations of Pb in Non-Air Media

Organisms may be exposed to Pb in soil, water, sediment, and other biota (via diet). Food, drinking water, and contact with contaminated soils are likely major routes of exposure for terrestrial wildlife. Ingestion and water intake are major routes of exposure for aquatic fauna. Inhalation is thought to be a minor pathway in wildlife, with the possible exception of exposures in proximity to Pb atmospheric point sources, such as smelters. Due to the presence of Pb in various environmental media, exposure to this metal can occur via multiple pathways.

To provide sufficient information to support development of air quality criteria for Pb that are protective of terrestrial and aquatic systems, it is important to gain a general understanding of current distribution and the concentrations of Pb in the environment. Information on environmental concentrations of Pb at U.S. locations is tabulated in Table 11-1. This table updates Table 6-2 in the 2013 Pb ISA U.S. EPA (2013) on Pb concentration in non-air media and biota. Sources of environmental concentration data in Table 11-1 were limited to regional or national-scale studies. Studies that reported concentrations in environmental media for one or a very small number of locations would be considered anecdotal for the purpose of this review. Measured concentrations of Pb in soils, sediment and water are not necessarily representative of the amount of Pb available to elicit a toxic effect. For Pb to interact with a biological membrane and be taken up into an organism, it must be in a bioavailable form (Section 11.1.6), which is dependent upon the physical, chemical, and biological conditions under which an organism is exposed at a particular geographic location. In addition, caution must be taken while comparing Pb concentrations in different studies of environmental media because reported concentrations of Pb may not be directly comparable across studies, in part due to differences in sampling, collection and measurement methods. For example, soil Pb measurements may vary between studies that used partial and complete acid digestion. Furthermore, complete acid digestion is likely to overestimate the amount of bioavailable Pb in many cases. In aquatic systems, measurements of dissolved Pb may vary among collection methods, notably due to different sample filtration sizes, while the composition of sediment samples of Pb is often influenced by sieving size. These are given as illustrative examples of how Pb observations may be affected by methods, but a comprehensive discussion of Pb sampling, collecting, and measuring methods is beyond the scope of this ISA.

Some surveys of Pb in environmental media in Table 11-1 predate the 2013 Pb ISA (U.S. EPA, 2013) and 2006 Pb AQCD (U.S. EPA, 2006). Although they may have used less optimal methods than more recent studies, these data are not excluded from the ISA in cases wherein they remain the best available information.

Table 11-1 Pb concentration in non-air media and biota

Media	Pb Concentration	Years Data Obtained	References
Soil	Conterminous U.S. 0–5 cm depth soil: Median: 18.1 ± 185 mg Pb/kg; range: <0.5–12,400 mg Pb/kg; IQR: 13.5–23.9 mg Pb/kg (dry weight) Conterminous U.S. A horizon soil: Median: 17.8 ± 46.6 mg Pb/kg; range: <0.5–2,200 mg Pb/kg; IQR: 13.2–23.2 mg Pb/kg (dry weight) Conterminous U.S. C horizon soil: Median: 14.9 ± 18.5 mg Pb/kg; range: <0.5–681 mg Pb/kg; IQR: 11.1–19.2 mg Pb/kg (dry weight)	2007–2010	Smith et al. (2013a)
	Northeastern U.S. forest floor soil mean: 151 ± 29 mg Pb/kg (dry weight)	1980	Richardson et al. (2014)
	Northeastern U.S. forest floor soil mean: 68 ± 13 mg Pb/kg (dry weight) (resurvey of 16 of 25 1980 sites)	2011	Richardson et al. (2014)
	Soil sampled at 54 sites in Los Angeles, Orange, San Bernardino, and Riverside counties in California Range: 5–70 mg Pb/kg Mean: 23.9 ± 13.8 mg Pb/kg	2019	Mackowiak et al. (2021)
Soil (freshwater wetlands and salt marshes)	Conterminous U.S. uppermost soil horizon mean: 20.15 ± 1.73 (95% CI) mg Pb/kg (dry weight)	2011	Nahlik et al. (2019)
Freshwater Sediment	Cores from 35 U.S. lakes 1970s Median: 115 mg Pb/kg (dry weight) 1990s Median: 73 mg Pb/kg (dry weight)	1996–2001	Mahler et al. (2006)
	National Water Quality Assessment of lotic systems Median: 28 mg Pb/kg (dry weight)	1991–2003	U.S. EPA (2006)
	National Water Quality Assessment of lotic systems grouped by river basin land use: Baseline (in low-population areas): median: 20 mg Pb/kg; range: 2–200 mg Pb/kg (dry weight) Agricultural sites: median: 20 mg Pb/kg; range: 6–310 mg Pb/kg (dry weight) Cropland sites: median: 19 mg Pb/kg; range: 8–310 mg Pb/kg (dry weight) Pasture sites: median: 20 mg Pb/kg; range: 6–49 mg Pb/kg (dry weight) Forested sites: median: 28 mg Pb/kg; range: 2–200 mg Pb/kg (dry weight) Rangeland sites: median: 18 mg Pb/kg; range: 6–330 mg Pb/kg (dry weight)	1991–2001	Horowitz and Stephens (2008)
	131 coastal conterminous U.S. rivers: Overall mean: 59 mg Pb/kg; median: 26 mg Pb/kg (dry weight) Atlantic rivers: mean: 110 mg Pb/kg; median: 36 mg Pb/kg (dry weight) Gulf rivers: mean: 32 mg Pb/kg; median: 24 mg Pb/kg (dry weight)	2010–2011	Horowitz et al. (2012)

Media	Pb Concentration	Years Data Obtained	References
	Pacific rivers: mean: 19 mg Pb/kg; median: 13 mg Pb/kg (dry weight)		
Saltwater Sediment	Global Range: 0.6–1,050 mg Pb/kg U.S. Range (from Puget Sound): 13.4–52.8 mg Pb/kg	Reported in studies dated 1977–1990	Sadiq (1992)
	U.S. Geometric Mean: 43 mg Pb/kg Global Geometric Mean: 43 mg Pb/kg Global Geometric Mean ("hot spot" data from contaminated sites removed): 34 mg Pb/kg	1984–1987	Cantillo and O'Connor (1992)
Fresh Surface Water (Dissolved Pb)	Median: 0.50 µg Pb/L Max: 30 µg Pb/L, 95th percentile 1.1 µg Pb/L	1991–2003	U.S. EPA (2006)
	8 Texas rivers Sabine: Mean: 0.04 ± 0.025 µg Pb/L Range: 0.013–0.098 µg Pb/L Neches: Mean: 0.036 ± 0.028 µg Pb/L Range: 0.01–0.099 µg Pb/L Trinity: Mean: 0.061 ± 0.067 µg Pb/L Range: 0.009–0.218 µg Pb/L Brazos: Mean: 0.02 ± 0.011 µg Pb/L Range: 0.008–0.061 µg Pb/L Colorado: Mean: 0.02 ± 0.009 µg Pb/L Range: 0.007–0.04 µg Pb/L Guadalupe: Mean: 0.049 ± 0.059 µg Pb/L Range: 0.005–0.202 µg Pb/L San Antonio: Mean: 0.356 ± 0.235 µg Pb/L Range: 0.177–0.919 µg Pb/L Nueces/Frio: Mean: 0.025 ± 0.034 µg Pb/L Range: 0.008–0.166 µg Pb/L	1997–1998	Jiann et al. (2013)
	Range: 0.0003–0.075 µg Pb/L (Set of National Parks in western U.S.)	2002–2007	Field and Sherrell (2003) Blett (2010)
	Appalachian headwater streams (4 sites located in second- or third-order streams within the Blue Ridge level III ecoregion) Mean: <0.28 µg Pb/L	2015–2017	Olson et al. (2019)
Fresh Surface Water (Particulate Pb)	8 Texas rivers Sabine: Mean: 27.76 ± 5.5 mg Pb/L Range: 21.81–38.17 mg Pb/L Neches: Mean: 32.4 ± 4.55 mg Pb/L Range: 26.48–39.23 mg Pb/L Trinity: Mean: 28.24 ± 3.82 mg Pb/L Range: 22.87–33.24 mg Pb/L Brazos: Mean: 22.45 ± 7.39 mg Pb/L Range: 12.18–40.06 mg Pb/L Colorado: Mean: 25.39 ± 12.33 mg Pb/L	1997–1998	Jiann et al. (2013)

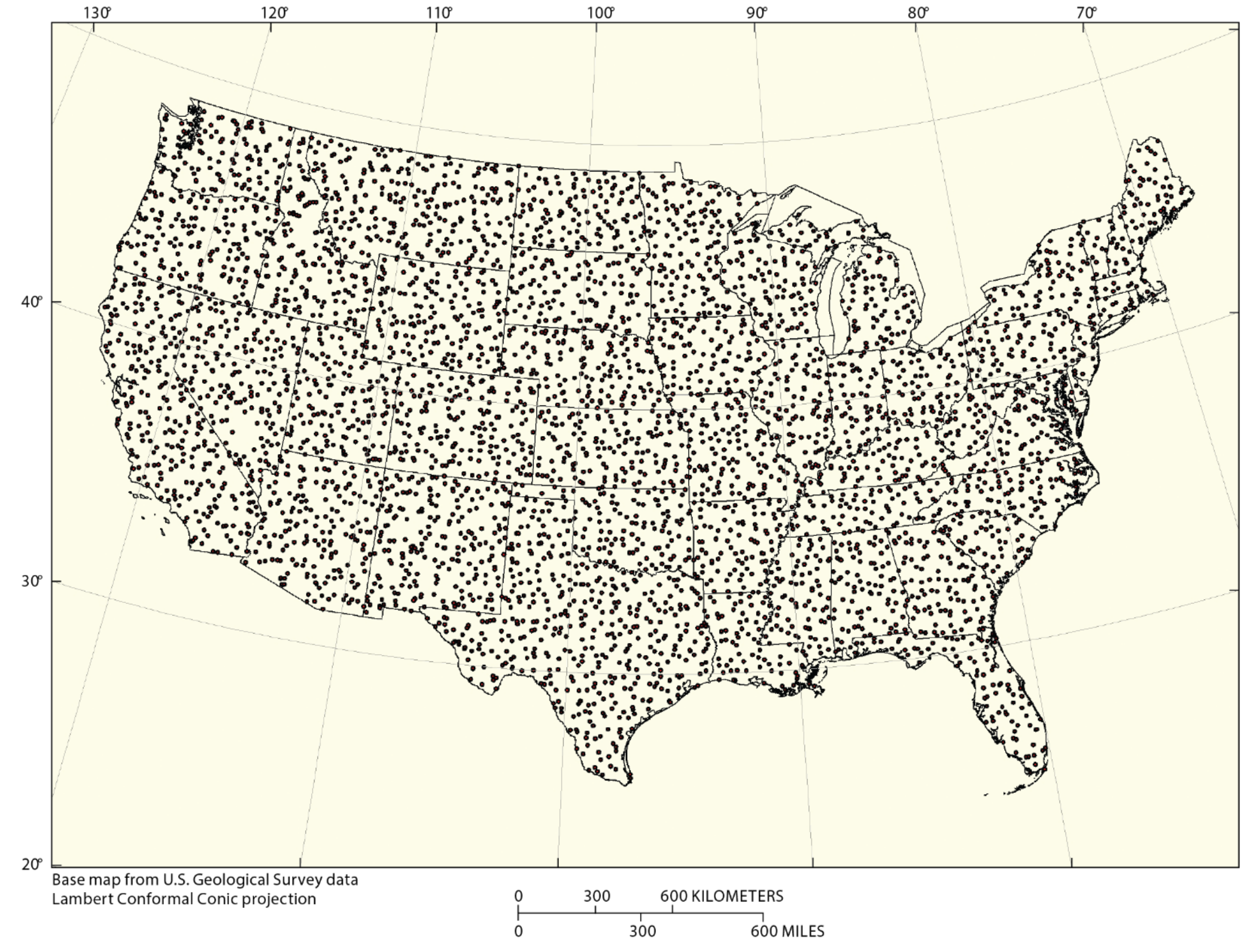
Media	Pb Concentration	Years Data Obtained	References
	Range: 13.4–72.92 mg Pb/L Guadalupe: Mean: 20.2 ± 5.17 mg Pb/L Range: 14.2–35.8 mg Pb/L San Antonio: Mean: 28.8 ± 5.23 mg Pb/L Range: 21.97–38.34 mg Pb/L Nueces/Frio: Mean: 22.33 ± 4.67 mg Pb/L Range: 14.05–32.27 mg Pb/L		
Saltwater	Global Range: 0.01–27 μg Pb/L Open-Ocean Range: 0.01–4.8 μg Pb/L	Reported in studies dated 1977–1990	Sadiq (1992)
Vegetation	Lichens: 0.3–5 mg Pb/kg (dry weight) (Set of National Parks in western U.S.)	2002–2007	Blett (2010)
	Leaves from woody shrubs and trees from 54 sites in Los Angeles, Orange, San Bernardino and Riverside counties in California *Adenostoma fasciculatum* Mean: 0.17 ± 0.08 (SE) mg Pb/kg *Artemisia californica* Mean: 0.16 ± 0.01 (SE) mg Pb/kg *Baccharis salicifolia* Mean: 0.22 ± 0.03 (SE) mg Pb/kg *Encelia farinosa* Mean: 0.20 ± 0.02 (SE) mg Pb/kg *Eriogonum* spp. Mean: 0.23 ± 0.03 (SE) mg Pb/kg *Heteromeles arbutifolia* Mean: 0.42 ± 0.17 (SE) mg Pb/kg *Malosma luarina* Mean: 0.38 ± 0.06 (SE) mg Pb/kg *Quercus agrifolia* Mean: 0.29 ± 0.04 (SE) mg Pb/kg	2019	Mackowiak et al. (2021)
Vertebrates	Fish (sampled from 111 sites in 9 river basins of large U.S. rivers): Mean: 0.07 mg Pb/kg (wet weight) (whole fish); Median: 0.10 mg Pb/kg (wet weight) (whole fish); 85th percentile: 0.27 mg Pb/kg (wet weight) (whole fish); Max: 9.29 mg Pb/kg (wet weight) (whole fish)	1995–2004	Hinck et al. (2009)
	Fish (96 sites in large U.S. rivers): Female bass (*Micropterus* spp.): median: 0.04 mg Pb/kg; mean: 0.06 ± 0.02 mg Pb/kg (wet weight) (whole fish) Male bass (*Micropterus* spp.): median: 0.03 mg Pb/kg; mean: 0.05 ± 0.01 mg Pb/kg (wet weight) (whole fish) Female carp (*Cyprinus carpio*): median: 0.10 mg Pb/kg; mean: 0.11 ± 0.01 mg Pb/kg (wet weight) (whole fish) Male carp (*Cyprinus carpio*): median: 0.09 mg Pb/kg; mean: 0.12 ± 0.01 mg Pb/kg (wet weight) (whole fish)	1995–2004	Hinck et al. (2008)

Media	Pb Concentration	Years Data Obtained	References
	Dolphinfish (*Coryphaena hippurus*) in southern Gulf of California (wet weight) (muscle tissue): Mean: 0.059 mg Pb/kg	2006–2015	Gil-Manrique et al. (2022)
	Fish (from a set of national parks in western U.S.): 0.0033 (fillet) to 0.97 (liver) mg Pb/kg (dry weight)	2002–2007	Blett (2010)
	Anna's hummingbirds (*Calypte anna)* surveyed in coastal, valley and Sierra Nevada foothills regions of northern California Mean: 0.23 ± 0.25 mg Pb/kg; range: 0.00–1.35 mg Pb/kg (body feathers; live) (dry weight) Mean: 3.00 ± 7.64 mg Pb/kg; range: 0.28–46.0 mg Pb/kg (body feathers; carcasses) (dry weight) Mean: 1.01 ± 3.10 mg Pb/kg; range: 0.01–16.9 mg Pb/kg (liver) (dry weight) Mean: 0.94 ± 2.07 mg Pb/kg; range: 0.03–12.43 mg Pb/kg (kidney) (dry weight) Mean: 8.17 ± 36.27 mg Pb/kg (combined feathers) (dry weight)	2015	Mikoni et al. (2017)
	Neotropic Cormorants (*Phalacrocorax brasilianus*) surveyed in Lake Livingston, Texas: Female mean: 4.92 ± 4.11 (SE) mg Pb/kg (breast feathers) (dry weight) Male mean: 1.68 ± 0.822 (SE) mg Pb/kg (breast feathers) (dry weight) In Richland Creek Wildlife Management Area, Texas: Female mean: 0.191 ± 0.044 (SE) mg Pb/kg (breast feathers) (dry weight) Male mean: 0.115 ± 0.015 (SE) mg Pb/kg (breast feathers) (dry weight)	2014	Mora et al. (2021)
Invertebrates	7 earthworm species in northeastern U.S. Overall mean: 29 ± 6 (SE) mg Pb/kg (dry weight) *Amynthas agrestis* mean: 21 ± 11 (SE) mg Pb/kg (dry weight) *Aporrectodea rosea* mean: 43 ± 5 (SE) mg Pb/kg (dry weight) *Aporrectodea tuberculata* mean: 30 ± 7 (SE) mg Pb/kg (dry weight) *Dendrobaena octaedra* mean: 43 ± 20 (SE) mg Pb/kg (dry weight) *Lumbricus rubellus* mean: 24 ± 5 (SE) mg Pb/kg (dry weight) *Lumbricus terrestris* mean: 14 ± 4 (SE) mg Pb/kg (dry weight) *Octolasion cyaneum* mean: 20 ± 8 (SE) mg Pb/kg (dry weight)	2013	Richardson et al. (2015b)
	Oysters (*Crassostrea virginica*) and mussels (*Mytilus edulis)* in east coast U.S. Range: 0.11–2.2 mg Pb/kg Pb (dry weight)	2003–2006	Shiel et al. (2012)

Media	Pb Concentration	Years Data Obtained	References
	Oysters (*Crassostrea gigas)* in west coast Canada Range: 0.05–0.22 mg Pb/kg Pb (dry weight)	2002–2004	Shiel et al. (2012)

CI = confidence interval; IQR = Interquartile range; Pb = lead; SE = Standard error.
This table updates Pb non-air media and biota concentration data from Tables 1-1 and 6-2 in the 2013 Pb ISA (U.S. EPA, 2013). Sources of concentration data are limited to regional or national-scale studies.

Several large-scale surveys of soil Pb concentrations were identified for inclusion in the ISA. The United States Geological Survey (USGS) North American Soil Geochemical Landscapes Project (NASGLP) (Smith et al., 2013a) is a recent soil survey that supplants Shacklette and Boerngen (1984), the national soil survey cited in the 2013 Pb ISA, because of the larger size and extent, use of modern geostatistical sampling methods, increased sampling resolution and documented data quality validation (Smith et al., 2013a). Shacklette and Boerngen (1984) collected 1,319 samples of Pb at a depth of 20 cm along U.S. roadways between 1961 and 1976. The NASGLP provides a more comprehensive survey of soil Pb in the conterminous United States because the survey employed a spatially balanced, sampling-location selection method and collected soil samples from multiple depths at each selected location. Samples were taken from depths of 0–5 cm in A-horizon and C-horizon soils at 4,857 sites systematically selected using a generalized random tessellation stratified design in 2007–2010 (Figure 11-1). Soil Pb concentrations were determined by inductively coupled plasma atomic emission spectroscopy and inductively coupled plasma mass spectrometry analyses. Measurements were validated using documented quality assurance and quality control procedures. A review of seven national-scale geochemical datasets compared the NASGLP survey design to that of Shacklette and Boerngen (1984) and discussed the methodological issues with other prior national-scale geochemical surveys that NASGLP was designed to address (Smith et al., 2013b). Summary statistics of conterminous U.S. soil Pb concentrations from Smith et al. (2013a) are provided in Table 11-1. Regional studies of soil Pb, including Richardson et al. (2014), which provides information on temporal trends of Pb concentrations in northeastern forest floor soils, and Mackowiak et al. (2021), which surveyed soil and vegetation Pb concentrations in four counties in southern California, are summarized in Section 11.2.3.



Source: Smith et al. (2013a).

Figure 11-1 Locations of the 4,857 soil sampling sites included in the U.S. Geological Survey North American Soil Geochemical Landscapes Project conducted from 2007 to 2010.

The 2006 Pb AQCD and 2013 Pb ISA reported representative Pb concentrations in fresh surface water (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L) and freshwater sediments (median 28 mg Pb/kg dry weight, range 0.5 to 12,000 mg Pb/kg dry weight) in lotic systems in the United States based on a synthesis of National Water Quality Assessment (NAWQA) data (U.S. EPA, 2013, 2006). Another analysis of the NAWQA data set provides additional detail to the prior 2006 Pb AQCD analysis by stratifying the summary of Pb concentrations in freshwater sediment by land use within river basins (Horowitz and Stephens, 2008). The baseline freshwater sediment concentration, comprising measurements taken in low-population areas only, is reported to have a median of 20 mg Pb/kg with a range of 2 to 200 mg Pb/kg. Land-use categories for agricultural, cropland, pasture, forested and rangeland sites are reported in Table 11-1. A more recent survey of Pb concentrations in freshwater sediment found higher concentrations in Atlantic rivers (mean 110 mg Pb/kg) compared with Pacific and Gulf of Mexico rivers (means of 19 and 32 mg Pb/kg, respectively) (Horowitz et al., 2012). This observed spatial variation in freshwater sediment Pb concentrations is likely driven by higher historical population density and industrial activity associated with Pb emissions in the eastern United States compared with the central and western regions of the country. Mahler et al. (2006) dated sediment cores and reported a decline in Pb concentrations in sediment deposited between the 1970s and the 1990s, which corresponds to the phasing out of widespread use of leaded gasoline. One additional regional survey of dissolved and particulate Pb in fresh surface water was identified for inclusion in this ISA. In a study of water quality in eight Texas rivers, Jiann et al. (2013) identified elevated particulate and dissolved Pb near areas with greater anthropogenic influence and noted that Pb concentrations were decreased downstream of dams and reservoirs, where slow-moving water causes suspended Pb to settle into sediment. Summary statistics of the rivers included in Jiann et al. (2013) are included in Table 11-1. Additional information on temporal trends observed in aquatic ecosystems is summarized in Sections 11.3.3 and 11.4.3.

No new surveys in coastal areas of the United States measuring dissolved Pb in saltwater or Pb in saltwater sediment were identified for inclusion in this Pb ISA, although concentrations measured from 1984 to 1987 are included in Table 11-1 to provide additional information on Pb concentrations in saltwater sediment (Cantillo and O'Connor, 1992). The 2013 Pb ISA (U.S. EPA, 2013) reported saltwater dissolved and sediment Pb concentrations from studies dated 1977 to 1990 summarized in Sadiq (1992), which reports a global range of 0.6 to 1,050 mg Pb/kg in saltwater sediment, although the authors noted that the maximum value reported was observed in an Australian inland saltwater lake. Observations from only one U.S. saltwater sediment study were reported in Sadiq (1992), in which Pb concentrations ranging from 13.4 to 52.8 mg Pb/kg from Puget Sound were recorded. Sadiq (1992) remains the only study identified for inclusion in the ISA in which global dissolved saltwater Pb concentrations are reported. Excluding observations from inland seas, open-ocean concentrations of dissolved Pb ranged from 0.01 to 4.8 μg Pb/L. Pb measurement methods have developed substantially in the last few decades, and measurements of dissolved Pb from older studies may be less accurate than those measured using modern methods. Table 11-1 summarizes the information available on concentrations of dissolved and sediment Pb observed in U.S. saltwater aquatic ecosystems.

Information on Pb concentrations observed in regional surveys of U.S. biota at sites located far from significant modern point sources of Pb have been collated in Table 11-1. The included surveys provide a range of reference values which may provide context for Pb concentrations observed in similar species and ecosystems. The Western Airborne Contaminants Assessment Project (WACAP) is the most comprehensive database on contaminant transport and depositional effects in U.S. sensitive ecosystems (U.S. EPA, 2013; Blett, 2010; Landers et al., 2010), although it only covers locations in the western part of the country. The project aimed to assess the locations where atmospheric pollutants were accumulating due to deposition in remote ecosystems in the western United States and identify the most likely sources of the identified pollutants. Pb (and other pollutants) was measured in sediment, snow, water, lichen, and fish at eight western U.S. national parks. For species sampled across multiple national parks, Pb concentrations in biota in terrestrial and aquatic ecosystems surveyed in this project were reported in the 2013 Pb ISA and are included in Table 11-1.

Recent regional surveys of Pb in terrestrial ecosystems published in the peer-reviewed literature include Anna's hummingbirds (*Calypte anna*) surveyed in the coastal, valley and Sierra Nevada foothills regions of northern California (Mikoni et al., 2017) and cormorants (*Phalacrocorax brasilianus*) sampled from two colonies in Lake Livingston and Richland Creek, Texas (Mora et al., 2021). A summary of feather Pb concentrations observed in each of these studies is included in Table 11-1. The study of Anna's hummingbirds is unique in its investigation of bioaccumulation of metals in a nectar-feeding bird species. The sources of Pb measured in hummingbird organs and feathers were not determined in this study, but the authors listed absorption from food sources including plant and insect species, particularly those living in urban environments, as the most likely routes of exposure (Mikoni et al., 2017). Mora et al. (2021) investigated the interaction between location and sex on Pb concentrations in cormorant feathers in the Trinity River watershed in Texas and found no statistically significant effect for either variable.

A study of seven species of earthworms at nine sampling sites in the northeastern United States was conducted alongside a concurrent soil survey that characterized the properties of the soil from which the earthworm specimens were collected (Richardson et al., 2015b). This study provides an example of how Pb from many sources in environmental media is distributed throughout a regional terrestrial ecosystem, observed in both earthworms and the soil they inhabit. Earthworm Pb concentrations were found to be poorly correlated with the Pb concentrations in the soil horizons they were sampled from, which is explained in part by the selectiveness of earthworms' feeding and the unknown fraction of bioavailable Pb in the measured soil Pb. Concentrations measured in earthworm species sampled in Richardson et al. (2015b) are summarized in Table 11-1.

Surveys of mussels (*Mytilus* sp.) and oysters (*Crassostrea* spp.) have been used to monitor Pb concentrations in coastal ecosystems. The U.S. national Mussel Watch project (discussed in aquatic temporal trends Section 11.4.3) has served as a biomonitoring network for Pb in coastal U.S. ecosystems (Kimbrough et al., 2008). An analysis of 2003–2006 Mussel Watch data including oysters (*Crassostrea gigas, Crassostrea virginica*) and mussels (*Mytilus edulis*) identified a higher range of Pb concentrations on the east coast of the United States relative to the west coast of Canada (Shiel et al., 2012) (Table 11-1). In this study, isotopic analysis and the covariance of cadmium (Cd) and zinc (Zn) were used to identify the sources of Pb. Higher concentrations of Pb in the oysters and mussels on the east coast are attributed to coal combustion and industries such as smelting and steelmaking.

The Large River Monitoring Network of the Biomonitoring of Environmental Status and Trends (BEST-LRMN) surveyed fish from nine U.S. river basins from 1995 to 2004. This survey is the most recent national-scale survey of Pb concentrations observed in biota in freshwater aquatic ecosystems, with results summarized in two studies. Hinck et al. (2008) measured species-dependent Pb concentrations in whole-fish common carp (*Cyprinus carpio*) and black bass (*Micropterus* spp.), and Hinck et al. (2009) presented average Pb concentrations measured across species including black bass, white bass (*Morone* spp.), catfish (*Ictaluridae*), northern pike (*Esox lucius)*, northern pikeminnow (*Ptychocheilus oregonensis*), burbot (*Lota lota)*, trout (*Salmonidae*), pikeperch (*Sander* spp.), and goldeneye (*Hiodon* *alosoides*) (Table 11-1; summary statistics of Pb observations are presented with each included species combined). The BEST-LRMN survey is the most comprehensive study of bioaccumulation of Pb in fish from U.S. ecosystems.

11.1.4 Concepts Related to Ecosystem Effects of Pb

Organism exposure and response to Pb in the various environmental media must be considered in the context of the ecosystem. An ecosystem is a functional unit consisting of living organisms, their nonliving environment, and the interactions within and between them (Allwood et al., 2014). The boundaries of what could be called an ecosystem are somewhat arbitrary, depending on the focus of interest or study. Thus, the extent of an ecosystem may range from very small spatial scales to, ultimately, the entire biosphere (Allwood et al., 2014). Ecosystems can be natural, cultivated, or urban (U.S. EPA, 1986) and may be defined on a functional or structural basis. "Function" refers to the suite of processes and interactions among the ecosystem components that involve energy or matter. Examples include water dynamics and the flux of trace gases such as rates of photosynthesis, decomposition, and nutrient cycling. Biotic or abiotic structure may also define an ecosystem. Abiotic structure includes climatic and edaphic components. Biotic structure includes species abundance, richness, distribution, evenness, and composition measured at the population, species, community, ecosystem, or global scale. A species (for eukaryotic organisms) is generally defined by a common morphology, genetic history, geographic range of origin, and ability to interbreed and produce fertile offspring. A population consists of interbreeding groups of individuals of the same species that occupy a defined geographic space. Interacting populations of different species occupying a common spatial area form a community (Barnthouse et al., 2008). Community composition may also define an ecosystem type, such as a pine forest or a tall grass prairie. Pollutants can affect the ecosystem structure at any of these levels of biological organization (Suter et al., 2005).

When an ecological receptor encounters Pb, this metal may affect uptake processes and/or interact with biological membranes. In some instances, depending on the form of Pb and prevailing environmental chemistry, Pb is taken up by biota which can then lead to a biological response. The alteration of cellular ion status (including disruption of Ca^{2+} homeostasis, altered ion transport mechanisms, and perturbed protein function through displacement of metal cofactors) appears to be the major unifying mode of action underlying all subsequent modes of action in plants, animals, and humans (U.S. EPA, 2013). Molecular mechanisms linked to oxidative stress may induce DNA damage and generation of reactive oxygen species (ROS), leading to protein modification, lipid peroxidation, and altered enzyme response. Initial perturbations such as cytological or biochemical changes associated with Pb exposure may cascade up to effects at higher levels of biological organization (i.e., from the subcellular and cellular level through the individual organism and up to ecosystem-level processes). In this ISA, biochemical (e.g., enzymes, stress markers) endpoints at the suborganism level of biological organization are grouped under the broad endpoint of "physiological stress." Organism-level effects include reproduction, growth, and survival. These endpoints also have the potential to alter population, community, and ecosystem levels of biological organization (Suter et al., 2004). Causality determinations for ecological effects of Pb in the 2013 Pb ISA used biological scale as an organizing principle to summarize effects on vegetation, invertebrates and vertebrates in terrestrial, freshwater and saltwater environments. The same approach is applied in this appendix, focusing especially on the organism-level endpoints of reproduction, growth, survival, and effects on ecosystems.

In natural environments, where many variables that may impact the effects of interest are left uncontrolled, partitioning the variability of responses and attributing observed effects to Pb unequivocally is difficult. The presence of confounding factors that is characteristic of field observational studies is also compounded by high natural variability in organismal genetics and in abiotic seasonal, climatic, water chemistry or soil-related factors (U.S. EPA, 2015). In natural environments, modifying factors affect Pb bioavailability and toxicity, and considerable uncertainties are associated with generalizing effects observed in controlled studies to effects at higher levels of biological organization. Differences in environmental chemistry may enhance or inhibit uptake of metal from the environment, thus creating a spatial patchwork of environments that are at greater risk than other environments. Similarly, organisms vary in their degree of adaptation to, or tolerance of, the presence of metals. Generally, the correct attribution of effects to Pb is expected to be most challenging in studies that examine its effects on entire ecosystems, as they incorporate all of the ecological interactions among the various populations and all of the chemical and biological processes that affect Pb bioavailability (Section 11.1.6). The fundamental principles of how metals interact with organisms and ecosystems are described in detail in U.S. EPA's Framework for Metals Risk Assessment (U.S. EPA, 2007).

11.1.5 Ecosystem Services

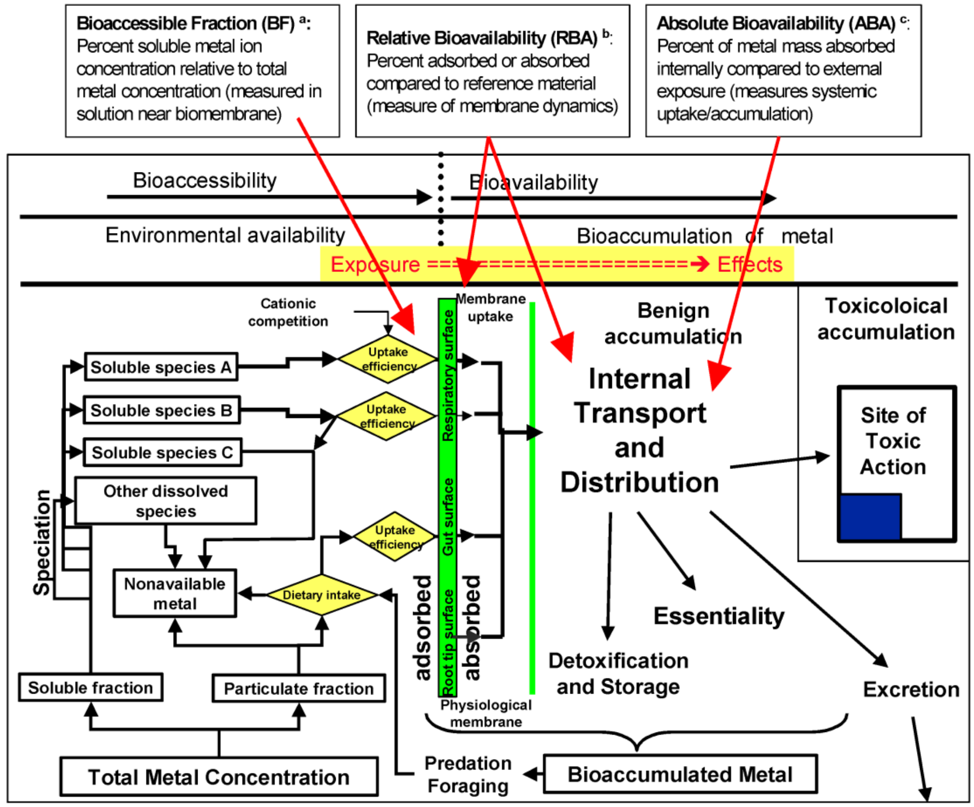
In general, both ecosystem structure and function play essential roles in providing goods and services. "Ecosystem services" refers to the concept that ecosystems provide benefits to humans, directly or indirectly (Costanza et al., 2017), and that ecosystems produce socially valuable goods and services deserving of protection, restoration, and enhancement (Boyd and Banzhaf, 2007). The concept of ecosystem services recognizes that human well-being and survival are not independent of the rest of nature, but rather that humans are an integral and interdependent part of the biosphere (Costanza et al., 2017). In some cases, ecosystem services analysis can result in attaching monetary values to ecosystem outcomes. However, because ecosystem services are often public goods, their benefits can be difficult to monetize. Although the ecosystem services literature has expanded since the 2013 Pb ISA, there are few publications that specifically link an ecological effect attributed to Pb to a change in an ecosystem service. No new studies were identified that explicitly address Pb effects on ecosystem services associated with terrestrial, freshwater, or saltwater systems.

11.1.6 Bioavailability

As discussed in prior AQCDs and sections 6.6.3 (terrestrial), 6.4.4 (freshwater) and 6.4.14 (saltwater) of the 2013 Pb ISA (U.S. EPA, 2013), bioavailability is a key concept for understanding Pb effects on the biotic components of ecosystems. U.S. EPA defines bioavailability as “the extent to which bioaccessible metals absorb onto, or into, and across biological membranes of organisms, expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time and under defined conditions” (U.S. EPA, 2007). This section presents a general overview of bioavailability and introduces modifying factors and models to estimate bioavailability. Chemical and biological modifying factors affecting bioavailability and subsequent toxicity to biota are considered in more detail in the following sections: Section 11.2.2 (terrestrial), Section 11.3.2 (freshwater) and Section 11.4.2 (saltwater).

Bioavailability increases with the amount of Pb available as free Pb ions (U.S. EPA, 2013). Factors affecting bioavailability and subsequent effects of Pb on biota include chemical factors that can be quantitatively linked to toxicity. In soils, these include but are not limited to pH, cation exchange capacity (CEC) and organic carbon (OC) content. In aquatic systems, water chemistry conditions including hardness, pH, alkalinity and colloidal or dissolved OC (DOC) as well as the presence of other metals affect the availability of Pb at sites of action on biological membranes. In saltwater, higher levels of ions additionally affect Pb bioavailability. In sediments, Pb bioavailability may be influenced by the presence of other metals, sulfides, iron (Fe) and manganese (Mn) oxides, and physical disturbance. In addition to chemical factors, biological factors (see Section 7.2.3, (U.S. EPA, 2006) and Section 6.4.9, (U.S. EPA, 2013)) affect bioavailability; however, they are more difficult to link quantitatively to toxicity.

The bioavailability of a metal is also dependent upon the fraction of metal that is bioaccessible. As stated in the Framework for Metals Risk Assessment (U.S. EPA, 2007), the bioaccessible fraction of a metal is the portion (fraction or percentage) of environmentally available metal that interacts at the organism’s contact surface and is potentially available for absorption or adsorption by the organism. The framework states that “the bioaccessibility, bioavailability, and bioaccumulation properties of inorganic metals in soil, sediments, and aquatic systems are interrelated and abiotic (e.g., OC) and biotic (e.g., uptake and metabolism) modifying factors determine the amount of an inorganic metal that interacts at biological surfaces (e.g., at the gill, gut, or root tip epithelium) and that binds to and is absorbed across these membranes. A major challenge is to consistently and accurately measure quantitative differences in bioavailability between multiple forms of inorganic metals in the environment.” A conceptual diagram presented in the Framework for Metals Risk Assessment (U.S. EPA, 2007) summarizes metals bioavailability and bioaccumulation in aquatic, sediment, and soil media (Figure 11-2).



[a]BF is most often measured using in vitro methods (e.g., artificial stomach), but should be validated by in vivo methods.
[b]RBA is most often estimated as the relative absorption factor, compared with a reference metal salt (usually calculated on the basis of dose and often used for human risk, but can be based on concentrations).
[c]ABA is more difficult to measure and used less in human risk; it is often used in ecological risk when estimating bioaccumulation or trophic transfer.
Source: ERG (2004) and U.S. EPA (2007).

Figure 11-2 Conceptual diagram for evaluating bioavailability processes and bioaccessibility for metals in soil, sediment, or aquatic systems.

The development and continued refinement of models that predict toxicity by incorporating factors affecting bioavailability in aquatic systems have advanced the field of risk assessment for metals (Adams et al., 2020). The physicochemical composition of the receiving water determines the bioavailability and thus the toxicity of metals to aquatic organisms. Therefore, aquatic bioavailability models must incorporate the effects of influential aspects of water chemistry on metal toxicity. The biotic ligand model (BLM) is a mechanistically based model for predicting the toxicity of single metals under a large range of water chemistry conditions that considers complexations with inorganic ligands and competition of active free metal ions with other cations, such as calcium (Ca) and magnesium (Mg), for the site of action (i.e., biotic ligand) (Niyogi and Wood, 2004; Paquin et al., 2002; Di Toro et al., 2001). It predicts both the bioaccessible and bioavailable fraction of Pb in the aquatic environment and can be used to estimate the importance of environmental variables such as DOC in limiting uptake by aquatic organisms (Alonso-Castro et al., 2009). The U.S. EPA-recommended freshwater ambient water quality criteria (AWQC) for copper (Cu) are based on the BLM. Deforest et al. (2017) proposed a BLM-based freshwater aquatic life criteria for Pb (Section 11.3.5).

Another recent approach to describing and predicting bioavailability and subsequent toxicity of metals in aquatic environments are empirically based multiple linear regression (MLR) models, which take into consideration a wide range of endpoints and water chemistry parameters from large empirical toxicity data sets (Brix et al., 2020). Since the 2013 Pb ISA, some studies have focused on further evaluating the suitability of bioavailability models for predicting the chronic toxicity of Pb to aquatic biota (Deforest et al., 2017; Nys et al., 2016b; Nys et al., 2014), while others have explored the development and evaluation of bioavailability models to predict the acute and chronic toxicity of metals mixtures, in which Pb is a component (Nys et al., 2017; Farley et al., 2015; Santore and Ryan, 2015). A detailed consideration of the advancements in metal bioavailability modeling approaches is beyond the scope of this ISA. A recent U.S. EPA report titled *Metals Cooperative Research and Development Agreement (CRADA) Phase I Report: Development of an Overarching Bioavailability Modeling Approach to Support U.S. EPA's Aquatic Life Water Quality Criteria for Metals* evaluates and compared BLM and MLR approaches for the purpose of updating the AWQC for Pb and other metals and advocated for the use of MLR models over the BLM in future AWQC for metals (U.S. EPA, 2022). A review of the current status and regulatory applications of metal bioavailability models is provided in (Mebane et al., 2020). For historical perspective, refer to (Adams et al., 2020) and see (Brix et al., 2020) for empirical bioavailability model development.

In terrestrial environments, predicting responses to Pb exposure under field conditions from exposure-response experiments that use soluble salts of Pb to spike study soils has met longstanding difficulties, chiefly because of the differences in the many interacting determinants of bioavailability and the difficulty of identifying and quantifying those interactions. Oorts et al. (2021) recently suggested that two bioavailability corrections to the results of those experiments may be sufficient: one to adjust for percolation and aging, and the other to correct differences in toxicity that arise from differing soil properties. The authors demonstrated the derivation of predicted no-effect concentrations (PNEC) according to the European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation European Parliament and Council (2006) using the two corrections and data that conformed to the REACH requirements.

11.1.7 Risk Screening Tools

Risk assessors have developed tools for identifying the concentrations of Pb in environmental media that are at or below the thresholds for effects on ecological receptors. The following sections present ecological screening criteria available for evaluating Pb in atmospheric deposition, soil, water, sediment, and biota.

11.1.7.1 Critical Loads for Atmospheric Deposition

The critical load concept is widely used as an organizing principle to relate atmospheric deposition to ecological endpoints that indicate impairment (Pardo et al., 2011; Bobbink et al., 2010; Porter and Johnson, 2007). The definition of a critical load is “a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge” (Nilsson and Grennfelt, 1988). No recently published critical loads for Pb from terrestrial ecosystems in the United States were identified for this ISA. Several critical load studies from Europe reviewed in the 2013 Pb ISA (de Vries and Groenenberg, 2009; Hall et al., 2006; Morselli et al., 2006) and a recent review study (Koptsik and Koptsik, 2022) noted uncertainties inherent in a critical load approach to Pb risk assessment, such as soil type, critical concentration of dissolved metal, adsorption coefficients of exposed soils, combined effects of different metals in multimetal mixtures and the influences of a changing climate. Since the 2013 Pb ISA, critical load studies for atmospheric deposition for aquatic systems have largely focused on eutrophication and acidification associated with nitrogen (N) deposition, with no detailed assessments for Pb in freshwater or coastal areas in Europe (RoTAP, 2012) or the United States. In the literature search for the current assessment, no published critical loads for atmospheric deposition of Pb were identified for U.S. inland or coastal waters.

11.1.7.2 Soil Screening Levels

Developed by U.S. EPA, ecological soil screening levels (Eco-SSLs) are maximum contaminant concentrations in soils that are predicted to result in little or no quantifiable effect on terrestrial receptors. The Pb Eco-SSL was completed in March 2005 and has not been updated since. Values for terrestrial birds, mammals, plants, and soil invertebrates are 11, 56, 120 and 1,700 mg Pb/kg soil (dry weight), respectively. These conservative values were developed so that contaminants that potentially present an unacceptable hazard to terrestrial ecological receptors are reviewed during the risk evaluation process while removing from consideration those that are highly unlikely to cause substantive effects. The studies considered for the Eco-SSLs for Pb and detailed consideration of the criteria for developing the Eco-SSLs are provided in the 2006 Pb AQCD (U.S. EPA, 2006). Preference is given to studies using the most bioavailable form of Pb to derive values. Soil concentrations protective with respect to avian and mammalian exposure through diet are calculated by first converting dietary concentration to dose (mg/kg body weight per day) for a critical study, then using food (and soil) ingestion rates and conservatively derived uptake factors to calculate a soil concentration that would result in unacceptable dietary doses. This approach frequently results in Eco-SSL values below the average background soil concentration (U.S. EPA, 2005a, 2003), as is the case with Pb for the birds Eco-SSL. Sample et al. (2019) used a re-analysis of some of the early studies included in the 2005 derivation of the avian Eco-SSL to propose a new value.

11.1.7.3 Ambient Water and Sediment Quality Criteria

AWQC represent surface water concentrations intended to be protective of aquatic communities, including recreationally and commercially important species. The most recent AWQC for Pb were developed in 1984 by the U.S. EPA Office of Water, which employed empirical regressions between observed toxicity and water hardness to develop hardness-dependent equations for acute and chronic criteria for the protection of aquatic biota (U.S. EPA, 1985a). These criteria are published pursuant to Section 304(a) of the Clean Water Act and provide guidance to states and tribes to use in adopting water quality standards for the protection of aquatic life and human health in surface water. The AWQC for Pb for aquatic life are expressed as a criterion maximum concentration (CMC) for acute toxicity and criterion continuous concentration (CCC) for chronic toxicity (U.S. EPA, 2009, 1985a). In freshwater, the CMC is 65 μg Pb/L and the CCC is 2.5 μg Pb/L at a hardness of 100 mg/L.

The current U.S. EPA AWQC for Pb in freshwater, published in 1984, are hardness-based and the chronic criteria were developed based on the acute-to-chronic ratio due to the lack of chronic toxicity tests in freshwater biota at that time. Since the AWQC for Pb were first published, additional acute and chronic toxicity data has become available and better characterization of factors that influence Pb bioavailability including development of a BLM for Pb. In view of this information, several researchers have proposed updated approaches for WQC derivation for this metal. Taking into account the range of surface water chemistry across the United States and the inclusion of newer toxicity data, Deforest et al. (2017) proposed a BLM-based acute Pb criteria range from 18.9 to 998 μg Pb/L and chronic BLM-based Pb criteria range from 0.37 to 41 μg Pb/L for freshwater (Section 11.3.5). The lowest criteria were for water with low DOC (1.2 mg/L), pH (6.7) and hardness (4.3 mg/L as calcium carbonate [$CaCO_3$]), and the highest criteria were for water with high DOC (9.8 mg/L), pH (8.2) and hardness (288 mg/L as $CaCO_3$). Compared to the current U.S. EPA AWQC for freshwater, the number of genera with acute toxicity data increased from 10 to 32, and the number with chronic toxicity increased from 4 to 13, which enabled the proposed chronic criteria to be based on bioassay data rather than an acute-to-chronic ratio. Furthermore, DOC and pH are represented in BLM; these water quality factors have a significant influence on Pb bioavailability and toxicity along with hardness and other water characteristics (Adams et al., 2020).

In comparison to the freshwater chronic criteria proposed by Deforest et al. (2017), Pb effect thresholds to protect 95% of freshwater species calculated by Van Sprang et al. (2016) for seven selected European freshwater scenarios were between 6.3 μg Pb/L and 31.1 μg Pb/L, based on chronic toxicity datasets. There were several differences in development of the European thresholds for chronic Pb toxicity compared with U.S. EPA guidelines, including the use of the 10% effect concentration (EC_{10}) rather than EC_{20} chronic toxicity data, selection of species mean values rather than genus mean values and consideration of toxicity data for plants and algae in combination with bioavailability models to derive effect thresholds. Furthermore, the range of water chemistries considered did not include the high bioavailability conditions evaluated in (Deforest et al., 2017).

For freshwater sediment, U.S. EPA guidance has not changed since the 2006 Pb AQCD, and a summary of the guidance is provided here. U.S. EPA has recommended sediment quality benchmarks for Pb that, although not truly regarded as criteria, are concluded to be protective of benthic organisms. Although sediment quality criteria have not been formally adopted, U.S. EPA has published an equilibrium partitioning procedure for developing sediment criteria for metals (U.S. EPA, 2005b). For freshwater sediment, the two approaches first summarized in the 2006 Pb AQCD, based on either bulk sediment or equilibrium partitioning, continue to be used and refined. The first approach is based on empirical correlations between metal concentrations in bulk sediment and associated biological effects to derive threshold effect concentrations (TEC) and probable effects concentrations (PEC) (MacDonald et al., 2000). The TEC/PEC approach incorporates numeric guidelines to compare bulk sediment concentrations of Pb. The equilibrium partitioning approach published by U.S. EPA for developing sediment criteria for metals (U.S. EPA, 2005b) considers bioavailability by relating sediment toxicity to the porewater concentration of metals. The amount of simultaneously extracted metal (SEM) is compared with the metals extracted via acid volatile sulfides (AVS), since metals that bind to AVS (such as Pb) should not be toxic in sediments where AVS occurs in greater quantities than SEM. The SEM approach was further refined in the development of the sediment BLM (Di Toro et al., 2005). An equilibrium partitioning sediment benchmark for cationic metals, including Pb, was derived by Burgess et al. (2013). The mechanistic-based sediment quality guideline was developed from the equilibrium partitioning theory, in which the dissolved phase of Pb in sediment interstitial water serves as a surrogate for bioavailable Pb. In the equation to derive the equilibrium partitioning sediment benchmark (Equation 11-1), AVS are subtracted from SEMs to determine the amount of metal that could become bioavailable. The equation takes into account interactions with both AVS and OC.

$$\frac{\mathrm{SEM} - \mathrm{AVS}}{f_{\mathrm{OC}}} = K_{\mathrm{OC}}\mathrm{FCV}$$

Equation 11-1

The final chronic value (FCV) (µg/L) in the equation is calculated with the following formula (Equation 11-2) using a conversion factor (CF) for Pb in freshwater (Equation 11-3). The FCV for Pb in saltwater is 8.1 µg/L.

$$\mathrm{FCV} = \mathrm{CF}\left[e^{1.273(ln(hardness))-4.705}\right]$$

Equation 11-2

$$\mathrm{CF} = 1.46203 - \left[0.145712\left(ln(hardness)\right)\right]$$

Equation 11-3

The most recent aquatic life AWQC for Pb in saltwater were released in 1984 (U.S. EPA, 1985a) by U.S. EPA's Office of Water. These criteria are published pursuant to Section 304(a) of the Clean Water Act and provide guidance to states and tribes to use in adopting water quality standards for the protection of aquatic life and human health in surface water. The AWQC for Pb are currently expressed as

CMC for acute toxicity and CCC for chronic toxicity (U.S. EPA, 2009). In saltwater, the CMC is 210 μg Pb/L and the CCC is 8.1 μg Pb/L.

Since the most recent update of the U.S. EPA AWQC for saltwater, there are considerably more acute and chronic toxicity data available for saltwater organisms, which reduce uncertainties related to Pb toxicity and regulatory thresholds. For example, the 1985 CCC for saltwater was calculated based on acute-to-chronic ratios from freshwater biota (Church et al., 2017; U.S. EPA, 1985a). The U.S. EPA's guidelines for derivation of AWQC indicate that when there are sufficient data, comparison of toxicity data sets from different taxa using species sensitivity distributions (SSDs) can be performed to estimate criteria values through a probabilistic approach and to set the level of protection (U.S. EPA, 1985). The minimum diversity required to develop SSDs has historically precluded this method for saltwater biota due to lack of toxicity data. Using EC_{10} acute toxicity data from sensitive early lifestages of 13 species representing 7 taxa (phytoplankton, polychaetes, bivalves, crustaceans, echinoderms, chordates, fish) inhabiting Atlantic European coastal ecosystems, Durán and Beiras (2013) derived an acute saltwater quality criterion for Pb of 25.3 μg Pb/L from SSD. This value, derived from the lower end of the 95% confidence intervals of the 5th percentile of the SSD, is intended to protect 95% of species in 95% of cases. Church et al. (2017) proposed an updated saltwater acute criterion of 100 μg Pb/L and chronic criterion of 10 μg Pb/L based on genus mean toxicity values following U.S. EPA methodology (U.S. EPA, 1985b) (Section 11.4.5). Church et al. (2017) derive regulatory values using species sensitivity distributions (SSDs) for which some of the toxicity values are from data sources not included in the ISAs, which are also not necessarily published in peer-reviewed literature (i.e., unpublished reports, university theses, memoranda).

Methods for establishing marine sediment guidelines and sediment quality values used globally were recently reviewed by Birch (2018). Sediment quality values for U.S. waters were generally in the range of the sediment quality threshold values reported by MacDonald et al. (1996), with a threshold effects level of 30 mg Pb/kg and a probable effects threshold of 112 mg Pb/kg. A low effects threshold of 46.7 mg Pb/kg sediment and median effects threshold of 218 mg Pb/kg sediment were the sediment quality guidelines developed for the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Program (NOAA, 1999).

11.2 Terrestrial Ecosystems

11.2.1 Summary of New Information on Effects of Pb in Terrestrial Ecosystems and Causality Determination Update Since the 2013 Pb ISA

Since the 2013 Pb ISA (U.S. EPA, 2013), evidence has continued to accrue for many of the effects of Pb on terrestrial ecosystems reported in the ISA and previous U.S. EPA assessments. This additional support includes investigations of effects on species and communities that had not been studied, but none of the additional evidence is sufficient to change any of the conclusions for terrestrial ecosystems that were reached at the time. **There are no changes to existing causality determinations for terrestrial biota or ecosystems from the 2013 Pb ISA** (Table 11-2).

Additional observational studies published after the 2013 Pb ISA (U.S. EPA, 2013), many of which were anthropogenic environmental gradient studies, have linked Pb exposure and effects on microbial community structure (e.g., abundance, diversity) and function (e.g., enzyme activities, respiration rates). Many found mixed (negative, positive, or null) relationships between total or bioavailable Pb soil concentration and the abundance of bacterial and fungal taxa. It remains difficult to disentangle the effects of Pb exposure on microbial communities from the effects of other soil contaminants using anthropogenic environmental gradients, as other heavy metals and soil physicochemical properties are significantly correlated with soil Pb concentration, and many of these factors also influence microbial processes.

Studies published since the 2013 Pb ISA (U.S. EPA, 2013) continue to support previous findings that plants tend to sequester larger amounts of Pb in roots as compared with shoots and that there are species, ecotype, and cultivar-dependent differences in the uptake of Pb from soil and the atmosphere, and in translocation of sequestered Pb. In the 2013 Pb ISA (U.S. EPA, 2013), the body of evidence was sufficient to conclude there is a causal relationship between Pb exposure and plant physiological stress and a causal relationship between Pb exposure and plant growth. Evidence was inadequate to determine causal relationships between Pb exposure and both plant survival and plant reproduction. Recent studies have continued to demonstrate various deleterious physiological effects of Pb exposure on plants, particularly oxidative stress. Strong uncertainties remain regarding the concentrations at which these effects would be observed in the environment. Recent studies have examined the protective effects of mycorrhizae and of some plant nutrients when added in excess of the minimal requirements of the plants.

In terrestrial invertebrates, the evidence reviewed in the 2013 Pb ISA (U.S. EPA, 2013) was sufficient to conclude that there is a causal relationship between Pb exposure and decreased survival and between Pb exposure and reproductive and developmental effects, a likely causal relationship between Pb exposure and decreased growth, neurobehavior effects and physiological stress, and the evidence is inadequate to conclude that there is a causal relationship between Pb exposure and hematological effects. Evidence collected since then provides additional support for the effects of Pb exposure on organismal and suborganismal responses including a decrease in survival, and decreased growth and fecundity. Recently published studies on physiological responses to Pb include decreases in protein and lipid content and increases in malondialdehyde (MDA) in earthworms. Acetylcholinesterase (AChE) activity decreased in response to Pb in snails and honeybees while the effects on protein, glycogen, other enzymes, and glutathione-S-transferase (GST) responses were variable depending on the site or species examined. Several new studies quantified behavioral changes to Pb exposure in bees. Evidence also suggests that in earthworms, Pb exposure can have lasting effects on growth even postexposure on earthworms and slow the time to maturation. Pb exposure delayed onset of the breeding season and shortened duration in isopods, as well as influenced mate selection in fruit flies. Evidence published after the 2013 Pb ISA (U.S. EPA, 2013) includes new organisms as well as modifying factors of organism response such as habitat, exposure history, and seasonality.

Effects of Pb commonly observed in terrestrial vertebrates include decreased survival, and reproduction, as well as effects on development and behavior (U.S. EPA, 2006). The 2013 Pb ISA (U.S. EPA, 2013) also provided evidence for Pb effects on hormones and other biochemical variables. In the 2013 Pb ISA (U.S. EPA, 2013) the body of evidence was sufficient to conclude that there is a causal relationship between Pb exposure and reproductive and developmental effects, and between Pb exposure and hematological effects, and a likely causal relationship between Pb exposure and decreased survival, physiological stress, and neurobehavioral effects for terrestrial vertebrates. The evidence was inadequate to conclude that the relationship between Pb exposure and growth is causal for terrestrial vertebrates. Studies published since the 2013 Pb ISA provide additional evidence for effects on suborganism- and organism-level endpoints, and specifically on hematological and physiological endpoints, but they do not affect determinations of causality. New studies have expanded upon the relationship between Pb exposure and ALAD activity by adding more species of birds, amphibians, and mammals to the evidence base. More evidence of oxidative stress has been gathered, as well as evidence of effects on corticosterone levels and immunity in birds. Literature since the 2013 Pb ISA continues to add to evidence relating to reproductive effects at both the organism and suborganism levels including effects on lifetime breeding success and some specific secondary sexual traits. New studies of behavioral effects included increased aggression in mockingbirds.

Systematic studies of the validity of using results of Pb salt-addition experiments for estimating effects of Pb exposure under field conditions have continued since the 2013 Pb ISA. As previously, experiments showed that the form of Pb, pH, CEC, OC, Fe and Mn oxides, percolation, aging, and soil composition are all strong modifiers of toxicity. Recent studies demonstrated additional interactions among those variables and showed that their effects are at times mediated by additional variables such as salinity. Those studies continue to support the conclusion that data from exposure-response experiments in terrestrial environments conducted using spiking of soils with soluble salts of Pb, are unlikely to generate accurate estimates of effects in contaminated natural environments. However, Oorts et al. (2021) suggested that two corrections to the results of exposure-response experiments conducted with additions of soluble salts of Pb to soil may be sufficient to derive predicted no-effect concentrations (PNEC) according to the European REACH Regulation European Parliament and Council (2006).

In the 2013 Pb ISA (U.S. EPA, 2013) the body of evidence was sufficient to conclude that there is a likely causal relationship between Pb exposure and terrestrial-community and ecosystem effects. Some new evidence of the effects of Pb at higher levels of biological organization is available, but it is insufficient to change the determination of causality. Species interactions between tree species and their pests, and between herbaceous plants and nectar robbers, worms and lepidopteran consumers were among the new community and ecosystem endpoints for which effects of Pb were observed. Several studies found negative relationships between Pb concentration along a pollution gradient and aspects of invertebrate community structure, specifically in soil mites, potworms, insect communities on kale and nematodes. Although evidence for effects on growth, reproduction, and survival at the individual organism level and in simple trophic interactions makes the existence of effects at higher levels of organization likely, direct evidence is relatively sparse and difficult to quantify. The presence of multiple stressors, especially including other metals, continues to introduce uncertainties in attributing causality to Pb at higher levels of organization.

Table 11-2 Summary of Pb causality determinations for terrestrial plants, invertebrates, and vertebrates

Level		Effect	Terrestrial[a]	
			2013 Pb ISA[b]	2024 Pb ISA
Community and Ecosystem		Community and Ecosystem Effects	Likely Causal	Likely Causal
Population-level Endpoints	**Organism-level Responses**	Reproductive and Developmental Effects – Plants	Inadequate	Inadequate
		Reproductive and Developmental Effects – Invertebrates	Causal	Causal
		Reproductive and Developmental Effects – Vertebrates	Causal	Causal
		Growth – Plants	Causal	Causal
		Growth – Invertebrates	Likely Causal	Likely Causal
		Growth – Vertebrates	Inadequate	Inadequate
		Survival – Plants	Inadequate	Inadequate
		Survival – Invertebrates	Causal	Causal
		Survival – Vertebrates	Likely Causal	Likely Causal
		Neurobehavioral Effects – Invertebrates	Likely Causal	Likely Causal
		Neurobehavioral Effects – Vertebrates	Likely Causal	Likely Causal
	Suborganismal Responses	Hematological Effects – Invertebrates	Inadequate	Inadequate
		Hematological Effects – Vertebrates	Causal	Causal
		Physiological Stress – Plants	Causal	Causal
		Physiological Stress – Invertebrates	Likely Causal	Likely Causal
		Physiological Stress – Vertebrates	Likely Causal	Likely Causal

[a]Conclusions were based on the weight of evidence framework for causal determination in Table II of the ISA Preamble (U.S. EPA, 2015) 2013 Pb ISA (U.S. EPA, 2013).
[b]Ecological effects observed at or near Pb concentrations measured in soil, sediment, and water in Table 6-2 of the 2013 Pb ISA were emphasized and studies generally within one to two orders of magnitude above the reported range of these values were considered in the body of evidence for terrestrial systems (Section 6.3.12) (U.S. EPA, 2013).

Previous AQCDs and the 2013 Pb ISA identified uncertainties with regard to the contribution of Pb from current deposition to soil Pb concentration and subsequent toxicity to terrestrial biota, as opposed to historic contributions. Historic Pb from gasoline and other sources as well as Pb from current air and non-air sources is present in terrestrial systems and moves through the different environmental media (e.g., soil, sediment, water, biota) confounding source apportionment. The contribution of atmospheric Pb to specific sites is not clear (U.S. EPA, 2013). Furthermore, as stated in the 2013 Pb ISA, many factors, including species and various soil physiochemical properties, interact strongly with Pb concentration to modify effects. In terrestrial ecosystems, where soil is generally the main component of the exposure route, Pb aging is a particularly important factor, and one that may be difficult to reproduce experimentally. Without quantification of those interactions, characterizations of exposure-response relationships would likely not be transferable outside of experimental settings (U.S. EPA, 2013). Key uncertainties with regard to Pb effects in terrestrial ecosystems in the last review included the uncertainties expected from widening the scope of inference from controlled laboratory studies to conditions in natural environments, where many modifying factors affect Pb bioavailability and toxicity. This also applies when going from studies at low levels of biological organization to effects at higher levels. Conversely, it is difficult to partition the variability of responses and to attribute observed effects to Pb unequivocally in natural environments, where many variables that may impact the effects of interest are left uncontrolled. The presence of confounding factors that is characteristic of field observational studies is also compounded by high natural variability in organismal genetics and in abiotic seasonal, climatic, water chemistry or soil-related factors (U.S. EPA, 2015). For instance, available studies on community and ecosystem-level effects are usually from contaminated areas where Pb concentrations are much higher than typically encountered in the environment and where multiple contaminants are present.

Studies that characterize bioavailability, uptake, bioaccumulation, and effects of Pb in terrestrial ecosystems or that decrease uncertainties identified in the prior Pb NAAQS review and were published since the 2013 Pb ISA (literature cutoff for inclusion in the 2013 Pb ISA was September 2011) are presented throughout the following sections. Brief summaries of conclusions from the 1977 Pb AQCD (U.S. EPA, 1977), 1986 Pb AQCD (U.S. EPA, 1986), 2006 Pb AQCD (U.S. EPA, 2006) and 2013 Pb ISA (U.S. EPA, 2013) are included where appropriate. Recent research on the bioavailability and uptake of Pb into terrestrial biota including plants, invertebrates and vertebrates is presented in Section 11.2.2. Environmental concentrations in terrestrial biota and ecosystems in the United States at different locations and over time are discussed in Section 11.2.3. The toxicity of Pb to terrestrial biota (Section 11.2.4) is followed by data from exposure-response studies (Section 11.2.5). Responses at the community and ecosystem levels of biological organization are reviewed in Section 11.2.6.

11.2.2 Factors Affecting Bioavailability, Uptake and Bioaccumulation and Toxicity in Terrestrial Biota

Long-range atmospheric transport of Pb and natural rock weathering are the primary sources of Pb in natural systems away from anthropogenic point sources. Non-urban terrestrial ecosystems potentially affected by Pb deposition include natural forests, managed forests, grasslands, pastures, and cropland. Once deposited, Pb can be resuspended into the air or transferred among other environmental media. Pb atmospheric inputs into terrestrial ecosystems include direct deposition as well as resuspension and transport of historically deposited Pb from nearby roads and contaminated soils (Appendix 1 https://assessments.epa.gov/isa/document/&deid=359536). In terrestrial systems, Pb is distributed between biota, soil, and soil porewater. Mobility of Pb into biotic components of the ecosystem is a function of the chemical speciation of Pb and subsequent bioavailability. Bioavailability of Pb in soils (Section 11.1.6) depends on local soil physicochemical properties including pH, CEC, organic matter (OM), inorganic compounds, salinity, clay content and aging. Uptake experiments with terrestrial plants and invertebrates generally show increases in Pb uptake with increasing Pb concentration in the medium but with strong effects from several interacting factors (U.S. EPA, 2013, 2006). Below, factors that affect bioavailability of Pb in terrestrial systems are summarized along with information that advances understanding of Pb uptake in terrestrial biota since the 2013 Pb ISA.

11.2.2.1 Factors Affecting Bioavailability of Pb in Terrestrial Biota

The 2013 Pb ISA described the bioavailable fraction of Pb in soil as being strongly dependent on the fraction of Pb dissolved in soil porewater, which is primarily controlled by processes related to partitioning of Pb between liquid and solid phases: (1) solubility equilibria; (2) adsorption-desorption relationship of total Pb with inorganic compounds (e.g., oxides of aluminum (Al), Fe, silicon (Si), Mn; clay minerals); (3) adsorption-desorption relationship reactions of dissolved Pb phases on soil OM; (4) pH; (5) CEC; and (6) aging (U.S. EPA, 2013). The 2013 Pb ISA summarized studies that confirmed the role each of these six factors plays in the sequestration and release of Pb in soil porewater (U.S. EPA, 2013). Total metal loading is described by the 2013 Pb ISA as the most influential factor controlling adsorption and desorption, with higher concentrations of Pb corresponding to an overall decrease in the fraction of Pb adsorbed to organic and inorganic surfaces (U.S. EPA, 2013). However, even as the adsorbed fraction decreases with increasing metal loading, the rate of that decrease and the fraction of adsorbed Pb will vary considerably between different soil types. This variability can be attributed to differences in soil physicochemical properties, pH, CEC, OM, inorganic compounds, salinity, and aging. These physicochemical properties are based on soil forming factors: climate, organisms, parent material, relief, time, and anthropogenic input. Soils that differ in these factors will subsequently have different physicochemical properties and considerable differences in the environmentally available fraction of Pb. In addition, although predictions of bioavailability and toxicity based on environmentally available fractions using extractable or porewater concentrations are still generally supported, evidence from recent studies suggests that there may be limitations in predicting toxicity from environmentally available concentrations represented as either porewater or calcium chloride ($CaCl_2$)-extractable concentrations (Lanno et al., 2019; Bur et al., 2012; Pauget et al., 2011).

11.2.2.1.1 pH and Cation Exchange Capacity

The 2013 Pb ISA cited a study conducted by Smolders et al. (2009) wherein models of metal bioavailability calibrated from 500+ soil toxicity tests on plants, invertebrates and microbial communities indicated pH and CEC were the most important factors governing both metal solubility and toxicity. Recent literature confirms these findings and continues to highlight the important influence that pH and CEC have on Pb bioavailability. To identify the main physicochemical factors controlling Pb bioavailability in earthworms, Tang et al. (2018) conducted toxicity experiments on earthworms exposed to 13 soils with low-level Pb contamination and varying physicochemical properties. Bioaccumulation factors (BAFs) were calculated for each of the 13 soils and stepwise MLR and path analyses were used to assess the relationships between soil physicochemical properties and BAFs. Results showed that the Pb BAFs of earthworms in soils with pH <5.5 were higher than those in other soils. OC, pH, and total Pb in soil were identified as the most important physicochemical parameters controlling Pb bioavailability. The authors concluded that their results confirmed that low pH increases Pb mobility, which promotes uptake and subsequent bioaccumulation (Tang et al., 2018). Romero-Freire et al. (2015) demonstrated the important influence of pH on bioavailability by measuring Pb toxicity to plants and bacteria exposed to aqueous extracts from seven soils with different physicochemical properties. Both Pb solubility and toxicity were significantly correlated with pH, CO_3 and OC. Of the seven soils that were assessed, sandy acidic soil with the lowest pH was associated with the highest extractable Pb concentration and the lowest half maximal effect concentration (EC_{50}) value for the plant bioassay. Wijayawardena et al. (2015) investigated the relationship between soil properties and relative bioavailability in swine exposed to 11 different soils spiked with Pb. Freundlich partition coefficients (K_d) were calculated for each soil, and stepwise regression analysis was used to evaluate the relationships between different soil properties and relative bioavailability as well as K_d partition coefficients. Regression models showed that pH and clay content were the most influential soil properties, accounting for 85% and 54% of variability in K_d and the relative bioavailability of Pb, respectively. Lanno et al. (2019) examined the effects of physicochemical properties on the toxicity of Pb to two different soil invertebrates, collembolans (*Folsomia candida*) and earthworms (*Eisenia fetida*), in seven different soils spiked with Pb salts at varying concentrations. EC_{50} values varied considerably amongst the different soil types, ranging from 35 to 5,080 mg/kg for earthworms and 389 to >7,190 mg/kg for collembolans. BAFs were also calculated for earthworms and varied with a >10-fold range across the different soil types. Effective CEC (eCEC) and soil properties related to eCEC including total C, exchangeable Ca and Mg and clay content had a significant effect on both Pb toxicity and bioaccumulation as well as the toxicity thresholds EC_{10} and EC_{50} in earthworms. However, there were no correlations between soil properties and Collembola toxicity threshold concentrations. The authors suggested that reduced toxicity in Collembola may be attributed to species-dependent differences in Pb uptake across epidermal surfaces, specifically the sclerotized cuticles of collembolans may reduce the uptake of Pb^{2+} across epidermal surfaces, limiting uptake to intestinal absorption from ingestion of soil porewater. The study also assessed whether variability in toxicity values was better explained using exposure estimates based on environmental available fractions (measured as

Pb^{2+} in porewater or as total dissolved Pb in porewater) rather than total Pb in soil. The results showed greater variation in EC_{50} values based on environmentally available fractions compared with EC_{50} values based on total Pb soil concentrations. These results combined with significant correlations between earthworm endpoints and eCEC, but not pH, may suggest that eCEC reduces Pb uptake by cation exchange of Pb^{2+} in both clay and OC coupled with competition for uptake between multiple cations at the surface of the earthworm epithelium. The competition for cation uptake at the epithelial surface may also extend to H^+, which may help explain why toxicity thresholds were not correlated with pH. Additional explanations for greater toxicity variability in porewater may also be due to unexplained chemical interactions between Pb^{2+} and soil porewater as well as the physiological mechanisms of earthworm absorption and metabolism. Similar results were reported in a study that examined Pb and Cd bioavailability in soils located in the vicinity of a smelter; uptake rate constants of Pb in earthworms were significantly greater at higher pH. Giska et al. (2014) suggested that higher pH may be associated with a decrease in competition between heavy-metal ions and H^+ ions for binding sites on biotic ligands.

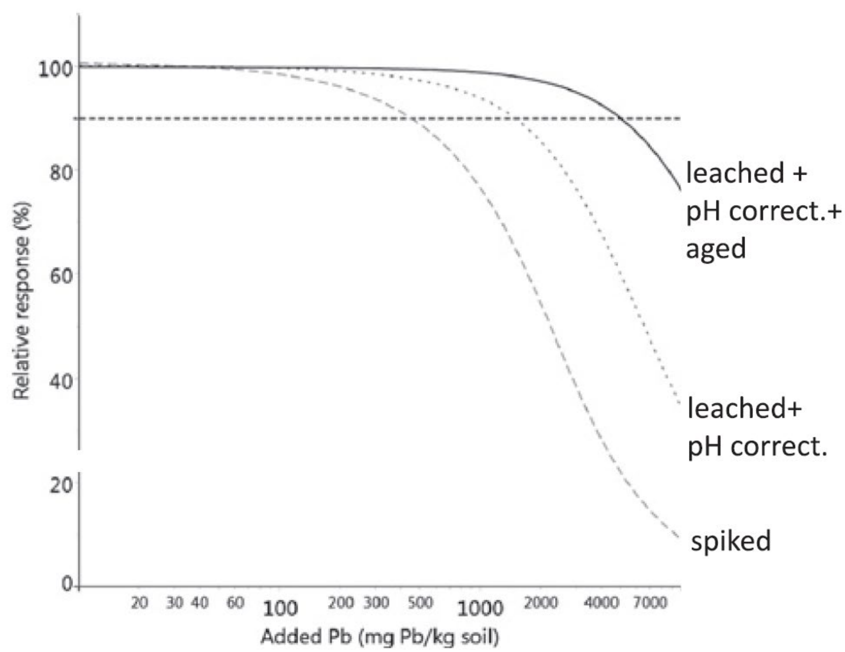
11.2.2.1.2 Organic Matter and Inorganic Compounds

The 2013 Pb ISA described the significant roles that both organic and inorganic soil constituents play in immobilizing Pb and decreasing bioavailability. Surfaces of both OM and inorganic materials (clays and sesquioxide minerals) contain negatively charged functional groups, which serve as sites of Pb adsorption. In addition, Pb can form immobile precipitates with CO_3, phosphate and sulfate that may also be present in soil porewater. Shaheen and Tsadilas (2009) noted that soils with higher clay content, OM, total $CaCO_3$ equivalent and total free sesquioxides also exhibited higher total Pb concentration, indicating that less Pb had been taken up by resident plant species.

While recent studies confirm findings from the 2013 Pb ISA regarding the roles of OM and inorganic surfaces in Pb immobilization, they also suggest that OM is capable of increasing or decreasing Pb mobility. Shahid et al. (2012) reviewed the role of humic substances on Pb phytoavailability and toxicity and concluded that the overall role of humic substances in Pb bioavailability is complex due to the heterogenous nature of humic substances and varying soil physicochemical properties. Depending on both of these factors, humic substances may exist as dissolved OM (DOM) capable of binding free Pb^{2+} in soil porewater, as solid constituents with high adsorption affinity for Pb or as DOM capable of increasing the extractable and bioavailable fractions of Pb. de Santiago-Martín et al. (2014) used bioassays with romaine and iceberg lettuce grown in calcareous Mediterranean soils with low levels of OM that were spiked with Pb, Cu, Cd and Zn to assess the contribution of soil physicochemical properties toward bioavailability. CO_3, OM and fine mineral fractions accounted for 85% of the variance in bioavailability, and OM was the most important variable explaining Pb and Cd bioavailability patterns. However, OM seemed to exert contrary effects on Pb and Cu bioavailability. At lower concentrations of the metals, OM and bioavailability were negatively correlated, but a positive correlation was observed at higher concentrations. The authors suggested that differences in the role OM had at different concentrations may be attributed to competitive binding between Pb and Cu onto humic acids, resulting in a larger bioavailable fraction at higher concentrations due to saturation of binding sites on humic acids (de Santiago-Martín et al., 2014). Similar results of the contradictory role that OM may have on bioavailability were reported by Zeng et al. (2011), whereby OM was observed to have a positive correlation with ethylenediaminetetraacetic acid (EDTA)-extractable chromium (Cr), Cu, Fe, Mn, Pb and Zn, and both positive and negative correlations with concentrations in rice straw grown in the contaminated soils. Pauget et al. (2011) evaluated the influence of pH, OM and clay content on chemical availability and bioavailability of Pb to land snails (*Cantareus aspersus*) exposed to nine contaminated soils, each differing by a single characteristic (pH, OM, or clay content). The results demonstrated that an increase in both pH and OM decreased Pb bioavailability to snails. However, clay did not have a significant influence. It is worth noting that the clay mineral used for this assessment was kaolinite. Kaolinite is 1:1 clay with no interlayer spaces and only external exchange sites at the edges of tetrahedral and octahedral sheets. As a result, kaolinite has a low CEC compared to other clay minerals. Other clay minerals (2:1) with both external and internal exchange sites in interlayer spaces may have had more influence on bioavailability. The authors of the study acknowledged this limitation and other studies have conveyed the important role that clay can have in decreasing metal mobility (de Santiago-Martín et al., 2013). Pauget et al. (2016) investigated the contributions of soil and lettuce to bioavailability in garden snails (*Cantareus aspersus*) and the influence of soil properties, pH, and OM on the contribution of each source. Results indicated that soil contributed to 90% of Pb bioavailability in snails exposed to both soil and lettuce, and increasing OM content further increased the contribution by an additional 6%. The authors suggested that increasing OM may have also resulted in increased DOM, which may have increased the soluble fraction of Pb through formation of DOM-Pb complexes in soil solution. An additional explanation suggested for the increased bioavailability in soil with higher OM may be an increase in ingestion rate caused by a decrease in nutrients following the addition of OM.

11.2.2.1.3 Salinity and Aging

In addition to the physicochemical properties described above, Pb mobility and bioavailability can also be influenced by salinity. Application of $CaCl_2$, MgCl or NaCl salts to field-collected soils containing 31 to 2,764 mg Pb/kg increased the proportion of the mobile metal fraction. As the strength of the salt application was increased from 0.006 to 0.3 M, the proportion of released Pb increased from less than 0.5% to over 2% for $CaCl_2$ and from less than 0.5% to over 1% for MgCl (Acosta et al., 2011). However, the majority of salinity-induced effects occurred in soils containing less than 500 mg Pb/kg, and the proportion of released Pb decreased with increasing total soil Pb concentrations. Recent literature continues to show that laboratory soils spiked with Pb^{2+} salts, which are commonly used in toxicity studies, may overestimate toxicity in corresponding field-contaminated soils (Figure 11-3) due to lack of aging as well as increases in salinity and acidification that occur after the soil has been spiked with Pb^{2+} salts (Smolders et al., 2015). Smolders et al. (2015) compared Pb toxicity between three groups of soils: (1) aged 5 years, leached and pH-corrected, (2) leached and pH-corrected, and (3) freshly spiked soils with no leaching or pH corrections. Leaching, pH correction and aging after spiking reduced toxicity to plant, microbial and invertebrate receptors by a factor of 8 (median value) based on EC_{10} values. EC_{10} values were often near background levels for freshly spiked soils, but after leaching, pH correction and 5 years of aging, the majority of EC_{10} values were above 1,000 mg/kg. The authors concluded that salinity stress, rather than acidification or aging, is the main factor explaining increased Pb toxicity in freshly spiked and unleached soils and suggested that researchers performing future toxicity tests consider spiking soils with lead monoxide (PbO) fine powder rather than $PbCl_2$ salt to exclude confounding salt effects. PbO fine powder would also be more representative of Pb that contaminates soil through atmospheric deposition. Similar results demonstrating the importance of aging were reported by Zalaghi and Safari-Sinegani (2014). In the study, soils were spiked with 0, 600, 1,200 and 1,800 mg/kb Pb as lead nitrate ($Pb(NO_3)_2$), and the environmentally available fraction of Pb and microbial toxicity were measured at select time increments across a 90-day period. The concentrations of Pb in the environmentally available fraction and microbial toxicity showed a considerable decrease over the 90-day period of the study. The authors concluded that this decrease in bioavailability was due to the transfer of Pb into CO_3 and residual fractions that occurred as a result of aging. Similar results demonstrating a decrease in Pb bioavailability following soil aging were reported by (Zhang and Van Gestel, 2019a).



Source: Smolders et al. (2015).

Figure 11-3 Change in toxicity expressed as relative responses (i.e., response relative to the mean of the corresponding control soil) for three different laboratory soil treatments: freshly spiked; spiked, leached and pH-corrected; and spiked, leached and pH-corrected with 5 years of aging.

11.2.2.1.4 Biological Factors

The severity of Pb effects on terrestrial biota depends in part upon species differences in metabolism, sequestration, and elimination rates. Because of the effects of soil aging and other bioavailability factors discussed above, in combination with differing species assemblages and biological accessibility, ecosystems may also differ in their sensitivity and vulnerability to Pb. The 2006 Pb AQCD and 2013 Pb ISA reviewed these factors, including nutritional factors, soil aging and bioavailability. Sensitivity to Pb exposure was found to vary widely among terrestrial species, even among closely related organisms. It was noted that in many species of birds and mammals, dietary factors can exert significant influence on the uptake and toxicity of Pb. Since the 2013 Pb ISA, new information on soil aging has further expanded understanding of factors that modify soil bioavailability under natural conditions.

To disentangle the effects of salinity, acidification, and aging on the sensitivity of microbial communities, plants, and invertebrates to Pb, Smolders et al. (2015) conducted an experiment in which toxicity to these groups was tested in soils spiked with Pb^{2+} salts, leached and aged. Uncontaminated soils were collected from grasslands and agricultural lands in Spain, the United Kingdom and Belgium and were exposed to 0, 250, 500, 1,000, 2,000, 4,000 or 8,000 mg Pb/kg using $PbCl_2$. Some of the soil was set aside (treatment: freshly spiked), while the rest was incubated for a week, leached using artificial rainwater and pH-corrected to maintain soil pH within 0.2 pH units within each Pb concentration using CaO (treatment: leached and pH-corrected). Five years prior to spiking soils with $PbCl_2$, additional soils were exposed to the same Pb gradient using $Pb(NO_3)_2$ and stored in perforated pots which were left outdoors to age. After 5 years, pH was corrected using CaO (treatment: aged, leached and pH-corrected). Soil solution Pb concentration, i.e., porewater Pb concentration, increased in a dose-dependent manner with spiked soils, followed by leached soils and finally aged soils containing the least soil solution Pb (except in aged soils from Spain). Toxicity was then tested in microbial communities, earthworms (*E. fetida*), Collembola (*F. candida*), tomato (*Lycopersicon esculentum*) and barley (*Hordeum vulgare*). Toxicity was highest in freshly spiked soils (mean ± S.E., EC_{50} for all organisms tested: 2,300 ± 145 mg/kb Pb), followed by leached and pH-corrected soils (6,500 ± 750 mg Pb/kg) and then aged soils (>10,000 mg Pb/kg); however, the effects of leaching with pH correction and aging with pH correction were inconsistent among organisms and toxicity tests. Depending on the origin of the soil, leaching and pH correction reduced toxicity based on EC_{10} values by a factor of 1.9–2.3 compared with freshly spiked soils, while aging and pH correction reduced toxicity by a factor of 2.7–13. Microbial activity (potential nitrification rate, substrate-induced nitrification, and respiration rate), invertebrate reproduction and plant growth were negatively correlated with total soil Pb concentration, porewater Pb concentration, Pb^{2+} ion activity and porewater ionic strength. With the exception of *E. fetida* reproduction, these factors were positively correlated with soil pH. Given porewater ionic strength had the strongest influence on toxicity across all tested organisms, the authors suggest that salt stress may modify the toxicity of Pb, as acidification and aging were unable to explain variation in toxicity.

11.2.2.1.5 Summary

In summary, studies published since the 2013 Pb ISA continue to substantiate the important role that soil geochemistry plays in sequestration or release of Pb and its bioavailability to organisms. Environmentally available concentrations, measured either in soil porewater or as extractable Pb, are generally still a useful predictor of bioavailability, although predictions cannot be transferred between experiments with soluble salts of Pb and field conditions. pH is still considered the most important factor influencing the concentration of Pb in this fraction due to its important role in Pb solubility. However, several studies have reported results that suggest limitations in using the environmentally available fraction to predict bioavailability and toxicity. These studies suggest species-dependent uptake and metabolism mechanisms as well as other soil physicochemical properties that may be involved in chemical interactions between soil porewater and biological receptors should be considered. Inorganic compounds, including clay minerals and sesquioxides, particularly Fe and Mn oxides are still considered to play important roles in Pb sequestration, and CEC is still a reliable measure of a soil's ability to sorb and exchange cations, which is an important function for Pb sequestration. The role of OM in Pb sequestration and mobility remains complex. Depending on the nature of the OM and soil physicochemical properties, Pb may bind to solid OM surfaces, decreasing Pb mobility. Alternatively, OM may enhance Pb release into soil solution through the formation of Pb-DOM complexes or following OM decomposition. Studies published since the 2013 Pb ISA also continue to highlight limitations in using laboratory soils spiked with Pb salts to predict toxicity in field-contaminated soils. Many of these studies have demonstrated that the use of Pb^{2+} salts in laboratory soils without adequate leaching, pH correction and aging greatly affects Pb bioavailability and leads to overestimating the toxicity that would be expected to occur in field-contaminated soils with similar concentrations of Pb.

11.2.2.2 Uptake and Bioaccumulation in Terrestrial Plants

Studies published since the 2013 Pb ISA continue to support previous findings that plants tend to sequester larger amounts of Pb in roots as compared with shoots, and that there are species-, ecotype-, and cultivar-dependent differences in uptake of Pb from soil and the atmosphere and translocation of the sequestered Pb (U.S. EPA, 2013, 2006, 1977). Further, many species of plants accumulate heavy metals in environments with extreme soil concentrations and are therefore used for phytoremediation at such sites. Although occasional phytoremediation studies may be informative with respect to the mechanisms of Pb uptake and tolerance, most do not add further evidence with respect to the effects of atmospheric Pb. The same applies regarding mosses and lichens as biomonitors of atmospheric Pb. Despite Pb not being a plant nutrient, it is taken up from soils through the symplastic route, the same active ion transport mechanism used by plants to take up water and nutrients and move them across root cell membranes (U.S. EPA, 2006). As with all nutrients, only the proportion of a metal present in soil porewater is directly available for uptake by plants. In addition, soil-to-plant transfer factors in soils enriched with Pb have been found to better correlate with bioavailable Pb soil concentration, defined as diethylenetriamine pentaacetate-extractable Pb, than with total Pb concentration (U.S. EPA, 2006).

Previous reviews (U.S. EPA, 2013, 2006, 1977) noted that terrestrial plants accumulate atmospheric Pb primarily via two routes: direct stomatal uptake into foliage and incorporation of atmospherically deposited Pb from soil into root tissue, followed by variable translocation to other tissues. It was recognized that most Pb taken up from soil remains in the roots and that distribution to other portions of the plant is variable among species. Most of the Pb absorbed from soil remains bound in plant root tissues either because (1) Pb may be deposited within root cell wall material or (2) Pb may be sequestered within root cell organelles, which may be a protective mechanism for the plant. Studies since the 2013 Pb ISA have generally confirmed that Pb taken up from soil largely remains in the roots (Naikoo et al., 2019; Zhou et al., 2019; Zhou et al., 2015; Meiman et al., 2012; Rossato et al., 2012).

Previous findings have shown that Pb translocation to stem and leaf tissues does occur at significant rates in some species, including some crops and herbaceous species (e.g., rattlebush, buckwheat, Chinese cabbage, pak-choi, and water spinach). There is broad variability in uptake and translocation among plant species, and interspecies variability has been shown to interact with other factors such as soil type. These results indicate significant interspecies differences in Pb uptake, as well as potential soil-dependent differences in Pb bioavailability (U.S. EPA, 2013).

Although exposures are often high, field studies carried out in the vicinity of Pb smelters and other industrial point sources have determined the relative importance of direct foliar uptake and root uptake of atmospheric Pb deposited in soils, with greater overall uptake corresponding to closer proximity to the source (Angelova et al., 2010; Hu and Ding, 2009; Cui et al., 2007). Hu and Ding (2009) concluded that metal accumulation in some leafy greens grown in the vicinity of a smelter were greater in shoot than in root tissue, which suggested both high atmospheric Pb concentration and direct stomatal uptake into the shoot tissue. Similarly, evidence since the last review shows substantial accumulation of Pb in needles in areas with high contributions of atmospheric Pb (Kandziora-Ciupa et al., 2016; Gandois and Probst, 2012). Studies also noted a significant difference between Pb concentrations in washed and unwashed leaves, indicating that aerial deposition and surface dust is likely a significant source of plant-associated Pb (Ugolini et al., 2013; El-Rjoob et al., 2008). Foliar Pb may include both incorporated Pb (i.e., from atmospheric gases or particles) and surficial particulate Pb deposition. The plant may eventually absorb the surficial component; however, the main importance of surficial Pb is its likely contribution to the exposure of plant consumers or to leaf litter. The consideration of these Pb exposures to humans via consumption of food crops is briefly discussed in Section 2.1.3 of Appendix 2.

Because of their long life spans, certain trees can provide essential information regarding the sources of bioavailable Pb. A Scots pine (*Pinus sylvestris*) forest in northern Sweden was found to incorporate atmospherically derived Pb pollution directly from ambient air, accumulating this Pb in the bark, needles, and shoots (Klaminder et al., 2005). More recent studies have also shown that accumulation in the bark of some species is a useful bioindicator of exposure to atmospheric Pb (Janta and Chantara, 2017; Palowski et al., 2016). Metal content can also vary in relation to altitude as a result of long-range transport. Korzeniowska et al. (2021) found that metal content in the moss (*Pleurozium schreberi* (Willd.) Mitten) and in Norway spruce (*Picea abies* (L.) H. Karst) in the Tatra National Park in the Carpathian Mountains of Poland was greater with increasing altitude.

Dendrochronology (tree-ring analysis) is an important tool for measuring the exposure of trees to environmental Pb (Watmough, 1999). While effectiveness may vary by species investigated, tree-ring studies reviewed in the previous AQCDs and ISAs showed that trees could be used as indicators of increasing environmental Pb concentrations with time (U.S. EPA, 2013, 2006, 1977). Trees accumulate and sequester atmospheric Pb in close correlation with the rate of point-source emissions (Guyette et al., 1991). Studies published since the 2013 Pb ISA continue to demonstrate dendrochronology is a useful tool for monitoring historical uptake of Pb into trees exposed to atmospheric or soil Pb (Sensuła et al., 2017; Dinis et al., 2016; Beramendi-Orosco et al., 2013; Doucet et al., 2012) (Section 11.2.3).

In the 2013 Pb ISA (U.S. EPA, 2013), plant-associated arbuscular mycorrhizal fungi (AMF) were found to protect the host plant from Pb uptake. Additional evidence indicates that the presence of AMF or bacteria hosts can influence Pb accumulation in and alleviate Pb stress on plants. Inoculation of David's mountain laurel (*Sophora davidii*, previously *Sophora viciifolia*) with the AMF *Funneliformis mosseae* resulted in lower concentrations of Pb in belowground and aboveground biomass (Xu et al., 2016a). *S. davidii* seeds collected from around the Qiandongshan Pb and Zn mine in northwest China were grown in pots receiving 0, 50, 500, or 1,000 mg Pb/kg (aqueous $Pb(NO_3)_2$). Half of the pots with *S. davidii* plants were inoculated with *F. mosseae*. After 4 months, mycorrhizal colonization, Pb accumulation, plant height, diameter, aboveground and belowground biomass, and root characteristics were recorded (Section 11.2.4.2). Vesicular, arbuscular, hyphal, and total root colonization of *S. davidii* decreased with increasing Pb treatment. Both mycorrhizal and nonmycorrhizal plants showed increasing Pb content in their roots and aboveground tissue in a dose-dependent manner, but belowground and aboveground Pb concentrations were lower for mycorrhizal plants. Pb concentration in aboveground tissue of mycorrhizal plants was 54%–66 % less Pb than that in nonmycorrhizal plants, while roots contained 15%–85 % less, depending on Pb exposure. The root-to-shoot Pb concentration of mycorrhizal plants increased with Pb exposure while nonmycorrhizal plant root-to-shoot concentration decreased with increasing Pb exposure, suggesting that Pb was sequestered in the root following inoculation with *F. mosseae*. Furthermore, transmission electron micrographs and X-ray microanalysis of *S. davidii* roots under different Pb and mycorrhizal treatments suggested Pb in the cytoplasm was sequestered in the cell walls and vacuoles of *F. mosseae*, while Pb was transported into the root cells and intracellular space of nonmycorrhizal plants.

Pot marigolds (*Calendula officinalis)* inoculated with *Glomus mossea* and *G. intradices* accumulated more Pb relative to nonmycorrhizal plants, yet experienced greater fitness-(Tabrizi et al., 2015). *Calendula officinalis* were grown in pots and received 0, 150, or 300 mg Pb/kg (aqueous $Pb(NO_3)_2$). Half of the plants were inoculated with a mixture of *G. mossea* and *G. intradices.* Root colonization, Pb accumulation, plant growth, reproduction flavonoid contents and nutrients were analyzed. Root colonization decreased with increasing Pb exposure in a dose-dependent manner, as root colonization in the control (0 mg Pb/kg) was 56% higher than in the high Pb treatment (300 mg Pb/kg). Pb concentration in the roots and the shoots (mg Pb/plant) increased with increasing Pb exposure. Inoculated *Calendula officinalis* had 10.3% more Pb in the roots compared with noninoculated plants, while shoots of inoculated and noninoculated plants contained the same amount of Pb. The interaction between Pb exposure and inoculation did not influence Pb uptake in aboveground or belowground biomass.

In another example, the AMF *Gigaspora margarita* increased bioaccumulation of Pb but reduced Pb-induced stress of silver banner grass (*Miscanthus sacchariflorus*) (Sarkar et al., 2018). *Miscanthus sacchariflorus* rhizomes and soil were collected from sites around the Ara River, Japan and placed in the greenhouse. The collected soil contained 0.12 mg Pb/kg. *Miscanthus sacchariflorus* received 0, 100, or 1,000 mg Pb/kg additional Pb (aqueous), and half of the plants were inoculated with *G. margarita.* After 4 months, root colonization, bioaccumulation of Pb and plant growth, survival, hormones, enzymes, nutrients, and chlorophyll content were characterized. Root colonization of *M. sacchariflorus* by *G. margarita* decreased with increasing Pb concentration for both inoculated and noninoculated plants. The Pb content of the belowground biomass of inoculated *M. sacchariflorus* was higher than the Pb content of noninoculated *M. sacchariflorus*. A similar pattern was observed for aboveground biomass, wherein inoculated plants contained equal or higher concentrations of Pb than noninoculated plants.

Inoculation of black alder (*Alnus glutonisa*) by an actinobacteria, *Frankia*, affected Pb uptake in roots and shoots (Bélanger et al., 2015). *Alnus glutonisa* seedlings were grown from seeds in the laboratory and half were inoculated with *Frankia alni* (ACN14a), isolated from *Alnus viridis* ssp. *crispa* in Québec, Canada. Half of the inoculated and noninoculated control plants were exposed to $Pb(NO_3)_2$ (0.10 mM). Pb exposure did not affect the nodule development of inoculated plants and Pb root concentration was 4.3 times lower in roots and 6.3 times higher in shoots compared with inoculated *A. gultonisa* not exposed to Pb.

In a recent study, (Gao et al., 2021) reported that the type of mycorrhizal fungi (AMF versus ectomycorrhizal fungi [EMF]) associated with seven tree species in an evergreen broadleaf forest in China does not affect uptake of Pb from roots to leaves. Foliar and root tissues were collected and analyzed for Pb concentrations as well as phosphorus (P), potassium (K), Ca, Mg, Fe, Mn, Cu, Zn, strontium (Sr), total C, and total N. Elemental concentrations in the tree were analyzed according to their mycorrhizal type (AMF versus EMF), plant organ (leaves versus roots) and an interaction term. Pb concentrations were significantly higher in the roots compared with the leaves. The elemental Pb concentrations between the roots and the leaves were uncorrelated for AMF-associated trees, EMF-associated trees, and all species, suggesting that mycorrhizal type does not influence Pb uptake in the roots or the leaves.

11.2.2.3 Uptake and Bioaccumulation in Terrestrial Invertebrates

At the time of publication of the 2006 Pb AQCD (U.S. EPA, 2006), little information was available regarding the uptake of atmospheric Pb pollution by terrestrial invertebrate species. Evidence in the 2013 Pb ISA indicated that invertebrates, especially snails and earthworms, can accumulate Pb via diet, exposure through soil, or from both exposure routes in the case of earthworms and snails. In the 2013 Pb ISA, snail Pb concentrations were reported to be lower than soil concentrations and uptake and bioaccumulation were reported to be lower than the corresponding values for other metals (U.S. EPA, 2013). Exposure routes for soil organisms are through food consumption and soil exposure; soil variables, such as pH and OM, influence uptake. Similarly, earthworm uptake is influenced by soil physicochemical properties, genus, and the vertical position earthworms occupy within the soil profile (i.e., epigeic, epi-endogeic, endogeic, anecic). Furthermore, earthworm activity in soil acts as a control on Pb bioavailability and its uptake by earthworms, potentially other soil organisms, and plants. In addition to providing supporting information on the uptake and availability of Pb to snails and earthworms, recent literature has examined the bioavailability and accumulation of Pb with many other invertebrates including lepidoptera, spiders and bees; in addition to soil factors (such as pH and OM), field characteristics, organism sex and season may also influence uptake and accumulation. Since new information has become available on organisms not discussed in previous assessments, these studies are included despite being non-U.S. based.

11.2.2.3.1 Snails

In support of the 2013 Pb ISA conclusions regarding Pb uptake by snails, recent literature continues to show snail tissue concentration is typically lower than soil concentration values. One recent study found that when Pb was examined in soil, leaves, and snail tissues at increasing distance to metal smelters, Pb in soils was, in general, highest closest to smelting plants and decreased with increasing distance. Pb content in stinging nettle leaves (*Urtica dioica*) followed the same general pattern of decreasing Pb concentration with distance as did European land snail (*Cepaea nemoralis*) digestive gland tissue. The concentration in plant tissue was positively correlated with soil level, and snail tissue concentration was positively correlated with plant tissue concentration. Patterns persisted over 4 months of exposure. Nettles are the preferred food source of *C. nemoralis* and exposure to Pb appears to be primarily through consumption. While bioaccumulation factors (BAF) were not calculated, Pb concentration in snail tissue was considerably lower than soil concentrations but was typically 2.5–3.5 times higher than plant tissues after 16 weeks of exposure (Boshoff et al., 2015) [see also (Nica et al., 2012)]. However, one recent study suggests some snail species may be greater accumulators than others. Vranković et al. (2020) sampled Roman snails (*Helix pomatia*) foot muscles and hepatopancreas tissue across a three-location urban gradient of soil Pb levels. Soil Pb varied from approximately 15 mg Pb/kg at the reference (forest), approximately 30 mg Pb/kg at the medium pollution site and approximately 110 mg Pb/kg at the high pollution site. Foot muscle and hepatopancreas tissue concentration increased with increasing exposure levels. More Pb was stored in the hepatopancreas than the foot tissue, and hepatopancreas levels were generally higher than soil contamination. BAF values were less than 1 for foot muscle (0.47, 0.9, and 0.42) and greater than 1 for hepatopancreas tissue at the low and medium pollution sites (1.61, 1.72, 0.76). The greater concentration found in the hepatopancreas indicates greater uptake via food. Concentrations reported within snail tissues in this study were higher than those reported in studies examining other snail species, suggesting uptake and accumulation are partly species-specific; see also (Mleiki et al., 2017).

New literature further supports that Pb uptake by snails is influenced by soil characteristics as well as being dose- and duration-dependent. The concentration in the digestive gland of the green garden snail (*Cantareus apertus*) increased with increasing exposure level after 1 week of exposure for low (25 mg Pb/kg), medium (100 mg Pb/kg) and high (2,500 mg Pb/kg, dietary values measured) exposure levels (Mleiki et al., 2016). However, tissue concentration was not significantly greater in the 2,500 mg Pb/kg treatment compared with the 100 mg Pb/kg treatment. Similarly, after 8 weeks of exposure, digestive gland tissue concentration was higher under Pb exposure compared with the control, but the highest concentrations were found under the 100 mg Pb/kg exposure. An observational field study examining the uptake and elimination kinetics of Pb by the common garden snail (*Cantareus aspersus*) found soil Pb concentration (positive), CEC (positive) and soil OC content (negative) have a multivariate effect on Pb bioavailability. Similarly, soil silt (positive), sand (positive) and OC content (negative) modulate Pb uptake by snails (Pauget et al., 2013b). In another study, soil Pb concentration was correlated with Pb concentration in juvenile *C. aspersus* but when OC content and Al and Fe oxides were included in the model, R^2 increased from 0.37 to 0.56. The most polluted plots (i.e., plots with the highest Pb concentration) did not have the highest Pb transfer to snails. OC content is known to influence metal mobility and bioavailability for soil organisms (Pauget et al., 2013a).

11.2.2.3.2 Earthworms

In the 2013 Pb ISA, studies of bioaccumulation of Pb in earthworms reported that many soil physicochemical properties, including pH, OM and CEC, affect metal bioavailability for these organisms; recent studies confirm these observations. Following 4 weeks in soil spiked with a solution of $Pb(NO_3)_2$ (40, 250, 500, 1,000, 2,500 mg Pb/kg, nominal soil values, concentration in worms was measured), juvenile *E. fetida* body Pb concentration increased with exposure concentration. BCFs ranged from 0.14 to 0.3, indicating either low bioavailability of Pb in the soil or low ability to accumulate Pb within tissues. After 4 weeks of recovery (no Pb exposure), earthworm body Pb was significantly lower than the value at the end of the exposure period but was still higher than the control and positively correlated with exposure values (Žaltauskaitė et al., 2020). A study on native *Eisenoides lonnbergi* earthworms in Maryland found *E. lonnbergi* can accumulate extraordinarily high levels of Pb, with a BAF of 83 recorded (Beyer et al., 2018). Accumulation was driven by soil Ca levels and indirectly by pH and clay content, not by soil Pb content or availability. In acidic, low Ca soils, Pb uptake and accumulation is greater. Over soil Ca concentrations ranging from 49 to 1,695 mg Pb/kg, *E. lonnbergi* can maintain body Ca concentrations between 4000 and 8,000 mg Pb/kg. Thus, even in Ca-poor soils, *E. lonnbergi* can uptake enough needed Ca to maintain necessary body concentrations. The Ca BCF was 3.3 in high Ca soils and 117 in low Ca soils. The Pb concentration factor was 1.02 in high Ca soils and 83 in low Ca soils, suggesting Pb is absorbed by the Ca transport system, which is known to occur in vertebrates (Beyer et al., 2018).

In *E. fetida* earthworms exposed to a range of soil Pb values from 125 to 350 mg Pb/kg across a range of pH, Pb concentration in the worms was higher in low pH (<5.5) soils than in neutral or alkaline soils with similar Pb concentrations (Tang et al., 2018). Following 4 weeks in soil spiked with a solution of Pb $(NO_3)_2$ (40, 250, 500, 1,000, 2,500 mg Pb/kg, nominal soil values, concentration in worms was measured), juvenile *E. fetida* body Pb concentration increased with exposure concentration. BCF varied from 0.14 to 0.3 indicating either low bioavailability of Pb in the soil or low ability to accumulate Pb within tissues (Žaltauskaitė et al., 2020). After 4 weeks of recovery (no Pb exposure), earthworm body Pb was significantly lower than at the end of the exposure period but was still higher than the control and positively correlated with exposure values. In another study examining earthworm Pb concentrations, BAFs in low pH soils were also higher than those in other soils but all BAFs were less than one (Richardson et al., 2015b). Soil Pb, OC, and pH together gave the best predictive model outcome on earthworm Pb concentration. Earthworm ecotype can influence Pb tissue concentrations as well. Endogeic and epigeic species were found to have higher Pb tissue concentration than epi-endogeic and anecic earthworms (Richardson et al., 2015b). A recent meta-analysis by Richardson et al. (2020) examined the influence of soil concentration, soil characteristics, earthworm genus and ecotype on trace metal uptake. They found soil concentration did not predict earthworm tissue concentration but ecophysiological group, earthworm genus, metal source, exposure duration, and soil OM were important predictors.

In studies cited in the 2013 Pb ISA, earthworm feeding and burrowing behavior altered the bioavailability, mobility, and uptake of Pb by earthworms and other soil biota. Recent studies further elucidate the effects of earthworms on soil Pb processes. One study examined the decomposition of *Amynthas agrestis* and *Lumbricus rubellus* earthworms and the subsequent release of Pb in different fractions within the soil column over 60 days (Richardson et al., 2016b). Both species had similar Pb tissue concentrations but due to the greater mass of *A. agrestis* added to experimental soils on a dry weight basis, *A. agrestis* contributed a larger pool of Pb to the soil column. Leachate from both earthworm treatments was significantly higher in Pb than leachate from control (no earthworm) soils. Exchangeable Pb pools were greater under both earthworm treatments but only at days 7 and 21. By day 60, there was only slightly more exchangeable Pb under the *A. agrestis* treatment compared with the control. The stable Pb pool was greater under earthworm treatments across all sampling dates and the majority of Pb under earthworm treatments was in the stable fraction. In a lab experiment using field-collected polluted soils, *Lumbricus terrestris* earthworms were exposed to a high Pb-contaminated soil (4,550 mg Pb/kg), a medium polluted soil (988 mg Pb/kg) and a low polluted soil (109 mg Pb/kg) for 28 days (Sizmur et al., 2011a). By the end of the exposure period, earthworms had consumed less than

2% of the bulk soil. Soil pH and water-extractable OC were higher in earthworm casts compared to control soils. Earthworm casts had greater extractable and residual Pb pools and lower reducible pools. Porewater from earthworm-inhabited highly contaminated soils had higher Pb concentrations compared with control soils. Under the medium contamination Pb soils, there was more Pb^{2+} and inorganic Pb, but less organic Pb compared with control soils. In low pollution earthworm soils, there was less Pb^{2+} but more organic and inorganic Pb compared with control soils. While earthworms only processed a small portion of the soil during the 28-day exposure treatment, the greater solubility of Pb from casts shows earthworms can alter Pb bioavailability and is tied to the changes in pH and OC of the casts.

The effect of two invasive, but widespread, species of earthworms in northeastern U.S. forests (*Amynthas agrestris* and *Lumbricus rubellus)* on litter decomposition, metal exchange, and metal bioaccumulation was examined in a laboratory experiment using forest floor material (collected from New Hampshire) with and without earthworms (Richardson et al., 2016a). Both species dwell at the soil surface either in or just below the litter layer. Pb levels in forest floor and soil were approximately 26 and 16 mg Pb/kg, respectively. After 80 days, litter mass, percent carbon, and carbon mass were all lower in the forest floor material when earthworms were present. Earthworm presence also resulted in lower exchangeable Pb fraction concentrations but there was no difference between earthworm treatments and control on the stable Pb fraction. Tissue concentration increased over time, with a BAF of 2.32 for *A. agrestris* after 80 days and 2.39 for *L. rubellus*. The BAF for the exchangeable fraction was only 104.2 for *A. agrestis* and 88.3 for *L. rubellus.* Both worms increased litter decomposition and carbon loss and lowered the exchangeable Pb fraction. However, the stable Pb pool did not respond to earthworm presence. Both earthworm species did accumulate Pb at greater concentrations than the forest floor and, as mentioned by the authors, at levels higher than the maximum tolerable level approved for poultry and mice feed, therefore posing a contamination risk to birds and small mammals. In an observational experiment in New England forests, Pb soil concentrations and pools were examined in the presence or absence of nonnative earthworms (*Aporrectodea rosea, Dendrobaena octaedra, Aporrectodea tuberculata* were most common) (Richardson et al., 2017). Like the previous study, Pb in New England soils sampled in this study represent background Pb levels in an area of the country with a history of metal enrichment via pollution. Pb concentration was lower in the Oa horizons at high abundance sites compared with low abundance sites; however, within the A and E horizons, Pb was higher at high abundance sites. Organic horizon Pb pools were negatively correlated with earthworm biomass, but total soil Pb pools showed no relationship with earthworm biomass.

In a study that examined earthworm effects on the bioavailability and mobility of metals in soil, leachates at the end of a 112-day exposure period had greater Pb concentration in the presence of *L. terrestris* earthworms (1.9 µg Pb/L) compared with control soils (1.0 µg Pb/L) (Sizmur et al., 2011b). Pb leachate from under *L. terrestris* consisted of 98.4% Pb^{2+} as free ions and 0.9% as fulvic-acid-complexed Pb compared with 95.7% and 4.0%, respectively, in control soil leachate. Soil pH was lower under all earthworm species at the end of the experiment compared with the control. Perennial ryegrass (*Lolium perenne*) was planted 28 days prior to soil sampling and harvested 21 days later. Ryegrass shoots had greater Pb concentrations when grown on columns with *L. terrestris* compared with grass grown in control soils. The dry mass of plant shoots did not differ between treatments. The results showed earthworms can increase Pb mobility and availability to plants, increasing sequestration. Over a 6-week experiment, there was no effect of Pb on lettuce growth but when grown in soils with earthworms, lettuce biomass increased with increasing concentrations (significantly higher at 3,730 mg Pb/kg concentration) (Leveque et al., 2014). Earthworms also increased lettuce Pb concentration but only at exposure concentrations of 2,822 and 3,730 mg Pb/kg.

11.2.2.3.3 Other Invertebrates

For the 2013 Pb ISA, studies of bioavailability and uptake comprised earthworms, snails, and arthropods including bees and beetles. Since the 2013 Pb ISA, new literature has examined additional invertebrate groups including spiders, and butterflies. Pollen, honey, and bees from 16 honeybee (*Apis mellifera*) apiaries were sampled twice a year for 2 years for Pb contamination across an urban-cultivated-hedgerow-natural environmental gradient in France (Lambert et al., 2012). Pb concentration in pollen was influenced by sampling season but not by landscape characteristics. Thirty percent of honey samples were below detection limits, and the rest had very low concentration values. Pb concentrations in honey from apiaries surrounded by a hedgerow matrix were two times higher than those in other landscapes measured, with honey from cultivated sites having the lowest concentrations (most were below the detection limit). Pb in honey was higher in the 2009 season compared with the 2008 season. Pb concentrations ranged from 0.001 to 1.896 mg Pb/kg in bees, from 0.004 to 0.798 mg Pb/kg in pollen and from 0.004 to 0.378 mg Pb/kg in honey. Seasonality may influence bee Pb concentration, as levels were higher in bees sampled during the June–October sampling period for one of the years studied. There was no clear relationship of contamination between the three biological compartments (pollen, bees, honey). In general, apiaries in urban and hedgerow locations had higher Pb contamination than apiaries in cultivated or island landscapes. There was variation across the year, and contamination was typically higher during the dry (summer) season. Honeybees are exposed to Pb contamination via direct contact with Pb atmospheric deposition on flowers and through food contamination. Pb contamination patterns in bees were similar to contamination levels in pollen, suggesting deposition contact contamination. Seasonal differences may be explained by changes in floral availability.

Following 20 km-pollution gradients away from active Zn or metal smelters in Russia and Poland, bumblebee (*Bombus* spp.) Pb levels (0.21–3.3 mg Pb/kg) and soil Pb levels (13.6–814.2 mg Pb/kg) both decreased with increasing distances from the pollution source (Szentgyörgyi et al., 2011). In another study, bee body, bee bread, propolis, and honey Pb content was examined across different geologic areas (Golubkina et al., 2016). Sites included an unpolluted control located in the Ribnitsa district in Moldavia (located away from industry or major highways), a selenium (Se)-deficient area in the Henty province of Mongolia and the Voskresensk district of Moscow region, which is an area of fertilizer production. Bee body Pb concentration was lowest at the unpolluted Moldavia location

(0.51 mg Pb/kg), higher in Mongolia (0.94 mg Pb/kg), 0.97 mg/kg away from fertilizer production area (Novoselki, Russia) and over 4 times higher near fertilizer production (2.16 mg Pb/kg, Lopatino). There was a positive correlation between Pb content in bees and bee bread for the Lopatino and Moldavia sites. Pb content in the propolis was highest in Mongolia (16.07 mg Pb/kg) and much lower in the other locations (2.08, 1.52, 3.18 mg Pb/kg, Moldavia, Novoselki, Lopatino, respectively) and was not as closely correlated with bee body content. Honey Pb content was low across all sites (approximately 0.2 mg Pb/kg or less).

Wolf spiders (Lycosidae) are common ground-dwelling arachnids and are known to accumulate metals. An observational study in Korea found that while Pb in soil did not differ by season (31.13 mg Pb/kg averaged across seasons), Pb was significantly greater in spiders from an autumn brood (7.83 mg Pb/kg) compared with that in a spring brood (1.52 mg Pb/kg). While overall BCF was below 1, the difference in brood accumulation suggests that while spiders accumulate Pb at low levels, seasonality may affect accumulation (Conti et al., 2018). Jung and Lee (2012) measured Ariadna spider Pb accumulation in Namibia in relation to uranium (U), Cu, and gold mines. Overall, Ariadna spiders do accumulate heavy metals in relation to their environment (in this case burrowing spiders and sand contamination), but Pb levels were higher in sand compared to the levels in spider bodies, indicating Pb is not readily bioaccumulated.

In the common cutworm (*Spodoptera litura*), Pb accumulation in body tissue generally increased with increasing Pb exposure concentration across all development stages (Shu et al., 2015). Larvae were exposed to increasing Pb concentration via diet at 0, 12.5, 25, and 50 mg Pb/kg (dietary values) and larvae were raised for five generations at each exposure concentration. Growth stage (larvae, pupae, adult), Pb exposure concentration, and their interaction explained Pb accumulation, but generation did not (F1 versus F5), nor were there any significant interactions with generation. Within development stages, Pb accumulation was highest during the 6th instar stage, second highest in adults, and lowest in pupae (Pb accumulation was only significantly higher at 50 mg Pb/kg treatment for pupae and adults). Within 6th instar larvae, Pb exposure and tissue type mattered but sex did not. Overall, Pb accumulated primarily within the midgut, and overall gut accumulation (mid, fore, and hindgut) was greater than that in the hemolymph, head, or body fat. Accumulation also increased with exposure. In a trophic uptake study, Pb accumulation in the roots, stems and leaves of mulberry (*Morus alba*) increased with increasing soil Pb exposure (0, 200, 400, 800 mg Pb/kg, dietary values) (Zhou et al., 2015). In turn, Pb in silkworm (*Bombyx mori*) larvae and moths as well as in feces and silk excretions increased with increasing Pb content in the mulberry leaves (in response to increasing Pb in soil). However, larvae (0.63, 4.08, 5.74, and 11.16 mg Pb/kg) and moths (0.6, 2.95, 4.39, 6.23 mg Pb/kg) had lower body content than leaves (5.54, 41.79, 51.21, 60.26 mg Pb/kg) while Pb in feces was higher than that in leaves (9.85, 187.96, 230.44, 279.8 mg Pb/kg), indicating that while silkworms accumulate more Pb in response to increasing exposure, Pb is not biomagnified, and the majority of Pb consumed is excreted instead.

A study that examined soil, plant, and grasshopper Pb concentrations at increasing distance to a Zn smelter in China Zhang et al. (2012) found Pb content in all compartments decreased with increasing distance. Soil Pb ranged from 49.9 to 973.5 mg Pb/kg. Plant Pb concentration ranged from approximately 5 to approximately 65 mg Pb/kg and varied by species (all species serve as a food source for grasshoppers). Leaf Pb content was greatest in Japanese millet (*Echinochloa crusgalli*), followed by Siberian elm (*Ulmus pumila*) and green foxtail (*Setaria viridis*). Grasshopper (*Locusta migratoria manilensis* and *Acrida chinensis*) Pb content ranged from 1.07 to 46.95 mg Pb/kg (8.83 average). Soil and plant contamination significantly decreased at 4000 m distance but Pb content in grasshoppers was significantly higher within only 2000 m to the smelter.

Whole-body Pb content in isopods (*Armadillidium granulatum*) was positively correlated with Pb food exposure (100, 500, 1,000 mg Pb/L, dietary values), but concentrations were much lower than food contamination levels, indicating isopods do not biomagnify Pb (Mazzei et al., 2013). Simon et al. (2016) examined soil, leaf litter, and beetle (*Carabus violaceus* and *Pterostichus oblongopunctatus*) Pb concentrations along an urbanization gradient in Hungary. Pb concentration in soils was highest in the urban locations but not different between rural and suburban locations. There was no difference in Pb concentration within beetle species across sites but *P. oblongopunctatus* (19.6 mg Pb/kg) had higher Pb concentrations compared with *C. violaceus* (not detected). Within *P. oblongopunctatus*, Pb concentration was higher in males compared with females (when pooled across sites). The BAF for *P. oblongopunctatus* was 1.26 in urban environments, 1.48 in suburban environments and 1.37 in rural environments.

Vinegar fruit flies (*Drosophila melanogaster*) also display Pb accumulation differences based on sex. Females had higher Pb accumulation compared with males (Peterson et al., 2017). Both sexes exposed to approximately 109 mg Pb/kg (250 μM Pb, nominal rearing medium values, concentration in flies was measured) had higher Pb body concentration (18.44 ng per female versus 7.32 for males) compared with controls (0.2 ng per male or female), but females had greater concentration values. Furthermore, exposure of either male or female parent did not lead to generational uptake effects. Pb loads in unexposed F1 generations with a Pb-exposed parent were no different from those in F1 adults with control-treated parents. However, in another study by Peterson et al. (2020), when *D. melanogaster* were reared in the same conditions but across an increasing gradient of Pb exposure of approximately 109, 217, and 434 mg Pb/kg (250, 500, and 1,000 μM Pb nominal rearing medium values, concentration in flies was measured), they found no effect of sex on Pb accumulation nor a sex-Pb exposure interaction. Body Pb accumulation did increase with increasing exposure concentrations, but the response was similar across both sexes. Additional work is needed to determine the effect organism sex has on Pb uptake and accumulation in *D. melanogaster*.

Overall, literature since the 2013 Pb ISA adds additional supporting evidence of the importance of soil variables on uptake and accumulation by soil invertebrates as well as new information on additional arthropod groups and modifying factors such as season, and possibly, generation. Snails typically accumulate Pb at lower concentrations than those found in soil or vegetation, but a higher concentration of Pb in the hepatopancreas compared with that in the snail foot show uptake via consumption leads to greater Pb accumulation than uptake through the soil-skin interface. Similarly, grasshoppers and silkworms readily accumulate Pb but at levels lower than those in both food and soil contamination. CEC and soil organic content interact with soil Pb concentration on driving uptake by the common garden snail while pH and Ca content influence uptake and accumulation in earthworms. Earthworm uptake also depends upon ecotype due to differences in feeding and burrowing behavior. As discussed in previous assessments, there is an abundance of information examining the effects of earthworms on Pb mobility and bioavailability due to these feeding and burrowing behaviors. Earthworm casts, for example, were found to have higher pH and water-extractable OC. Literature since the 2013 Pb ISA provides new information on the uptake and accumulation of Pb by spiders and butterflies, and additional information on bees. Generally, Pb concentration is higher in bee bodies compared to honey and pollen. Two spider genera examined show low accumulation levels in relation to soil contamination, suggesting spiders do not readily bioaccumulate Pb. Lastly, there appear to be interactions of generation and sex on Pb uptake by common cutworms and fruit flies, but the results are variable and the overall effects remain unclear.

11.2.2.4 Uptake and Bioaccumulation in Terrestrial Vertebrates

The 2013 Pb ISA provided evidence of the accumulation of Pb in blood, bones, and a variety of different tissues in birds and mammals. In studies of birds in the 2013 Pb ISA, the focus was mainly on ingestion of manmade materials (e.g., Pb shot). In mammals, multiple species were found to accumulate Pb from contaminated soils as well as from plants grown in contaminated soils. In birds, low dietary Ca^{2+} concentrations were linked to increased accumulation of Pb in liver, bone, kidney, muscle, and brain tissues.

New information has become available on the uptake of Pb in terrestrial reptiles and amphibians since the 2013 Pb ISA. A study of northern pine snakes (*Pituophis melanoleucus melanoleucus*) in the pine barrens of New Jersey found that Pb was accumulated in a wide variety of tissues including liver, kidney, muscle, skin, heart, as well as in blood, with the highest mean Pb concentration in muscle (0.393 ± 0.131 µg/g wet weight) (Burger et al., 2017). The pathway of exposure was not determined in this study, but the authors suggested that consumption of prey items was the most likely pathway, as pine snakes are a top predator in their food web. Pb was found to accumulate in the blood of giant toads (*Rhinella marina*) captured at industrial, urban, and rural sites in Mexico (Ilizaliturri-Hernández et al., 2013). Blood Pb levels ranged from 10.8 to 70.6 µg/dL and were found to increase with increasing soil Pb levels.

Since the 2013 Pb ISA, new studies have been published that support findings of Pb accumulation in different mammalian tissues. Tête et al. (2014) and Camizuli et al. (2018) both found evidence of Pb accumulation in the kidneys and livers of wood mice *(Apodemus sylvaticus*). Kidney concentration ranged from values under the limit of detection to 268.3 µg/g dry weight, and liver concentrations ranged from values under the limit of detection to 281.7 µg/g dry weight. Another study on Pb accumulation in mammalian tissues evaluated brain tissue from nine mesocarnivore species in Europe (Kalisinska et al., 2016). Eurasian otters (*Lutra lutra*), badgers (*Meles meles*), pine martens (*Martes martes*), beech martens (*Martes foina*), European polecats *(Mustela putoris*), red foxes (*Vulpes vulpes*), feral and ranch American minks *(Neovison vison*), raccoons (*Procyon lotor*), and raccoon dogs (*Nyctereutes procyonoides*) were all sampled during this study. Brain tissue Pb was highest in raccoons (0.47 mg/kg dry weight) and lowest in ranch American minks (0.072 mg/kg dry weight). The study's authors speculated that carrion with hunting ammunition is likely to be an important source of Pb for omnivores and partial scavengers, while organic Pb incorporated in the diet and Pb contained in the soil, earthworms, and dusted food may also be possible sources of exposure.

Studies of bioaccumulation and uptake in birds tend to support information provided in the 2013 Pb ISA and provide additional evidence for Pb accumulation in a variety of different tissues. Soil remains an important source of Pb exposure in many bird species. French et al. (2017) identified soil consumption as one of the most common routes of Pb exposure in American woodcocks *(Scolopax minor*). Woodcocks use their long bills to probe the soil for earthworms, with their dietary intake comprising as much as 10% ingested soil, indicating that Pb-contaminated soil may be an important exposure pathway. Additionally, the consumption of earthworms is another pathway of exposure, as earthworms can bioaccumulate metals from the soil. Other species with similar feeding habits to woodcock such as American robins *(Turdus migratorius*) may be exposed to Pb through these same pathways.

Birds of prey such as bald eagles (*Haliaeetus leucocephalus*) and California condors (*Gymnogyps californianus*) have also been shown to accumulate Pb in blood and different tissues. A study of bald eagle nestlings in the western Great Lakes region found blood Pb concentrations ranging from below the limit of detection to 26.4 µg/dL wet weight and feather Pb concentrations ranging from below the limit of detection to 371 µg/g wet weight (Bruggeman et al., 2018). The authors speculated that Pb air pollution, as well as Pb shot and Pb paint may all be sources of exposure. A study of California condors found that between 1997 and 2010, the annual percentage of condors with blood Pb levels higher than 0.1 µg/mL (originally reported as 100 ng/mL) ranged from 50% to 88% (Finkelstein et al., 2012). However, this study found that the majority (79%) of condors had blood Pb isotope ratios that were not significantly different from Pb-based ammunition. This indicates that Pb ammunition is likely the primary source of Pb exposure in California condors. Behmke et al. (2015) examined bone Pb as a measure of chronic exposure and Pb in liver as an indicator of more recent exposure in American black vultures (*Coragyps atratus*) and turkey vultures (*Cathartes aura*) collected in Virginia. Bone Pb was significantly higher than Pb in liver in both species indicating that Pb in the birds was primarily associated with long-term exposure. Possible sources of Pb in these long-lived birds based on comparison of Pb isotope ratios in femur bones and Pb isotope ratios associated with Pb sources included ammunition, coal-fired power plants, leaded gasoline, and zinc smelting operations.

In summary, literature since the 2013 Pb ISA (U.S. EPA, 2013) adds support to existing evidence of Pb accumulation in blood, bones, and a variety of different tissues in terrestrial vertebrates. Pine snakes accumulated Pb in liver, kidney, muscle, skin, and heart tissue, with the highest concentrations found in the muscles. In toads, Pb was found to accumulate in blood and increased with increasing soil Pb levels. New evidence continues to support findings of the accumulation of Pb in tissues from a wide range of mammalian species. Pb ammunition continues to be a prevalent source of Pb contamination in both mammals and birds. Consumption of prey species has also been found to be an important route of Pb exposure especially in species that consume earthworms such as woodcocks and robins.

11.2.2.5 Uptake and Bioaccumulation Through Food Web

In the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013), various studies suggested that Pb might be transferred through terrestrial food webs, with lower Pb concentrations occurring in each successive trophic level. Having data on bioavailable or bioaccessible concentrations of Pb at every trophic level would lead to more accurate estimates of trophic transfer within food webs. Since the 2013 Pb ISA (U.S. EPA, 2013), there have been more observational and experimental examples of gradual attenuation of Pb concentrations with increasing trophic level; however, this depends on Pb concentration, the presence of other heavy metals, ecosystem, and organism sensitivity to Pb exposure. Although most of the following studies were conducted in non-U.S. locations or in proximity to point sources, they further elucidate biotransfer processes for Pb.

Pb was transferred through a soil, nettle, snail food web in Antwerp, Belgium (Boshoff et al., 2015). In a microcosm field experiment, adult European land snails (*Cepaea nemoralis*) from an uncontaminated site were exposed to sites varying in distance from the Umicore Precious Metal Refinery, a nonferrous smelter in Antwerp, Belgium. The snails were sampled along with nettle (*Urtica dioica*), one of their food sources. *Cepaea nemoralis* were placed in microcosms at each site and allowed to feed on soil, litter, and vegetation for 16 weeks. A subset of snails was collected at weeks 0, 1, 2, 4, 8, and 16 for metal analysis (Pb, arsenic [As], Cd, Cu, Zn, nickel [Ni]) and morphological and physiological biomarker response (Section 11.2.4.4). Nettle (*U. dioica*) samples were collected three times throughout the experiment for trace metal analysis. Pb concentration in the soil was the only significant factor explaining Pb concentration in *U. diocia*. Pb concentrations in the digestive glands of the *C. nemoralis* varied spatially and temporally, as there was a statistically significant interaction between site and time. Pb concentration in the soil was higher than that in *U. diocia*, while the concentrations of Pb in the digestive glands of *C. nemoralis* were similar to or higher than Pb concentrations in *U. diocia.*

Detoxification may be an important mechanism behind biodilution of Pb with trophic level in the food web. Silkworms (*Bombyx mori*) were shown to excrete Pb when fed Pb-exposed mulberry (*Morus alba*) (Zhou et al., 2015). Soils collected from an agricultural field in China were exposed to nominal concentrations of 0, 200, 400, or 800 mg Pb/kg via Pb $(NO_3)_2$. Pb concentrations were also measured in soil, mulberry, and silkworms. *Morus alba* was planted in the Pb-spiked soils for 3 months, and the leaves were collected and fed to fifth instar larvae of *B. mori*. The available fraction of Pb in the soils, the total concentration in mulberry leaves, shoots and roots, and *B. mori* larvae, silk, feces, and adult moth increased with increasing soil Pb addition in a dose-dependent manner. Roots sequestered the most Pb, followed by stems, and leaves. The translocation factor was highest for the transfer of Pb from the soil to the root in the 400 mg Pb/kg treatment, followed by 1.60 in the 800 mg Pb/kg treatment, and 1.13 in the 200 mg Pb/kg treatment. All other translocation factors between the soil and plant (root–soil, stem–soil, leaves–stem, stem–root, leaf–root, leaves–stem) were below 1.0 or near 1.0 for the control (0 mg Pb/kg). Across all treatments, the subcellular distribution of Pb in the leaves was greatest in the cell wall, followed by the soluble fraction, and organelles. Pb treatment did not affect silkworm survival or mean weight, but increasing Pb treatment negatively affected the silkworm growth rate. Specifically, the body weight of silkworms was significantly lower at the end of the experiment in the 800 mg Pb/kg treatment compared to the control and the 200 mg Pb/kg treatment. Pb concentration in the silkworm increased with increasing treatment. Pb concentration in the feces was the greatest, followed by the concentration in the peel, the larvae, the silk moths and finally the silk. Metallothionein synthesis increased in *B. mori* when fed with Pb-treated leaves. Metallothionein content in the midgut was more sensitive to lower Pb exposure (200 mg Pb/kg) than metallothionein in the posterior of the silk gland and in the fat body, both of which increased in the high Pb exposures (400 and 800 mg Pb/kg). These results suggest that *B. mori* can detoxify Pb through excretion and homeostasis.

Field studies published since the 2013 Pb ISA (U.S. EPA, 2013) provide additional evidence for biodilution in terrestrial food webs. Oil rapeseed (*Brassica napus*) and insects were collected from 35 agricultural sites in Southwest Poland (Orłowski et al., 2019). These agricultural sites varied in size, habitat fragmentation, and percent cover by forests and were characterized by percent arable land, permanent vegetation, linear woody features, dirt or unpaved roads, and wooded areas. *Brassica napus* and the insect community (grouped into guilds: pollinators, consumer/herbivores, saprovores, predators, and parasitoids) were analyzed for Pb and other trace elements. The concentration of Pb in *Brassica napus* (mean: approximately 2 mg Pb/kg) was higher than those in all insects examined (range: 0.77 to 2.31 mg Pb/ kg), and Pb concentration generally decreased with increasing trophic level, suggesting Pb is diluted in this food web. As the size of the field area increased, the Pb concentration in pollinators decreased, suggesting that even under low Pb levels, larger areas with more diversified landscapes could reduce Pb body burden for pollinators.

The presence of other heavy metals in the soil, specifically Cd, can affect the uptake and trophic transfer of Pb. In an agricultural system in Pakistan (Aslam et al., 2015), alfalfa (*Medicago sativa*) seeds were grown in control, Pb (0 mg Pb/kg, 200 mg Pb/kg or 400 mg Pb/kg), Cd (0 mg Cd/kg, 4 mg Cd/kg or 8 mg Cd/kg) or Pb and Cd-enriched soil (200 mg/kb Pb + 4 mg/kg Cd and 400 mg/kb Pb + 8 mg/kb Cd). Soils were treated with $Pb(NO_3)_2$ and $Cd(NO_3)_2$ salts, resulting in 1.45 ± 0.23 mg/kb Pb (mean ± S.E.) for control, 112.0 ± 2.43 mg Pb/kg for 200 mg Pb/kg and 237.4 ± 2.79 for 400 mg Pb/kg at the end of the experiment for Pb-treated soils. Rabbits (*Oryctolagus cuniculus*) were placed in chambers and fed with metal-treated *M. sativa* for 10 days. Soil, *M. sativa* root and shoot, and *O. cuniculus* blood and fecal Pb and Cd concentrations increased with increasing concentrations of metal treatment. *Medicago sativa* BAF in the roots increased with increasing Pb concentration and in combined treatments with Cd relative to Pb exposure alone. Specifically, the Pb BAF associated with the 200 mg Pb/kg treatment was 0.87, while the BAF resulting from the 400 mg Pb/kg + 8 mg Cd/kg treatment was 0.96. Conversely, Pb contents in the shoots and leaves of *M. sativa* showed higher BAF in the Pb treatments relative to the combined Pb + Cd treatments. Only a small portion of Pb was transferred to the shoots, as all BAFs were below a threshold of 1.0. Although not explicitly tested, *O. cuniculus* blood and feces Pb levels were similar between Pb-only and Pb + Cd treatments (e.g., fecal concentration in 200 mg Pb/kg treatment: 3.86 ± 0.73 mg Pb/kg [mean ± S.E.], 200 mg/kb Pb + 4 mg/kg Cd treatment: 2.89 ± 0.67 mg Pb/kg), suggesting Pb uptake and accumulation is not influenced by the presence of Cd in *O. cuniculus*. Combined, this study suggests that although Pb bioaccumulation is higher in *M. sativa* roots and lower in the shoots in the combined Pb + Cd treatment relative to Pb exposure alone, it does not affect the uptake of Pb by herbivores such as *O. cuniculus*.

Although many observational studies examining BAFs across multiple trophic levels have found evidence for biodilution of Pb, some studies have observed bioaccumulation. For example, soil samples (0–15 cm), berseem plants (*Trifolium alexandrinum*), aphids (*Sitobion avenae*), grasshopper (*Aiolopus thalassinus*) and ladybird beetle larvae (*Coccinella septempunctata*) were collected from five agricultural sites in Punjab, Pakistan and analyzed for accumulation of Pb, Cd and Zn. In this study, Pb was not significantly correlated with any other soil physicochemical variables or metals (percent sand, percent silt, percent clay, soil OM, CEC, Zn, Cd, or pH). Pb concentrations in the soil were low and similar among all sites (3.08 ± 0.53 mg Pb/kg, mean ± S.D.). BAFs were greater than 1.0 for *Trifolium alexandrinum* (BAF for soil – berseem: 2.26 ± 0.42), *Sitobion avenae* (BAF for berseem – aphids: 1.40 ± 0.41), *Aiolopus thalassinus* (BAF for berseem – grasshoppers: 14.64 ± 3.42). and *Coccinella septempunctata* (BAF for aphid – beetle: 2.94 ± 1.31). Overall, this system does exhibit bioaccumulation of Pb, but the concentrations of Pb in soil were very low. There was no significant correlation between Pb soil concentration and *T. alexandrinum* Pb concentration, between *S. avenae* and *T. alexandrinum*, between *T. alexandrinum* and *A. thalassinus* and between *C. septempunctata* and *S. avenae*.

In summary, Pb generally shows patterns of biodilution through terrestrial food webs; however, some observational studies have shown bioaccumulation of Pb. Furthermore, the rate at which Pb biodilutes or accumulates in food webs depends on the presence of cadmium, the sensitivity of the organism to Pb exposure and ecosystem type.

11.2.3 Environmental Concentrations of Pb in Terrestrial Biota and Ecosystems in the United States at Different Locations and Over Time

Studies that present long-term trends of Pb concentrations observed in terrestrial ecosystems are summarized in this section. National and regional studies that summarize Pb concentrations in soils and biota on decadal timescales are included.

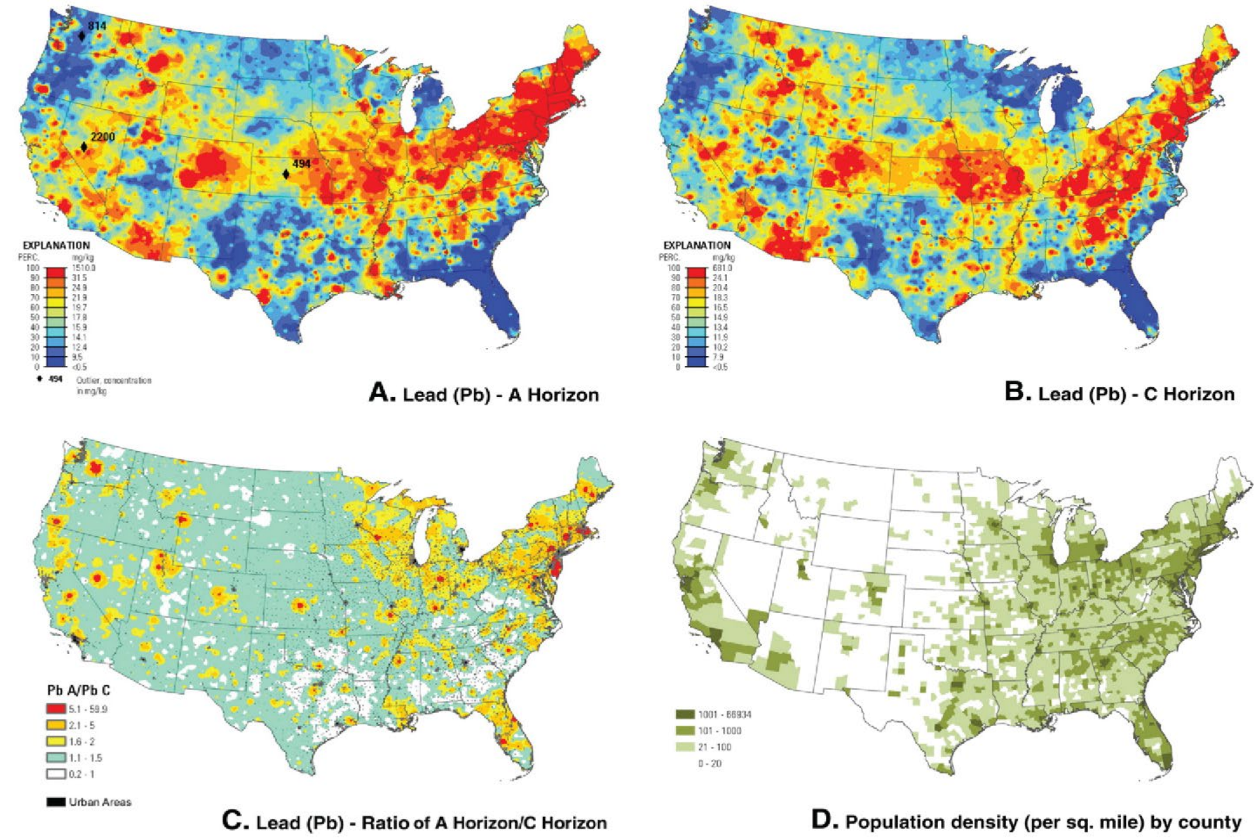
11.2.3.1 Pb in Soils

Pb concentrations in soils vary across the United States due to a variety of anthropogenic and natural factors. In general, areas with higher population density and intensity of industrial activity have higher soil Pb concentrations relative to rural areas. This pattern was observed in the following studies of national and regional soil Pb concentrations.

A regional survey of forest floor soils sampled in the northeastern United States provides a time series of Pb concentrations from 1980 to 2011. The region has a large amount of urban and industrial development associated with high historical anthropogenic Pb emissions. Soils were sampled at 25 sites in 1980 and sampled again at 16 of those sites in 1990, 2002 and 2011. Sites were located across northeastern states including Pennsylvania, New York, Connecticut, Massachusetts, Vermont, and New Hampshire. Across all sites, mean soil Pb concentrations decreased from 151 ± 29 (SE) mg Pb/kg in 1980 to 68 ± 13 (SE) mg Pb/kg in 2011 (Richardson et al., 2014) (summarized in Table 11-1). The authors explained the observed reduction in forest floor Pb concentrations by the dilution effect of added organic material containing less Pb than in older forest floor organic soil as well as by the leaching of Pb from upper soil horizons into the underlying soil. Isotopic analysis of Pb samples indicated that gasoline was the dominant source of the measured soil Pb and that it persisted in forest floor soils until at least 2011, and likely later. In another analysis of the data set of 1980–2011 northeastern U.S. forest floor soils, Pb concentrations were estimated to decline 2.0 ± 0.3% per year (Richardson et al., 2015a).

A 2019 survey of peri-urban soil Pb from 54 sampling sites in southern California counties including Los Angeles, Orange, San Bernardino, and Riverside found that soil Pb was elevated relative to the southwestern U.S. region, but lower than concentrations found at contaminated sites near point sources of Pb, with a mean of 23.9 ± 13.8 mg Pb/kg (Mackowiak et al., 2021) (summarized in Table 11-1). The mean is considerably lower than the forest floor mean observed in the Richardson et al. (2014) surveys and the results of this study are illustrative of the regional variance in U.S. soil Pb concentrations. Foliage samples from eight shrub and tree species collocated with soil samples were collected from the sampling sites of Mackowiak et al. (2021). No correlation was identified between foliar bioaccumulation and soil Pb concentrations in the study.

Measuring the ratio of Pb concentrations between different soil horizons can provide information on the relative contribution of anthropogenic Pb to total Pb observed in the soil. In the recent NASGLP soil survey of the conterminous United States Smith et al. (2013a) (summarized in Section 11.1.3 and Table 11-1), samples were collected from multiple soil horizons. Stratified sampling enabled the comparison of Pb concentrations from bedrock to those in upper-horizon soil. In areas with historic depositional input of Pb, the concentration of Pb observed in upper-horizon soils was often higher than that in the bedrock. Figure 11-4C. shows the ratio of A-horizon to C-horizon Pb concentrations mapped in Woodruff et al. (2015), using inverse-distance weighting methods derived from the NASGLP survey (Smith et al., 2013a). This map displays areas with increased concentrations of Pb in A-horizon soils relative to lower horizons, hinting at the lasting effect of depositional Pb pollution. The mapped ratio of A-horizon to C-horizon soils from Woodruff et al. (2015) may serve as an indicator for soil in areas where historical Pb deposition may have a relatively higher effect on people and ecosystems. Patterns of elevated A- to C-horizon soil Pb concentrations in Figure 11-4C are conspicuous in areas with historical anthropogenic sources of Pb. This pattern is observed in the northeastern United States, with a historically high population density and intensity of industrial development. Likewise, mapping highlights former Pb smelting and mining sites, for instance in areas near smelters in Everett and Tacoma, Washington or the Doe Run smelter in Herculaneum, Missouri (the last Pb smelter in the United States, which closed in 2013). Areas near mining sites, including near Leadville, Colorado, Cooke City, Montana, and northern Utah, also have a high ratio of A- to C-horizon Pb. Woodruff et al. (2015) emphasized that no known natural geological process would otherwise explain elevated A-horizon soils relative to the underlying layers.



Source: Woodruff et al. (2015).

Figure 11-4 Maps of Pb sampled from A-horizon (A) and C-horizon (B) soils, the ratio of Pb observed in A-horizon to C-horizon soils (C) and a map of U.S. population density (D).

Recent national and regional surveys of soil Pb document the spatial and temporal patterns of residual pollution from decades of Pb emissions. Data made available from the NASGLP provide the most comprehensive information on the distribution of Pb across the conterminous United States (Smith et al., 2013a). Regional studies of soil Pb provide valuable information on temporal trends and relate observed soil Pb concentrations to Pb in biota collocated with soil sampling locations. Elevated upper soil horizon Pb concentrations relative to the underlying soil with greater substratum content observed across the conterminous U.S. in (Woodruff et al., 2015) and over four decades in the northeast in (Richardson et al., 2014) demonstrate the persistence of historical Pb contamination in U.S. soils.

11.2.3.2 Pb in Tree Rings

Dendrochronology can be used to reconstruct historical trends of Pb in air pollution as tree rings record an annual record of ambient environmental conditions across a tree's lifespan, although radial transport of Pb within the tree may reduce the precision of historical Pb concentrations reconstructed from tree rings. Because trees primarily uptake Pb through their roots, there may be a 10–15-year delay in tree-ring Pb compared with air Pb concentrations as Pb deposition leaches through the soil and is absorbed by the tree (U.S. EPA, 2013).

Several studies conducted after the 2013 Pb ISA report temporal trends in Pb as observed in tree rings, three from Canada and one from Mexico. A study of white spruce trees (*Picea glauca*) located in the Northern Athabasca Oil Sands Region of western Canada near oil sands mining operations reconstructed Pb concentrations from 1878 to 2009. Tree-ring records of Pb concentrations increased beginning in 1922, peaked in 1968–1973, then decreased until 2009 (Dinis et al., 2016). In eastern Canada, a study reconstructed Pb trends from 1880 to 2007 in red spruce (*Picea rubens*), beech (*Fagus grandifolia*), white pine (*Pinus strobus*), and white cedar (*Thuja occidentalis*). The beech trees located in both Montreal and Georgian Bay exhibited a decline in concentrations after a 1970–1985 peak. The authors attribute the lack of an observed temporal trend in Pb concentrations in white pine to the radial mobility of Pb within the tree (Doucet et al., 2012). Another study of tree-ring Pb concentrations in white cedar in Québec dated concentrations from 1850 to 2010 and recorded increased concentrations from 1950 to 2000 near a Pb smelter. The increasing trend at a control site further from the smelter was delayed to 1990–2010. Concentrations across most sites in this study decreased from 2000 to 2010 (Arteau et al., 2020). In contrast to the trends observed in the Canadian studies, a study of *Prosopis julifora* tree rings dated from 1903 to 2007 located near a copper smelter in San Luis Potosi, Mexico found increasing Pb concentrations from 1990 to 2007 (Beramendi-Orosco et al., 2013).

Although trends in reconstructed Pb concentrations varied across tree species and regions, studies identified a temporal pattern of Pb that increased after 1850–1900 and, in some cases, peaked in 1970–1985, then decreased afterward. Tree-ring studies with temporal patterns in exception to this pattern were conducted near persisting industrial point sources of Pb pollution.

11.2.4 Effects of Pb in Terrestrial Systems

This section focuses on studies of the biological effects of Pb on terrestrial biota published since the 2013 Pb ISA. First, new information on factors that affect biological sensitivity to Pb is discussed, followed by subsections on effects on vegetation, microbes, invertebrates, and vertebrates. The biological effects of Pb in the 2013 Pb ISA and in this appendix are generally presented in increasing order of the complexity of biological organization, from suborganismal responses (i.e., enzyme activities, changes in blood variables) to endpoints relevant to the population level and higher (growth, reproduction, and survival), up to effects on ecological communities and ecosystems.

11.2.4.1 Effects on Terrestrial Microbes

Several field and laboratory studies have examined the relationship between soil Pb concentration and microbial community structure and processes. Cell viability of bacteria grown in Pb-contaminated media was unaffected, and bacteria were able to take up Pb in studies reported in the 1977 AQCD (U.S. EPA, 1977). Furthermore, in other studies reported in the 1977 AQCD (U.S. EPA, 1977), 1986 AQCD (U.S. EPA, 1986), and the 2013 Pb ISA (U.S. EPA, 2013), soil Pb concentration was correlated with decreases in the diversity and function of soil microorganisms. New studies since the 2013 Pb ISA added a gradient of Pb to the soil and showed negative relationships between Pb concentrations and bacterial abundance. Most new studies since the 2013 Pb ISA were observational and leveraged natural environmental gradients of pollutants. In these cases, Pb was not the sole contaminant in the soil, contributing some uncertainty to their interpretation. Observational field studies showed mixed associations between soil Pb concentration and microbial abundance and diversity metrics. Additionally, there has been substantial research on how Pb affects the interactions between microbes and their hosts, specifically, plants and mycorrhizal associations (Section 11.2.4.2).

Pb contamination slightly affected microbial diversity and significantly affected the abundance of certain bacteria phyla and genera in an agricultural system (An et al., 2018). Soil in an agricultural field in China was supplemented with nominal concentrations of 0, 175, or 350 mg Pb/kg using $Pb(NO_3)_2$ and permitted to age for 3 months while maintaining soil moisture. After 3 months, soil physicochemical variables and bacterial community structure were analyzed. Measured available Pb and total Pb concentration in the soil varied with Pb treatment level (available Pb in the control (mean ± S.D.): 3.97 ± 0.08 mg Pb/kg, 150 mg Pb/kg treatment: 126.6 ± 4.98 and 350 mg Pb/kg treatment: 254.46 ± 7.13). Total and available Pb concentrations were highly correlated. Some soil physicochemical variables differed between the control and Pb-spiked soils; soil OM was lower in Pb-spiked soils compared with the control, while soil moisture was the lowest in the 150 mg Pb/kg treatment. Soil pH, available P, available K, and available N were similar among all treatments. Pb exposure marginally affected microbial Operational Taxonomic Unit (OTU) richness and diversity, as well as the abundance-based coverage estimator (ACE), Chao and Shannon's diversity indices were highest in the 175 mg Pb/kg treatment compared with the control and the 350 mg Pb/kg treatment (statistics not reported, error bars do overlap). The abundances of certain genera were affected by Pb treatment; *Bacillus, Lactobacillus,* and *Truepera* abundances were negatively correlated with Pb concentration, while *Streptococcus* and *Arhtorbacter* were highest under the low Pb treatment. *Bosea* and *Aquicella* increased in abundance with Pb treatment. Total Pb concentration was correlated with the abundance of *Planctomycetes* and *Gemmatimonadetes* and marginally correlated with *Nitrospirae*.

Microbial enzyme activity was significantly negatively affected in soils collected from a research station in northwestern Iran, exposed to nominal concentrations of 0, 100, 200, 300, 400, or 500 mg Pb/kg using aqueous Pb nitrate and incubated for 2 weeks (Shirzadeh et al., 2022). After 3, 15, 30, 90 and 180 days, microbial enzyme activities and microbial indices, including acid and alkaline phosphomonoesterase, nitrate reductase, urease, soil microbial biomass carbon, soil basal respiration were characterized. Nominal Pb concentration, incubation time and the interaction between Pb concentration and incubation time significantly affected all enzyme activities and microbial indices. In general, higher concentrations of Pb and longer incubation times resulted in a commensurate reduction in enzyme activities and microbial indices.

The root nodule allocation by the actinobacteria *Frankia* on Alder (*Alnus glutonisa*) was unaffected by Pb treatment, while *Frankia* microbial respiration was significantly affected by Pb treatment (Bélanger et al., 2015). The authors suggested that large difference between the maximum tolerable concentration (MTC), the highest metal concentration when *Frankia* has 95% of its relative respiration capacity (<0.01 mM) and the minimum inhibitory concentration (MIC), when under 5% of relative respiration capacity occurs (10.0 mM), may be due to sequestration or binding of Pb by *Frankia*, which has been shown to occur with other heavy metals.

Bacteria and archaeal abundance and diversity have been found to be affected by soil Pb concentration in several observational studies. Beattie et al. (2018) examined the relationship between Pb and other soil heavy metals as well as bacterial and archaeal communities in Oklahoma. Picher, an abandoned mining town, is located near the Picher mine field (PMF), which was declared a U.S. EPA Superfund Site in 1983 (Tar Creek Superfund Site). Soil samples were analyzed for trace metals and soil physicochemical properties (Pb, Al, Ar, B, Cd, Cr, cobalt [Co], Cu, Fe, Mg, Mn, molybdenum [Mo], Ni, K, sodium [Na], tellurium [Te], titanium [Ti], tungsten [W], vanadium [V], Zn, soil pH and soil moisture) and soil bacterial and archaeal abundance and diversity using 16S rRNA gene copies. Pb soil concentration was 76.39 ± 1.37 mg Pb/kg (mean ± S. E.) and ranged from 3.0 mg/kb Pb to 1115.2 mg/kg (Beattie et al., 2017). Bacterial abundance (16S rRNA gene copies) was found to be negatively correlated with soil Pb concentration, while archaeal abundance and the bacteria:archaea ratio were not. In addition to soil Pb concentration, bacterial copy numbers were significantly correlated with Cd, Zn and Mg. Out of four metals tested (Pb, Al, Cd and Zn), Pb was the only metal to significantly affect microbial diversity. Shannon-Wiener diversity and Simpson's evenness indices were negatively correlated with Pb concentration, while the Simpson diversity index was positively correlated, and the Shannon evenness index was not correlated with Pb concentration. The authors suggested that these conflicting results might be due to how the indices were calculated or the presence of an outlier. Given that the other metals analyzed (Al, Cd, Zn) were not correlated with microbial diversity, the authors suggested that the microbial community had already reached a stable equilibrium with long-term heavy-metal exposure. Using CCA to determine the relationship of Pb, Cd, Zn and Al with OTU abundance, 1150 OTUs were found to be significantly correlated with Pb. A total of 2,591 OTUs out of 27,082 were significantly correlated with one of the four metals (Al, Cd, Pb or Zn), and 60% of these OTUs correlated with two or more metals while 28% correlated with all four metals. Finally, distance-based linear modeling and redundancy analysis were used to determine which environmental factors best explained variation in the soil microbial community. Soil Pb explained 6.96% of the variance in community structure, with only Al and Zn explaining more (Al = 7.99%, Zn = 7.64%).

Long-term exposure to Pb and other heavy metals influence microbial community structure, as heavy-metal-tolerant fungi have been isolated in forested areas in the United States (Torres-Cruz et al., 2018). Fungi were isolated from soil collected from N-fertilized and unfertilized plots in Duke Forest, North Carolina. Fungi tolerant to Pb were isolated from the rest of the fungal community by adding diluted soil to malt extract agar supplemented with antibiotics and Pb stock solutions (100 or 500 ppm $Pb(NO_3)_2$). Fungal isolates were identified using OTUs and used in phylogenetic analyses and next generation sequencing was conducted to determine the abundance of heavy-metal-tolerant taxa. The number of isolated OTUs tolerant to Pb were higher compared with the number of isolates tolerant to other heavy-metal stock solutions analyzed in this study, including Al, Cr, Fe, Ni, Cu, Cd and Zn, and the largest number of isolates were obtained from Pb (30% of all isolates) followed by Zn (14% of isolates). The genera *Trichoderma, Penicillium, Umbelopsis, Pochonia,* and *Saitozyma*, all have isolates tolerant to Pb stress. The most common taxa, *Trichoderma* and *Penicillium*, were detected in all metal-enriched samples, and the authors hypothesized this gives them a competitive advantage across a wide range of polluted conditions.

Other field studies have found mixed relationships between soil Pb concentration and bacterial abundance and community structure. For example, Větrovský and Baldrian (2015) examined the relationship between bacteria and actinobacterial biomass and diversity and soil heavy-metal content (Pb, Cd, Cu, and Zn) across sites ranging in distance from a polymetallic smelter in Příbram, Czech Republic. Pb soil concentrations ranged from 160.5 ± 3.9 mg Pb/kg (mean ± S.E.) to 1713.5 ± 123.4 mg Pb/kg at the most contaminated site. Pb concentration in the soil was significantly correlated with Cd, Cu and Zn, but not oxidizable C, total N content, C/N, and pH. Bacterial biomass, actinobacteria biomass and the ratio of actinobacteria:bacteria were not significantly correlated with Pb concentration. Finally, the Shannon-Wiener diversity index increased with increasing heavy-metal contamination.

Although abundance and diversity indices are commonly reported in observational studies examining the relationship between Pb, other soil metals and microbial communities, some studies have reported additional effects including average cell wall color development (AWCD) or average carbon source utilization, microbial growth rate and enzyme activities. These effects can act as surrogates for microbial activity and diversity. Specifically, Boshoff et al. (2014) used BIOLOG® EcoPlates™ to assess microbial capacity to metabolize a variety of carbon substrates in two grassland sites that varied in their distance from an active metal refinery in Antwerp, Belgium. Average carbon utilization AWCD, the number and variety of utilized substrates (functional richness (R') and the functional diversity (H')) were analyzed. Unlike pH, OC, particle size distribution, Cd, Ni and Zn concentration in the soil, Pb concentration differed significantly between the soils of the two sites, ranging from 147.10 mg Pb/kg to 1,373 mg Pb/kg across all subplots. Additionally, soil moisture, temperature, As and Cu differed between the two grassland sites. Overall, pseudototal Pb and Cu concentration, which was measured by adding hydrochloric acid and nitric acid to the samples (as well as As and Cu) was negatively correlated with AWCD, R' and H'; however, when an analysis of covariance was performed to understand the effect of metal pollution on microbial responses, Pb was not a significant factor driving variation for AWCD, R' or H', while sampling site and As concentration were significant predictors.

In many observational field studies, total Pb soil concentration is often used when analyzing soil microbial communities; however, some studies attempt to determine bioavailable Pb in addition to total soil Pb. The relationship between total and bioavailable concentrations of heavy metals (Pb, Zn, Cu, Cd), soil physicochemical properties (pH, total N, available P, available K and OM) and soil microbial communities was explored from soil collected near an abandoned ore-dressing plant in Hezhang County, China (Wang et al., 2018a). Total soil Pb concentrations ranged from 67.4 ± 1.6 mg Pb/kg (mean ± S.D., n = 3) to 759.3 ± 11.4 mg Pb/kg, while bioavailable Pb, measured as 0.1 M HCl-extractable Pb (HCl-Pb) ranged from 33.0 ± 1.9 mg Pb/kg to 681.0 ± 33.9 mg/kb Pb. In this study, neither total soil Pb nor HCl-Pb was correlated with microbial enzyme including fluorescein diacetate hydrolysis activity, an indicator of soil microbial activity and urease activity. Additionally, Pb was not significantly correlated with any microcalorimetric parameters examined; however, when bioavailable Pb (HCl-Pb) was used instead of total Pb, the direction of these trends changed. Pb and HCl-Pb showed mixed relationships with bacterial abundances. For example, *Thiobacillus, Anaerolineaceae*, and *Xanthobacteraceae* abundances were significantly positively correlated with HCl-Pb, HCl-Cu, and Cu, while uncultured *Acidmicrobiales* showed significant negative correlation with Pb and HCl-Zn.

Previous exposure to pollution in soil may affect the sensitivity of microbial communities in the rhizosphere to Pb stress (Zhang et al., 2019b). Ferns (*Athyrium wardii*) were collected from either a site exposed to mining (mining ecotype or ME) or a reference site (nonmining ecotype [NME]) in Sichuan Province, China. Collected *A. wardii* were then grown in uncontaminated soil for several generations and subsequently exposed to one of five experimental Pb levels: 0, 200, 400, 600 or 800 mg Pb/kg (aqueous $Pb(NO_3)_2$). After 50 days, soil Pb concentration, soil respiration, microbial biomass carbon (MBC), aboveground and belowground biomass, soil physicochemical characteristics (total and available N and P, pH, and OM), and heavy metals were analyzed. Total and available Pb in the rhizosphere increased significantly with experimental Pb exposure, while OM, TN, available N, available P, available K, and pH were similar across all Pb treatments. Total Pb was 9.74 ± 0.11, 210.27 ± 0.41, 412.24 ± 0.60, 607.17 ± 0.65 and 811.74 ± 0.44 mg Pb/kg (mean ± S.D.), and available Pb was 2.15 ± 0.24, 72.23 ± 0.28, 166.30 ± 0.38, 242.94 ± 0.19 and 382.17 ± 0.60 mg Pb/kg, respectively. The rhizosphere of *A. wardii* ME had significantly higher concentrations (12–4.8 times) of Pb compared with that of the NME. Microbial activity, characterized through soil respiration and MBC, was reduced under increasing Pb concentration for both ecotypes; however, the microbial community in the rhizosphere of NME experienced a greater reduction in MBC when exposed to high Pb treatments (400–800 mg Pb/kg) than ME plants (NME 28.4%–68.2% versus ME: 21.2%–60.9% less MBC than control). Additionally, the MBC of soils in the rhizosphere of the NME was significantly lower than that of ME for *A. wardii* exposed to Pb. Finally, the soil metabolic quotient or soil qCO_2 increased with increasing Pb exposure; however, plant ecotype did not affect soil qCO_2. The authors suggested that in general, the microbial community in the rhizosphere of the ME was more adapted to Pb stress than the community in the rhizosphere of the NME, as soil respiration and MBC are less affected by Pb exposure.

Since the 2013 Pb ISA (U.S. EPA, 2013), additional observational studies, many of which were natural environmental gradient studies, have linked microbial community structure (e.g., abundance, diversity) and function (e.g., enzyme activities, respiration rates). Many studies found mixed (negative, positive, and null) relationships between total or bioavailable Pb soil concentration and the abundance of bacterial and fungal taxa (Zappelini et al., 2015), diversity (Aleksova et al., 2020; Kerfahi et al., 2020; Gołębiewski et al., 2014; Tipayno et al., 2012), microbial C and N (Zeng et al., 2020), and respiration and nitrification (Smolders et al., 2015). Unfortunately, it is difficult to disentangle the effects of Pb exposure on microbial communities from the effects of other soil contaminants using environmental gradients, as other heavy metals and soil physicochemical properties are significantly correlated with soil Pb concentration, and many of these factors also influence microbial processes.

11.2.4.2 Effects on Terrestrial Plants and Lichen

In terrestrial plants, Pb is known to induce oxidative stress and impair plant growth, root elongation, seed germination, transpiration, chlorophyll production, lamellar organization in the chloroplast, and cell division. However, the extent of these effects varies and depends on the Pb concentration tested, the duration of exposure, the intensity of plant stress and co-stressors, the stage of plant development, and the particular organs studied. Plants have developed various mitigations when exposed to toxic metal exposures including selective metal uptake, excretion, complexation by specific ligands, and compartmentalization. At sufficiently high Pb exposure, the plant’s antioxidant capacity is exceeded, and peroxidation of lipids and DNA damage follows, eventually leading to accelerated senescence and potentially, death. In the 2013 Pb ISA, the body of evidence was sufficient to conclude there are causal relationships between Pb exposure and both plant physiological stress and reduced plant growth, and inadequate to infer causal relationships between Pb exposure and both plant survival and plant reproduction (U.S. EPA, 2013).

Previous AQCDs recognized declines in photosynthesis and damage to mitosis as effects of Pb toxicity in plants (U.S. EPA, 2006, 1986, 1977). The 2013 Pb ISA added additional experimental studies showing photosynthesis impairment in plants exposed to Pb, and studies of damage to photosystem II due to alteration of chlorophyll structure, as well as decreases in chlorophyll content in plants, lichens, and mosses. Recent studies have continued to demonstrate decreases in photosynthetic performance due to Pb exposure (Alkhatib et al., 2019; Silva et al., 2017a; Rodriguez et al., 2015) as well as documented damage to chlorophyll structure caused by Pb (Tokarz et al., 2020; Alkhatib et al., 2019; Rodriguez et al., 2015). A substantial amount of evidence of oxidative stress in response to Pb exposure has also been produced and documented in the 2013 Pb ISA and previous AQCDs. Monocot, dicot, and bryophytic taxa grown in Pb-contaminated soil or in experimentally spiked soil all responded to increasing exposure with increased antioxidant activity. Recent studies continue to confirm increased antioxidant activity in plants in response to Pb stress (Kaur et al., 2015; Reis et al., 2015; Rossato et al., 2012), as well as the genotoxic effects of Pb exposure (Silva et al., 2017b), albeit at concentrations that greatly exceed Pb measured in soils (Table 11-1). Finally, studies of the effects of Pb on terrestrial plants published since the 2013 Pb ISA continue to support the previous known findings of declines in plant growth under controlled exposures of Pb (Muradoğlu et al., 2016; Kaur et al., 2012; Rossato et al., 2012).

Although Pb exposure is associated with various responses in plant and lichen species, most effects seen in terrestrial plants occur at exposures that are generally at higher environmental concentrations than those outside of the boundaries set for consideration in this ISA (Section 11.1.1). Additionally, while studies find that exposure to Pb has effects on terrestrial plants that could, depending on a number of factors, then contribute to community- or ecosystem-scale effects, the exposure methods typically used make it difficult to compare these effects to what might occur under the uncontrolled conditions encountered in natural environments. Overall, these experiments demonstrate the effects of Pb exposure in terrestrial plants and the underlying physiological and biochemical mechanisms, but strong uncertainties remain regarding the natural concentrations at which these effects would be observed.

One novel area of research is the existence of sex-dependent differences in the effects of Pb in poplar (*Populus* spp.) trees. In a study of sexual differences in *Populus cathayana* exposed to Pb in soil or applied to the leaves, singly and in combination with drought conditions, Han et al. (2013) found significantly different effects between male and female trees. Male trees exhibited a greater ability to bioconcentrate Pb in the root systems, a higher heavy-metal tolerance and photosynthesis plasticity, and less-damaged cell ultrastructure. When Pb was applied to the leaf alone and in both combined treatments, there were significant effects on dry mass production, photosynthetic activity, long-term water use efficiency, potential quantum yield of photosystem II and cellular ultrastructure, and greater effects were observed in females than in males. Drought worsened Pb stress in both sexes; however, the effects were larger on female trees. A second study examined sex-dependent responses to Pb stress in the congeneric *Populus deltoides* (Xu et al., 2016b). Pb-induced negative effects on *P. deltoides* root growth were sex-related and branch order-specific. Compared with plants in control conditions, Pb decreased total root length, total surface area, root diameter and biomass, and the effects were significantly greater in female trees than in males. This agrees with the findings of Han et al. (2013) that female poplar trees exhibit greater Pb sensitivity. Xu et al. (2016b) found that males of *P. deltoides* could sequester Pb in the roots of lower orders and suppress transportation of Pb to high-order roots, which may partially explain the greater Pb tolerance in males when evaluating tree physiological variables.

Recent studies have also examined the protective effects of certain plant nutrients as well as the influence of mycorrhizal inoculation on the effects of Pb in terrestrial plants. In a hydroponic experiment with two different ecotypes of *Elshotzia argyi* (one from an agricultural site and one from an abandoned Pb mine in China), plants were exposed to 50 μM Pb with normal Zn levels (0.5 μM) and high Zn (20 μM) for 12 days (Islam et al., 2011). Application of Pb with normal Zn had negative effects on the overall growth and antioxidant capacity of both ecotypes; however, the effects were more pronounced in agriculturally sourced plants. The addition of high Zn improved the growth and antioxidant capacity of both ecotypes under Pb stress. Finally, a study using Pb exposures on *Torreya grandis* seedlings (0, 700 and 1400 mg Pb^{2+}/kg) examined the possible protective effects of the addition of 1040 mg/kg Mg^{2+}(Shen et al., 2016). The addition of Mg^{2+} improved the growth of the Pb-stressed seedlings, increased chlorophyll content, enhanced chloroplast development and improved both the photosynthetic rate and maximum quantum efficiency in Pb-stressed plants. In addition, Mg^{2+} addition increased root growth and oxidative activity and protected the root ultrastructure. These studies showed that some mineral nutrients, when added beyond the minimal plant requirements, can increase plant tolerance of Pb stress. This is particularly true of Mg addition. Ling and Hong (2009) hypothesized that Pb^{2+} may replace either Mg^{2+} or Ca^{2+} in chlorophyll or the oxygen-evolving center, inhibiting photosystem II function through an alteration of chlorophyll structure.

Mycorrhizal inoculation also appears to protect terrestrial plants from the effects of Pb. One study examined the effects of AMF (*Funneliformis mosseae*) on the growth and Pb uptake of *Sophora viciifolia* (Xu et al., 2016a). As expected, the AMF altered root growth and architecture (increasing root length, fork number, tip number, surface area and volume), and these effects are also present under high Pb stress (1,000 μg/g). Examining roots under transmission electron microscope and X-ray spectroscopy revealed that Pb was deposited not only in plant cells but also the cell walls and vacuoles of the AMF intracellular hyphae, meaning that AMF uptake some of the Pb, alleviating the effects on the plant. Whether the protective effect of mycorrhizae is species-dependent or not is unknown.

In summary, recent studies have continued to demonstrate various deleterious physiological effects of Pb exposure, particularly oxidative stress, though uncertainties remain regarding the environmental concentrations at which these effects would be observed. Additionally, recent studies have examined the protective effects of mycorrhizae in some plants and of some plant nutrients when added in excess of plants' minimal requirement. There is still very little evidence addressing the relationship between Pb exposure and plant survival and reproduction, especially at exposures to concentrations of interest for this ISA.

11.2.4.3 Effects on Terrestrial Invertebrates

For terrestrial invertebrates, exposure to Pb generally increases mortality, decreases growth, and can have detrimental effects on behavior as summarized in previous U.S. EPA reviews of this metal. In studies from the 2006 AQCD, Pb caused antioxidant effects, reductions in survival and growth, as well as decreased fecundity in soil invertebrates (U.S. EPA, 2006). In the 2013 Pb ISA, there was also evidence for neurobehavioral aberrations and, in some cases, decreasing fecundity via changes in the endocrine system (U.S. EPA, 2013). Second-generation effects were reported in some invertebrate species. Recent literature expands the evidence base for suborganism-level and organism-level endpoints and further supports effects on physiological endpoints in additional invertebrate groups, as well as multigenerational effects of Pb exposure. In addition, recent literature provides new information on the effects of Pb on organisms not included in the 2013 Pb ISA such as honeybees. Similarly, while soil nematodes are aquatic organisms—living in the water-filled pore spaces between particles and in water films on soil particles—they are included in the terrestrial section since they are exposed to soil Pb concentrations. Accordingly, adherence to aquatic concentration guidelines was not strict when effects were examined in laboratory conditions.

11.2.4.3.1 Suborganism-Level Response

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a likely causal relationship between Pb exposure and suborganism physiological level responses in terrestrial invertebrates (U.S. EPA, 2013). Changes in enzyme activities and oxidative stress markers were reported in terrestrial invertebrates, including earthworms, snails, and nematodes. Additional studies published since the 2013 Pb ISA, primarily in earthworms and snails, provide additional supporting evidence for perturbation of biomarkers of physiological stress associated with Pb exposure.

Available studies in earthworms have assessed a suite of physiological responses including protein and lipid content following Pb exposure. In field-collected earthworms (*Aporrectodea caliginosa*) from metal-polluted soils across northern France, protein content in earthworm was negatively correlated with easily extractable Pb ($CaCl_2$ extractable), and stepwise model selection further correlated protein content positively with soil clay content (Beaumelle et al., 2014). Lipid content was also negatively correlated with Pb and was positively correlated with silt content. Glycogen was not related to any metal or soil parameter measured. Total Pb soil concentration varied from 19.6 to 491 mg Pb/kg. It is important to note that Pb did not occur alone in these soils and is an example of natural pollution conditions. The authors suggested that energy responses to Pb may be due to demands for mediating oxidative stress mechanisms or regulation.

Several studies with the earthworm *E. fetida* assessed changes in biomarkers of physiological stress following exposure to Pb. In adult *E. fetida* exposed via soil (40, 250, 500, 1,000, 2,500 mg Pb/kg, nominal values, values in organism were measured post-exposure) for 4-weeks followed by a 4-week recovery period, MDA was higher in Pb-exposed earthworms during both the exposure and recovery periods (Žaltauskaitė et al., 2020). MDA was positively correlated with soil Pb exposure, and while MDA concentrations were lower during the recovery period compared with the exposure period, the levels were still higher than control levels at the end of the recovery period (1.2–1.9 times higher). While MDA levels did decrease in Pb-exposed worms during the recovery period, the lack of complete recovery of MDA levels shows worms are not able to recover from Pb-induced oxidative stress within 4 weeks and that either a longer recovery period is needed or MDA response to Pb has a delayed effect. Juvenile *E. fetida* earthworms exposed to Pb had higher levels of MDA, which increased by 25%–54% as soil Pb increased (40, 250, 500, 1,000, 2,500 mg Pb/kg, nominal values, values in organism were measured post-exposure)

(Žaltauskaitė and Sodienė, 2014). In another study, *E. fetida* exposed to 5 mg Pb/kg of Pb had lower protein content than control worms but there was no difference at the 50 and 500 mg Pb/kg exposure levels (nominal values) (Wu et al., 2012a). Cellulase activity, however, was higher across all Pb exposure levels compared with control. DNA damage in coelomocytes (phagocytic leukocytes) was measured by changes in olive tail moments, tail length, tail DNA content, and tail moment using a comet assay. There was no effect of Pb on olive tail moments or tail length. Tail moments increased but only in the 50 mg Pb/kg treatment, as did tail DNA contents. The authors concluded since cellulase activity is involved in the breakdown of cellulose, an increase in cellulase activity suggests Pb may increase *E. fetida*'s ability to degrade plant matter within the soil profile. Pb exposure at 50 mg Pb/kg appeared to lead to more DNA damage of coelomocytes but not at 500 mg Pb/kg, indicating more research is needed to elucidate the effect of Pb exposure on the earthworm immune system via DNA damage, and given that the exposures were nominal, the putative effects should be quantified with measured exposures in more complete experiment.

For snails, after 7 days of exposure to Pb via diet, AChE activity in the digestive gland of the green garden snail (*Cantareus apertus*) decreased with increasing Pb exposure (nominal dietary exposure values reported, values in snail tissue measured) (Mleiki et al., 2015). Activity was 200 µmol/nm/mg in control snails, approximately 75 µmol/nm/mg at 25 mg Pb/kg exposure and about 25 µmol/nm/mg at 2,500 mg Pb/kg Pb exposure. After 60 days of exposure, the activity level was lower across all groups but followed the same decreasing pattern with increasing exposure. AChE activity in the foot also followed a similar pattern to the digestive gland, with decreasing activity at day 7 with increasing exposure. After 60 days, differences across treatments were not significant in the foot. Overall, Pb caused a decrease in AChE activity in both the foot and digestive gland, but the effect was stronger in the short term compared with the long term. In another snail study, metal concentrations in soil, stinging nettle (*Urtica dioica*), and the digestive gland of *Cepaea nemoralis* snails were assessed in relation to the pollution source (metal smelter in Belgium), with various physiological biomarkers also measured (Boshoff et al., 2015). Soil Pb concentrations varied from approximately 50 mg/kg to 1300 mg/kg and generally decreased with increasing distance from the pollution source. Pb in leaves followed the same general pattern. European land snails prefer nettle leaves as a food source, and Pb concentrations in the digestive gland followed the same pattern as those in soil and leaves each week of the experiment, with the pattern becoming more pronounced over time with far greater concentrations at the pollution source location (orders of magnitude greater than other sites). Metal concentration in plants was positively correlated with soil concentrations, and concentrations in the snail digestive gland were positively correlated with plant concentrations. Protein, glycogen, GST, and total energy levels measured within the digestive gland showed no clear pattern in relation to Pb and instead depended on interactions between the specific site, exposure time, and different heavy metals. There were also no correlational changes in shell morphology.

Physiological stress response linked to Pb exposure was reported in a few additional terrestrial invertebrates. Overall, gut enzyme activities, with the exception of alpha-glucosidase, were higher in honeybees (*A. mellifera*) within urban-located hives in Nigeria compared with wild beehives.

Carbohydrases (amylase and cellulase) were higher than lipase and proteinase across both nesting habitats. However, there was no difference in Pb concentration in bees between habitats, and differences in enzyme activities showed no direct correlation to Pb specifically (Lawal et al., 2014). In another study, honeybees in a laboratory setting were fed a sucrose solution with Pb concentrations of 10, 1, 0.1, and 0 mg Pb/L over a 48-hour period. GST enzyme activity and gene expression were examined, along with AChE activity. No effect of Pb was observed at any exposure concentration on GST activity or gene expression after 48 hours. AChE activity was lower at 0.1 mg Pb/L and higher at 10 mg Pb/L concentrations (Nikolić et al., 2019). In a trophic study examining Pb uptake by mulberry trees (*M. alba*) and subsequent transfer to silkworms (*B. mori*), Pb content in silkworms and silkworm excretions (feces and silk) increased with increasing Pb treatment (0, 200, 400, and 800 mg Pb/kg soil treatments, nominal values) across lifestages (larvae and moth). Additionally, metallothionein was higher in the midgut in all Pb treatments compared with control larvae and was higher in the 800 mg/kg treatment compared with the 200 and 400 mg Pb/kg treatments. Metallothionein was also higher in silk-glands and body fat in the 400 and 800 mg Pb/kg treatments (Zhou et al., 2015).

11.2.4.3.2 Organism-Level Response

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a likely causal relationship between Pb exposure and neurobehavioral responses in terrestrial invertebrates (U.S. EPA, 2013) (and see Table 11-2 of this appendix). Evidence was primarily from feeding studies in snails and altered behaviors in nematodes (*Caenorhabditis elegans*). Several new studies have assessed behavior modification following Pb exposure in soil organisms and flying insects; most were conducted at nominal dietary Pb concentrations, whereby known nominal amounts of Pb were fed to the organisms as a method of achieving a gradient of tissue concentrations that was in turn measured.

Additional studies in nematodes lend further support to Pb neurotoxicity in these organisms. In a behavioral food preference and food-finding lab study using agar plates, nematodes (*C. elegans*) avoided contaminated food and chose uncontaminated food spots at 1 mg Pb/L, but at 129 mg Pb/L (50% lethal concentration; LC_{50}), Pb contamination interfered with food-finding ability, and there was no difference in movement toward either contaminated or uncontaminated food (Monteiro et al., 2014). Another study using *C. elegans* found that feeding activity decreased as Pb concentration increased. EC_{50} for feeding behavior was approximately 15 mg Pb/L (54 μM). Pb also increased damage to the dopaminergic neurons (Tang et al., 2019). The study also examined the effects of Cd and Mn, the effects when Pb was mixed with either metal, or the effects of a treatment containing all three metals. The effects on *C. elegans* feeding behavior were greater than the additive effect in binary Pb mixtures at $fa < 0.85$ (fraction of organism system affected) but less-than-additive at $fa > 0.9$. The ternary combination had greater-than-additive effects at $fa < 0.75$ and less-than-additive effects at $fa > 0.8$.

New studies in honeybees suggest Pb exposure alters feeding and foraging behaviors. Soil Pb contamination (approximately 47.3 mg Pb/kg) did not change the number of honeybee, bumblebee, or megachilid visits to sunflowers but soil contamination did change the foraging behavior of bees (Sivakoff and Gardiner, 2017). Bumblebees visited uncontaminated grown sunflowers 5.4 times, honeybees 3.7 times and megachilidae 3.6 times longer than sunflowers grown in contaminated soils. Structural equation modeling analysis shows a direct negative effect of Pb soil contamination on bee visit duration for bumblebees and honeybees but direct effects of floral traits or indirect effects of Pb on floral traits were not significant, suggesting Pb contamination directly explains bee visit duration when floral traits are held constant. In a behavioral lab experiment, *A. mellifera* were exposed to a range of Pb concentrations (0.07, 0.66, 6.61, 661 mg Pb/kg, dietary values) in a sucrose solution to examine the effect of Pb contamination on feeding behavior. Only at the highest Pb concentration did bees reduce sucrose solution intake. By measuring neuron response to sucrose in antennal gustatory sensilla, the authors determined this response was due not to detection of the Pb but rather due to a decrease in sucrose perception when Pb was added to the solution. Furthermore, bees readily ingested the Pb-contaminated solution within a range of 0.075 to 0.75 mg Pb/kg, which the authors reported as comparable to concentrations found in flowers (1.1 to 1.735 mg Pb/kg) (Monchanin et al., 2022). In another behavioral honeybee experiment, the effects of Pb (0.07 and 0.66 mg Pb/kg, dietary values) on bee cognitive flexibility were tested. Bees exposed to 0.66 mg Pb/kg contaminated food over 70 days showed less flexibility in response to changing flower rewards. This response was positively correlated with bee body Pb concentration. Furthermore, higher Pb exposure during the larval state correlated with lower body weight and head size (Monchanin et al., 2021).

A behavioral experiment examined whether there was a difference in foraging behavior between cabbage white butterflies (*Pieris rapae*) reared on a Pb-contaminated diet versus those raised on an uncontaminated diet (Philips et al., 2017). Larvae were fed either a 4 mg Pb/kg (dietary values) or control (approximately 0.17 mg Pb/kg) diet. Behavioral testing following Pb exposure involved yellow sponges soaked in honey (rewarding) or water-soaked blue sponges (nonrewarding). Butterflies reared on Pb as larvae were more likely as adults to interact with sponges (approximately twice as many adults interacted with the sponges compared with control-reared butterflies). Of the butterflies that did interact with the sponges, there was no difference between treatment groups in the proportion that completed five consecutive landings on the rewarding sponge. There was also no difference in the duration it took for butterflies to complete the test (time taken to land five times in a row on yellow sponges). The authors suggested this species may already have adapted to low levels of Pb in their diets because brassicas (natural food source for larvae of *P. rapae*) mature quickly and are often found in disturbed locations where Pb may be present. Therefore, the 4 mg Pb/kg concentration may not have been high enough to induce a different response between treatments in the laboratory-exposed butterflies.

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a likely causal relationship between Pb exposure and growth endpoints in terrestrial invertebrates (U.S. EPA, 2013) (see Table 11-2 of this appendix). Evidence in the 2013 Pb ISA was primarily from concentration-dependent inhibition of growth in earthworms raised in Pb-amended soil, and, to a more limited extent, for reduced growth in snails (dietary studies) and nematodes. New evidence continues to show growth related effects in invertebrate soil organisms.

Additional studies in earthworms since the 2013 Pb ISA continue to support findings of Pb on growth. Žaltauskaitė et al. (2020) examined the effects of Pb exposure on earthworm (*E. fetida*) weight, growth, and recovery postexposure. During 4 weeks of soil exposure (40, 250, 500, 1,000, 2,500 mg Pb/kg, nominal values, values in organism were measured post-exposure), no effect on weight loss was found, but Pb decreased growth rate with a difference of 15.8%–40% lower fresh weight compared with control worms. Following exposure, earthworms were given a 4-week recovery period with no Pb exposure. While earthworms recovered some weight, they did not reach equal weights compared with non-exposure worms (11%–17.6% lower than control at end of recovery period). Fresh weight was negatively correlated with increasing Pb soil concentration during both the exposure and recovery periods. Growth and recovery rate varied with concentration, with earthworms exposed to 40 mg Pb/kg having the greatest growth rate compared with other Pb concentrations. Earthworms grew slower during the recovery period compared with the exposure period except for those exposed to 2,500 mg/kg, which showed equal growth rates during exposure and recovery. MDA was also positively correlated with Pb levels. During recovery, MDA concentrations were lower but did not reach the same levels as control worms. Weight response to Pb exposure and recovery suggests Pb inhibits earthworm growth and may have a short-term legacy or lag effect as recovery did not reach 100% within the same time frame. Increased MDA concentration is indicative of oxidative stress, which may explain the reduced growth since MDA concentrations were still comparatively high after the recovery period. Another earthworm study by Žaltauskaitė and Sodienė (2014) examined juvenile earthworm growth and time to maturation across nominal soil Pb concentrations of 40, 250, 500, 1,000, 2,500 mg Pb/kg, values in organism were measured post-exposure. There was no overall effect on weight loss, but juveniles exposed to Pb were smaller than control worms. The EC_{50} for juvenile growth increased with increasing time of exposure—at 3 weeks, the EC_{50} was approximately 100 mg Pb/kg but after 14 weeks the EC_{50} for reduced weight was 179 mg Pb/kg. The time of maximum growth in the 40 mg Pb/kg exposure group was during the 8–10-week period, while maximum growth was delayed in higher Pb treatments. Pb significantly lengthened the time to sexual maturation. The minimum time to maturation was 9 weeks for the control and Pb treatment groups, and the minimum weight at this development point was 0.182 g in the 40 mg Pb/kg treatment group. Since increasing Pb concentrations reduced the growth rate, the time needed to reach the minimum maturation size would increase with increasing Pb; therefore, the time needed at 250 mg Pb/kg would be 16 weeks. The total number of earthworms that reached maturity by the end of the experiment was negatively correlated with Pb concentrations, with only 5%–7% of worms reaching maturity in the 250 mg Pb/kg treatment group.

Adding to the evidence for growth effects in snails from the 2013 Pb ISA, studies on green garden snail (*Cantareus apertus*) bioaccumulation and growth in response to increasing Pb dietary concentrations (25, 100, and 2,500 mg Pb/kg, dietary values) over 1 week and 8 weeks of exposure found the wet weight of snails increased with time across all Pb treatments, and the effect was dose-dependent in Pb-treated snails (Mleiki et al., 2016). The weight of snails was significantly lower than the weight of control snails by week 2 in the high Pb-treatment group, by week 3 for medium Pb-treatment snails and by week 7 for snails in the low Pb-treatment group. The cumulative growth rate followed a similar pattern but was lower by week 1 for the high Pb-treatment snails, by week 3 for medium treatment and by week 7 for low Pb treatment. Overall, dietary Pb decreased growth in green garden snails, with a lowest observed effect concentration (LOEC) of approximately 25 mg Pb/kg food within several weeks. A trophic snail study found soil Pb levels varied from approximately 6 mg Pb/kg to 52 mg Pb/kg across a gradient of polluted sites in Romania (Nica et al., 2012). Shell height was negatively correlated with Pb in nettle leaves (food source), and relative shell height was positively correlated with snail hepatopancreas Pb levels. Pb in soil was also correlated with other metals (Zn and Cd). Heavy metals are known to accumulate in snail shells and can often lead to changes in shell size and geometry.

The growth effects of Pb reported for earthworms, snails and nematodes are augmented by studies in a few additional terrestrial invertebrates. In a generational study with tobacco cutworms (*Spodoptera litura*) reared on artificial diets with increasing Pb concentration, both Pb and generation effects were observed on relative growth rate, pupation rate, and eclosion rate (Shu et al., 2015). First-generation pupae experienced no effects of Pb stress on pupation rate or relative growth rate. Eclosion rates did decrease in the 100 and 500 mg Pb/kg treatments groups (dietary values) (eclosion rates were 51.48% and 28.89%, compared with approximately 70% for all other treatments). Fifth generation larvae showed significantly lower eclosion and pupation rates at 25 and 50 mg Pb/kg compared with 12.5 mg Pb/kg and control treatments. The relative growth rate of fifth generation pupae declined as well for the 25 and 50 mg Pb/kg treatments. Differences between generations occurred at the 50 mg Pb/kg treatment, with 50 mg Pb/kg having stronger negative effects in the fifth generation compared with the first. There was no effect of Pb (4 mg Pb/kg) on cabbage white butterfly (*Pieris rapae*) development time or body size regardless of Pb concentration or butterfly sex (Philips et al., 2017). Kenig et al. (2013) reared fruit flies (*Drosophila subobscura*) in the lab for eight generations at low and high Pb exposure (10 µg Pb/mL and 100 µg Pb/mL, dietary values) from two wild-caught populations with a difference in Pb exposure history (298.6 mg Pb/kg and 25.7 mg Pb/kg soil). Flies from the population originally collected from the site with high pollution levels exhibited a decrease in development time over generations reared at control (no Pb) lab conditions, a decrease in development time when reared at low Pb-exposure lab conditions and an increase when reared at high Pb-exposure conditions. Flies from the low historic contamination site exhibited an increase in development time at control conditions, a decrease at low Pb exposure, and a decrease at high exposure. Across all levels of Pb exposure in the lab, there were population, generation, and population × generation effects on fruit fly development time. Overall, the flies from the high Pb-exposure contamination group had faster development time across both lab exposure Pb concentrations compared with the low historic contamination population responses. The authors suggest this response in development time in the high historic exposure population may be an ancestral adaptation response to allow for growth and reproduction to occur before Pb toxicity occurs.

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship between Pb exposure and reproduction in terrestrial invertebrates (U.S. EPA, 2013) (see Table 11-2 of this appendix). Reproduction endpoints examined in the 2013 Pb ISA included brood size and hatching success. Additional studies in soil invertebrates published since the 2013 Pb ISA continue to report Pb effects on reproduction and development, adding to the evidence base for this endpoint.

Pb exposure (40, 250, 500, 1,000, 2,500 mg Pb/kg, nominal values, concentration in tissue measured) significantly lengthened time to sexual maturation for juvenile *E. fetida* earthworms (Žaltauskaitė and Sodienė, 2014). The minimum time to maturation was 9 weeks for the control and Pb treatment groups, and the minimum weight at this development point was 0.182 g in the 40 mg Pb/kg treatment group. Since increasing Pb concentrations reduced growth rate, the time needed to reach the minimum maturation size would increase with increasing Pb; therefore, the time needed at 250 mg/kg would be 16 weeks. The total number of earthworms that reached maturity by the end of the experiment was negatively correlated with Pb concentrations, with only 5%–7% of worms reaching maturity in the 250 mg/kg treatment group. In addition, cocoons were only found at the lowest treatment of 40 mg Pb/kg, and the number of cocoons was less than half of the number of cocoons produced in control soils.

In a multigeneration vinegar fruit fly (*D. melanogaster*) study, females that were reared under no Pb conditions preferentially mated with control males (60% of the time) over males reared in Pb conditions (108 mg Pb/kg) (Peterson et al., 2017). In the same study, Pb-reared females preferentially mated with Pb-reared males over control males (65% of the time). Second-generation females did not show a significant preference for either second-generation male group (Pb-reared mother or control-reared mother). Males across treatments showed no mate preference, and second-generation male body Pb content was not related to parental Pb content. Despite the behavioral response of females in mate preference, a principal component analysis of male and female pheromones showed no significant difference between either male or female treatment groups. Furthermore, there was no difference in multiple male courtship song variables. While the mechanisms for mate preference remain unclear, there appears to be no generational effect on fitness. There was no difference between Pb treatments in the parental generation on either parental or second-generation responses in dry body weight, fecundity, or time to reach either 50% or 80% mortality. Pb accumulates in fruit fly bodies and this accumulation appears to influence female but not male mate choice but does not lead to any differences in ability, success, or fecundity of the flies or their offspring. Another study with *D. melanogaster* observed that vinegar fruit flies accumulate Pb linearly with Pb exposure concentration and that the number of eggs laid on Pb-treated media varied with Pb treatment (Peterson et al., 2020). Control-reared females laid fewer eggs on Pb-contaminated media than Pb-reared females at both approximately 109 and 217 mg Pb/kg (250 and 500 µM, PbAc). However, females reared on the highest Pb treatment of approximately 434 mg Pb/kg (1,000 µM) laid fewer eggs than the other Pb treatment females. These results suggested females reared in a Pb-free environment avoid laying eggs in Pb-contaminated areas whereas females raised in a Pb-contaminated environment did not show this preference for egg site. The authors suggested this may be due to a loss of this specific avoidance behavior due to developmental exposure or possibly due to changes in microbial composition. The microbial composition influences oviposition site selection, with females choosing a site with a composition more similar to the one in which they grew. Pb acetate was used as the source of Pb contamination in this study. Pb acetate may directly change the microbial community, which could also explain why Pb-reared females did not discriminate in laying their eggs in a Pb-contaminated site.

Kenig et al. (2013) isolated *Drosophila subobscura* adults from wild populations collected at two sites with different Pb contamination histories (high pollution site 298.6 mg Pb/kg soil average and low pollution site of 25.7 mg Pb/kg). Gravid females from both populations were used to establish separate population breeding lines. Flies were then reared for multiple generations on either a control substrate (no Pb contamination), a low Pb contamination substrate (10 μg Pb/mL, dietary values) and a higher Pb contamination substrate (100 μg Pb/mL, dietary values). Reproduction response variables were measured at the F2, F5, and F8 generations for each of the two population lines. Both populations reared under control conditions in the laboratory across eight generations exhibited an increase in the number of eggs laid between the F2 and F5 generation. This was followed by a decrease in egg production by the F8 generation but only for the population with a lower historic Pb exposure. Under low Pb-exposure lab conditions, both populations showed the same pattern of increasing number of eggs from F2 to F5 followed by a decrease in production to F8, though this pattern was less pronounced for the low historic exposure population. Under high exposure conditions, both populations saw egg production decrease by the F8 generation. Egg viability for the high historic exposure population decreased from F2 to F5/F8 under control conditions, and the low exposure population saw an increase from F2 to F5 followed by a decrease to F2 viability levels by generation F8. Under low exposure conditions, both populations followed the same pattern they showed under control conditions. Under high Pb lab conditions, neither population showed a change in egg viability across generations but the egg viability of the population from low historic exposure conditions had overall lower egg viability than the population that experienced historically high exposure. Individuals from the historic high exposure showed higher viability and fecundity when exposed to higher Pb concentrations in all generations compared with those from the historically low exposure population, exhibiting higher tolerance to heavy-metal exposure.

Mazzei et al. (2013) examined isopod *Armadillidium granulatum* reproductive response to metal contamination of food. According to the authors, isopod heavy-metal concentration factors vary widely across species as does their breeding patterns. In this study, Pb concentration (100, 500, 1,000 mg Pb/kg, dietary values) in food led to an alteration of reproductive patterns in *A. granulatum*. Increasing concentrations led to a delayed onset in breeding season while also reducing the duration of the season. Breeding season onset did not differ between control and 100 mg Pb/kg treatments. Breeding season occurred 1 week later in the 500 mg/kg treatment group and 6 weeks later in the 1,000 mg Pb/kg treatment group. The length of the breeding season decreased from 79 days (control) to 59 (500 mg Pb/kg) and 46 (1,000 mg Pb/kg) days. There was no effect of Pb on incubation period (approximately 23 days), and the percent gravid rate of females increased from 97.2% (control) and 95.8% (100 mg Pb/kg) to 100% for higher Pb treatments. However, while gravid rate increased, brood number declined (from 1.22 to 1). Lastly, the number of juveniles for each brood increased with 500 mg Pb/kg treatment. Overall, contamination at 100 mg Pb/kg did not influence any reproductive endpoint examined for *A. granulatum* but higher levels led to changes in breeding seasonality and the number of juveniles.

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship between Pb exposure and survival in terrestrial invertebrates (U.S. EPA, 2013) (see Table 11-2 of this appendix). Additional evidence continues to show Pb effects on mortality in some terrestrial invertebrates, while others appear to be unaffected. In a laboratory study examining green garden snail (*Cantareus apertus*) response to increasing Pb dietary concentrations (25, 100, 2,500 mg Pb/kg, dietary values) over a period of 1 to 8 weeks, cumulative mortality was greater in all Pb treatments than the control after 6 weeks of exposure, with the high treatment having significantly greater mortality after 1 week (Mleiki et al., 2016). At the end of the experiment, cumulative mortality was below 30% for all treatments. An observational study of *C. apertus* exposed to multiple metal-polluted soils with Pb concentrations ranging from 28.1 to 4574 mg Pb/kg found only 6.5% of snails died after 28 days of exposure (Pauget et al., 2013b). Studies in the 2006 Pb AQCD found earthworm LC_{50} for 14 and 28-day exposure fell within a range of 2,400–5,800 mg Pb/kg. A study reported in the 2013 Pb ISA evaluated *E. fetida* earthworms exposed to field-collected soils with Pb concentrations up to 390 mg Pb/kg and found no effect on earthworm survival (Delistraty and Yokel, 2014). In support, juvenile *E. fetida* earthworms exposed to a range of Pb concentrations (40, 250, 500, 1,000, 2,500 mg/kg, nominal values, concentration in tissue measured) over 14 weeks found mortality increased in the 500, 1,000 and 2,500 mg Pb/kg treatments, with mortality reaching 90% in the highest treatment (Žaltauskaitė and Sodienė, 2014). Juvenile mortality increased with the time of exposure in these treatment groups, with an LC_{50} of 911 mg Pb/kg for 14 weeks of Pb exposure. Juvenile mortality did reach 10% by week 3 for the 40 and 250 mg Pb/kg treatments but did not increase any further over time. However, in another earthworm exposure experiment using adults of *E. fetida*, across only 4 weeks of exposure (40, 250, 500, 1,000, 2,500 mg Pb/kg, nominal values , concentration in tissue measured), there was no significant effect on survival (Žaltauskaitė et al., 2020). In cabbage white butterflies (*P. rapae*) raised from eggs from wild-caught females, no effect on survival was observed in a laboratory study with a diet of 4 mg Pb/kg (Philips et al., 2017).

In terrestrial invertebrates, literature since the 2013 Pb ISA provides additional support on the effects of Pb exposure on organismal and suborganismal responses including a decrease in survival and reduced growth and fecundity. Recently published studies on physiological responses to Pb included decreases in protein and lipid content and increases in MDA in earthworms. AChE activity decreased in response to Pb in snails and honeybees while protein, glycogen, other enzymes, and GST responses were variable depending on modifying site factors or species examined. There are several new studies quantifying behavioral changes to Pb exposure in bees. Soil Pb contamination altered foraging behavior, and at high levels (above 600 mg Pb/kg), also altered sucrose intake. However, at low concentrations (0.66 mg Pb/kg), honeybees showed lower flexibility in response to changing flower rewards, suggesting

Pb may lead to lower nectar and pollen supply and subsequently slower colony development or winter survival. New literature on growth endpoints suggests Pb can have lasting effects even postexposure on earthworms. Growth, eclosion, and pupation rates of the common cutworm were all lower under Pb exposure, and fruit fly development time increased within eight generations in populations with historic Pb pollution exposure. In addition to previously assessed endpoints of Pb on brood size and hatching success, new literature shows Pb exposure slows time to maturation in earthworms, delays onset to and duration of breeding season in isopods and influences mate selection in fruit flies. While the literature since the 2013 Pb ISA has primarily provided additional support on previously examined organisms and endpoints, there has been new information on new organisms as well as on modifying factors on organism response including habitat, exposure history, seasonality, and duration of effects.

11.2.4.4 Effects on Terrestrial Vertebrates

In observational and experimental studies, commonly observed effects of Pb on terrestrial vertebrates include decreased survival, reproduction, and growth, as well as effects on development and behavior (U.S. EPA, 2006). The 2013 Pb ISA (U.S. EPA, 2013) also provided evidence for Pb effects on hormones and other biochemical variables (U.S. EPA, 2013). Recent studies provide additional support to suborganism-level and organism-level endpoints and expand on the effects on hematological and physiological endpoints.

11.2.4.4.1 Suborganism-Level Response

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship between Pb exposure and hematological effects in terrestrial vertebrates (U.S. EPA, 2013) (see Table 11-2 of this appendix). Since the 2013 Pb ISA, numerous new studies have continued to support the connection between Pb exposure and hematological effects. The relationship between Pb concentrations and aminolevulinic acid dehydratase (ALAD) activity has been explored in the literature, across a broad assortment of different vertebrate species including songbirds (Beyer et al., 2013), house sparrows (Cid et al., 2018), Japanese quail (Beyer et al., 2014), griffon vultures (Espín et al., 2015), eagle owls (Espín et al., 2015), common ravens (Herring et al., 2018), turkey vultures (Herring et al., 2018), Canada geese (van der Merwe et al., 2011), mallards (Binkowski and Sawicka-Kapusta, 2015), coots (Binkowski and Sawicka-Kapusta, 2015), giant toads (Ilizaliturri-Hernández et al., 2013), cattle (Rodríguez-Estival et al., 2012), and sheep (Rodríguez-Estival et al., 2012).

Beyer et al. (2013) investigated blood, liver, and kidney concentrations of Zn, Cu, Pb, and Cd and ALAD activity in northern cardinals (*Cardinalis cardinalis*) and American robins (*Turdus migratorius*) living in Pb-contaminated mining sites in southeast Missouri. Birds from contaminated locations had ALAD activity levels that were decreased by between 58% and 82% compared with those from noncontaminated locations. Another field study that examined the relationship between Pb and ALAD activity found similar results in griffon vultures (*Gyps fulvus*) and eagle owls (*Bubo bubo*) (Espín et al., 2015). Blood samples were taken from birds near an industrial area (electric power plants, explosives, and ship-building factories) and a historic Pb-Zn mine. The study found a significant negative relationship between blood Pb levels and ALAD activity in griffon vultures and in eagle owls, with ALAD inhibition of up to 94% and 79%, respectively.

Herring et al. (2018) examined the effects of Pb exposure on ALAD activity in two species of free-living scavengers in the Pacific Northwest: common ravens (*Corvus corax*) and turkey vultures (*Cathartes aura*). The authors speculated that environmental Pb exposure in these species was most likely associated with a variety of sources including hunting, Pb-based paint, soil, and sediment Pb, and mining and smelting activities. Both species exhibited decreased ALAD activity (mean = 5.9 ± 1.4 SE) in birds with blood Pb concentrations greater than 0.2 µg/g (the subclinical toxicity benchmark) when compared with birds with blood Pb concentrations below this benchmark (mean = 9.9 ± 0.6 SE).

Binkowski and Sawicka-Kapusta (2015)is another field study that examined the relationship between blood Pb levels and ALAD activity in free-living birds published since the 2013 Pb ISA. This study investigated free-living mallards (*Anas platyrhynchos*) and Eurasian coots (*Fulica atra*) in Poland. In both species, there was a significant negative correlation between Pb concentrations in blood and ALAD activity. The authors suggested that Pb exposure mainly occurred through Pb shot. van der Merwe et al. (2011) also found evidence of a relationship between Pb concentrations and ALAD inhibition in waterfowl. Geese from the tri-state mining district of Kansas, Oklahoma, and Missouri and multiple different metal concentrations were measured (silver [Ag], As, barium [Ba], Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Se, Ti, V, Zn). This study found that ALAD activity was inversely correlated with tissue Pb concentrations in all tissue except muscle.

Multiple laboratory studies have examined this relationship. Cid et al. (2018) exposed house sparrows (*Passer domesticus*) to sublethal oral doses of Pb acetate solution (1.3, 3.5, 5.5, 7.0, 14.0 µg/g animal/day) for 5 days. This resulted in a gradual decrease in ALAD activity between 3.5 and 7.0 µg Pb/g animal/day, with the 7.0 and 14.0 µg Pb/g animal/day doses producing greater α-ALAD activity inhibition (82% less activity than control group). This study also examined the effects of Pb exposure in drinking water for 15 or 30 days. Inhibition of ALAD activity was similar between the two groups, with an approximately 35% decrease when comparing the mean value of both treatment groups and the controls.

Beyer et al. (2014) studied the effect of Pb-contaminated soil on captive Japanese quail (*Coturnix japonica*) to examine the relationship between Pb exposure and hematological effects and to determine benchmark doses associated with different percentages of ALAD reduction. Quail were fed experimental diets containing 0% to 12% contaminated soil by weight (0.12 to 382 mg Pb/kg, dry weight) for 6 weeks. All quail groups exposed to Pb-contaminated soil had a significantly lower mean ALAD activity than the control group. ALAD activity also decreased with increasing dosage, with control quail having the highest amount of activity and the 12% contaminated soil group having the lowest. The benchmark doses of Pb associated with a 50% reduction in ALAD activity were 0.62 mg Pb/kg in the blood, dry weight, and 27 mg Pb/kg in the diet.

Although there is limited new evidence on the effects of Pb on ALAD activity in other terrestrial vertebrates since the 2013 Pb ISA (U.S. EPA, 2013), two nonbird studies examined this relationship. Rodríguez-Estival et al. (2012), investigated this relationship in both cattle and sheep from livestock farms in Spain. Blood Pb level was found to be negatively correlated with ALAD reaction ratio in both cattle and sheep. Blood Pb level also had a negative effect on ALAD activity. Ilizaliturri-Hernández et al. (2013) examined the relationship between blood Pb levels and ALAD inhibition in giant toads (*Rhinella marina*) in Veracruz, Mexico. Blood Pb levels ranged from 10.8 to 70.6 μg/dL and were significantly higher in industrial sites. Toads at industrial sites also had a 78% decrease in ALAD activity when compared with those at rural sites. Examining the relationship between blood Pb levels and ALAD, a strong inverse relationship was identified. The authors stated that Pb exposure was most likely from pollution released into the air and water by chemical and petrochemical companies in the area.

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a likely causal relationship between Pb exposure and physiological stress for terrestrial vertebrates(U.S. EPA, 2013) (see Table 11-2 of this appendix). Since then, multiple new studies have added to this evidence base. Many different factors are included in physiological stress, including oxidative stress, corticosterone (CORT) levels, and immune response, all of which are discussed here.

Two different studies investigated CORT levels in response to Pb exposure. Meillère et al. (2016) evaluated the relationship between feather Pb levels and feather CORT levels in wild common blackbirds (*Turdus merula*) along an urbanization gradient. Male adult blackbirds were found to have an average feather Pb concentration of 1.00 ± 0.76 μg/g, dry weight, which was positively correlated with the degree of urbanization. Feather CORT levels were found to be significantly and positively related to both the degree of urbanization and feather Pb levels. Herring et al. (2018) also investigated CORT levels in birds. Examining the relationship between fecal CORT levels (FCORT) and blood Pb levels in common ravens (*Corvus corax*), it was found that blood Pb significantly affected FCORT levels only when there was simultaneous exposure to mercury (Hg). FCORT was either not related or negatively correlated with blood Pb when blood Hg concentrations were below 0.2 μg/g, wet weight. Above this blood Hg concentration, the FCORT response increased with increasing blood Pb concentrations.

Another aspect of physiological stress that has been linked to Pb exposure is oxidative stress. Espín et al. (2014) assessed oxidative stress related to Pb in the Eurasian eagle owl (*Bubo bubo*). One study in three different subareas in Murcia, southeastern Spain (rural, industrial, and mining areas) evaluated the relationship between Pb exposure and oxidative stress biomarkers in blood. Glutathione peroxidase (GPx) activity had a significant inverse correlation with Pb concentrations. Catalase (CAT) activity was inversely related to Pb concentration as well. Both GPx and CAT are antioxidant enzymes that catalyze the breakdown of free radicals and indirectly support the antioxidant defense system. Espín et al. (2016) also examined these oxidative stress biomarkers in relation to blood Pb concentrations with different results. In two different gull species, Audouin's gull (*Ichthyaetus audouinii*) and slender-billed gulls (*Chroicocephalus genei*), total glutathione (GSH) content, antioxidant enzymes activities (GPx, superoxide dismutase (SOD), CAT, GST), and lipid peroxidation (thiobarbituric acid reactive substances) were analyzed to determine whether blood Pb concentrations had any effect on these oxidative stress biomarkers. The only significant linear regression on Pb was the positive effect of Pb on GSH levels in Audoin's gulls. The authors speculated that this could reflect the necessity to up-regulate GSH to balance increased oxidative stress caused by metals. A laboratory study of female Japanese quail (*Coturnix japonica*) also examined these effects, as well as other effects including liver histology and lipid metabolism (Kou et al., 2020). Quail were fed one of five experimental concentrations of Pb solution (0, 50, 250, 500 and 1,000 ppm) for 49 days. Pb exposure of 250, 500, and 1,000 ppm induced severe histopathological damages (liver lipid vacuoles and accumulation, hepatic cytoplasmic hyalinization and vacuolization, hepatocyte necrosis, hepatic sinusoid congestion). It also led to a significant decrease in GPx, SOD, and CAT activities in the liver.

Immune response has also been linked to Pb exposure, for example, in the following two studies. Vermeulen et al. (2015) examined the effects of Pb exposure on the innate immunity of great tit (*Parus major*) nestlings in populations along a metal pollution gradient. Average Pb concentration in red blood cells was significantly higher in the populations closest to the pollution source than the farthest population. There were significant differences in lysis scores among the populations, with lysis varying inversely to Pb concentrations. Meissner et al. (2020) used the ratio of heterophils to lymphocytes (H/L ratio) in mute swans (*Cygnus olor*) to determine physiological stress levels. A higher H/L ratio indicates a higher immune response, thus higher physiological stress. Mean blood Pb concentration was 0.239 µg/g (range: 0.028–0.675 µg/g). H/L ratio was found to increase with blood Pb level, indicating that birds with higher blood Pb levels had higher physiological stress.

11.2.4.4.2 Organism-Level Response

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship between Pb exposure and reproduction and developmental endpoints in terrestrial vertebrates (U.S. EPA, 2013) (see Table 11-2 of this appendix). Since the 2006 AQCD (U.S. EPA, 2006) and 2013 Pb ISA (U.S. EPA, 2013), several field studies have examined the relationship between Pb exposure and reproduction. Fritsch et al. (2019) found that the lifetime breeding success of free-living female European blackbirds (*Turdus merula*) in Northwest Poland decreased with increasing levels of Pb in tail feathers (average tail feather Pb = 6.7 µg Pb/g dry weight). This same study also examined the relationship between breeding success, lifespan, and Pb exposure. In birds with the greatest exposure and highest breeding success, there is likely a trade-off between breeding effort and survival, as their lifespans tended to decrease as Pb exposure increased. Chatelain et al. (2016) also studied how Pb exposure affected reproduction. Adult feral pigeons (*Columba livia*) were dosed with one of four exposure treatments: Pb only (1 ppm Pb acetate in tap water), Zn only (10 ppm $ZnSO_4$ in tap water), Pb and Zn (1 ppm Pb acetate + 10 ppm Zn sulfate in tap water), or control (tap water with no metal addition) every other day for 2 weeks. One-day old nestlings of parents exposed to Pb (Pb and Pb +Zn groups) weighed significantly less than the nestlings from other treatments (control and Zn groups) (mean 14.94 ± 0.72 and 17.20 ± 0.67 g, respectively). Additionally, eggs from parents exposed to Pb had significantly thinner eggshells than those from the other groups (mean: 0.47 ± 0.00 and 0.49 ± 0.01 mm respectively).

While Fritsch et al. (2019) examined reproduction at the organism level, Hargitai et al. (2016) examined suborganismal level responses to Pb exposure in relation to reproduction. Hargitai et al. (2016) found that in great tit (*Parus major*) eggs from both woodland and urban habitats in the Pilis Mountains of Hungary, egg yolk lutein and retinol levels were negatively related to the concentrations of Pb in the eggshell. Lutein and retinol are both important antioxidants related to embryo viability in birds.

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a likely causal relationship between Pb exposure and neurobehavioral effects in terrestrial vertebrates (U.S. EPA, 2013) (see Table 11-2 of this appendix). Several additional studies in birds have since been published that assess Pb effects on behavioral endpoints in birds. One new study of the neurobehavioral effects of Pb-exposure evaluated the relationship between the behavior of free-living Northern mockingbirds (*Mimus polyglottos*) and the soil Pb concentrations in their habitats in New Orleans, LA (McClelland et al., 2019). Birds living in neighborhoods with high soil Pb concentrations had higher Pb concentrations in their blood and feathers than those from the neighborhood with low soil Pb concentrations. This study used simulated territory intrusions to examine the level of aggression displayed by individuals from different neighborhoods. Birds from the high Pb neighborhoods exhibited a more aggressive response to simulated intrusions than birds from the low Pb neighborhood.

Another study of the effects of Pb exposure on behavior examined how early-life dietary Pb exposure in great tits (*Parus major*) affected both physical and neurological development (Ruuskanen et al., 2015). Wild birds in selected nests were given an oral dose of Pb acetate in distilled water (4 µg/g body weight for high exposure and 1 µg/g body weight for low exposure) every day for 12 days, starting at 3 days after hatching. At 15 days old, the birds were brought into captivity and kept there for the remainder of the experiment to assess their development after Pb exposure. Early-life Pb exposure was found to have no effect on activity, exploration, neophobia, or success in learning and spatial memory tasks.

Commonly observed effects of Pb on terrestrial vertebrates include decreased survival, reproduction, and growth, as well as effects on development and behavior (U.S. EPA, 2006). The 2013 Pb ISA (U.S. EPA, 2013) also provided evidence for Pb effects on hormones and other biochemical variables. New studies have expanded upon the relationship between Pb exposure and α-ALAD activity by adding more species of birds, amphibians, and mammals to the evidence base. More evidence of oxidative stress has been gathered, as well as evidence of effects on CORT levels and immunity in birds. Literature since the 2013 Pb ISA continues to add to evidence relating to reproductive effects at both the organism and suborganism levels including effects on lifetime breeding success and some specific secondary sexual traits. New studies of behavioral effects included increased aggression in mockingbirds.

11.2.5 Exposure and Response of Terrestrial Species

As previously reported in the (U.S. EPA, 1977), the 1986 Pb AQCD (U.S. EPA, 1986), the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013), a large number of experimental studies have exposed a wide variety of terrestrial organisms to gradients of Pb exposures and reported a broad assortment of responses, including growth, reproduction, survival, antioxidant levels and markers of oxidative stress. More than 80 such additional experimental studies conducted since the 2013 Pb ISA were identified. Organisms subjected to these exposure-response experiments have included various wild plants including reeds and ferns, cultivated crops, microbes, lichens, fungi including mycorrhizae, bacteria, nematodes, worms, collembolans, beetles, spiders, rodents, and birds. The 2006 AQCD and 2013 Pb ISA (U.S. EPA, 2013, 2006) reported that variation in exposure is generally associated with commensurate variation in growth, reproduction, survival, antioxidant activity and more. Such coupling of exposure and response is considered a strong indicator of causality (U.S. EPA, 2015), and exposure-response studies with Pb thus continue to provide evidence supporting the causality of Pb for the effects they investigate, as highlighted in the sections of this appendix dedicated to specific groups of terrestrial organisms.

With very few exceptions, experimental exposure-response studies of terrestrial organisms generate multiple level of exposure through addition of various soluble salts of Pb to the culture medium (natural or artificial soil or hydroponic solution) or to food in the case of some animals. This makes it possible to create a gradient that is easy to quantify and manipulate and is isolated from confounding, nuisance and interacting variables. In principle, these attributes are desirable, as they allow for a more accurate measurement and modeling of exposure-response relationships. They may introduce limits on the scope of inference, but can nonetheless lead to credible, accurate predictive estimates of response, within an acceptable range of natural conditions wherein factors other than exposure are left to vary freely. However, in the particular case of terrestrial organisms and estimates of their response that are obtained through experiments in which exposure is accomplished using salts of Pb, this may not be the case. These experiments are informative for establishing causality, but not for deriving accurate predictive estimates of response under natural conditions.

Section 11.2.2.1 discussed environmental variables that have a strong impact on bioavailability in soils. They include pH, CEC, salinity, aging, OM, soil type and the presence of other metals. The use of soluble salts of Pb brings pH, CEC, salinity, and aging into ranges far removed from those found in natural environments following exposure to Pb emissions. Predicted effects derived from those experiments cannot be expected to be accurate in environmental conditions, not only because the experimental conditions of pH, CEC, salinity and aging diverge too far from those present in the environment, but, more intractably, because in both the experiments themselves and in the environments in which a prediction is attempted, the measurement of Pb concentration may sharply diverge from the concentration actually affecting the organism. These difficulties were discussed in the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013), as well as in studies explicitly designed to clarify these issues, such as Smolders et al. (2009), Cheyns et al. (2012) and Dayton et al. (2006). In 2009, following extensive toxicity testing with both spiked soils and contaminated field soils, Smolders et al. (2009) concluded that "despite all of the efforts made, a large proportion of the difference between the toxicity observed in field-contaminated soils and that in laboratory-amended soils remains unexplained." Smolders et al. (2009), further demonstrated that not only are the effects of pH, for example, more complex than previously thought, but pH, CEC, DOM, Fe and Mn oxides, aging and soil type are all powerful modifiers of Pb toxicity to soil-dwelling organisms. Underscoring the complexity of modifying effects, Cheyns et al. (2012), for instance, showed that in tomato and barley plants, soil type is a major modifier of toxicity, but that once pH is controlled, toxicity can be mediated by the nutrient deficiencies that stem from reactions of Pb with essential nutrients in the soil solution, whereby the apparent effects of Pb are caused by nutrient deficiencies from Pb robbing the plants of P, for example, by forming Pb phosphates.

The following more recent studies have continued to untangle the respective roles of the various factors that complicate predictive estimation of the effects of Pb in terrestrial organisms from exposure-response studies (Table 11-3). With enough knowledge of the effects of these factors on the exposure-response relationship, it could, in principle, become possible to use some of those experiments to generate useful estimates of concentrations associated with responses of interest from experiments. Experimental procedures might be adjusted, for example by aging or leaching soils prior to exposing organisms, or the modeling of the relationship itself might be modified, for example by adding correction coefficients to the exposure.

Among other questions, Zhang and Van Gestel (2019b) investigated the effects of 18 months of aging, form of Pb and percolation (leaching) on the toxicity of Pb to the worm *Enchytraeus crypticus* in natural standard soil spiked with nine levels of Pb between 0 and 3,200 mg Pb/kg dry soil using $Pb(NO_3)_2$ and between 0 and 1,000 mg Pb/kg dry soil using PbO. Among the complex interactions between these variables, they found that while leaching dramatically decreased porewater concentration of Pb in fresh and aged soils, and more so for $Pb(NO_3)_2$ than for PbO, it did not affect Pb uptake, which was greater for the more soluble form ($Pb(NO_3)_2$). LC_{50} and LC_{10}, estimated from logistic regression on all nine levels was higher following leaching for $Pb(NO_3)_2$ but not for PbO. The authors concluded that generally, the effect of percolation on the toxicity of Pb-spiked soils was dependent on the chemical form used for spiking as well as on aging, and porewater Pb concentration could not explain Pb toxicity. For survival, leaching decreased the toxicity of $Pb(NO_3)_2$ but did not affect the toxicity of PbO. For effects on reproduction, leaching had a greater influence in freshly spiked soils than in aged soils. This suggests that manipulating or accounting for aging and form of Pb might be useful in generating effect predictions in natural environments from spiking experiments, but that manipulating leaching may not be.

The same authors also included variation in the length of the aging period in another exposure-response experiment (Zhang and Van Gestel, 2019a). Using the same materials and methods as in Zhang and Van Gestel (2019b), they incubated the soil samples for five periods from 0 to 18 months, after spiking and before exposure of the worms. Toxicity increased with aging when soils were spiked with PbO but not with $Pb(NO_3)_2$, as did availability when estimated via $CaCl_2$ extraction. This may conflict with (Smolders et al., 2015), who found that lethality declined with five years of aging, but in outdoors conditions that included leaching by rain rather than laboratory incubation. Including aging in the translation from experiment to field thus appears warranted, but not without also including the form of Pb and leaching.

Finally, Zhang et al. (2019a) investigated the effects of soil properties toxicity to *Enchytraeus crypticus* using the same materials and methods as Zhang and Van Gestel (2019b) and six standard natural soils, using $Pb(NO_3)_2$ but not PbO treatments, and not varying aging or percolation. The soils varied in OM content, pH, CEC, water-holding capacity, dissolved OC, and composition. Soil type had very large effects on survival of earthworms in the presence of Pb even though no effect was observed on the internal Pb concentration of worms, with effects ranging from no survival at the midrange of Pb concentration, to complete survival even at the highest concentration. However, soil type had only weak effects on survival when exposure was measured as porewater Pb and no effect on survival when measured as $CaCl_2$-extractable Pb. Similarly strong effects of soil type were seen on the exposure-response relationship of Pb concentration and earthworm reproduction. However, the same weak effects of Pb as for survival were observed for reproduction when using porewater Pb concentrations, and no effects were observed when using $CaCl_2$-extractable Pb. Furthermore, measuring exposure as $CaCl_2$-extractable Pb resulted in accurate and precise predictions of responses regardless of soil type. In contradiction with other studies cited above, such as Cheyns et al. (2012) or Smolders et al. (2009), the authors suggested that despite soil type having a strong effect on toxicity when exposure is measured as simple soil concentration using $CaCl_2$-extractable Pb as a metric of exposure may be sufficient when estimating the effects of Pb on worms, since using that metric supported accurate and precise prediction of earthworm responses regardless of soil type, and the exposure-response relationship was then insensitive to soil type.

Romero-Freire et al. (2015) assessed the respective influence of soil properties in laboratory toxicological assays, with the same aim of making experimental exposure-response studies with spiked soils usable for environmental risk assessment. Seven natural soils of varying pH, conductivity, texture, OC, water-holding capacity, CEC, specific area, carbonate content and metal oxides were spiked with five levels of $Pb(NO_3)_2$ and incubated for 4 weeks. The authors observed that pH and $CaCO_3$ content were the soil properties with the highest influence on Pb extractability and interacted strongly with total Pb concentration, with extractability most affected at higher concentrations of Pb. However, they also found that retention via organic complexation kept most of the Pb from being bioavailable and that texture (silt/sand/clay proportions) and Fe and Mn oxides also had major effects on extractability. In three tests of toxicity—one with lettuce seeds, one with a strain of marine bacterium, and one measuring microbial soil respiration—soil type strongly modified overall toxicity in all tested organisms and the relative effects of each concentration of Pb (in other words, the slope of the response curve). In addition, the magnitude of these modifying effects differed among the three tests. The authors did not attempt to partition the effects of every soil property beyond the most salient effects on extractability noted above. They concluded that soil properties in the particular locations and land use where risk is to be assessed must be taken into consideration when conducting risk assessment, including at minimum, pH, OM and carbonate.

Many variables distinguish natural soils from each other with regard to influence on Pb toxicity, as enumerated in the experiments cited here. As noted by Romero-Freire et al. (2015), Zhang et al. (2019a), Smolders et al. (2009) and others, given practical limitations on the number of soils that can be included in one experiment, it is not possible to definitively separate the effect of each of the variables that define soil type, let alone quantify their interactions. It is possible however to separate some variables that affect the exposure-response more strongly from those that have little or no influence, and it may be possible to identify measures of exposure under which the exposure-response relationship is insensitive to soil type, but nonetheless support accurate and precise estimation of toxic effects.

Another study of the factors that contribute most strongly to differences between responses occurring in natural environments and those observed in Pb-spiking experiments was conducted by Smolders et al. (2015). The study was aimed at assessing the relative magnitude of the effects of salinity, acidification, and aging on the toxicity of Pb to invertebrates, plants, and microbes. Samples of three natural soils were spiked with seven levels of Pb ranging from 0 to 8,000 mg Pb/kg as $Pb(NO_3)_2$ and as $PbCl_2$. Some samples were used unleached and unaged, some were leached and pH-corrected, and some were leached, pH-corrected and aged for five years, a much longer period than in most aging studies. Tomato and barley seedlings were grown in all nine treatments, and biomass was measured after 21 days. Nitrification and soil respiration were measured to assess microbial activity, and the reproduction of the worm *E. fetida* and the collembolan *F. candida* was likewise measured for the nine treatments. Relative to the unaged, unleached treatment, the increase in EC_{10} with leaching and pH correction, aging or leaching, pH correction and aging, showed very wide variation between endpoints. All endpoints demonstrated strong toxicity relative to controls at all levels of added Pb in all three unaged, unleached soils. The EC_{50} for all endpoints increased with leaching and pH correction except for earthworm reproduction in one soil, again with wide variation among endpoints. Finally, aging for five years combined with leaching and pH correction increased EC_{50} to such a degree for all endpoints that its value could not be estimated for any of them. Earthworm reproduction was the endpoint for which EC_{50} increased the least. Smolders et al. (2015) attempted to identify which variables among total Pb concentration, porewater Pb, Pb^{2+} ionic activity, pH and porewater ionic strength were most strongly correlated with endpoints. Overall, porewater ionic strength was the variable most strongly correlated with toxicity. Based on this correlation, the authors suggest that increased salinity, i.e., salt stress compounding true Pb toxicity in freshly spiked soils, is likely the greatest modifying factor of toxicity. They found the effect of pH to be inconclusive due to limitations of their experimental protocol, and perhaps surprisingly, caution about giving too much weight to the effects of aging despite its seemingly large effect. They re-emphasized the limitations of the experimental protocol, specifically the leaching that preceded aging. For plants, they noted a deficiency of P, with both increased Pb concentration and aging as the more direct factor explaining the effects on plant growth. The authors concluded that regardless of the mechanisms behind their observations, this study offered "…a strong confirmation that acute dosing of soluble Pb^{2+} salts does not appear to be an appropriate model for environmental sources of Pb where Pb gradually enters soils via atmospheric deposition as PbO, PbS, and $PbSO_4$…"

In 2021, Oorts et al. (2021) proposed two corrections to the results of exposure-response experiments conducted with addition of soluble salts of Pb to soil and used them to derive some examples of ecological soil standards. They suggested first that a single correction factor can be applied to the toxicity results of fresh, i.e., unleached, unaged, spiking experiments to adequately convert the results to the values that would have been observed following leaching and aging. They further proposed to demonstrate that this conversion generates values that correspond to the toxicity levels that would be observed in corresponding hypothetical field conditions. The second correction was intended to adjust differences in toxicity that arise from differing soil properties. Although as referenced previously, multiple properties of soils have been shown to affect Pb toxicity in both spiking experiments and field conditions, the authors argued that adjusting for CEC is sufficient. The authors demonstrated the derivation of predicted no-effect concentrations (PNEC) according to the European REACH Regulation European Parliament and Council (2006), using the two corrections above and data that conformed to the REACH requirements. In contrast with Eco-SSL values, none of the derived standards were lower than background soil Pb concentration.

A few methodological developments in analyzing and using Pb exposure-response experiments have also been explored since the 2013 Pb ISA (U.S. EPA, 2013), although they may not be of immediate applicability to risk assessment or standard setting. Zhang and Van Gestel (2017) used one standard natural soil spiked with seven levels of $Pb(NO_3)_2$ between 0 and 3,200 mg/kg soil to study the toxicokinetics and toxicodynamics of uptake, elimination, and survival in the worm *Encytraeus crypticus*. Uptake and toxicity were measured at seven time intervals and elimination at six. The measurement and statistical modeling of the time course of uptake, elimination and survival demonstrated that accumulation and toxicity were dependent on exposure duration, and that once the time course of exposure was taken into consideration, the internal concentration of Pb in worms may be a better predictor of survival than soil concentration. Using the model organism *C. elegans* exposed to five levels of Pb between 0 and 2000 ppm as Pb acetate, Sudama et al. (2013) combined chromatographic metabolite profiling and principal component analysis to show that changes in the purine pathway and its metabolites can be detected after exposure to extremely low concentrations of Pb.

Finally, the applicability of Species Sensitivity Distribution analysis was investigated by Ding et al. (2016) using 21 natural soils spiked with four levels of Pb between 0 and 350 mg/kg soil as $Pb(NO_3)_2$ and 12 cultivars each of carrot (*Daucus carota*), radish (*Raphanus sativus*), and potato (*Solanum tuberosum*), to show that Species Sensitivity Distribution analysis could be a reliable approach to determining safety thresholds, as long as the threshold values are derived from experiments designed for that purpose. However, exposure was from soluble salt, and the safety thresholds the authors investigated were for the safety of human consumers of vegetables grown in heavily polluted sites. They therefore measured only accumulation in the plants and not the effects on the plants themselves.

Table 11-3 Studies of factors that affect the interpretability of exposure-response experiments in terrestrial biota, since the 2013 Pb ISA

Organism	Experimental conditions		Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
Barley *(Hordeum vulgare)* Tomato *(Lycopersicon esculentum)*	**Form of Pb:** $PbCl_2$ **Medium:** Six topsoils from five European countries Hydroponic system **Exposure method:** Salt mixed with soil Salt in hydroponic solution	**pH:** Soils 7.4, 6.5, 6.7, 5.7, 5.2, 4.7 (pH $CaCl_2$ (0.01 M) Adjusted with CaO) Hydroponics 6.1 **CEC:** Soils 14.7, 27.1, 8.7, 4.2, 7.6, 41.7 ($cmol_c$/kg soil) Hydroponics N/A **OC:** Soils 14, 31, 10, 15, 21, 310 (gC/kg soil) Hydroponics N/A **Aging/leaching:** Soils. Leached by immersion and draining after 1 wk incubation. Three 1-wk periods of moist incubation separated by 1-wk periods of dry storage and one dry storage period of up to 20 wk Hydroponics N/A	Soils Measured: 6 levels × 6 soils = 36 values between 47 and 12,700 mg/kg, plus 1 control × 6 soils with background Pb between 4.7 and 135 mg Pb/kg soil Hydroponics 1, 3.2, 10, 32, 100, 320 mM	Soil (location of origin) Soil P content 44, 48, 67, 89, 90, 121 mg P/kg soil Hydroponics Gradually increasing P supply for 17 days to maintain growth rate and avoid precipitation AND 7 levels of P supply based on P content in plant tissue (0.10%–0.32% P in plant tissue)	Tomato growth: decreasing with increasing Pb in all soils Barley growth: no effect of Pb in three soils, decreasing with increasing Pb in three soils	Tomato shoot dry weight NOEC for six soils: 4,400, 750, <250, 440, 260, 1,100 mg Pb/kg soil EC_{50}: 6,000, 6,500, 2,200, 2,700, 1,600, 5,400 mg Pb/kg soil Barley shoot dry weight NOEC for six soils: >7,200, >5,000, 2,000, >3,400, 260, 1,100 mg Pb/kg soil EC_{50}: >7,200, >5,000, 4,900, >3,400, 1,900, 8,300 mg Pb/kg soil	Strong interaction effect of soil type and Pb concentration on growth P content in plants was strongly influenced by Pb and explained the effect of Pb across soils and in hydroponic experiment	Cheyns et al. (2012)

Organism		Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
Potworm (*Enchytraeus crypticus*)	**Form of Pb:** $Pb(NO_3)_2$ PbO **Medium:** LUFA 2.2 standard soil **Exposure method:** Soil spiked with powdered salt	**pH:** Nominal pH 5.49 **CEC:** 9.10 cmolc/kg **OC:** not reported **Aging/leaching:** Spiked soils were aged for 0, 3, 6, 12 and 18 mo. No leaching	Nominal concentrations of: $Pb(NO_3)_2$ 0, 50, 100, 200, 400, 600, 800, 1,600 and 3,200 mg Pb/kg dry soil PbO 0, 78, 156, 312, 625, 1,250, 2,500, 5,000 and 10,000 mg Pb/kg dry soil	Aging and chemical form of Pb	*E. crypticus* mortality increased with increasing Pb soil concentration	$Pb(NO_3)_2$: $CaCl_2$ extractable Pb 0, 3, 6, 12, 18-mo LC_{50} = 2.18, 3.06, 2.49, 2.28, 1.72 mg Pb/kg EC_{50} = 0.149, 0.125, 0.090, 0.103, 0.093 mg Pb/kg Porewater Pb 0, 3, 6, 12, 18-mo LC_{50} = 0.247, 0.346, 0.328, 0.366, 0.583 mg Pb/L EC_{50} = 0.020, 0.016, 0.019, 0.015, 0.046 mg Pb/L Internal Pb 0, 3, 6, 12, 18-mo LC_{50} = 76.2, 76.4, 77.1, 73.4, 76.8 mg Pb/kg dry body weight EC_{50} = 22.2, 24.7, 30.0, 31.5, 20.1 mg Pb/kg dry body weight	The dose-response curves and toxicity values (LC_{50} and EC_{50}) based on total Pb concentrations differed widely between the two forms of Pb $Pb(NO_3)_2$ was more toxic than PbO in freshly spiked soils, but the toxicity of PbO increased with aging, while the toxicity of $Pb(NO_3)_2$ remained constant $CaCl_2$-extraction provided the best estimate of Pb toxicity and bioaccumulation	(Zhang and Van Gestel, 2019a)

Organism	Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
					PbO: $CaCl_2$ extractable Pb 0, 3, 6, 12, 18-mo LC_{50} = 3.02, 3.15, 2.36, 2.66, 2.45 mg Pb/kg EC_{50} = 0.170, 0.135, 0.098, 0.138, 0.101 mg Pb/kg Porewater Pb 0, 3, 6, 12, 18-mo LC_{50} = 0.262, 0.312, 0.286, 0.302, 0.391 mg Pb/L EC_{50} = 0.025, 0.048, 0.050, 0.023, 0.048 mg Pb/L Internal Pb 0, 3, 6, 12, 18-mo LC_{50} = 78.0, 77.7, 74.4, 78.4, 78.7 mg Pb/kg dry body weight		

Organism	Experimental conditions		Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
						EC_{50} = 23.7, 18.1, 19.8, 16.9, 12.0 mg Pb/kg dry body weight		
Potworm (*Enchytraeus crypticus*)	**Form of Pb:** $Pb(NO_3)_2$ PbO **Medium:** LUFA 2.2 standard soil **Exposure method:** Soil spiked with powdered salt.	**pH:** Aged Soil: pH_{pw}: 5.61 pH_{CaCl2}: 5.14 Freshy Spiked: pH_{pw}: 5.93 pH_{CaCl2}: 5.65 **CEC:** not reported **OC:** not reported **Aging/leaching:** One soil form was spiked and then aged for 18 mo, while the other soil form was used without aging as freshly spiked soil Half of each set of soils were leached with deionized water equal	Nominal concentrations of: $Pb(NO_3)$ 0, 50, 100, 200, 400, 600, 800, 1,600 and 3,200 mg Pb/kg dry soil PbO 0, 78, 156, 312, 625, 1,250, 2,500, 5,000 and 1,000 mg Pb/kg dry soil	Percolation, chemical form of Pb and aging	*E. Crypticus* mortality increased with increasing Pb soil concentration	$Pb(NO_3)_2$: $CaCl_2$-extractable Pb aged, aged+leached, freshly spiked and freshly spiked+leached. LC_{50} = 1.72, 2.42, 2.07 and 2.78 mg Pb/kg EC_{50} = 0.093, 0.173, 0.044 and 0.109 mg Pb/kg Porewater Pb aged, aged+leached, freshly spiked and freshly spiked+leached. LC_{50} = 0.583, 0.201, 0.686 and 0.148 mg Pb/L EC_{50} = 0.046, 0.063, 0.012 and 0.033 mg Pb/L	When exposure was measured as total soil Pb, aging increased toxicity for both forms of Pb and leaching had no meaningful effect. However, all effects of form, aging or leaching disappeared when exposure was measured as $CaCl_2$-extractable Pb	(Zhang and Van Gestel, 2019b)

Organism	Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
	to two times the base moisture content				Internal Pb aged, aged+leached, freshly spiked and freshly spiked+leached. LC_{50} = 76.8, 84.4, 77.3 and 83.6 mg Pb/kg dry body weight EC_{50} = 20.1, 22.1, 25.5 and 32.7 mg Pb/kg dry body weight <u>PbO:</u> $CaCl_2$ extractable Pb aged, aged+leached, freshly spiked and freshly spiked+leached. LC_{50} = 2.45, 2.01, 2.79 and 2.16 mg Pb/kg EC_{50} = 0.101, 0.160, 0.123 and 0.168 mg Pb/kg Porewater Pb aged, aged+leached, freshly spiked		

Organism	Experimental conditions		Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
						and freshly spiked+leached. LC_{50} = 0.391, 0.233, 0.197 and 0.097 mg Pb/L EC_{50} = 0.048, 0.043, 0.047 and 0.031 mg Pb/L Internal Pb aged, aged+leached, freshly spiked and freshly spiked+leached. LC_{50} = 78.7, 76.4, 83.3 and 84.5 mg Pb/kg dry body weight EC_{50} = 12.0, 14.5, 41.1 and 38.6 mg Pb/kg dry body weight		
Potworm *(Enchytraeus crypticus)*	**<u>Form of Pb:</u>** $Pb(NO_3)_2$ **<u>Medium:</u>** LUFA 2.2 standard soil	**<u>pH:</u>** Nominal pH of 5.49 **<u>CEC:</u>** 9.10 cmolc/kg **<u>OC:</u>** not reported	Nominal concentrations of 0, 100, 200, 400, 800, 1,600 and 3,200 mg Pb/kg dry soil Measured Concentrations of 16, 114, 202,	Exposure duration	Toxicity was dependent on both the concentration and duration of exposure. Pb toxicity developed more slowly than uptake,	Days 4, 7, 10, 14 and 21 <u>Total concentration in soil:</u> LC_{50} = 2,336, 2278, 1,220, 756 and 558 mg Pb/kg dry soil	Strong interaction effect of duration and Pb concentration on mortality	(Zhang and Van Gestel, 2017)

Organism	Experimental conditions		Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
	Exposure method: Soil spiked with aqueous salt solution	**Aging/leaching:** After 14-d exposure in spiked soils, the surviving *E. crypticus* were transferred to clean soil for the 14-d elimination phase	391, 793, 1,601 and 3,585 mg Pb/kg dry soil		with final LC_{50} not yet reached after 21 d	Internal concentration: LC_{50} = >287, >270, 161, 76.6 and 76.4 mg Pb/kg dry body weight		
Potworm (*Enchytraeus criticus*)	**Form of Pb:** $Pb(NO_3)_2$ **Medium:** Five standard soils (LUFA standard soil 2.1, 2.2, 2.3, 2.4, 5 M) and one soil from a soccer field in the Netherlands **Exposure method:** Soils spiked with aqueous solution	**pH:** 4.86, 5.66, 5.38, 6.87, 6.99, 6.85 **CEC:** 2.23, 7.59, 4.04, 20.1, 10.1 and 20.0 cmolc/kg **OC:** DOC 45.7, 61.7, 34.4, 72.0, 51.2 and 189 mg/L **Aging/leaching:** Soil equilibrated for 14-d.	Nominal concentrations of 0, 100, 200, 400, 600, 800, 1,200, 1,600, 2,400 and 3,200 mg Pb/kg dry soil	Soil type, soil properties: OM, DOC, pH, CEC, water-holding capacity, composition	Reproductive toxicity and mortality increased with Pb concentration in soil	LUFA standard soil 2.1, 2.2, 2.3, 2.4, 5 M and soccer field, respectively. Total Pb: LC_{50} = 246, 1,192, 655, 3,125, 2,875 and >3,092 mg Pb/kg dry soil EC_{50} = 81.4, 238, 205, 948, 1,008 and 991 mg Pb/kg dry soil $CaCl_2$ extractable Pb: LC_{50} = 2.35, 2.11, 1.86, 1.64, 2.11 and >1.39 mg Pb/kg dry soil	Correlation of single soil properties with endpoints, followed by simple regression, followed by stepwise multiple regression suggested that pH_{CaCl2} was the best explanatory factor for LC_{50} values based on total Pb concentration The differences between soil toxicity were not present when exposure was measured as $CaCl_2$-extractable Pb concentration	(Zhang et al., 2019a)

Organism	Experimental conditions			Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
							EC_{50} = 0.329, 0.193, 0.107, 0.180, 0.241 and 0.115 mg Pb/kg dry soil <u>Porewater Pb:</u> LC_{50} = 0.308, 1.25, 0.335, 0.334, 0.933 and >0.754 mg Pb/L EC_{50} = 0.044, 0.127, 0.117, 0.169, 0.046 and 0.105 mg Pb/L <u>Internal Pb:</u> LC_{50} = 95.7, 83.0, 87.0, 84.3, 81.7 and >47.7 mg Pb/kg dry body weight EC_{50} = 13.6, 34.1, 26.0, 39.9, 27.2 and 32.6 mg Pb/kg dry body weight		
Tomato (*Lycopersicon esculentum*)	**<u>Form of Pb:</u>** $PbCl_2$ $Pb(NO_3)_2$	**<u>pH:</u>** 6.1–7.4 **<u>CEC:</u>** 8.2–27.1 cmolc/kg soil		Nominal concentrations of, 250, 500, 1,000, 2,000, 4,000 and 8,000 mg Pb/kg	Leaching combined with pH correction, aging combined with	All effects increased with increasing Pb in freshly spiked (unaged,	EC_{50}s calculated for tomato growth, barley growth, nitrification rate, nitrification 28-d, respiration, *E.*	Strong interaction effect of leaching, aging and Pb concentration on all	Smolders et al. (2015)

Organism	Experimental conditions		Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
Barley (*Hordeum vulgare*) Collembola (*Folsomia candida)* Earthworm (*Eisenia fetida*)	**Medium:** Soils gathered from topsoils in Spain, the United Kingdom and Belgium **Exposure method:** Soil spiked with salt	**OC:** 10–43 g C/kg soil **Aging/leaching:** Soils were given three different treatments. Treatment A: freshly spiked. Treatment B: leached and pH-corrected. Treatment C: leached, pH-corrected and aged for 5 yr		leaching and pH correction	unleached) soils	*Fetida* reproduction and *F. candida* reproduction in each soil, respectively. Spain: Freshly spiked EC_{50} = 2,900, 2,380, 3,240, 7,190, 8,720, 480 and 712 mg Pb/kg soil Leached and pH-corrected EC_{50} = 6,370, 7,190, 2,200, 7,120, 12,300, 1,182 and n.s. mg Pb/kg soil Aged 5 yr EC_{50} = 12,600, n.s, n.s, n.s, 7,020, 1,270 and n.s. mg Pb/kg soil United Kingdom: Freshly spiked EC_{50} = 6,140, 6,750, 2,820, 1,750, 9,970,	responses. Leaching combined with pH correction decreased toxicity for all effects. Aging following leaching and pH correction further decreased toxicity for most effects but not all. Authors suggest decreased ionic strength (salt stress) and changes in pH are the main drivers of decreasing toxicity	

Organism	Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
					2,400 and 4,530 mg Pb/kg soil Leached and pH-corrected EC_{50} = 6,420, 5,020, 4,920, n.s., 6,160, 1,700 and 5,020 mg Pb/kg soil Aged 5 yr EC_{50} = n.s., n.s., n.s., n.s., n.s., 3280 and n.s. mg Pb/kg soil <u>Belgium:</u> (no test for *E. fetida*) Freshly spiked EC_{50} = 1,240, 1,710, 1,470, 1,410, 1,680 and 1,710 mg Pb/kg soil Leached and pH-corrected EC_{50} = 1,430, 4,580, 1,640, 2,820, 8,150 and		

Organism	Experimental conditions		Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
						2,700 mg Pb/kg soil Aged 5 yr EC_{50} = 4480, n.s., n.s., n.s., n.s. and n.s. mg Pb/kg soil		
Lettuce (*Lactuca sativa*) Bacterium (*Vibrio fischeri*)	**Form of Pb:** $Pb(NO_3)_2$ **Medium:** Seven soils representing the main soil groups in Spain **Exposure method:** Spiked with aqueous solution.	Reported for soils H1–H7 respectively **pH:** 7.96, 8.67, 8.79, 6.74, 7.20, 5.87 and 7.03 **CEC:** 21.4, 9.83, 2.94, 9.91, 25.9, 3.83 and 15.5 cmolc/kg **OC:** 5.43, 0.42, 0.38, 0.61, 8.22, 0.49 and 0.66% **Aging/leaching:** Soils were incubated for 4 wk after spiking	Nominal concentrations of 500, 1,000, 2,000, 4,000 and 8,000 mg Pb/kg soil	Soil type (location of origin)	All effects increased with increasing Pb in all soils	Reported for soils H1–H7, respectively *L. sativa*: EC_{10} = 499, 1,363, 254, 1,097, 3,452, 498 and 344 mg Pb/kg soil *V. fischeri*: EC_{10} = >8,000, 5,337, 2,901, 386, 2,473, 8 and 744 mg Pb/kg Soil Respiration: EC_{10} = >8,000, 3,128, 5,951, 90, >8,000, 122 and 45 mg Pb/kg	Strong interaction effect of soil type and Pb concentration on all responses. Authors suggest that the main soil properties that affected toxicity were pH, carbonate content and OC	Romero-Freire et al. (2015)

$CaCl_2$ = calcium chloride; CaO = calcium oxide; CEC = cation exchange capacity; DOC = dissolved organic carbon; EC_{50} = half maximal effect concentration; LC_{50} = 50% lethal concentration; LUFA = Landwirtschaftliche Untersuchungs- und Forschungsanstalt; mo = months; N/A = not available; NOEC = no-observed-effect concentration; n.s. = nonsignificant.

Organism	Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference

OC = organic carbon; OM = organic matter; P = lead; Pb = lead; $Pb(NO_3)_2$ = lead nitrate; $PbCl_2$ = lead chloride; PbO = lead(II) oxide; pH_{pw} = pH of porewater; pH_{CaCl2} = pH via calcium chloride; wk = week(s); yr = year(s).

11.2.6 Terrestrial-Community and Ecosystem Effects

In the 2013 Pb ISA the body of evidence was sufficient to conclude there is a likely causal relationship between Pb exposure and terrestrial-community and ecosystem effects (U.S. EPA, 2013). In the 2006 Pb AQCD (U.S. EPA, 2006), terrestrial ecosystems near stationary Pb sources exhibited decreased species diversity, changes in floral and faunal composition, and a reduction in vegetation fitness. In the 2013 Pb ISA (U.S. EPA, 2013), a study reported decreased population growth of earthworms. Additional studies in the 2013 Pb ISA examined how the presence of AMF or earthworms affect plant Pb uptake and fitness. Recent evidence of the effects of Pb at the community and ecosystem levels includes several studies of the relationship between Pb soil concentration and species interactions and invertebrate community structure. Specifically, studies conducted since the 2013 Pb ISA have reported that Pb affects plant-insect interactions and is correlated with invertebrate community structure. Considering that Pb rarely occurs as the only contaminant in terrestrial ecosystems it is difficult to attribute effects observed at higher levels of biological organization solely to Pb.

In an experimental study, Jiang et al. (2020) demonstrated trophic transfer of Pb can affect the chemical defenses of larch seedlings (*Larix olgensis*) against an economically important pest, the Asian gypsy moth (*Lymantria dispar*), in China. Larch seedlings were enriched with Pb at 0, 500, or 1500 mg Pb/kg. Second instar *L. dispar* larvae raised from field-collected egg masses were placed on *L. olgensis* seedlings for 7 days. Pb content in *L. dispar* larvae were significantly higher than *L. olgensis* needles for the 500 mg Pb/kg and 1500 mg Pb/kg treatments, and Pb bioaccumulated in this experiment, as the transfer coefficients were 0.97 for the 0 mg Pb/kg treatment, 5.43 for the 500 mg Pb/kg treatment and 6.03 for the 1500 mg Pb/kg treatment. Pb treatment reduced *L. olgensis* total biomass (40.36% reduction in the 1500 mg Pb/kg compared with control) and *L. dispar* larval weights (by 34.44%–52.05%) and survival rates (by 30.91%–59.28%) in a dose-dependent manner compared with the control. Antioxidants (peroxidase and SOD) of *L. olgensis* increased under 500 mg Pb/kg treatment and were reduced under 1500 mg Pb/kg. Phytochemical defenses, protease inhibitors (trypsin inhibitor and chymotrypsin inhibitor) and the secondary metabolites (total phenolic acids) were significantly increased under the low dose of Pb (500 mg Pb/kg) compared with the control, while all phytochemical defense chemicals, including condensed tannins, decreased significantly under high Pb stress (1500 mg Pb/kg). *Lymantria. dispar* fed with *L. olgensis* seedlings had higher antioxidase activities in the fourth instar (SOD and CAT), while nonenzymatic antioxidants were significantly decreased (glutathione content and ascorbic acid content), suggesting that the reduction of antioxidants might lead to the oxidative stress experienced by *L. dispar* larvae. Finally, MDA content increased with Pb exposure.

Heavy-metal concentration along a pollution gradient in Romania affected soil mite (Acari: Mesostigmata) community structure (Manu et al., 2019; Manu et al., 2017). Manu et al. (2017) examined soil mite communities in relation to soil metal content and physicochemical properties in 12 grasslands. Some heavy metals (Pb, As, Cu and Zn) influenced the soil mite community in highly polluted sites, while altitude and soil humidity played larger roles in less polluted sites. Pb soil concentration ranged from 28.21 ± 4.62 mg/kb Pb to 421.12 ± 71.62 mg/kb Pb. The sites with the highest Pb were closest to the pollution source. Canonical correspondence analysis (CCA) determined that heavy metals (Cu, Zn and Pb) as well as the C/N ratio, humidity, total N, altitude, and slope were the strongest determinants of species composition, and Pb soil concentration showed association with the abundance of *Zercon berlesei*. In another study, Manu et al. (2019) collected soil from a pollution gradient surrounding the Certej ore deposit and characterized heavy-metal concentration and soil mite communities. Pb concentrations ranged from 153.68 to 292.35 mg Pb/kg across five sites (mean concentration). The relationship between mite abundance and heavy metals was examined using CCA, and the first axis accounted for 50.67% of the variation in mite community and was highly correlated with Pb (correlation = 0.81), Cu, As and Mn. The abundance of *Arctoseius cetratus* showed the strongest relationship with Pb.

Potworm (Enchytraeidae) diversity, but not herbaceous plant diversity, was negatively correlated with soil Pb concentration across 41 sites near a Zn-Pb mining site in South Poland (Kapusta and Sobczyk, 2015). Pb soil concentration varied across sites, ranging from 300 ± 300 mg Pb/kg (mean ± S.D.) to 9,600 ± 14,100 mg Pb/kg at sites closer to the smelter, and water-soluble Pb showed a similar pattern, with higher Pb concentrations found closer to the smelter site (range: 0.103 ± 0.068 mg Pb/kg to 0.477 ± 0.212 mg Pb/kg Pb). Pb concentration was positively correlated with silt content, OC, total Cd, total Zn, exchangeable Cd, water-soluble Cd, and water-soluble Zn and negatively correlated with distance from the smelter. Water-soluble Pb was positively correlated with distance from the smelter, OC, and water-soluble Zn. Total Pb was significantly negatively correlated with Enchytraeid species richness, genus richness, and density in 2010, but not density in 2009, while water-soluble Pb showed no significant relationships with species richness, genus richness, or density in 2009 or 2010. Plant community species richness and herbaceous cover showed no correlation with total Pb in the soil or water-soluble Pb.

The abundance of insects on Pb-contaminated kale (*Brassica oleracea* L. var. *acephala*) was higher than control *B. oleracea* plants in a field experiment in Brazil (Morales-Silva et al., 2022). *Brassica oleracea* plants were grown in control soil (background Pb concentration: 25.9 mg Pb/kg) or in soil spiked with $Pb(NO_3)_2$ to nominal concentrations of 144, 360, or 600 mg Pb/kg and exposed to natural insect populations. Lepidoptera and their associated parasitoids, as well as aphids and their predators and parasitoids, were collected from plants. At the end of the experiment, plant biomass was unaffected by Pb soil contamination, while plants exposed to 600 mg Pb/kg had significantly higher concentrations of Pb in the leaves compared with plants in the control, 144, and 360 mg Pb/kg treatments. *Brassica oleracea* plants in the control treatment had significantly higher abundance of insects compared with the contaminated plants, regardless of Pb level.

Longer-lived nematodes with lower fecundity are most affected by experimental Pb exposure (Park et al., 2016). Tomatoes (*Lycopersicon esculentum*) were grown in pots of soil collected from an agricultural field in Korea and exposed to Pb via irrigation. Measured Pb concentrations of the soil were 16.97 ± 0.24 mg Pb/kg (mean ± S.D) for the control soil, 15.19 ± 0.55 mg Pb/kg, 15.54 ± 0.42 mg Pb/kg, 18.08 ± 0.67 mg Pb/kg and 34.98 ± 2.57 mg Pb/kg. Soil nematode communities were characterized before *L. esculentum* were planted and after 18 weeks of growth. Nematode community structure was analyzed using a variety of metrics, from trophic guilds to maturity indices to the abundance of colonizers and persister (cp-1 = colonizer to cp-5 = persister). Pearson's correlation coefficients between Pb and nematode community indices were largely nonsignificant, except for the negative relationship between Pb and the richness of cp-3 as well as the maturity index and the positive relationship between Pb and the abundance of fungivores as well as the abundance of cp-2. There was a significant decrease in nematode abundance in omnivores-predators (OP) and cp-4 at the highest concentrations of Pb. Nematode richness decreased at higher concentrations of Pb, particularly for OP, cp-4, and cp-5. The authors suggested that these groups are likely most sensitive to environmental stress, as they have longer-lifecycles and lower reproduction rates.

In another nematode study, the diversity and abundance of nematode communities were correlated with soil Pb concentration near a ferroalloy manufacturer in North Slovakia (Salamún et al., 2011). Soil samples near the factory and downwind of the factory were analyzed for heavy metals, including Pb. The total Pb concentration ranged from 0.815 ± 0.471 mg Pb/kg to 1.766 ± 0.082 mg Pb/kg (mean ± S.D). Soil Pb concentration was positively correlated with the abundance of certain trophic guilds and ecological indices of nematodes, specifically, predators, root-fungal feeders, and maturity index (MI2-5). Maturity index (2-5) is used as a measure of functional diversity, which incorporates the abundance of *r* and *K*-strategists in a community. Pb was not significantly correlated with any other trophic group or ecological index (bacterial feeders, fungal feeders, omnivores, plant feeders, maturity index, plant-parasite index, genera richness, Shannon-Weaver index, Simpson index or abundance). In a follow-up study, Šalamún et al. (2012) examined nematode community structure in relation to the total element concentration of Pb, Zn, Cu Cr, Ca, and As, in another region of Slovakia using an HNO_3 extraction and mobilization fraction Na_2EDTA extraction. Unlike Salamún et al. (2011), in which Pb was positively correlated with certain trophic groups, total soil Pb concentration was negatively correlated with the abundance of omnivorous nematodes, MI2-5, structure index and genera richness.

Since the 2013 Pb ISA (U.S. EPA, 2013), several studies have found evidence that Pb affects species interactions, including chemical defenses (Jiang et al., 2020) and pollinator foraging behavior (Xun et al., 2018). Additionally, several studies found negative relationships between Pb concentration along a pollution gradient and aspects of the invertebrate community structure, specifically in soil mites (Manu et al., 2019; Manu et al., 2017), potworms (Kapusta and Sobczyk, 2015), insect communities on kale (Morales-Silva et al., 2022), and nematodes (Salamún et al., 2011).

11.3 Freshwater Ecosystems

11.3.1 Summary of New Information on Effects of Pb in Freshwater Ecosystems and Causality Determination Update Since the 2013 Pb ISA

Recent evidence further supports the findings of the previous Pb AQCDs and 2013 Pb ISA that waterborne Pb is toxic to freshwater plants, invertebrates, and vertebrates, with toxicity varying with species and lifestage, duration of exposure, form of Pb, and water quality characteristics (U.S. EPA, 2013, 2006, 1986, 1977). In natural environments it is difficult to attribute observed effects solely to Pb due to the presence of confounding factors such as other pollutants, and additional modifying factors that affect Pb bioavailability and toxicity. Furthermore, the portion of Pb from atmospheric sources is usually not known. The majority of the available studies of Pb exposures in freshwater biota are laboratory toxicity tests on single species in which an organism is exposed to a known concentration of Pb, and the effect on a specific endpoint is evaluated. These studies provide evidence for a temporal sequence between Pb exposure and an effect, an aspect important in judging causality. Concentration-response data from freshwater organisms indicate that there is a gradient of response to increasing Pb concentration and that some effects in sensitive species are observed at or near the upper limit of Pb concentrations quantified in U.S. surface waters (Table 11-1). New evidence for freshwater biota (Table 11-5) continue to support the existing causality determinations from the 2013 Pb ISA summarized in Table 11-4 of this document. In most cases, new evidence expands somewhat the evidence for endpoints that were already established as causal in the 2013 Pb ISA. Some studies have reported effects at lower effect concentration than in the 2013 Pb ISA. **There are no changes to existing causality determinations for freshwater biota or ecosystems from the 2013 Pb ISA** (Table 11-4).

For physiological stress endpoints in freshwater plants, invertebrates, and vertebrates, new evidence continues to support the likely to be causal determination from the 2013 Pb ISA. A small subset of studies that report molecular or cellular perturbations of Pb concurrently assess an effect on reproduction, growth, or survival. Few studies were identified since the 2013 Pb ISA that quantified ALAD response in freshwater invertebrates or vertebrates; hence there is not sufficient evidence to warrant a reconsideration of any of the causality relationships for the hematological effects of Pb.

Neurobehavioral effects of Pb were concluded to have a likely to be causal relationship for Pb exposure for freshwater invertebrates and vertebrates in the 2013 Pb ISA. For invertebrates, a few new studies in amphipods, bivalves and gastropods further support the 2013 finding of a likely to be causal relationship between Pb exposure and neurobehavioral endpoints (Section 11.3.3). Effects on locomotion were observed in adult amphipods, *G. fossarum,* following Pb sublethal exposure (analytically verified concentrations were 2.1 and 2.7 µg Pb/L in two separate studies, one conducted for 24 hours, another conducted for 5 days) (Lebrun and Gismondi, 2020; Lebrun et al., 2017). Alteration of neurotransmitter (AChE) activity was reported for two freshwater bivalve species including *Parreysia corrugata*, in which

AChE activity was significantly induced at 26 µg Pb/L in 21-day aqueous exposure. Impaired foot movement was also observed in this species at a similar concentration (Brahma and Gupta, 2020). AChE activity was significantly induced in the freshwater snail *B. aeruginosa* during 28-day exposure to Pb-spiked sediment (29.7 mg Pb/kg dry weight) (Liu et al., 2019b).

The 2013 conclusion of a likely to be causal relationship between Pb exposure and neurobehavioral effects in freshwater vertebrates is bolstered in this current ISA by multiple studies with zebrafish (*D. rerio*) as an animal model for human health effects including developmental and neurological changes associated with Pb exposure (Section 11.3.4.4). Effects on behavioral endpoints such as locomotion and social interactions in larval zebrafish were reported at lower effect concentrations than studies in the 2013 Pb ISA, with some effects reported at ≤ 20 µg Pb/L; a subset of these studies analytically verified Pb in the exposure water (Kataba et al., 2020; Zhao et al., 2020; Wang et al., 2018b; Zhu et al., 2016). Neurological responses of fish to Pb exposure were first reported in the 1986 Pb AQCD (U.S. EPA, 1986). The likely to be causal determination in the 2013 Pb ISA was based primarily on altered behaviors, such as reduced locomotion and prey capture ability, observed in fish following Pb exposure. These included a decrease in zebrafish larval startle response to mechanosensory and visual stimuli following nominal exposure to Pb (2.0 and 6.0 µg Pb/L) (Rice et al., 2011), and reduced prey capture in assays with 10-day old fathead minnows born from adult fish exposed to 120 µg Pb/L for 300 days then subsequently tested in a breeding assay for 21 days (Mager et al., 2010). In another study in the 2013 Pb ISA with fathead minnows, swimming performance measured as critical aerobic swim speed was significantly impaired in minnows in 24-hour acute (139 µg Pb/L) and chronic 33- to 57-day (143 µg Pb/L) exposures; however, no significant difference in swim speed was observed in chronic exposures to 33 µg Pb/L (Mager and Grosell, 2011). The evidence in the 2013 Pb ISA and previous AQCDs also included effects on molecular targets; however, these experiments were typically conducted at Pb concentrations that greatly exceeds environmental concentrations.

In the 2013 Pb ISA, there was a conclusion of a causal relationship between Pb exposure and reduced survival in both freshwater invertebrates and vertebrates. Newly available evidence continues to support these causal determinations. For invertebrates, several studies provide further characterization for known effects on survival in a few sensitive species of freshwater invertebrates at <20 µg Pb/L (Section 11.3.5). In the gastropod *L. stagnalis,* survival was significantly decreased at 8.4 µg Pb/L after 21-day exposure to the end of a 56-day full lifecycle assessment (Munley et al., 2013). In a chronic 42-day bioassay with the amphipod *H. azteca*, the EC_{20} for survival was similar under two different experimental diets administered concurrently (LC_{20} = 15 µg Pb/L and LC_{20} = 13 µg Pb/L) (Besser et al., 2016). For freshwater vertebrates, studies in fish provided the basis for causality determination in the 2013 Pb ISA (Section 11.3.5). Additional fish bioassays conducted in varying water chemistry conditions report effects on survival at Pb concentrations similar to those reported in the 2013 Pb ISA. For larval zebrafish (*D. rerio*), 96-hour LC_{50} values varied with water hardness; LC_{50} = 52.9 µg Pb/L in soft water and LC_{50} = >590 µg Pb/L in hard water (Alsop and Wood, 2011). Several studies considered the role of Pb and other trace metals on the decline of the white sturgeon in U.S. waters, and one study examined endpoints in westslope cutthroat trout. In 96-hour acute toxicity assays conducted with two lifestages of white sturgeon (*A. transmontanus*), the lowest 96-hour LC_{50} was 177 µg Pb/L for 8 dph larvae (Vardy et al., 2014).

For growth effects in freshwater organisms associated with Pb exposure, recent studies continue to support the findings in the 2013 Pb ISA. There was a likely to be causal relationship between Pb exposure and reduced plant growth concluded in the 2013 Pb ISA. Most primary producers experience EC_{50} values for growth at concentrations that greatly exceed Pb concentrations typically found in U.S. surface waters. One new study reported growth rates in three commonly tested algal species (*P. subcapitata, C. kesslerii,* and *C. reinhardtii*) at lower effect concentrations than previously reported. *P. subcapitata* was the most sensitive in 72-hour bioassays, with an EC_{50} = 83.9 µg Pb/L, EC_{20} = 45.7 µg Pb/L and EC_{10} = 32.0 µg Pb/L based on filtered Pb concentration. Varying the pH resulted in greater sensitivity (De Schamphelaere et al., 2014). In the 2013 Pb ISA, there was a causal relationship concluded to exist between Pb exposure and reduced growth in invertebrates. Since then, additional studies have supported previous findings of Pb effects on the growth of snails (*L. stagnalis*) in the low µg Pb/L range (Crémazy, 2018, 6708984} (Munley et al., 2013; Brix et al., 2012; Esbaugh et al., 2012). Reduction in weight gain and specific growth rate were observed in juvenile Oriental river prawn (*M. nipponense*) exposed to 25 µg Pb/L in chronic 60-day trials. No growth effects were observed in prawns at 12 µg Pb/L (Ding et al., 2019). The evidence remains inadequate to infer a causality relationship for Pb exposure and reduced growth in freshwater vertebrates. One study reported a threshold of 160 µg Pb/L for tadpole growth in dark-spotted frogs (*P. nigromaculata*) (Huang et al., 2014).

Reproductive and developmental effects were concluded to be causally related to Pb exposure for freshwater invertebrates in the 2013 Pb ISA. This remains the case in newer studies. Recent evidence further supports previous observations of Pb effects on reproductive endpoints at low µg/L concentrations in sensitive species of gastropods, cladocerans and rotifers, especially under chronic exposure scenarios (Section 11.3.5) (see Table 11-5). In *L. stagnalis,* a gastropod known to be sensitive to Pb at low µg Pb/L concentration, NOEC <1.0 µg Pb/L and LOEC = 1.0 µg Pb/L were determined for the number of egg masses and time until the first egg mass in a 56-day lifecycle bioassay (Munley et al., 2013). In this species, the egg capsule and embryo diameter were significantly reduced after 7 days of development at 2.7 µg Pb/L (the highest concentration in which reproduction was observed in the study). For the cladoceran *C. dubia,* 7-day EC_{20} values for reproduction ranged from 12 to 223 µg Pb/L in assays conducted in a variety of natural waters across the United States with different water chemistries; 7-day-EC_{50} values ranged from 20 to 573 µg Pb/L in the same test waters (Esbaugh et al., 2012). Using the same sampled waters from across the United States, reproduction (as population growth) was also assessed in rotifer *P. rapida* over a 4-day exposure period. Chronic EC_{20} and EC_{50} in this species ranged from 3 to 103 µg Pb/L and from 10 to 154 µg Pb/L, respectively.

Several studies in fish in which Pb concentration was analytically verified further support the causal determination reported in the 2013 Pb ISA between Pb and reproductive and developmental effects for freshwater vertebrates (Section 11.3.4.4). For example, hatching success rates in zebrafish embryos were reduced at 4.5, 9.6 and 18.6 µg Pb/L aqueous exposure; at 72 hpf, the hatching success rates at all three concentrations were significantly decreased compared with the control, indicating that Pb caused a hatching delay. This effect persisted until the end of the experiment at 96 hpf (Zhao et al., 2020). Endocrine disruption (significant reduction in thyroid hormones T3 and T4) was observed in zebrafish larvae following exposure to 30 µg Pb/L, although there was no effect on the hatching success rate (Zhu et al., 2014). These studies in fish are bolstered by several analytically verified studies in amphibians (Section 11.3.4.4.3).

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a likely to be causal relationship between Pb exposure and freshwater-community and ecosystem effects, and recent evidence continues to support this finding (Section 11.3.6). Reductions in species abundance, richness or diversity associated with the presence of Pb in freshwater habitats are reported in the literature, usually in heavily contaminated sites where Pb (and other metal) concentrations are higher than typically observed environmental concentrations. Most evidence is from sediment-associated macroinvertebrate communities. Observational and experimental studies published since the 2013 Pb ISA continue to show negative associations between sediment and/or porewater Pb concentration and macroinvertebrate communities. The evidence is expanded somewhat with studies reporting associations with Pb and periphyton abundance. Uptake of Pb into aquatic and terrestrial organisms and subsequent effects on mortality, growth, developmental and reproduction at the organism level can cascade up to ecological populations and communities and lead to ecosystem-level consequences, and thus provide consistency and plausibility for causality in ecosystem-level effects. Although the evidence is strong for the effects of Pb on growth, reproduction, and survival in certain species in experimental settings at or near the range of Pb concentrations reported in surveys of U.S. freshwater systems, considerable uncertainties exist in generalizing effects observed at a smaller scale, particular conditions up to predicted effects at the ecosystem level of biological organization. In many cases, it is difficult to characterize the nature and magnitude of effects and to quantify relationships between ambient freshwater concentrations of Pb and ecosystem response due to the presence of multiple stressors, variability in field conditions and differences in Pb bioavailability at that level of organization.

Table 11-4 Summary of Pb causality determinations for freshwater plants, invertebrates, and vertebrates

Level		Effect	Freshwater[a]	
			2013 Pb ISA[b]	2024 Pb ISA
Community and Ecosystem		Community and Ecosystem Effects	Likely Causal	Likely Causal
Population-level Endpoints	**Organism-level Responses**	Reproductive and Developmental Effects – Plants	Inadequate	Inadequate
		Reproductive and Developmental Effects – Invertebrates	Causal	Causal
		Reproductive and Developmental Effects –Vertebrates	Causal	Causal
		Growth – Plants	Likely Causal	Likely Causal
		Growth – Invertebrates	Causal	Causal
		Growth – Vertebrates	Inadequate	Inadequate
		Survival – Plants	Inadequate	Inadequate
		Survival – Invertebrates	Causal	Causal
		Survival – Vertebrates	Causal	Causal
		Neurobehavioral Effects – Invertebrates	Likely Causal	Likely Causal
		Neurobehavioral Effects – Vertebrates	Likely Causal	Likely Causal
	Suborganismal Responses	Hematological Effects – Invertebrates	Likely Causal	Likely Causal
		Hematological Effects – Vertebrates	Causal	Causal
		Physiological Stress – Plants	Likely Causal	Likely Causal
		Physiological Stress – Invertebrates	Likely Causal	Likely Causal
		Physiological Stress – Vertebrates	Likely Causal	Likely Causal

[a]Conclusions were based on the weight of evidence framework for causal determination in Table II of the ISA Preamble (U.S. EPA, 2015)I. Ecological effects observed at or near Pb concentrations measured in sediment and water in Table 6-2 of the 2013 Pb ISA were emphasized and studies generally within one to two orders of magnitude above the reported range of these values were considered in the body of evidence for freshwater (Section 6.4.12) (U.S. EPA, 2013).

Inputs of Pb into freshwater ecosystems include air-related sources and non-air sources (Appendix 1: https://assessments.epa.gov/isa/document/&deid=359536). Atmospherically derived Pb can enter aquatic systems through direct wet or dry deposition and erosional transport or resuspension of Pb from terrestrial systems (Section 11.1.2). Receiving water bodies include lakes (lentic systems) and rivers and streams (lotic systems). Freshwater wetlands, some of which may be inundated occasionally or constantly, also provide habitat for aquatic biota. The focus of this section is on Pb bioavailability, bioaccumulation, and the effects of Pb on freshwater organisms including algae, aquatic plants, microbes, invertebrates, vertebrates, and other biota with an aquatic lifestage (e.g., amphibians).

The following sections review the recent literature published since the 2013 Pb ISA on effects of Pb on freshwater ecosystems. The new evidence is considered along with the ecological findings of previous Pb assessments. The 2013 Pb ISA developed causality determinations for freshwater biota based on the weight of evidence for Pb effects on specific endpoints and taxonomic groups (Table 11-4). In the 2013 Pb ISA, the body of evidence was sufficient to conclude that there was a causal relationship between Pb exposure and reproductive and developmental effects in freshwater invertebrates and vertebrates, reduced growth and survival of invertebrates, reduced survival of vertebrates, and hematological effects in vertebrates. Relevant concentrations for causality judgments for the welfare effects of Pb in the 2013 Pb ISA were determined considering Pb concentrations "generally within one or two orders of magnitude above those which have been observed in the environment and the available evidence for concentrations at which effects were observed in plants, invertebrates, and vertebrates" (U.S. EPA, 2013). Of these causal relationships concluded for freshwater ecosystems, effects on reproduction, growth, and survival in sensitive freshwater invertebrates are well characterized from controlled studies at concentrations at or near Pb concentrations occasionally encountered in U.S. fresh surface waters. The 2013 Pb ISA concluded there is a likely to be causal relationship between Pb exposure and physiological stress in freshwater biota. For hematological effects, there was a likely to be causal relationship for freshwater invertebrates. Effects on neurobehavioral endpoints were likely to be causal for freshwater invertebrates and vertebrates. Pb effects on plant growth were likely to be causal and were only reported at relatively high concentrations compared with effects on invertebrates. There was also a likely to be causal relationship between Pb exposure and community and ecosystem-level effects. For all effects in freshwater biota, the toxicity of Pb varied with species and lifestage, duration of exposure, form of Pb, and water quality characteristics. Key uncertainties from the last review for freshwater ecosystems included the uncertainties associated with generalization of effects observed in controlled laboratory studies to conditions in streams, rivers, and lakes where many modifying factors affect Pb bioavailability and toxicity. For example, there is a discrepancy between the sensitivity of aquatic insect taxa in laboratory studies compared with longer-term field studies. In a meta-analysis of study findings, longer-term studies suggest that aquatic insect taxa are more sensitive to metals than indicated in acute exposure scenarios (Brix et al., 2011). In aquatic ecosystems affected by Pb, exposures are most likely characterized as low-dose, chronic exposures, whereas the majority of available toxicological data for this metal is from acute laboratory exposures, typically conducted at higher concentrations. There are considerable uncertainties associated with generalizing effects observed in controlled studies to effects at higher levels of biological organization. Furthermore, available studies on community and ecosystem-level effects are usually from contaminated areas where Pb concentrations are much higher than typically encountered in the environment and multiple contaminants are present. At the time of the 2013 Pb ISA, the connection between air concentration of Pb and ecosystem exposure was poorly characterized for aquatic habitats (U.S. EPA, 2013). Furthermore, the previous review noted that the level at which Pb elicits a specific effect is difficult to establish in freshwater systems, due to the influence of other environmental variables (e.g., pH, OM) on both Pb bioavailability and toxicity, and due to substantial species differences in Pb sensitivity. Evidence indicated that Pb is bioaccumulated in biota; however, the sources of Pb in freshwater organisms have only been identified in a few studies, and the relative contribution of Pb from all sources, including atmospheric deposition, is usually not known.

Studies published since the 2013 Pb ISA that characterize bioavailability and uptake of Pb, and its effects in freshwater organisms and ecosystems, that identify additional uncertainties, or decrease uncertainties identified in the prior NAAQS review of this criteria air pollutant are presented throughout the following sections. Brief summaries of conclusions from the 1977 Pb AQCD (U.S. EPA, 1977), the 1986 Pb AQCD (U.S. EPA, 1986), the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013) are included where appropriate. Recent research on the bioavailability and uptake of Pb into freshwater organisms including plants, invertebrates and vertebrates is presented in 11.3.2. Information on environmental concentrations in freshwater biota and ecosystems in the United States at different locations and over time is presented in Section 11.3.3. Toxicity of Pb to freshwater flora and fauna including growth, reproductive and developmental effects (Section 11.3.4) are followed with data on the exposure and response of freshwater organisms (Section 11.3.5). Responses at the community and ecosystem levels of biological organization are reviewed in Section 11.3.6.

11.3.2 Factors Affecting Bioavailability, Uptake and Bioaccumulation and Toxicity in Freshwater Biota

Toxicity of Pb to aquatic life varies with the physicochemical properties of surface waters (U.S. EPA, 2013). Factors affecting the bioavailability and subsequent toxicity of Pb to biota include chemical factors (primarily water hardness, DOC, pH) and biological factors (e.g., lifestage, development of tolerance, organism interactions). Water hardness, DOC, and pH can be quantified, are directly related to the toxic effects and are used in bioavailability models to predict acute and chronic toxicity (Adams et al., 2020) (Section 11.1.6). Biological factors discussed in prior Pb AQCDs or the 2013 Pb ISA that may influence organism response to Pb exposure include the lifestage of an organism, genetics, and nutrition (see Section 7.2.3, 2006 AQCD (U.S. EPA, 2006) and Section 6.4.9, 2013 Pb ISA (U.S. EPA, 2013)). These factors are more difficult to link quantitatively to toxicity. Often, species differences in metabolism, sequestration and elimination rates control the relative sensitivity and vulnerability of exposed organisms. The organism route of exposure also influences Pb toxicity. Uptake of Pb by aquatic invertebrates and vertebrates may preferentially occur via exposure routes other than direct absorption from the water column, such as ingestion of contaminated food and water, uptake from sediment porewater, or incidental ingestion of sediment (U.S. EPA, 2013, 2006). Fewer studies assess uptake, bioaccumulation, and subsequent toxicity of Pb via diet than via aqueous exposure. Of the available Pb feeding studies in freshwater biota, only a few pair the same concentration of waterborne exposure with dietary exposure to compare the relative importance of dietary versus aqueous uptake pathways (Alsop et al., 2016; DeForest and Meyer, 2015). Studies published since the 2013 Pb ISA on chemical factors (water hardness, DOC, pH, temperature, and other metals) and biological factors discussed in this section further enhance understanding of Pb uptake and subsequent toxicity in freshwater systems. Biological factors include those that were well characterized in previous AQCDs and the 2013 Pb ISA, (e.g., lifestage), and factors not previously considered, such as the role of parasites in modulating Pb bioaccumulation.

11.3.2.1.1 Water Hardness

The role of water hardness (the amount of Ca^{2+} and Mg^{2+} ions) in Pb uptake and subsequent toxicity was reported in previous Pb AQCDs and the 2013 Pb ISA. Furthermore, U.S. EPA's existing Pb AWQC are hardness-based (Section 11.1.7.3) (U.S. EPA, 1985a). Generally, as water hardness increases, there is less Pb uptake due to competition of Ca^{2+} and Mg^{2+} for binding sites. Newer literature has continued to examine the role of Ca^{2+} and Mg^{2+} and other cations commonly present in surface waters (e.g., K^+, Na^+) in modulating Pb bioaccumulation and toxicity. For example, in a study of the amphipod *Gammarus pulex* exposed 2 days to 10 µg Pb/L and a range of environmentally relevant cation concentrations (Na^+, Mg^{2+} or Ca^{2+}), both Na^+ and Mg^{2+} had no significant effect on Pb uptake while increasing Ca^{2+} concentrations inhibited Pb uptake (Urien et al., 2015). In a study reviewed in the 2013 Pb ISA, Ca^{2+} influenced Pb accumulation and toxicity in the fathead minnow (*Pimephales promelas)* during waterborne exposure (Grosell et al., 2006a). In a newer study in fish, Ca^{2+}, Mg^{2+} or H^+ significantly decreased Pb accumulation and toxicity in zebrafish larvae *Danio rerio*, while K^+ and Na^+ showed no effect (Feng et al., 2018) (see Section 11.3.2 for further discussion of water hardness and Pb toxicity).

As described in prior AQCDs and the 2013 Pb ISA, the effect of water hardness is variable; generally, both the acute and chronic toxicity of Pb increase with decreasing water hardness as Pb becomes more soluble and bioavailable and less Ca^{2+} and Mg^{2+} ions are available to compete with Pb for binding sites. Studies available since the 2013 Pb ISA are also illustrative of the varying influence of water hardness on the toxicity of Pb. In reproductive toxicity tests with *C. dubia*, 7-day EC_{50} was 81.2 µg Pb/L at 10 mg/L Ca (0.25 mM) and 130 µg Pb/L at 70 mg/L Ca (1.75 mM), showing that the daphnids tested in the soft water were more sensitive to Pb toxicity (Nys et al., 2014). However, in a bioassay with the rotifer *Brachionus calyciflorus,* Ca was not protective in a chronic (48-hour) exposure (Nys et al., 2016b). In bioassays with zebrafish larvae, Pb was more toxic in soft water (11.7 mg $CaCO_3$/L) compared with hard water (141 mg $CaCO_3$/L) (Alsop and Wood, 2011).

11.3.2.1.2 Dissolved Organic Matter and Dissolved Organic Carbon

In studies cited in the 2013 Pb ISA, DOC was shown to have a protective effect on Pb toxicity in freshwater invertebrates and fish Esbaugh et al. (2011); Mager et al. (2011a); Mager et al. (2011b), and newer studies continue to support these observations. Esbaugh et al. (2012) compared the relative importance of water chemistry variables including DOC, Ca, and pH in the toxic response of freshwater cladoceran (*Ceriodaphnia dubia*), mollusk (*Lymnaea stagnalis*) and rotifer (*Philodina rapida*) to a range of Pb concentrations in bioassays conducted in a variety of natural waters from across North America. The greatest toxicity to the cladoceran and snail species was observed in low-DOC waters, and toxicity was found to be correlated with DOC using multilinear regression modeling analysis. This was not the case in rotifer *P. rapida*, where toxicity was most closely correlated with Ca and pH, not DOC. In contrast, in the rotifer *B. calyciflorus*, high DOC was protective against Pb chronic reproductive toxicity; however, when expressed as free-ion activity, toxicity increased with increasing fulvic acid concentration (Nys et al., 2016b). The authors suggest that fulvic acid-Pb complexes may also contribute to Pb bioavailability in *B. calyciflorus*. Taking metal speciation into consideration, Dong et al. (2014) calculated the Comparative Toxicity Potential of Pb (described as the ecotoxicological impact associated with a unit emission of substance to defined ecological receptors via different pathways of exposure). Pb had the highest Comparative Toxicity Potential in water with low DOC, moderate pH and hardness, and the lowest Comparative Toxicity Potential in water with moderate DOC, high pH, and hardness. Pb typically has high affinity to DOC, resulting in a low fate factor (residence time) and bioavailability factor (fraction of truly dissolved metal within total metal) (Dong et al., 2014). Additionally, Zhang et al. (2021) found that modeling Pb and other heavy metals was improved when incorporating total OC and AVS.

Since the 2013 Pb ISA, studies have further elucidated the relationship between the characteristics of humic substances and Pb bioavailability, such as molecular weight (MW) or other additional effects associated with solar irradiation. In lake sediments, Pb-humic acid complexes are more stable when the MW of the humic acid is lower. In particular, humic acids with MW lower than 10 kDa could increase the biosorption capacity of Pb (Bai et al., 2019). While Pb-humic acid complexes are discussed in the 2013 Pb ISA, the study by Kostić et al. (2013) suggests a mechanism for the binding of Pb to humic acid may be the "acid-like" nature of Pb(II). Pb(II)-ions strong affinity for humic acid may be explained by its borderline acid properties and by how humic acids behave as weak acid polyelectrolytes. Humic acids carry a variety of oxygen-containing functional groups such as carboxylic, hydroxyl, phenolic and carbonyl groups with oxygen as a donor atom, which helps them form strong bonds with Pb(II). This is also supported by the study by Liu et al. (2022), which found Pb(II) caused greater quenching (the decrease of fluorescence by the metal addition) in humic-like DOM compared with protein-like DOM. The finding was likely due to humic-like components complexing with Pb(II) through carboxyl and hydroxyl (-COOH and -OH) groups, which generally bonds to Pb(II) preferentially over protein-like DOM that contains significant amounts of the amino group ($-NH_2$).

The bioaccumulation capacity for Pb in algae is influenced by the presence of organic acids. Que et al. (2020) found that adding organic acids, such as malic acid or citric acid prolonged the adsorption equilibrium time of the algae-Pb binary system. Citric acid showed a greater bioaccumulation capacity for Pb in algae than malic acid, due to ternary complex formation. The binding capacity of Pb to OM is also influenced by solar or UV-B radiation. Pb complexation with representative humic substances (Suwannee River humic acid and Suwannee River fulvic acid) decreased with increasing simulated solar radiation (Spierings et al., 2011). This may be due to an increase in the relative abundance of the carboxyl groups in the photoaltered humic substances and from decreased aromaticity (and thus less electronegativity) with increasing irradiation doses. The presence of Pb^{2+} can also increase the photodegradation of microcystin and thus reduce microcystin accumulation in sediments and in certain fish (Dai et al., 2017). Reduced amounts of humic acid were adsorbed to the freshwater microalga *Chlorella kesslerii*, which then reduced Pb bioavailability to the microalgae because the humic substances increase the bioavailability of Pb to microalgae by adding supplementary binding sites and because Pb uptake by *C. kesslerii* is controlled by transport across the biological membrane rather than by diffusion in the medium (Spierings et al., 2011). However, there was no correlation with an increase in free Pb ions and algal intracellular Pb content, likely due to the formation of additional binding sites on the photoaltered humic acids. In additional tests using Elliott humic acid under simulated solar radiation, free Pb ions were released from the metal-DOM complex as the irradiation dose increased, and there was a 33% increase in intracellular Pb concentration in *Chlamydomonas reinhardtii* at high irradiance (Worms et al., 2015).

11.3.2.1.3 pH

As described in prior AQCDs and the 2013 Pb ISA, uptake and subsequent toxicity of Pb to freshwater biota can be affected by pH, either directly or indirectly. Generally, at low pH, there is more Pb^{2+} available to bind to the biotic ligand. As pH increases, there is increased formation of Pb organic (DOC) and inorganic (OH^-, CO_3^{2-}) complexes, which decrease Pb bioavailability. Since the 2013 Pb ISA, several studies have further characterized Pb complexation and adsorption under changing pH conditions. There are more binding sites for Pb to humic acids at pH 6 than at pH 4, likely due to the higher content of dissociated functional groups in humic acids at higher pH, and more favorable electrostatic attraction when binding surfaces become deprotonated at higher pH (Bai et al., 2019). Xu et al. (2018) found that the binding dynamics of DOM groups in response to Pb(II) addition were regulated by both pH and ionic strength. Specifically, at lower pH and ionic strength (e.g., pH 4.7 and ionic strength 0.01 M), as Pb(II) was added, aryl C-H and carboxyl C = O groups gave the fastest response, followed by polysaccharide C-OH and chromophoric groups at 265 nm (CDOM265). However, when pH was raised to 6.0, the opposite binding sequence was found, in that the CDOM265 group was bound first, followed by the polysaccharide C-OH and carboxyl C = O, and finally the aryl C-H groups. Hua et al. (2013) found that Pb absorption to biofilms was greatest at pH 9, which was 3.5 times greater than that at the minimum adsorption (pH = 7).

Several studies since the 2013 Pb ISA have tested the effects of changing pH on Pb toxicity to biota. In the freshwater algal species *Pseudokirchneriella subcapitata,* as pH increased from 6.0 to 7.6, the 72-hour EC_{50} decreased from 72.0 to 20.5 µg filtered Pb/L (De Schamphelaere et al., 2014). Further, Antunes and Kreager (2014) observed greater toxicity (more bioavailability) for common duckweed (*L. minor*) at higher pH; this was due to less H^+ and competition at the macrophyte binding sites. The apparent increase in Pb^{2+} toxicity at pH >7.0 coinciding with a changing ratio of $[Pb^{2+}]/[Pb(OH)^+]$ (due to the marked increase in $[Pb(OH)^+]$) suggests that $Pb(OH)^+$ also contributed to the toxicological response.

In some freshwater invertebrates, recent studies generally support previous understanding that higher pH is protective; however, these findings vary by the duration of the toxicity bioassays and by taxa. In a series of chronic reproductive toxicity tests with daphnia *C. dubia* conducted at different pH values, high pH was protective of Pb toxicity. At the lowest pH tested (pH 6.4), the EC_{50} = 99.8 µg Pb/L, while at the highest pH (pH 8.2), the EC_{50} = 320 µg Pb/L (Nys et al., 2014). Similarly, decreasing toxicity of Pb to *D. magna* with higher pH was observed by Qin et al. (2014); as pH increased from 5.0 to 9.0, the 24 h-LC_{50} increased from 784 µg Pb/L to 9,473 µg Pb/L, and the predicted proportion of free Pb^{2+} ion was 99.75% at pH 5.0 and 2.9% at pH 9.0. High pH was also protective in chronic reproductive toxicity tests with rotifer *B. calyciflorus*. Both the population growth rate and population size generally decreased with increasing pH in bioassays conducted at pH values ranging from 6.4 to 8.2 (Nys et al., 2016b). Wang et al. (2016b) found that for crustaceans, Pb toxicity increased with increasing pH, but for mollusks and worms, toxicity decreased with increasing pH. For fish, toxicity was least at neutral pH and increased at lower or higher pH levels. The toxicity of Pb can increase at higher pH when there is less competition between H^+ and metal binding sites on cell-surface ligands. However, there may be higher toxicity at lower pH due to increased solubility and altered Pb speciation, which can increase Pb bioavailability for certain animals. Uptake studies in natural environments have also pointed to the importance of pH in uptake of Pb. A field study conducted in 36 headwater streams in the Lake District of England reported statistically significant correlations between total dissolved Pb in stream water and body burdens in the sampled aquatic insect taxa (*Leuctra* spp., Simuliidae, *Rhithrogena* spp., Perlodidae) (De Jonge et al., 2014). In the streams, H^+ ion activity was the overriding factor influencing Pb body burden, while DOC was not a significant factor.

In fish, the effects of pH on toxicity were variable in studies cited in the 2013 Pb ISA. For example, lower pH was shown to result in increased sensitivity to Pb in juvenile fathead minnows following 30-day exposure to Pb at varying concentrations (Grosell et al., 2006a). Additionally, Birceanu et al. (2008) determined that fish (specifically rainbow trout) were more susceptible to Pb toxicity in acidic, soft waters, characteristic of sensitive regions in Canada and Scandinavia. Hence, fish species endemic to such systems may be more at risk from Pb contamination than fish species in other habitats. In a study published after the 2013 Pb ISA, Esbaugh et al. (2013) compared three methods used to acidify laboratory bioassay water on LC_{50} values in fathead minnow. Pb toxicity varied significantly depending upon the acidification method used in the experiment. The authors recommended direct acid-base addition rather than CO_2 or 3-(N-morpholino)propanesulfonic acid buffer. In an approach that linked metal accumulation with toxicity through a BLM-aided toxicokinetic-toxicodynamic model, Gao et al. (2015) demonstrated that increasing concentrations of H^+ in test media significantly reduced Pb accumulation in zebrafish larvae within the exposure duration of >4–72 hours. In the same study, increasing [H^+] significantly decreased the mortality of the larvae at >12–96 hours.

11.3.2.1.4 Water Temperature

In the 2013 Pb ISA, water temperature was noted as a factor affecting the toxicity of Pb to aquatic organisms, with higher temperatures generally leading to greater response; a few recent studies reported variable responses to Pb with temperature. Isopods *Asellus aquaticus* exposed for 10 days to one of two water temperatures (15 ± 1°C and 20 ± 1°C) and three concentrations of Pb (0.0353 µmol/L, 7.3 µg Pb/L), 0.353 µmol/L (73 µg Pb/L) and 0.882 µmol/L (181 µg Pb/L) exhibited distinct responses at the two temperature treatments (Van Ginneken et al., 2019). At 15°C, respiration decreased as Pb concentration in the isopods increased. In the higher temperature treatment, feeding and respiration rates were higher and were positively correlated with Pb uptake and accumulation. Park et al. (2020) assessed survival, malformation and heart rate in zebrafish embryos exposed to three analytically verified concentrations of Pb (2, 10 and 17 µg Pb/L) at two temperatures (26°C and 34°C). At 26°C, the survival rate decreased early in the 7-day exposure at the two highest concentrations, reaching 73% at 10 µg Pb/L and 57% at 17 µg Pb/L by the end of the experiment, with no significant effect at 2 µg Pb/L. At 34°C, the survival rate decreased significantly in all concentrations and to a greater extent in the highest concentration; at 7 days, embryo survival at 17 µg Pb/L was 30% that of the control. Malformations such as spinal curvature were observed in all tested concentrations at both temperatures. At 34°C, heart rate was significantly decreased at all Pb concentrations, while at 26°C, heart rate was significantly decreased at the two highest tested concentrations.

11.3.2.1.5 Other Metals

Multiple metals are present simultaneously in aquatic environments and may interact with one another, influencing Pb uptake and resulting in antagonistic, synergistic, or other toxic effects. Recent advances in in multimetal research since the 2013 Pb ISA have included development and evaluation of bioavailability models to predict the toxicity of acute and chronic metal mixtures, of which Pb is one component (Nys et al., 2017; Farley et al., 2015; Santore and Ryan, 2015). Since the 2013 Pb ISA, considerable research beyond the scope of this document (Section 11.1.1) has focused on metal mixture assessment, including how uptake and bioaccumulation are affected in freshwater biota in the presence of multiple metals. The mechanisms of metal interactions may include competition for the same metal transporter at the biological membrane or displacement of one metal by another metal on DOM, which leads to changes in the free metal ion concentration in water (Crémazy et al., 2019). The effects of metals on Pb uptake and toxicity vary by metal. In the juvenile freshwater snail *L. stagnalis,* Ni and Zn had no effect on Pb uptake, but a small but significant inhibitory effect was observed with Ag (Crémazy et al., 2019). In the isopod *A. aquaticus* exposed to Cd and Pb simultaneously, synergistic interactions occurred with metal uptake as well as on growth rates and mortality rates when compared with single-metal studies (Van Ginneken et al., 2015). In juvenile rainbow trout (*Oncorhynchus mykiss*) uptake studies of binary mixtures with Pb paired with other metals, Pb uptake into gill tissue was significantly inhibited in a noncompetitive manner by Ag, Cd and Cu, while Ni and Zn had no effect on Pb uptake (Brix et al., 2017). In another study with juvenile rainbow trout, there was no effect on ionoregulation at a low Pb concentration of 5.4 µg Pb/L (26.1 nmol/L) (Clemow and Wilkie, 2015). However, in combination with Cd, there was greater-than-additive toxicity, likely due to differences in the underlying mechanism of action, with some shared binding sites between the two metals. In 5-day postfertilization zebrafish larvae exposed to Pb alone (10 µg Pb/L), Cd alone (5 µg Pb/L) or Pb + Cd since 4 hours postfertilization, the respective mean concentrations of Pb and Cd in tissue were statistically significantly lower in the co-exposure group than in the groups exposed to Pb or Cd alone (Liao et al., 2021). There were differences in behavioral outcomes in the three treatment groups; Pb primarily affected locomotor activity, Cd affected circadian behavioral rhythm and the two compounds in combination were antagonistic for both locomotor activity and behavioral rhythm. The bioavailability of Pb is also affected by the formation of complexes with various Fe (oxyhydr)oxides, such as ferrihydrite, schwertmannite, jarosite, goethite, hematite, and magnetite (Shi et al., 2021). Fe (oxyhydr)oxides influence the speciation, partitioning and transport of Pb through adsorption and coprecipitation, and this can vary by acidity, alkalinity, temperature, and oxic conditions.

11.3.2.1.6 Lifestage

The differential sensitivity of early lifestages of aquatic biota to contaminants is well-established in the scientific literature, such that national and international entities (e.g., U.S. EPA, Organisation for Economic Co-operation and Development, European Union) have standardized laboratory toxicity assay protocols that call for testing with embryo, larval or juvenile organisms to assess effects at the most sensitive lifestages. Differences in susceptibility to Pb at distinct lifestages for freshwater invertebrates and fish are discussed in Section 6.4.9.4 of the 2013 Pb ISA. Recent studies conducted with freshwater organisms reviewed in Sections 11.3.4 and 11.3.5 continue to demonstrate that lifestage is an important determinant of increased sensitivity to Pb. For example, endangered white sturgeon (*Acipenser transmontanus)* were three and a half times more sensitive when exposed to Pb at 8 days posthatch (dph) than at 40 dph (Vardy et al., 2014).

11.3.2.1.7 Species Sensitivity

As described in previous U.S. EPA reviews of Pb, sensitivity to this metal can vary by several orders of magnitude across freshwater biota. Pb elicits responses in some species at low (<5 to 10 µg Pb/L range under some water conditions) concentrations while others appear to be unaffected at concentrations greatly exceeding 1,000 µg Pb/L. In a study reported in the 2013 Pb ISA, a series of SSD showed the greatest sensitivity to Pb in crustaceans, followed by cold water fish, and warm water fish and aquatic insects, which exhibited a similar sensitivity (Brix et al., 2005). A comparison of cladoceran and copepod freshwater species curves generated by Wong et al. (2009) indicated that cladoceran species, as a group, were more sensitive to the toxic effects of Pb than were copepods, with respective hazardous concentration values for 5% of the species of 35 and 77 µg Pb/L. Following the 2013 Pb ISA, Deforest et al. (2017) used acute and chronic toxicity data across a range of freshwater species and genera, taking into account the differences in sensitivity to Pb, to propose updated aquatic life AWQC for Pb (Section 11.3.5).

Some uncertainty is associated with the extrapolation of toxicity values generated from laboratory-based single-metal acute exposure assays to chronic exposure to multiple metals and other contaminants in field studies. (Brix et al., 2011) provided examples of acute laboratory exposures with aquatic insects that suggested the insects are relatively insensitive to metals, in contrast to field studies that report sensitivity. The authors conducted a meta-analysis of laboratory and field studies that generally supported the finding of greater sensitivity of aquatic insects in chronic exposure field conditions. However, the majority of available field studies involve multimetal exposures. The authors speculated there could be a difference in the mechanism of toxicity between acute exposure and chronic exposure in aquatic insects or that dietary metal exposure is another important contributing factor to toxicity in these organisms.

11.3.2.1.8 Development of Tolerance

Tolerance to prolonged Pb exposure may develop over time in some organisms as they physiologically adapt and survive under low variations of various environmental stresses, including Pb. Evidence for genetic selection in the natural environment has been observed in some aquatic populations exposed to metals in studies, as reviewed in the 2006 AQCD. Fewer laboratory-based assays have examined the development of Pb tolerance. In a study reviewed in the 2013 Pb ISA, multigenerational exposure to Pb appears to confer some degree of metal tolerance to *Chironomus plumosus* larvae; however, metal-tolerant larvae were significantly smaller than larvae reared under clean conditions (Vedamanikam and Shazilli, 2008). In a more recent multigenerational test with *D. magna* exposed to an analytically verified concentration of 50 µg Pb/L, the LC_{50} (= 430 µg Pb/L at the F0 generation) increased to 2,110 µg Pb/L in the F9 generation. The LC_{50} of control organisms in the F9 generation varied from 430 µg Pb/L to 890 µg Pb/L suggesting that the Pb-exposed organisms developed some tolerance to Pb over time (Araujo et al., 2019). In a comparative study of adult amphipods *Gammarus fossarum*, either freshly collected from the field and exposed to 2.1 µg Pb/L for 24 hours or chronically exposed to the same concentration for 10 weeks, there were differences in response to Pb. In the freshly collected amphipods, both locomotion and respiration were significantly decreased compared with unexposed organisms, whereas in the chronically exposed amphipods, no statistically significant response to these endpoints was observed, suggesting that the compensatory response developed over time (Lebrun and Gismondi, 2020). In another study with *G. fossarum* and the amphipod *Gammarus pulex,* a history of metal exposure did not affect Pb bioaccumulation parameters, as accumulation and elimination parameters were similar between reference and pre-exposed populations collected from field sites and exposed to Pb in microcosms (Urien et al., 2017). Amphipods were exposed to water spiked with an analytically verified concentration of 10 µg Pb/L for 7 days, then transferred to mineral water for depuration for 7 days. The net bioaccumulation of Pb was quantified by subtracting the basal concentrations of Pb from the total Pb concentration after exposure. There was no interpopulation variability or difference in the pattern of accumulation or elimination between *G. pulex* and *G. fossarum*.

The peak Pb body concentration was slightly higher in pre-exposed populations relative to the reference populations for both species.

11.3.2.1.9 Seasonality

In the 2013 Pb ISA, several studies reported seasonal alterations in aquatic plant Pb tissue concentrations, suggesting that species-dependent seasonal physiological changes may control Pb uptake in aquatic macrophytes (Section 6.4.9.1) (U.S. EPA, 2013). Several studies published since the 2013 Pb ISA further describe changes in Pb bioavailability linked to season. In a study examining the interacting effects of macrophytes and season, metal concentration in small fish inhabiting the phytoplankton-dominated northern zone of Lake Taihu, China was significantly greater in summer than in small fish collected from the southern zone of the lake characterized by a high density of macrophytes (Zeng et al., 2012). These differences in metal concentration in small fish collected from the two regions of the lake disappeared in winter, suggesting that the presence of algae and macrophytes modified trace metal concentrations during the summer months, resulting in two distinct ecological regions that differed in their potential for metal exposure. Differences in metal accumulation in larger fish from the two lake zones varied with season in some tissues, but no significant differences were reported in carnivorous fish. Chen et al. (2019) quantified seasonal differences in Pb mobility in lake sediments from phytoplankton-dominated and macrophyte-dominated areas of Lake Taihu. In the phytoplankton-dominated region, labile and dissolved Pb in sediment was highest in April and July and lowest in October and January. The opposite pattern was observed for the macrophyte-dominated region. In littoral anoxic sediment, the periodic drying and rewetting process can increase the bioavailability of Pb to aquatic organisms (Liu et al., 2020). Even though high total OC content in the sediment facilitates the formation of anoxic conditions, periodic drying oxidizes the sediment and leads to sulfide oxidation, which increases the mobility and bioavailability of Pb because it is less firmly bound to sediment in these conditions.

11.3.2.1.10 Parasites

The combined effects of endoparasites and other stressors, such as metals, modulate uptake and toxicity to host organisms (Marcogliese and Pietrock, 2011). Multiple studies have reported differences in Pb accumulation between parasitized and nonparasitized organisms including studies in fish (Brázová et al., 2015; Filipović Marijić et al., 2014; Sures et al., 2003; Sures and Siddall, 1999) and snails (Mostafa et al., 2014). These studies suggest that the effects of parasites on host organisms in the presence of Pb are complex. In a recent synthesis of parasite-host studies, Pb was accumulated to a higher degree in parasites than in tissues of host species, and Pb accumulation in infected hosts was consistently lower compared with uninfected conspecifics (Sures et al., 2017).

11.3.2.1.11 Bioturbation/Association with Sediment

Since the 2013 Pb ISA, several studies have examined how the activities of sediment-associated benthic invertebrates influence Pb transfer to the water column and subsequent bioavailability to other aquatic organisms. A statistical Random Forest model that took into account riverine invertebrate community traits such as feeding strategy, respiration and locomotion to predict metal bioaccumulation from environmental compartments (water column, sediment, suspended particulate matter) showed that the strongest predictor of metal bioaccumulation in the organisms was the degree to which taxa live in or directly on sediment (Peter et al., 2018). In mesocosms with two (Amphipod, Bivalve) or three (Amphipod, Bivalve, Oligochaete) sediment-associated species combinations, water, and tissue concentrations of Pb (and other trace elements primarily associated with organic colloids) increased as the number of bioturbating organisms present increased (Andrade et al., 2020). One set of experiments Blankson et al. (2017); Blankson and Klerks (2017, 2016a, 2016b) used oligochaete worm *Lumbriculus variegatus* in Pb-spiked mesocosms as a model organism for bioturbation in freshwaters and demonstrated that concentration of Pb in water column and water turbidity increases with increased density of sediment organisms. Bioturbation activity was also affected by increasing Pb concentration, a decline in bioturbation was observed with worms exposed to 681.9 mg/kg and 3396.2 mg/kg (Blankson et al., 2017). The amount of Pb transferred to the water column varied with sediment characteristics (Blankson and Klerks, 2017). For transfer of Pb to the water column, the most important variables were silt/clay content and sediment pH; Pb bioaccumulation in the worms was influenced by OM in the sediments and the pH of the porewater. Overall, bioturbation by oligochaetes could bring about the transport of Pb from sediments to the water column. This means that the presence of these bioturbators can enhance Pb availability to organisms in the water column and potentially cause toxic effects in planktonic and nektonic organisms.

11.3.2.1.12 Intraspecific Interactions

Additional research published since the 2013 Pb ISA provides experimental evidence that interactions among individuals of the same species may affect sensitivity to metals. The influence of intraspecific competition on Pb (13 to 236 μg Pb/L) toxicity was explored by Gust et al. (2016) using single daphnia exposures conducted concurrently with assays of multiple daphnia (proportionally scaled assays of 20 *D. magna* per beaker) and at two different feeding regimens (low-feed ration and high-feed ration). After 14-day exposure to Pb, the LC_{50} was threefold higher in assays with single daphnia (232 [156–4810] μg Pb/L) compared with assays with multiple individuals (68 [63–73] μg Pb/L) at the lower feeding ration. Similar results were obtained with the higher feeding ration experiment with multiple daphnia per experimental unit (LC_{50} = 79 (74–84) μg Pb/L) and the single-animal treatment (LC_{50} = 236 μg Pb/L) (no 95% confidence interval could be calculated). Moreover, reproduction (neonate production) decreased with intraspecific competition at 9 and 14 days in both feeding ration groups compared with assays with single daphnia where no negative effects on reproduction were observed at any concentration tested. The authors proposed that individual daphnia modulate their life-history response in the presence of others of the same species through chemical cues, and this has a modifying effect on toxicity.

11.3.2.1.13 Predator-Stress and Metal Mixture Effects

Research published since the 2013 Pb ISA tested the effects of multiple stressors on Pb uptake and toxicity. Predator stress and the presence of other metals affected the accumulation and sensitivity of the aquatic sowbug (*A. aquaticus*) to Pb stress (Van Ginneken et al., 2018). Individual *A. aquaticus* collected from a stream in Belgium were placed in a control, 0.0232 µmol/L, (4.8 µg Pb/L), 0.276 µmol/L (57 µg Pb/L) or 3.08 µmol/L (638 µg Pb/L) solution with two black alder (*Alnus glutinosa*) leaf discs. Each Pb treatment and metal mixture (Cu + Pb, Cd + Pb and Cu + Cd + Pb) was crossed with one of two treatments, either a heterospecific predator cue or conspecific alarm cue. To create the heterospecific predator cue solution, one damselfly larva (*Calopteryx splendens*) was placed in a container of water for 72 hours. Next, one adult three-spined stickleback (*Gasterosteus aculeatus*) and the ninespine stickleback (*Pungitius pungitius*) were placed in water for 24 hours. After removing the predators from the water, equal parts of stimulus water were mixed to create the heterospecific predator cue. To create the conspecific alarm cue, one *A. aquaticus* was homogenized in solution. Either water (control) or predator or the conspecific alarm cue solution was added to the control and Pb-contaminated containers with *A. aquaticus* every day for 10 days. Afterward, *A. aquaticus* Pb concentration, growth rate, feeding rate, percent active time, survival and respiration rate were recorded. Overall, there were no significant effects of either heterospecific or conspecific predator cues on Pb accumulation in *A. aquaticus*, although respiration rates did increase when exposed to predator cues. Pb accumulation in the isopods was positively correlated with Pb free-ion activity. There were no significant effects of predator stress on isopod body burdens. Metal mixture significantly affected Pb accumulation, as the slope of the relationship between Pb treatment and Pb body burden decreased when Cu and Cd + Cu were added. Respiration rates were affected by both Pb exposure and predator stress. Differences in respiration rates between predator-stress and control treatments were greater when isopods had greater Pb body burdens. Activity levels decreased as Pb body burden increased, but there was no difference between predator treatments and the interaction between Pb treatment and predator stress. Growth rate (mg/day) was negatively correlated with Pb free-ion activity in the water but was not found to vary with predator stress or body burden. Although Pb body burden did not influence feeding rates (mg/mg/day), the Pb body concentration of *A. aquaticus* exposed to the Pb + Cd mixture had the greatest effect on feeding rate compared with Pb, Pb + Cu and Pb + Cu + Cd. Finally, activity decreased with increasing Pb body burden, but was unaffected by predator stress and Pb-metal mixtures.

11.3.2.2 Uptake and Bioaccumulation in Freshwater Plants and Algae

Studies on bioavailability of Pb in aquatic plants and algae published since the 2013 Pb ISA continue to support previous findings that plants tend to sequester larger amounts of Pb in roots as compared with shoots and that there are species-specific differences in uptake of Pb from water and sediments, as well as compartmentalization of that sequestered Pb (U.S. EPA, 2013, 2006). Further, it has previously been established that many plants accumulate heavy metals in environments with high concentrations and are used for phytoremediation at such sites; additional studies on this topic have little relevance in the current assessment.

Very little new information is available on the bioavailability of Pb in freshwater algae at levels that are within the concentrations of interest in this ISA (Section 11.1.1). One study contains data on bioavailability and partitioning between water and sediment correlated with toxic harmful algal blooms (HABs), which are of concern in many freshwater bodies. This study, conducted in a freshwater reservoir in Portugal, examined in situ interactions between Pb and *Microcystis aeruginosa*, a HAB-forming cyanobacterium found in the United States (Baptista et al., 2014). The metal content of water and sediments from both the reservoir and an upstream reference site were monitored monthly for 16 months, during which time *M. aeruginosa* bloomed twice, firstly forming a scum, and later with colonies scattered throughout the reservoir. No correlation was found between Pb in the water column and algal blooms. When blooms occurred, a significant increase of metal levels in the sediment occurred simultaneously (average Pb concentration was measured at 43.2 mg/kg); however, quantification of the exchangeable metal fraction during this algal bloom indicated that this Pb was probably not bioavailable. The authors speculate shallow water depth would have allowed the cells of *M. aeruginosa* to deposit upon the sediments rapidly, and the presence of the cyanobacteria in the sediment might have contributed to an increase in metal content, meaning that algae may be an important biotic compartment for Pb during such blooms. In three Scottish lakes receiving varying inputs of metals solely from atmospheric deposition changes in phytoplankton biomass, cellular Pb and the P content of cells were measured simultaneously. The results showed that algal bloom events in the lakes diluted the mass-specific Pb in the phytoplankton (Gormley-Gallagher et al., 2016). As total cellular P increased, there was a corresponding increase in phytoplankton growth, and the concentration of Pb declined.

In freshwater floating macrophytes, there is also very little new information on the bioavailability of Pb. These life forms are important because their roots dangle in the water column instead of being buried in substrate, and thus, Pb uptake occurs solely through the interface with the water column. One U.S. study examined the uptake and distribution of metals by a floating macrophyte, water lettuce (*Pistia stratiotes* L.), in storm water impoundments in Florida (Lu et al., 2011). Two stormwater impoundment ponds were divided into two plots, a control without *P. stratiotes* and one with enough young plants to initially cover 1/20th of the water surface. While the authors stated that water Pb levels were mostly low (below the Maximum Daily Limits), they did not provide the concentrations. Even at these low concentrations, reported BCFs of Pb from the water column into plant roots were higher than 104. Lead was found inside and adsorbed to plant roots, with approximately 60% of Pb within the root tissue. Another study by Chen et al. (2019) found that submerged macrophytes in lakes can accumulate Pb, which is absorbed either from the sediments through roots or from the water by leaves.

Although the U.S. EPA Framework for Metals Risk Assessment states that the latest scientific data on bioaccumulation do not currently support the use of BCFs and BAFs when applied as generic threshold criteria for the hazard potential of metals (U.S. EPA, 2007), such metrics are useful to provide information about the amount of uptake of metals into plants, compartmentalization into different plant tissues, and differences between species. In a series of field studies undertaken in Sicily, spanning a gradient of affected wetlands, Pb concentrations in soil, water, and plant tissues of several wetland species were quantified (Bonanno et al., 2018; Bonanno and Cirelli, 2017; Bonanno and Vymazal, 2017; Bonanno et al., 2017; Bonanno, 2013). These studies affirmed that metal uptake is species-specific despite similar ecology, anatomy, and life form, and that Pb is mainly compartmentalized in root tissue in freshwater plants.

11.3.2.3 Uptake and Bioaccumulation in Freshwater Invertebrates

This section expands on the findings from the 1986 Pb AQCD (U.S. EPA, 1986), the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013) on the bioaccumulation and sequestration of Pb in freshwater invertebrates. Uptake and subsequent bioaccumulation of Pb varies greatly between species and across taxa, as characterized in previous U.S. EPA reviews of this metal. In invertebrates, Pb can be bioaccumulated from multiple sources, including the water column, sediment and dietary exposures, and factors such as the proportion of bioavailable Pb (Section 11.1.6) lifestage, age and metabolism can affect the accumulation rate. As reviewed by Wang and Rainbow (2008) and supported by subsequent studies, there are considerable differences between species in the amount of Pb taken up from the environment and in the levels of Pb retained in the organism.

Uptake studies generally show that aquatic invertebrates accumulate Pb from water in a concentration-dependent manner and may reach an equilibrium depending on the organism's ability to eliminate or store Pb. In a study reviewed in the 2013 Pb ISA, the tissue concentration of Pb in adult Eastern Elliptio mussel (*Elliptio complanata*) increased for the first 14 days in an aqueous exposure at an exposure-dependent rate then did not change significantly for the remainder of the 28-day exposure (Mosher et al., 2012b) In another study with the same species conducted after the 2013 Pb ISA, Pb was measured in hemolymph every 7 days during a 28-day exposure, and distinct patterns of response were observed with Pb concentration. At the lowest concentrations (≤ 6 μg Pb/L), Pb gradually increased in the hemolymph but did not exceed the exposure concentration, at midrange concentration (up to 66 μg Pb/L), the mussels appeared to regulate Pb by day 14, whereas at the highest concentration tested (251 μg Pb/L), Pb in hemolymph increased throughout the exposure period (Mosher et al., 2012a). Pb in tissue was highly correlated with the exposure concentration at the end of the experiment. The lowest exposure concentration of 0.9 µg Pb/L resulted in an average tissue concentration of 1.5 µg Pb/g dry weight.

Studies in the 2006 Pb AQCD and 2013 Pb ISA generally showed that the tissue distribution of Pb in aqueous exposures of freshwater invertebrates is primarily sequestered in the gills, hepatopancreas and muscle. Recent short-term (3–4-hour) aqueous uptake studies with juvenile snail *L. stagnalis* showed no significant difference in Pb accumulation among foot, mantle, digestive tract and remaining soft tissues, suggesting uptake occurred directly across the skin (Crémazy et al., 2019). *L. stagnalis* was previously identified as one of the aquatic invertebrates most sensitive to Pb exposure (Grosell and Brix, 2009; Grosell et al., 2006b).

There is some evidence to suggest patterns of tissue distribution differ when uptake of Pb is from sediment. In 28-day exposure to Pb-spiked sediments (205 ± 9 and 419 ± 16 mg/kg dry mass) the freshwater bivalve *Hyridella australis* accumulated Pb in both the low (2.2 ± 0.2 mg/kg dry mass) and high treatments (4.2 ± 0.1 mg/kg dry mass) in the order labial palps>mantle>gill>visceral mass>muscle (Marasinghe Wadige et al., 2014). Labial palps accumulated significantly more Pb than other tissues, consistent with the sediment-burrowing activities of this species. After 28-days, 83%–91% of the accumulated Pb in hepatopancreas of the bivalves was in the biologically detoxified fraction, primarily sequestered in MRG. Concurrently, the relative proportion of Pb sequestered in the metallothionein-like protein fraction (13% to 32%) decreased with Pb exposure. The biologically active metal fraction significantly increased with increased Pb exposure, and the highest percentage of Pb was associated with the mitochondrial fraction.

The 2006 Pb AQCD recognized the potential importance of the dietary uptake pathway as a source of Pb exposure for invertebrates. Additionally, several studies reviewed in the 2013 Pb ISA quantified water versus dietary uptake of Pb in aquatic invertebrates (Komjarova and Blust, 2009; Borgmann et al., 2007; Besser et al., 2005). Since the 2013 Pb ISA, the relative importance of dietary versus aqueous uptake pathways has been further discerned for some biota. Camusso et al. (2012) applied a biologically based Biodynamic Model to previously published data and additional unpublished data on uptake of trace metals in *L. variegatus* from field-collected sediments to assess the main uptake route in this sediment-dwelling organism. The modeled data suggest that for Pb, both free dissolved concentration in porewater and dietary uptake contributed to body burden, and the amount of Pb taken up in the gut appears to be controlled by how tightly Pb is bound to sediment. In *D. magna* fed under two different dietary regimens (regular diet = 3 × 105 *Raphidocellis subcapitata* algal cells/mL; restricted diet = half algae concentration), Pb uptake from water was gradual in individuals with restricted food intake and faster under regular feeding, suggesting that a portion of Pb uptake occurred via diet (Araujo et al., 2019). Hadji et al. (2016) used a series of microcosms in which the amphipod *G. pulex* was exposed to Pb for 6 days in the water column only (0.36, 0.71. 3.62, 6.75 µg Pb/L) or water column (0.31, 0.57, 3.07, 5.02 µg Pb/L) with access to food (poplar leaves *Poplus nigra* pretreated for 1 week in Pb concentrations ranging from 0.5 to 10 µg Pb/L). In the water-column-only microcosms, Pb-treated poplar leaves were present but were enclosed in mesh bags so that the gammarids could not feed. At the end of the study, Pb was significantly higher in amphipods with access to Pb-contaminated leaves than in amphipods exposed to Pb via the water column alone. The dietary contribution ranged from 29% to 31% in the tested concentrations. In an 8-day depuration period, there were no significant differences in elimination regardless of exposure route.

Few studies have assessed the relationships between Pb speciation, water chemistry and biouptake in aquatic invertebrates in situ. In aquatic insect taxa (*Leuctra* spp., Simuliidae, *Rhithrogena* spp, Perlodidae) sampled from 36 headwater streams in the Lake District of England, pH was the prevalent factor influencing Pb uptake, and there were statistically significant correlations between total dissolved Pb in stream water and insect body burdens (De Jonge et al., 2014). For prediction of observed body burdens, Windermere Humic Aqueous Model modeling of stream chemistry and Pb chemical speciation that took into account competition among cations for uptake in biota resulted in a better model fit than "metal accumulation as a function of total dissolved metal levels or the free ion alone"(De Jonge et al., 2014).

11.3.2.4 Uptake and Bioaccumulation in Freshwater Vertebrates

In freshwater vertebrates, Pb uptake in tissues generally increases with increasing concentration of Pb in exposure media (U.S. EPA, 2013); recent studies continue to support these observations. Evidence in the 2013 Pb ISA supported the 2006 AQCD conclusions that the gill is a major site of Pb uptake in fish and that there are species differences in the rate of Pb accumulation and distribution of Pb within the organism. In dietary studies reviewed in the 2013 Pb ISA, the anterior intestine was identified as a target of Pb in fish. New uptake studies continue to show distinct patterns of Pb tissue distribution in water versus dietary exposures. As reviewed in Lee et al. (2019), some studies in fish reported higher rates of Pb accumulation in gill tissues from waterborne exposure compared with dietary exposure. Pb typically accumulates in metabolically active organs including kidney, liver, and intestine in both aqueous and dietary exposure.

In a study designed to investigate the relative influence of waterborne and dietary Pb on accumulation by rainbow trout *(O. mykiss*), juvenile trout were exposed to Pb (8.5, 20, 60 or 110 µg Pb/L), for 7 weeks via waterborne Pb only, dietary Pb only in the form of live prey (worms *L. variegatus* pre-exposed for 28-days to the same concentration of Pb as the fish) or simultaneously to waterborne and dietary Pb (Alsop et al., 2016). Accumulation of Pb in fish was significantly higher via the waterborne exposure pathway compared with dietary exposure in all tissues except in the gut, which accumulated similar amounts of Pb regardless of the exposure route. When fish were exposed to Pb from both water and their diet, whole-body Pb was reduced up to 61% at 110 µg Pb/L, and Pb accumulation was significantly reduced at a threshold of ~50 µg Pb/L, with significantly lower concentrations in the liver and carcass but not the gill or gut. The authors noted that Pb may have altered the nutrient quality of the prey; carbohydrates and lipid levels in the worms were significantly decreased even at the lowest Pb concentration.

11.3.2.5 Uptake and Bioaccumulation Through Food Web

In the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013), transference of Pb through the food web was generally found to be low, with lower Pb accumulation at higher trophic levels; however, some studies found bioaccumulation of Pb at higher trophic levels. Recent evidence supporting little bioaccumulation through freshwater food webs is reviewed here.

In a review published since the 2013 Pb ISA, Cardwell et al. (2013) compiled laboratory and field studies published prior to the 2013 Pb ISA to examine the transfer of Pb and other heavy metals through aquatic food webs. The concentrations of Pb decreased with increasing trophic position in food web studies examining trophic transfer between phytoplankton, cladocera and fish. In most of the field studies reviewed, no evidence was found for biomagnification of Pb across trophic levels in freshwater systems. Specifically, 17 studies examined trophic transfer of heavy metals through aquatic lake or stream food webs; while 10 of these studies found no evidence of Pb biomagnification, one study found possible evidence, and six studies did not examine Pb or did not present data on Pb. More recent studies are presented below.

In a high-elevation lake in the Alps, Pastorino et al. (2020b) examined the accumulation of heavy metals, including Pb, in sediment, chironomids, and fish. Surface sediment, benthic macroinvertebrates, and fish were sampled from a glacial-origin lake, Dimon Lake, in Northeast Italy. While there is only a single fish species in this lake, i.e., the European bullhead (*Cottus gobio*), the benthic macroinvertebrate community consists of midges (Diptera Chironomidae), worms (Oligochaeta), and leeches (Hirudinea). The only prey found in the stomachs of *C. gobio* was Diptera Chironomidae, and therefore only these specimens were used for trace-element analysis. Surface sediment Pb was 109.6 ± 1.2 mg Pb /kg, whole-body Diptera Chironomidae Pb concentration was 49 ± 0.5 mg Pb/kg, and Pb concentration in *C. gobio* was 0.06 ± 0.03 mg Pb/kg in the muscle and 0.03 ± 0.4 mg Pb/kg in liver. The BAF and trophic transfer factor (TTF) in Diptera Chironomidae and *C. gobio* muscle and liver samples were less than 1.0 for Pb, indicating biodilution. The BAF in Diptera Chironomidae was 0.45 and the BAF in fish muscle and liver was 0.0005 and 0.003, respectively. The TTF in *C. gobio* was 0.002 in muscle and 0.007 in liver. In a similar study, Pastorino et al. (2020a) examined BAFs for all the benthic macroinvertebrates from Dimon Lake (Chironomidae, Oligochaeta and Hirudinea) and from another nearby lake, Balma Lake (Chironomidae, Oligochaeta). In this analysis, Dimon Lake surface sediment was 110 ± 1.1 mg Pb/kg (mean ± S.D.), while Balma Lake had considerably less Pb (41 ± 1.2 mg/kb Pb). In addition to lower Pb concentration in the surface sediments, Balma Lake had a lower pH (mean ± S.D.; summer: 6.70 ± 0.34; autumn: 7.64 ± 0.09) than Dimon Lake (summer: 8.77 ± 0.12; autumn: 9.44 ± 0.05). The lower pH was a result of Balma Lake's granite bedrock whereas Dimon Lake covers volcanic rock and sandstone. No correlation was found between the sediment trace-element concentrations and the benthic macroinvertebrates. BAFs were calculated using the mean Pb sediment concentration from each lake across the summer and the fall. In this study, BAFs for Dimon Lake Chironomidae were similar to results found in Pastorino et al. (2020b) for Chironomidae at 0.45. Additionally, Dimon Lake BAFs were 0.42 for Oligochaeta and 0.1 for Hirudinea, suggesting biodilution. In Balma Lake, however, BAFs were above 1.0, suggesting bioaccumulation for the benthic macroinvertebrate community (1.61 for Chironomidae and 1.66 for Oligochaeta).

Some studies use stable-isotope analysis to characterize trophic position in a food web. Using stable isotopes, Pb accumulation was found to decrease with increasing trophic level in Korean wetlands (Kim and Kim, 2016). The Upo wetlands consist of four smaller wetlands (Upo, Mokpo, Sajipo, and Jokjibul), which have different water inflow sources and consequently abiotic condition and biotic communities. Sediment and biota (primary producers: water caltrop [*Trapa japonica*], primary consumers: leaf beetle [*Galerucella nipponensis*] and secondary consumers: water strider [*Gerris* sp.] and wolf spider [*Arctosa* sp.]) were collected and characterized for metal content (Pb, Cd, Cu, and Zn). Afterward, δ13C and δ15N isotopes were used to characterize the food web. Sediment Pb concentrations ranged from approximately 35 to 50 mg Pb/kg and differed significantly among sites. In general, the plant and leaf beetle had lower δ13N and δ15N signatures than water striders and wolf spiders. Concentrations of Pb in the leaves of *T. japonica* were the highest compared with the other organisms analyzed at all sites. Pb concentrations in *G. nipponensis* were significantly lower than those in *T. japonica*. Pb accumulation in the secondary consumer *Arctosa* sp. was lower than Pb accumulation in *Gerris* sp. Overall, Pb concentrations decreased significantly as trophic level increased (plant <leaf beetle≤water strider<wolf spider); Pb has low biomagnification potential, as the biomagnification factors (BMF) were below 1.0 for all sites.

Pb concentrations in aquatic insects could potentially transfer to terrestrial ecosystems as shown in several new studies from the United States. For example, aquatic insects, which are consumed by spiders, might incorporate metals and other contaminants from water systems into terrestrial food webs. In 35 subalpine streams across the Colorado Mining Belt, Colorado, U.S., which was predominately affected by hard-rock mining in the past, Pb concentrations (mg Pb/kg) were positively correlated in water, aquatic vegetation, aquatic insect larvae, aquatic insect adults, and riparian spiders (Kraus et al., 2021). The existence of the positive correlations suggested there was no decoupling of Pb concentration between the aquatic vegetation and insects and terrestrial spiders. Unlike the other metals, Pb may be retained during the insect metamorphosis phase, and the spiders might be an important link in terrestrial transfer from aquatic environments. In another study, risk quotients for Pb were calculated for communities of birds along the Emory River in Tennessee based on Pb concentrations in, as riparian spiders, which can represent a significant portion of the diet, especially for nestlings (Beaubien et al., 2020). Riparian orb-weavers (*Tetragnatha elongata*), which feed primarily on adult aquatic insects, had wet-weight Pb concentrations ranging from 0.03 ± 0.003 mg Pb/kg to 0.045 ± 0.045 mg Pb/kg (mean ± S.D.). Risk quotients for Pb and other heavy metals were calculated for bird species using the contaminant exposure or the reach-specific spider mean metal concentration, divided by the toxic threshold for each study reach. Pb chronic risk quotients calculated for the Emory River study area ranged across species, with the highest risk quotients found for 1 and 12-day Chickadee nestlings (*Poecile* spp.) (range: 0.81–1.52; percentage of diet consisting of spiders: 25.0%), Eastern Bluebird 2-day nestlings (*Sialia sialis*) (range: 0.81–0 1.21; percentage of diet consisting of spiders: 30.9%), and Red-cockaded Woodpecker 9–12-day nestlings (*Picoides borealis*) (range: 0.80–1.20, percentage of diet consisting of spiders: 60%). All Pb acute risk quotients reported were 0.00. Chronic spider-based avian wildlife values for adult and nestling birds ranged from 0.03 mg Pb/kg for 1-day nestlings for *Poecile* spp. to 1.347 mg Pb/kg for Setophaga discolor (prairie warbler) 12-day nestlings.

In another example of aquatic insect transfer of Pb to the surrounding environment, Fletcher et al. (2022) found that 80%–95% of Pb in dragonfly species was shed with emergence. Ten dragonfly species were collected from a constructed wetland at the Savannah River Site, a National Environmental Research Park in South Carolina, United States, where materials for nuclear weapons are produced. Although sediment and freshwater concentrations were not reported in this study, average Pb concentrations in the shed exuviae of 10 dragonfly species (*Brachymesia gravida, Libellula auripennis, Libellula luctuosa, Orthemis ferruginea, Plathemis lydia, Pachydiplax longipennis, Perithemis tenera, Pantala flavescens, Pantala hymenaea,* and *Tramea lacerata*) ranged from 2.94 to 10.7 mg Pb/kg, which was significantly higher than Pb concentrations in the tenerals, or the freshly molted adult insect (< 0.4 mg Pb/kg), suggesting that Pb in the exuviae was 17–96 times higher than the concentrations in the teneral.

New observational studies and literature reviews since the 2013 Pb ISA (U.S. EPA, 2013) generally confirm that many freshwater food webs exhibit reduced accumulation of Pb in higher trophic levels (Pastorino et al., 2020b; Kim and Kim, 2016; Cardwell et al., 2013), although one study reported the bioaccumulation of Pb (Pastorino et al., 2020a). Additional studies demonstrated that Pb can transfer between aquatic food webs and terrestrial ecosystems via aquatic insect emergence and predation by and of riparian spiders (Fletcher et al., 2022; Kraus et al., 2021; Beaubien et al., 2020).

11.3.3 Environmental Concentrations of Pb in Freshwater Biota and Ecosystems in the United States at Different Locations and Over Time

Few U.S. studies have examined national or regional-scale trends of Pb in freshwater biota. The 1986 AQCD reported the results of Lowe et al. (1985), a nation-wide survey of metal concentrations in fish from 1979 to 1981. At 112 monitoring stations, they found an average (geometric mean) of 0.19 µg Pb/g wet weight for the period 1978 to 1979 and 0.17 µg Pb/g wet weight for 1980 to 1981 (U.S. EPA, 1986). In the 2006 AQCD, a representative median and a range of Pb concentrations were reported in surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L), sediments (median 28 mg Pb/kg dry weight, range 0.5 to 12,000 mg Pb/kg dry weight) and fish tissues (geometric mean 0.54 mg Pb/kg dry weight, range 0.08 to 23 mg Pb/kg dry weight [whole body]) in the United States based on a synthesis of

National Ambient Water Quality Assessment data (U.S. EPA, 2006). The 2013 Pb ISA reported survey results from the Western Area Contaminants Assessment Project (2002–2007), which included the concentration of Pb in fish tissue (0.0033 [fillet] to 0.97 [liver] mg Pb/kg [dry weight]) from a set of national parks in the western United States (Blett, 2010; U.S. EPA, 2008b). No recent studies examining spatial or temporal trends in Pb concentration in freshwater fish or invertebrates from locations across the United States were identified in this ISA. Many individual studies report Pb concentrations in aquatic ecosystems and biota from specific sites across the United States; compilation of those data is outside the scope of this ISA. Pb concentrations in water, sediment and other environmental media are available in Section 11.1.3 and summarized in Table 11-1.

Since the 2013 Pb ISA, a few regional-scale studies, including a study in Canada, have assessed trends in Pb concentrations in vegetation (peat bogs) or the water column. Peat bogs deposit and preserve stable layers of accumulated moss and other plant material that can be used to reconstruct a record of spatial and temporal distribution patterns of air Pb concentrations. Six peat cores collected in 2013 and 2014 in northern Alberta, Canada Shotyk et al. (2016) record the rates of atmospheric Pb deposition dated from 1910 to 2014 using ^{210}Pb and ^{14}C dating in models, linking sample depth to age. Peak accumulation rates were observed between the years 1950 and 2000 in each sample, and overall decreasing rates of Pb accumulation were observed from 1980. Although this study was not in the United States, decreased Pb accumulation rates coincided with the introduction of unleaded gasoline in the United States and Canada in the mid-1970s and nearby potential point sources of Pb air pollution (industrial development including bitumen mines and upgraders) are not attributed to the increase in Pb accumulation. The uppermost, most recent, peat layers show near-zero modern atmospheric Pb deposition in the Alberta peat bogs.

In a 2015–2017 water quality survey of four Tennessee headwater Appalachian streams Olson et al. (2019), the maximum observed Pb concentration and sole detectable measurement of this metal was less than 1 µg Pb/L. Reported mean concentration values at each site were less than the minimum detection limit of 0.28 µg Pb/L. These observations from remote streams without upstream anthropogenic Pb sources suggest that long-range atmospheric deposition is not a major source of Pb contamination to this region. Limited evidence from regional studies of temporal trends in freshwater aquatic ecosystems published since the 2013 Pb ISA suggest that modern atmospheric deposition of Pb is not a major contributor to Pb concentrations in freshwater aquatic biota and ecosystems in remote locations.

11.3.4 Effects of Pb in Freshwater Systems

This section focuses on studies of the biological effects of Pb on freshwater plants and algae, microbes, invertebrates, and vertebrates published since the 2013 Pb ISA. The biological effects of Pb in the 2013 Pb ISA and in this appendix are generally presented in increasing complex levels of biological organization from suborganismal responses (i.e., enzyme activities, changes in blood parameters) to endpoints relevant to the population level and higher (growth, reproduction, and survival) up to effects on ecological communities and ecosystems. Exposure-response studies that report toxicological dose descriptors (e.g., LC_{50}, EC_{50}, lowest observed adverse effect level [LOAEL]) for effects on growth, reproduction or survival endpoints are reported in Section 11.3.5.

11.3.4.1 Effects on Freshwater Microbes

The effects of Pb on microbial communities in freshwater ecosystems were not reviewed in detail in the 2013 Pb ISA (U.S. EPA, 2013), except for a report that Pb could alter bacterial infection in the fish *Channa punctatus* (Pathak and Gopal, 2009). In the 2006 Pb AQCD (U.S. EPA, 2006), it was reported that Pb could adsorb to biofilms, depending on pH, water hardness, polarity of matter, and amount of Fe or Mn in the water and that methylation by microbes may result in Pb remobilization in aquatic ecosystems; however, few studies directly report effects on microbes from Pb exposure. Since the 2013 Pb ISA (U.S. EPA, 2013), several experimental and observational studies have examined the relationship between Pb concentration in the sediment and effects on freshwater microbes, as summarized below. Several of these studies report negative relationships between sediment Pb concentration and microbial abundance or community structure, while some report no relationship or positive associations.

In a study from the United States, porewater and sediment Pb concentrations were negatively correlated with bacterial RNA abundance, but not diversity or richness in Lake DePue, Illinois (Gough and Stahl, 2011). Lake DePue is a shallow lake on the Illinois River located near a U.S. EPA Superfund Site (the DePue/New Jersey Zinc/Mobil Chemical Site). Although the Zn smelting facility and phosphate fertilizer plant are no longer operational, Lake DePue has received metal-contaminated sediments from this site for over 80 years. Sediment Pb concentration in the lake was on average 180 mg Pb/kg (range: 68.6 and 541 mg Pb/kg). Porewater and sediment Pb were correlated with a low abundance of archaeal, bacterial, and eukaryotic terminal restriction fragments (TRF). Overall, there were some differences in overall community structure with regard to metal contamination observed using terminal restriction fragment length polymorphism analysis of 16S rRNA genes, although variation in bacterial diversity, richness and composition across a metal gradient was not detected. In a follow-up study using the same samples, Kang et al. (2013) further explored the bacterial communities using a different approach, a function gene microarray (GeoChip). Overall, the diversity of functional gene variants was similar across all five sites, suggesting that heavy-metal concentrations in the sediments did not significantly affect bacterial community structure; however, some individual gene categories were correlated with certain porewater metal concentration, including Pb. Using a CCA, Pb, Zn, and Cd were all found as important predictors for sulfate-reducing bacteria communities. Although significant correlations with Pb existed, functional gene variants had similar relationships with other porewater metal concentrations, including As, Cd, Cr, and Zn.

In another U.S. study, observational evidence suggests that the exchangeable Pb fraction decreases microbial community diversity, while oxyhydroxide Pb concentration was correlated with an increase in diversity in the mining district of Lake Coeur d'Alene, Idaho (Moberly et al., 2016). Pb concentrations in the sediment were high in the lake, ranging from 1,540 to 3,422 mg Pb/kg, while the St. Joe River delta reference site sediment Pb concentration was 29 mg Pb/kg. More than 70% of the Pb was associated with the exchangeable/carbonate phase, which is thought to the be most bioavailable phase. Pb in the exchangeable/carbonate fraction was negatively correlated with the abundance of Aquificae and Synergistes and positively correlated with candidate phylum LD1PA abundance (a phylum without many cultured representatives); furthermore, this pattern is similar for Fe and Mn oxyhydroxides, as Pb exchangeable/carbonate concentrations are highly correlated. Bacteroidetes OTU abundance was negatively correlated with Pb-exchangeable/carbonate and positively correlated with Pb-(oxy)hydroxide. These results suggest that the phase of Pb is integral in determining the relationship between Pb concentration in the sediment and microbial communities, as seasonal changes in Pb speciation could affect microbial diversity.

To understand how heterotrophic bacteria in river sediments are affected by Pb, sediments were collected from sites along three tributaries of the Nagara River in Japan, varying in land use types (agricultural, industrial, or forested) and contamination (Du et al., 2018). Overall, Pb (100, 1.000, and 10.000 μg/L) did not have significant effects on heterotrophic bacteria density, activity, and community structure after 30 days of a sequencing batch incubation experiment.

In contrast, the Pb enrichment factor, along with other heavy metals, was found to influence bacterial community structure in the Poyang Lake river system, China (Zhang et al., 2018). Mean Pb concentration in the sediments from five rivers ranged from 29.52 to 40.06 mg Pb/kg. The Pb enrichment factor, which takes into account Fe as the normalizer element, along with the As and Cd enrichment factors, pH, OC, and degree of contamination were the main variables affecting bacterial community structure (redundancy analysis). The Pb enrichment factor, as well as Cd enrichment factor and the degree of contamination, was strongly associated with higher abundances of Acidobacteria, suggesting tolerance of the phyla.

In another study in freshwater systems in China, Pb concentration in the sediment was found to negatively correlate with the relative abundance of major bacterial groups, but not with bacterial diversity (Li et al., 2020). Pb concentration in the sediment was 17.3 ± 7.3 mg Pb/kg (mean ± S.D.) and ranged from 1.9 to 25.4 mg Pb/kg across 12 sites in Huangjinxia Reservoir in Shaanxi Province, China. Sediment Pb concentration was highly correlated with Cr, Zn, and Ni but not significantly correlated with microbial diversity indices (ACE, Chao1, Shannon, and Simpson's index). However, Pb sediment concentration was significantly negatively correlated with the relative abundance of Bacteroidota, Nitrospirota, and Verrucomicrobia and positively correlated with the relative abundance of Chloroflexi..

Variation in bacterial community composition along an elevation gradient in Yangtze River, China, was driven by OM, elevation, urbanization, and Pb concentration (Zhang et al., 2020). Pb concentration in the sediment ranged from 14.40 ± 0.80 mg Pb/kg to 87.01 ± 8.00 mg Pb/kg. Elevation (meters above sea level) was negatively correlated with Pb concentration in the sediment as were many other physicochemical parameters and metal concentrations. OM was the most significant variable, followed by elevation (10.4%), urbanization rate (9.0%) and Pb (9.5%). Bacterial community structure between 50 and 400 masl was most correlated with Pb, and below 50 masl community structure was most correlated with urbanization rate. Above 400 masl, Pb concentration and OTU abundance were significantly correlated, while the correlations between Pb and the Shannon index and evenness were not significant. Below 400 masl, the opposite pattern emerged: the relationship between Pb and OTU abundance was negative and the relationships between Pb and the Shannon index and evenness were nonsignificant. Finally, Pb concentration was positively correlated with the abundance of certain bacterial genera, negatively correlated with others, and not correlated with most dominant bacteria taxa.

In summary, since the 2013 Pb ISA (U.S. EPA, 2013), several observational and experimental studies examining the effects of Pb concentrations in freshwater sediment and porewater found negative associations with bacterial or archaeal abundance, but not diversity (Li et al., 2020; Kang et al., 2013; Gough and Stahl, 2011), while others found mixed associations between Pb and microbial diversity (Moberly et al., 2016) or no relationship (Du et al., 2018).

11.3.4.2 Effects on Freshwater Plants and Algae

The toxicity of Pb to freshwater algae and plants has been recognized in earlier U.S. EPA reviews of the metal and the findings are briefly summarized here. In the 1977 Pb AQCD, differences in sensitivity to Pb among different species of algae were observed, and concentrations of Pb within the algae varied among genera and within a genus (U.S. EPA, 1977). The 1986 Pb AQCD (U.S. EPA, 1986) reported that some algal species (e.g., Scenedesmus sp.) were found to exhibit physiological changes when exposed to high Pb concentrations in situ. Effects of Pb on algae reported in the 2006 Pb AQCD included decreased growth, deformation, and disintegration of algae cells, and blocking of the pathways that lead to pigment synthesis, thus affecting photosynthesis. Most studies on effects of Pb in freshwater algal species reviewed in the 2013 Pb ISA and the AQCDs were conducted with nominal media exposures and effect concentrations greatly exceeded Pb reported in surface water. In studies in which Pb was quantified, effect concentrations for growth (EC_{50}) for freshwater algae and macrophytes were much higher than currently reported environmental Pb. Growth endpoints in freshwater algae reviewed in the 2013 Pb ISA included significant inhibition of chlorophyll a content at 210 µg Pb/L and higher in *Wolffia arrhiza* (Piotrowska et al., 2010). An increase in biomass was reported in *L. minor* exposed to 100 or 200 µg Pb/L, with inhibition observed at higher concentrations (Dirilgen, 2011). There were also numerous studies conducted at nominal Pb concentration that reported effects on enzyme activities and protein content in freshwater aquatic plant species. Exposure-response relationships in which increasing concentrations of Pb lead to increasing effects were consistently observed for freshwater aquatic plants. In the 2013 Pb ISA, the body of evidence was sufficient to conclude there were likely to be causal relationships between Pb exposure and freshwater plant physiological stress and between Pb exposure and reduced freshwater plant growth. The body of evidence was inadequate to conclude there are causal relationships between Pb exposure and freshwater plant reproduction and between Pb exposure and freshwater plant survival.

New information on freshwater algae since the 2013 Pb ISA addresses the deficit of analytically verified chronic toxicity data for these organisms. De Schamphelaere et al. (2014) conducted 72-hour bioassays in standard test media to assess growth rate in three commonly tested algal species; *P. subcapitata, C. kesslerii*, and *C. reinhardtii*. *P. subcapitata* was the most sensitive, with EC_{50} = 83.9 µg Pb/L, EC_{20} = 45.7 µg Pb/ and EC_{10} = 32.0 µg Pb/L based on filtered Pb concentration. Furthermore, in subsequent tests with *P. subcapitata* at varying pH, 72-hour EC_{50} decreased from 72.0 µg filtered Pb/L at pH 6.0 to 20.5 µg filtered Pb/L at pH 7.6. The authors noted that this species exhibited greater sensitivity to Pb than two of the most chronically Pb-sensitive aquatic invertebrates (the crustacean *C. dubia* and the snail *L. stagnalis*) at pH > 7.4 based on model-predicted chronic EC_{50} values.

Additionally, new information on Pb effects on the emergent freshwater macrophyte, the common reed (*Phragmites australis*), shows an alteration in growth form and propagation strategy under Pb exposure. In a phytotron experiment, reed plants were exposed to five Pb levels in sediment (measured 5.9 ± 0.2, 304 ± 4.38, 508 ± 7.89, 1513 ± 37.28, 3020 ± 120.41 mg Pb/kg) (Zhang et al., 2015a). In addition to decreases in total biomass, photosynthesis and rhizome growth, the addition of Pb caused a significant alteration in growth form. The numbers of axillary shoot buds and daughter apical rhizome shoots were increased by Pb addition at the highest concentrations, and the bulk (80%) of daughter shoots were from daughter axillary shoots. This clonal propagation strategy of increased formation and output of axillary shoot buds, called the phalanx pattern, is an adaptive response to maintain population stability at the lowest energetic cost. This same growth pattern alteration was also found in an additional study on the effects of Pb and drought in *P. australis* by the authors (Zhang et al., 2015b), but clonal modular growth and reproductive ability were significantly inhibited by the interaction between drought and Pb. These propagation effects would cause a decline in *P. australis* populations in a dry environment under Pb pollution.

In summary, information published since the 2013 Pb ISA does not substantially change what was previously known about Pb effects on freshwater plants and algae. A few new studies assessed the sensitivity of freshwater algal growth to Pb exposure and found a significantly negative effect in certain species. New information on Pb effects on common reed (*P. australis*) shows significant decreases in total biomass, photosynthesis, and rhizome growth as well as alterations in growth form and propagation strategy under Pb exposure. The growth and reproductive ability of common reed have also been shown to be significantly inhibited by an interaction between Pb exposure and drought, which may have implications for future drought events. There is still little information on the relationships between Pb exposure and freshwater plant or algal survival, particularly at exposure levels below the thresholds used in this ISA.

11.3.4.3 Effects on Freshwater Invertebrates

Freshwater aquatic invertebrates are generally more sensitive to Pb exposure than other taxa. Controlled studies at concentrations near the upper range of representative concentrations of Pb available from surveys of U.S. surface waters (median: 0.50 µg Pb/L; range 0.04 to 30 µg Pb/L, 95th percentile 1.1 µg Pb/L) (U.S. EPA, 2006) (Table 11-1) reviewed in the 1986 AQCD, the 2006 Pb AQCD and the 2013 Pb ISA provide evidence for the effects of Pb on reproduction, growth and survival in sensitive freshwater invertebrates. Freshwater invertebrate taxa that exhibit sensitivity to Pb include some species of gastropods, amphipods, cladocerans and rotifers, although the toxicity of Pb is highly dependent upon water quality variables such as DOC, hardness, and pH. Key studies reported in the 1986 AQCD include increased mortality as low as 19 µg Pb/L for the snail *Lymnaea palustris* (Borgmann et al., 1978) and reproductive impairment at 30 µg Pb/L (nominal values) for Daphnia sp. (Biesinger and Christensen, 1972). In a 42-day chronic study reviewed in the 2006 Pb AQCD, the LOEC for reproduction was 3.5 µg Pb/L in the amphipod *H. azteca* receiving both waterborne and dietary Pb (Besser et al., 2005).

In the 2013 Pb ISA, additional studies provided evidence for Pb effects on freshwater invertebrates at low µg Pb/L concentration. The growth of juvenile freshwater snails (*L. stagnalis*) was inhibited at an EC_{20} of <4 µg Pb/L (Grosell and Brix, 2009; Grosell et al., 2006b). In fatmucket mussel, *L. siliquoidea* juveniles, a chronic value (geometric mean of no-observed-effect concentration [NOEC] and LOEC) of 10 µg Pb/L was obtained following 28-day exposures (Wang et al., 2010). In a 7-day exposure of the cladoceran *C. dubia* to 50 to 500 µg Pb/L, increased DOC led to an increase in mean EC_{50} for reproduction ranging from approximately 25 µg Pb/L to >500 µg Pb/L (Mager et al., 2011a). The 48-hour LC_{50} values for the cladoceran *C. dubia* tested in eight natural waters across the United States varied from 29 to 1,180 µg Pb/L and were correlated with DOC (Esbaugh et al., 2011). The freshwater rotifer *E. dilatata* 48-hour LC_{50} was 35 µg Pb/L using neonates hatched from asexual eggs (Arias-Almeida and Rico-Martínez, 2011). The EC_{20} for reduced growth and emergence of the midge *C. dilutus* was reported to be 28 µg Pb/L, observed in a 55-day exposure study, while the same species had a 96-hour LC_{50} of 3,323 µg Pb/L (Mebane et al., 2008) The EC_{10} for molting in the mayfly *B. tricaudatus* was 37 µg Pb/L (Mebane et al., 2008). These studies provided evidence in the 2013 Pb ISA supporting determinations of causal relationships between Pb exposure and growth, reproductive effects, and survival in freshwater invertebrates (Table 11-4).

11.3.4.3.1 Suborganism-Level Response

The key studies described above from the 2013 Pb ISA and earlier AQCDs report effects on reproduction, growth, and survival in freshwater invertebrates. Additional endpoints for Pb toxicity in aquatic invertebrates considered in the 2013 Pb ISA and previous AQCDs included suborganism-level effects such as enzyme function and oxidative stress. These suborganism-level effects were considered together in the 2013 Pb ISA as “physiological stress” and the body of evidence was sufficient to conclude that there is a likely to be causal relationship between Pb exposure and altered response. Although stress responses are correlated with Pb exposure, they are nonspecific and may be altered with exposure to any number of environmental stressors. An additional suborganism-level endpoint in the 2013 Pb ISA was "hematological effects," which included changes to ALAD expression or the hematopoietic system associated with Pb exposure. For this endpoint, the body of evidence was sufficient to conclude that there is a likely to be causal relationship between Pb exposure and hematological effects in freshwater invertebrates in the 2013 Pb ISA. These suborganism-level responses may serve as biomarkers for effects at the organism level and higher; however, only a subset of studies that quantified response at the suborganismal level concurrently assessed effects on growth, reproduction, development, or survival. Only a few of the many studies identified in the literature search on suborganism-level response to Pb exposure in freshwater invertebrates were conducted in the low µg Pb/L range and hence met the criteria for inclusion in the ISA.

Recent literature supports the previous evidence for Pb effects on enzymes and antioxidant activity in freshwater invertebrates. New studies on physiological stress endpoints include changes in the activities of antioxidant defense enzymes with aqueous exposure to Pb. SOD and GPx activities were significantly reduced, and MDA levels were significantly increased in juvenile Oriental river prawn (*Macrobrachium nipponense*) exposed to 25 µg Pb/L for 60 days. CAT activity in the hepatopancreas increased at 12 µg Pb/L and decreased in the 25 µg Pb/L treatment (Ding et al., 2019). In the same study, reductions in weight gain and specific growth rate were observed in prawns exposed to 25 µg Pb/L in chronic 60-day exposure tests. No growth effects were observed in prawns at 12 µg Pb/L (see Section 11.3.5).

Physiological stress in freshwater invertebrates was also assessed during sediment exposure to Pb. Exposure of larval midge *Chironomus riparius* to Pb-spiked sediment (132 mg Pb/kg dry weight and 505.5 mg Pb/kg dry weight) for 16 days resulted in an antioxidant response (increase in metallothionein) and cellular damage (increase in MDA) (Arambourou et al., 2013). There was no significant change to protein concentration, lipid was depleted while glycogen increased with increasing Pb in the sediment. In the same organisms, Pb exposure via sediment did not result in statistically significant effects on growth, survival, or number of mentum (mouthpart) deformities. In a separate study in *C. riparius* in Pb-spiked sediment ranging from 18.1 to 456.9 mg Pb/kg dry weight, no significant differences were observed in the frequency of mouthpart deformities (Arambourou et al., 2012). In freshwater snail *Bellamya aeruginosa* exposed for 28 days to Pb-spiked sediment, CAT activity and metallothionein were significantly induced at the lowest concentration tested (29.7 mg Pb/kg dry weight) (Liu et al., 2019b). In the bivalve *Hyridella australis* also exposed 28-days to Pb-spiked sediments (205 ± 9 and 419 ± 16 mg Pb/kg dry mass), the body burden of accumulated Pb was low (2.2 ± 0.2 mg Pb/kg dry mass and 4.2 ± 0.1 mg Pb/kg dry mass, respectively); however, total antioxidant capacity significantly decreased, while ROS and MDA increased with Pb exposure compared with controls (Marasinghe Wadige et al., 2014).

As reported in the 2013 Pb ISA, inhibition of ALAD enzyme activity, an important rate-limiting enzyme needed for heme production, is a recognized biomarker of Pb exposure in some freshwater invertebrate species that have hemoglobin. Previous studies have indicated considerable species differences in ALAD activity in response to Pb. For example, the concentration at which 50% ALAD inhibition was measured in the freshwater gastropod *Biomphalaria glabrata* (23 to 29 µg Pb/L) was much lower than that in the freshwater oligochaete *L. variegatus* (703 µg Pb/L), based on nominal exposure data (Aisemberg et al., 2005). No recent studies quantifying ALAD activity in freshwater invertebrates at environmentally relevant concentrations of Pb were identified for inclusion in this ISA. Furthermore, no significant ALAD activity was detected at baseline metabolic conditions in hemolymph or tissue of the freshwater unionoid mussel *E. complanata*, suggesting this is not a viable biomarker for the species (Mosher et al., 2012a).

11.3.4.3.2 Organism-Level Response

Organism-level endpoints include effects on behavior linked to Pb neurotoxicity. In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a likely to be causal relationship between Pb exposure and neurobehavioral effects in freshwater invertebrates (U.S. EPA, 2013) (see Table 11-4 of this appendix). In limited studies available on worms and snails, there is evidence that Pb may affect the ability to escape or avoid predation. For example, in the tubificid worm *T. tubifex,* the 96-hour EC_{50} for immobilization was 42 µg Pb/L based on nominal exposure (Khangarot, 1991). Some organisms exhibit behavioral avoidance while others do not seem to detect the presence of Pb (U.S. EPA, 2006). Additional behavioral endpoints reported in the Great Lakes Environmental Center draft *Ambient Aquatic Life Water Quality Criteria for Lead* document U.S. EPA (2008a) include an EC_{50} of 140 µg Pb/L for feeding inhibition in the freshwater cladoceran *C. dubia*. In a study published since the 2013 Pb ISA, adult amphipods, *G. fossarum* exposed to Pb for 5 days at a concentration at which survival was unaffected (2.7 µg Pb/L) exhibited sublethal behavioral and physiological responses. Locomotion was significantly decreased over time (assessed 24, 48 and 120 hours) and respiration rate was significantly lower at 120 hours compared with unexposed amphipods (Lebrun et al., 2017). In a separate study with *G. fossarum,* both locomotion and respiration were significantly decreased following exposure to 2.1 µg Pb/L for 24-hour (Lebrun and Gismondi, 2020).

Alterations in neurotransmitter regulation and release may be an underlying mechanism for the behavioral effects of Pb. Few studies in freshwater invertebrates have reported effects on neurotransmitters at lower Pb concentrations. In prereproductive freshwater bivalve *Lamellidens jenkinsianus obesa* exposed for 21 days to either 68 or 763 µg Pb/L, AChE activity (assessed on days 1, 7, 15 and 21 of the experiment) was significantly inhibited at each timepoint compared with control (Brahma and Gupta, 2020). Several locomotor behaviors (movement in the form of gliding, foot-siphon extension) were significantly reduced or ceased completely in the Pb-exposed individuals compared with the control during a separate 5-day exposure to either 69 or 776 µg Pb/L. In the same study, reproductive-age individuals of another bivalve species, *P. corrugata,* were exposed to either 26 or 302 µg Pb/L for 21 days. AChE activity was significantly induced at 26 µg Pb/L and significantly inhibited compared with control at 302 µg Pb/L at all timepoints. Behavioral response in the form of impaired movement with Pb exposure (25 and 304 µg Pb/L) was also observed in this species. In 28-day chronic exposure of freshwater snail *B. aeruginosa* to Pb-spiked sediment, the activity of the neurotransmitter AChE was significantly induced starting at day 7 in the lowest concentration (29.7 mg Pb/kg dry weight) (Liu et al., 2019b).

In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship between Pb exposure and growth in freshwater invertebrates (U.S. EPA, 2013) (see Table 11-4 of this appendix). The growth of freshwater snail *L. stagnalis* was identified as one of the most sensitive organisms and endpoints for Pb toxicity. At the time of the 2013 Pb ISA, the hypersensitivity of this species to Pb was hypothesized to be from Pb inhibition of Ca^{2+} uptake. Subsequent experiments by Brix et al. (2012) observed that effects on growth occur prior to effects on net Ca^{2+} flux, inhibition of carbonic anhydrase activity in the snail mantle also showed no effect with Pb; therefore, the mechanism of Pb in these highly sensitive organisms remains elusive. Additional studies reported in Section 11.3.5, Exposure and Response of Freshwater Species, support Pb effects on the growth of *L. stagnalis* in the low µg Pb/L range (Crémazy et al., 2018; Brix et al., 2012).

Exposure-response studies discussed in Section 11.3.5 also add to the existing body of evidence in the 2013 Pb ISA for a causal relationship between Pb exposure and reproductive effects as well as survival in freshwater invertebrates. In summary, studies in freshwater invertebrates for suborganism-level and organism-level endpoints are confirmatory with findings in the 2013 Pb ISA, with evidence in additional species for some effects.

11.3.4.4 Effects on Freshwater Vertebrates

The 1977 Pb AQCD reported Pb effects in both fish and waterfowl. The available Pb studies on waterfowl investigated exposure to Pb via accidental poisoning or ingestion of Pb shot (U.S. EPA, 1977). Studies on aquatic vertebrates reviewed in the 1986 Pb AQCD were limited to hematological, neurological, and developmental responses in fish (U.S. EPA, 1986). In the 2006 Pb AQCD, effects on freshwater vertebrates included consideration of the role of water quality parameters on toxicity to fish, as well as limited information on the sensitivity of turtles and aquatic stages of frogs to Pb (U.S. EPA, 2006). In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship between Pb exposure and hematological effects, reproduction and survival in freshwater vertebrates (based primarily on evidence from fish) (U.S. EPA, 2013) (see Table 11-4 of this appendix). There were also likely to be causal relationships concluded between Pb exposure and physiological stress and neurobehavioral effects. Newly available studies on the effects of Pb in fish and other freshwater vertebrates are summarized below.

11.3.4.4.1 Fish

11.3.4.4.2 Suborganism-Level Response

A large body of evidence supports sublethal biomarker perturbations with Pb exposure in freshwater vertebrates; however, few studies were identified for this ISA that reported physiological response at more environmentally relevant concentrations of Pb (≤ 10 µg Pb/L; Section 11.1.1) or concurrently assessed response at organism-level endpoints (i.e., from the cellular and subcellular level to effects on growth, reproduction or survival). Various biomarkers of oxidative stress assessed in carp (*Carassius auratus gibelio*) after 96 hours and 21 days were significantly altered at analytically verified concentration of 10 and 30 µg Pb/L (Khan et al., 2015). For the acute exposure, CAT activities (liver and kidney) were significantly reduced, and SOD was significantly upregulated in brain, kidney, and muscle tissue. GP_X activity in the liver and gill increased significantly, while activity in the muscle and kidney was significantly reduced. Biomarker response in chronic exposure showed significant reduction in CAT (liver, gill, muscle) at 10 and 30 µg Pb/L, whereas CAT was upregulated in the kidney. There was a decline in GP_X (liver and gill) as well as in SOD (liver, kidney, muscle), while the brain showed an increase. Acetylcholine, a biomarker of neurotoxic stress, was significantly inhibited following chronic exposure to 30 µg Pb/L. Clemow and Wilkie (2015) observed no significant effect on respiratory stress, mean cell hemoglobin concentration, plasma Ca^{2+} or Na^{2+} ion concentration or plasma protein in juvenile rainbow trout (*O. mykiss*) over a 5-day exposure to 5.4 µg Pb/L (26.1 nmol/L). In fingerling rainbow trout, used in the same study for unidirectional Na^+ flux measurement, there was an initial Na^+ loss after 48 hours of exposure that recovered by 72 hours with exposure to 8.3 µg Pb/L (40.2 nmol/L).

Hematological effects of Pb on fish reported in the 2013 Pb ISA and AQCDs include a decrease in red blood cells and inhibition of ALAD with elevated Pb exposure under various test conditions. Inhibition of ALAD is also reported in environmental assessments of metal-impacted habitats. For example, as reported in the 2013 Pb ISA, lower ALAD activity has been significantly correlated with elevated blood Pb concentrations in wild-caught fish from Pb-Zn mining areas, although there are differences in species sensitivity (Schmitt et al., 2007; Schmitt et al., 2005). Few studies were identified since the 2013 Pb ISA that quantify ALAD response in freshwater fish at concentrations considered for this ISA (Section 11.1.1). In a field study of brown trout (*Salmo trutta*) collected from a lake in Norway contaminated with Pb (14 µg Pb/L) from an abandoned shooting range, ALAD activity in the trout population was approximately 20% of that of a relatively uncontaminated reference lake (0.76 µg Pb/L) (Mariussen et al., 2017).

11.3.4.4.3 Organism-Level Response

In the 2013 Pb ISA, studies supporting a likely to be causal relationship between neurobehavioral endpoints in freshwater vertebrates and Pb exposure included research from early U.S. EPA reviews of the metal. In the 1977 Pb AQCD, behavioral impairment of a conditioned response (avoidance of a mild electric shock) in goldfish was observed at concentrations as low as 70 μg Pb/L (Weir and Hine, 1970). In the 2006 Pb AQCD, several studies were reviewed in which Pb was shown to affect predator-prey interactions, including alteration in prey size choice and delayed prey selection in juvenile fathead minnows following 2-week pre-exposure to 500 μg Pb/L (Weber, 1996). Prey capture ability was decreased in 10-day old fathead minnow larvae born from adult fish exposed to 120 μg Pb/L for 300 days, then subsequently tested in a 21-day breeding assay (Mager et al., 2010).

Since the 2013 Pb ISA, there have been additional studies on neurobehavioral response in freshwater vertebrates, particularly in zebrafish *D. rerio.* As a widely used model organism in environmental toxicology, the zebrafish genome shares a high degree of homology with the human genome (Dai et al., 2014; Howe et al., 2013). Endpoints assessed in some zebrafish assays, such as decreased locomotor activity and altered social interactions used as surrogates for autistic behaviors in humans, can affect organism fitness in natural environments. Furthermore, some of these studies link changes in gene expression, neurotransmitter levels or other molecular and cellular responses to the observed behavioral outcomes. Experiments conducted in the low μg/L range are particularly representative of environmental concentrations (Table 11-1); therefore, zebrafish behavioral assay studies conducted at low concentrations of Pb are reviewed below.

In zebrafish embryos exposed to 5.0, 9.7 or 19.2 μg Pb/L there were no significant effects on dorsal axon length up to 144 hours postfertilization (hpf); however, there was a significant reduction in swimming speed at the highest Pb concentration tested (Zhu et al., 2016). Alterations in the neurotransmitter gamma-aminobutyric acid (GABA) were observed during development of zebrafish embryos exposed to Pb (nominally to 10, 50 and 100 μg Pb/L up to 72 hpf and then Pb uptake was subsequently quantified in embryos) (Wirbisky et al., 2014). The levels of this neurotransmitter varied with the dose of Pb and developmental stage, with all three treatments resulting in a significant decrease in GABA by the end of embryogenesis (72 hpf). Newly hatched larval zebrafish exposed to Pb since 2.5 hpf exhibited neuromuscular responses (increased muscular twitching) at concentrations of 49.6 and 100.7 μg Pb/L at 72 hpf. No twitches were observed at lower concentrations or in the control group (Kataba et al., 2022). In another study, locomotor and social behavior responses were assessed in zebrafish larvae exposed to 4.5, 9.6 or 18.6 μg Pb/L at 6 days postfertilization (dpf) during a dark and light photoperiod (Zhao et al., 2020). During the dark period, swimming activity was significantly decreased at 18.6 μg/L, and at both 9.6 and 18.6 μg/L, there was a decrease in clockwise turning; social contact time was significantly higher in the light period at the highest Pb concentration. Downregulation of genes involved in brain neutrophic factor signaling was observed in the Pb-exposed larvae, suggesting an underlying mechanism for the observed responses. Hyperactivity (increased distance covered and speed) during the light period was observed in larval zebrafish exposed to Pb (3.2, 93 or 252.6 μg Pb/L) for 30 minutes in alternating light and dark intervals of 10 minutes (Kataba et al., 2020).

Studies in zebrafish have also considered the neurobehavioral effects of Pb at multiple lifestages. Wang et al. (2018b) assessed swimming behavior in larval (15 dpf)) and juvenile (30 dpf) zebrafish that were continually exposed to Pb (analytically verified concentration of 10 µg Pb/L or 100 µg Pb/L) from maternal exposure through egg fertilization and subsequent larval development. Larval responses to Pb exposure included decreases in measures of locomotion such as angular velocity, turn angle and inter-fish distance, a measure of social behavior. Juvenile zebrafish exhibited similar behavioral responses to Pb; however, the inter-fish distance increased, and there were increases in the percentage of fish moving up to the top of the tank. The expression of key genes linked to behaviors, Ca channels and the metabolism of environmental contaminants were altered with Pb exposure.

There is some evidence for parental transfer and transgenerational effects on fish learning and avoidance behavior following Pb exposure. Zebrafish larvae (15 dpf) hatched from adult females previously exposed to 19.5 µg Pb/L were used as a model to test autism-like behaviors (Wang et al., 2016a). Behaviors assessed included measures of locomotion, and repetitive, social and anxiety behaviors. Analysis of larval swimming activity recorded on video indicated significant increases in distance moved and swimming velocity compared with control larvae. No significant differences were observed in inter-fish distance, angular velocity or turn angle. Additionally, changes in the expression of several genes associated with autism-like behaviors were detected in the larvae hatched from the Pb-exposed fish.

In the 2013 Pb ISA, evidence was inadequate to establish a causal relationship between Pb exposure and growth effects in freshwater vertebrates. Since the 2013 Pb ISA, a few additional studies in fish have assessed the effects on growth following dietary or aqueous exposure to Pb. In chronic dietary exposure (24 months) to 8–49 mg Pb/kg in food pellets, there were no significant differences in fish body weights or the survival of Prussian carp *C. gibelio* females (Łuszczek-Trojnar et al., 2013). In another study with adult female carp *C. carpio* exposed to Pb via diet (68.4 mg Pb/kg dry weight in food pellets), there were no significant differences in mean body weights at the end of the study (three exposure seasons), although Pb-exposed fish weighed significantly less than control fish after the first exposure season (Łuszczek-Trojnar et al., 2016). This is consistent with dietary studies reviewed in the 2013 Pb ISA (Alves and Wood, 2006). In aqueous exposure studies, zebrafish embryos exposed to Pb (19.3 µg Pb/L) to 6 dpf (144 hpf) showed no significant differences in hatching success, body length or body weight compared with the control (Chen et al., 2016b). Similarly, exposure of zebrafish embryos to Pb (5.0, 9.7, 19.2 µg Pb/L) up to 144 hpf did not affect growth rate or survival (Zhu et al., 2016). No differences in head length, head width or total body length were observed in 72 hpf embryos exposed nominally to one of three concentrations of Pb (10, 50 or 100 µg Pb/L and then Pb uptake was subsequently measured in the embryos)(Wirbisky et al., 2014).

For the effects of Pb on reproduction and development in freshwater vertebrates, the weight of evidence for the causal relationship in the 2013 Pb ISA was primarily from studies with fish. Pb AQCDs have reported developmental effects in fish, specifically spinal deformities in brook trout (*Salvelinus* *fontinalis*) exposed to 119 μg Pb/L for three generations (U.S. EPA, 1977), as well as in rainbow trout exposed to concentrations as low as 120μgPb/L (U.S. EPA, 1986). In the 2006PbAQCD (U.S. EPA, 2006), decreased spermatocyte development in rainbow trout was reported at 10 μg Pb/L, and testicular damage occurred in fathead minnow at 500 μg Pb/L. In the 2013 Pb ISA, a 300-day chronic toxicity study was conducted by Mager et al. (2010) in fathead minnows treated with both 31 and 112 μg Pb/L with HCO_3 and with 130 μg Pb/L with DOC. The total reproductive output was decreased, and average egg mass production increased as compared with egg mass size in controls and in low HCO_3 and DOC treatments with Pb. Other supporting evidence for the causal determination in the 2013 Pb ISA for reproductive effects in aquatic vertebrates included alteration of steroid profiles and additional reproductive parameters, although most of the available studies were conducted using nominal concentrations of Pb. Additionally, a study in frogs in the 2006 AQCD showed Pb delayed metamorphosis, decreased larval size and caused skeletal malformations at nominal concentration of 100 μg Pb/L; however, tissue concentrations quantified in frogs following exposure fell within the range of tissue concentrations in wild-caught tadpoles (Chen et al., 2006).

Several new early lifestage fish studies add to the existing evidence for Pb effects on endocrine and developmental endpoints. In a study that quantified Pb in the exposure water, hatching success rates in zebrafish embryos were reduced at 4.5, 9.6 and 18.6 μg Pb/L. At 72 hpf, the hatching success rates in all three concentrations were significantly decreased compared with the control, indicating that Pb caused a hatching delay, which was also observed at the end of the experiment at 96 hpf (Zhao et al., 2020). . In another zebrafish study, endocrine disruption in larvae was assessed by quantifying changes in thyroid hormone following exposure to Pb (analytically verified concentration of 2, 5, 10, 15, 20, 30 μg Pb/L) in embryos from 2 hpf to 144 hpf (Zhu et al., 2014). Triiodothyronine (T3) and thyroxine (T4) levels were significantly reduced at 30 μg Pb/L. Pb did not significantly affect the percentage of hatched larvae; however, Pb exposure significantly increased malformations and reduced survival at 30 μg Pb/L compared with the control. In comparison to these studies showing reproductive and endocrine responses in fish early lifestages, no endocrine disruption was observed in adult male common carp (*C. carpio*) at 7, 14 or 21 days of Pb exposure, even at the lowest analytically verified concentration (120 μg Pb/L) (Korkmaz et al., 2022).

Reproductive and endocrine effects of exposure to Pb via diet were assessed in dietary exposure with female Prussian carp *C. gibelio.* At 12 months, there was a significant increase in luteinizing hormone (LH) secretion after hormonal stimulation at the two highest analytically verified concentrations (24 and 49 mg Pb/kg), whereas (8 mg Pb/kg) spontaneous LH secretion significantly decreased at the lowest dose tested (Łuszczek-Trojnar et al., 2014). At 24 months, differences in LH secretion between treatment groups were not significant. There were also differences in oocyte size and maturation. At 12 months, oocytes in the 8 mg Pb/kg treatment group were significantly larger than those in the control and other treatment groups. After 24 months, oocyte maturity and oocyte diameter were not significantly different between the control and Pb-treated fish.

11.3.4.4.4 Birds

A new study in mallards (*A. platyrhynchos*) expands existing information on Pb effects in birds frequenting aquatic habitats contaminated with Pb and other metals. Prior AQCDs and the 2013 Pb ISA include evidence for changes in ALAD activity and other oxidative stress biomarkers. A positive relationship between the lipid peroxidation index and blood Pb in female mallards sampled in northeastern Spain adds to this evidence. Lysozyme levels were negatively correlated with blood Pb concentrations (Vallverdú-Coll et al., 2016). Additionally, in male mallards, there were significant relationships between blood Pb and beak and leg hue. In mallards, male leg and beak color typically ranges from orange-red to yellow-orange and from yellow-orange to green, with redder beaks and yellower legs typically being more attractive to females. In this study, the leg redness of males had a significant negative relationship with blood Pb levels, as did beak yellowness. This indicates that male mallards with higher blood Pb levels are likely to be less attractive to females, and therefore could potentially have lower reproductive success. Another study from the same author investigated how blood Pb levels in mallard chicks can affect multiple suborganismal and organismal-level effects (Vallverdú-Coll et al., 2015). This study on the same population of mallards in northeastern Spain found that ducklings with blood Pb levels above 180 ng/mL showed reduced body mass and died during the first week posthatching. Additionally, cellular immune function at day 15 in ducklings was negatively correlated with Pb levels in blood on the same day.

11.3.4.4.5 Amphibians

Since the 2013 Pb ISA, new laboratory studies on the effects of Pb exposure on freshwater amphibians have focused on tadpole growth, development, and survival. Asiatic toads reared in water with different concentrations of Pb (0, 9.85, 48.73, 97.69, 497.34, and 998.27 µg Pb/L analytically verified concentrations for 0, 10, 50, 100, 500, 1,000 µg Pb/L) showed a significant increase in total length and body mass at 50 µg Pb/L and a significant decrease in snout-to-vent length at 1000 µg Pb/L on day 10 compared with controls. However, farther along in development at day 20, there was a significant decrease in snout-to-vent length at 100 and 500 µg Pb/L compared with controls (Yang et al., 2019).

Huang et al. (2014) examined the effect of Pb on these endpoints in dark-spotted frogs (*Pelophylax nigromaculata*). Tadpoles were reared in different concentrations of Pb 38.2, 79.3, 158.4, 318.7, 638.1, 1278.9 µg Pb/L (analytically verified concentrations for 40, 80, 160, 320, 640, 1280 µg Pb/L) from heartbeat to complete tail reabsorption. The threshold concentrations for effects on body mass, snout-vent length, forelimb length, and hindlimb length were 160, 160, 160, and 320 µg Pb/L, with total malformation rate increasing linearly with Pb concentration. Metamorphosis time was significantly affected by Pb concentration and exhibited a linear increase with increasing Pb concentration (0 µg Pb/L = 76.4 ± 0.5 days, 160 µg Pb/L = 90.8 ± 0.5 days, 1280 µg Pb/L = 118.4 ± 0.5 days). Pb concentration also significantly affected the survival rate, which decreased with increasing Pb concentration (0 μg Pb/L = 98.3 ± 1.7%, 160 μg Pb/L = 93.3 ± 1.7%, 1280 μg Pb/L = 80.0 ± 0.3%).

Other than the studies in fish described above and in the following section on exposure-response, there is limited new information regarding Pb toxicity in freshwater vertebrates. For fish, studies are largely confirmatory with studies in the 2013 Pb ISA. Additional research with zebrafish augment existing understanding of Pb effects on neurobehavior and reproductive endpoints.

11.3.5 Exposure and Response of Freshwater Species

Evidence regarding exposure-response relationships and potential thresholds for Pb effects on aquatic populations can provide tools for quantitative analyses of risks in freshwater ecosystems (Section 11.1.7.3). Exposure-response data for the reproduction, growth, and survival of freshwater biota (including microalgae, invertebrate, amphibian, and fish species) were summarized in Table 6-5 of the 2013 Pb ISA (U.S. EPA, 2013). Additionally, the Annex of the 2006 Pb AQCD (U.S. EPA, 2006) summarized data on exposure-response functions for invertebrates (Table AX7 2.4.1) and fish (Table AX7 2.4.2) available at the time. For Pb exposure-response, there is significant new research reporting results from bioassays of freshwater algae, invertebrates and fish based on measured rather than nominal concentration of Pb. In some cases, effects were observed in sensitive species at concentrations comparable to or lower than those reported in the 2013 Pb ISA (Table 11-5) or earlier U.S. EPA reviews of Pb. Some of the studies report LC_{10} and LC_{20} toxicity values and/or calculate the free-ion concentration.

In the 2006 AQCD and 2013 Pb ISA, available exposure-response data for freshwater plants and algae did not indicate any effects on growth or survival at environmentally relevant concentrations. In the 2006 AQCD, EC_{50} values for growth inhibition in various freshwater algal and aquatic plant species were between approximately 1,000 and >100,000 μg/L and were mostly based on nominal concentration data (U.S. EPA, 2006). An important advancement since the 2013 Pb ISA is the availability of bioassay data for algal growth rate in several freshwater species based on measured Pb concentration instead of nominal concentration, which strengthens confidence in the findings for the concentrations assessed (De Schamphelaere et al., 2014). In chronic 72-hour bioassays in standard test media to assess the growth rate in three commonly tested algal species (*P. subcapitata, C. kesslerii*, *C. reinhardtii*), *P. subcapitata* was the most sensitive, with EC_{50} = 83.9 μg Pb/L, EC_{20} = 45.7 μg Pb/L and EC_{10} = 32.0 μg Pb/L based on filtered Pb concentrations (De Schamphelaere et al., 2014). Furthermore, in subsequent tests with *P. subcapitata* at varying pH, the 72h EC_{50} decreased from 72.0 μg filtered Pb/L at pH 6.0 to 20.5 μg filtered Pb/L at pH 7.6. Inhibitory concentration (IC) values calculated using a specific growth rate at 72 hours with a linear interpolation method for *Raphidocelis subcapitata* (formerly *P. subcapitata*) were IC_{10} = 0.15 μM, (31 μg Pb/L), IC_{25} = 0.39 μM (81 μg Pb/L) and IC_{50} = 0.78 μM (161 μg Pb/L) (Alho et al., 2019).

In addition to freshwater algae, there is new toxicity information based on measured Pb concentration for freshwater plants. The toxicity of Pb to duckweed *Lemna minor* expressed as percent net root elongation was assessed in chronic bioassays of seven U.S. surface waters with different water chemistries (Antunes and Kreager, 2014). The 20% IC in 7-day static renewal tests with the waters ranged from 306 nM to >6920 nM (63 µg Pb/L to >1,433 µg Pb/L) expressed as total dissolved Pb indicating that Pb speciation, solubility, subsequent bioavailability, and toxicity varied under the range of water hardness, pH, and DOC in the tested waters.

For freshwater invertebrates, effects in sensitive species of amphipods, gastropods, cladocerans and mussels were reported at low µg Pb/L concentrations in exposure-response studies reviewed in the 1986 AQCD, the 2006 AQCD and the 2013 Pb ISA. Additional toxicity data for these taxonomic groups discussed below support and expand upon what was known in the previous Pb assessment in terms of the relative sensitivity of these freshwater biota to Pb.

Toxicity testing with amphipods reported in the 2006 AQCD and 2013 Pb ISA indicate a response to Pb at <10 µg Pb/L under some water conditions. At higher pH and water hardness, these organisms are less sensitive to Pb (U.S. EPA, 2006). For example, a 7-day LC_{50} of 1 µg Pb/L was observed in soft water with the amphipod *H. azteca* (Borgmann et al., 2005). In this same species, the 96-hour LC_{50} for Pb at pH 5 was 10 µg Pb/L (Mackie, 1989). In 42-day chronic exposures of *H. azteca* exposed to Pb via water and diet, the LC_{50} was 16 µg Pb/L (Besser et al., 2005). In a chronic 42-day bioassay with *H. azteca*, published after the 2013 Pb ISA, survival was similar to that observed by Besser et al. (2005) under two different experimental diets conducted concurrently (LC_{20} = 15 µg Pb/L and LC_{20} = 13 µg Pb/L) and support the findings of effects in amphipods in the low µg/L range (Besser et al., 2016).

Some species of freshwater gastropods have exhibited sensitivity to Pb at <20 µg Pb/L. In the 1986 AQCD, Borgmann et al. (1978) found increased mortality at Pb concentration as low as 19 µg Pb/L in the freshwater snail *Lymnaea palutris* exposed from hatching to reproductive maturity (approximately 120 days). To follow-up on the set of studies reviewed in the 2013 Pb ISA (Grosell and Brix, 2009; Grosell et al., 2006b) that identified the freshwater snail *L. stagnalis* as highly sensitive to Pb (EC_{20} = <4 µg Pb/L in 30-day exposure experiments) several additional chronic studies have since been undertaken with this species. In growth bioassays conducted in a variety of natural waters across the United States with different water chemistries 14-day EC_{20} and EC_{50} values ranging from 1.5 to 49.5 and from 3.6 to 244.6 µg Pb/L, respectively, were reported for *L. stagnalis* (Esbaugh et al., 2012). Munley et al. (2013) conducted full lifecycle bioassays with a duration of 56 days to assess the effects on survival, growth, reproduction, and development in *L. stagnalis* and determine if there was any recovery from growth inhibition effects reported in the 30-day exposures. Survival was significantly decreased at the highest concentration of Pb tested (8.4 µg Pb/L) after 21-days of exposure until the end of the experiment, for a final NOEC = 2.7 µg Pb/L and LOEC = 8.4 µg Pb/L. Consistent with the earlier 30-day exposures, growth was significantly decreased at day 28, even at the lowest tested concentration (1.0 µg Pb/L), for

NOEC < 1.0 μg Pb/L and LOEC = 1.0 μg Pb/L. By day 56, growth remained significantly lower than that of the controls in the 2.7 and 8.4 μg Pb/L concentration; however, snails exposed to 1.0 μg Pb/L surpassed the growth rates of the unexposed snails. Inhibition of the specific growth rate at the 2.7 μg Pb/L exposure was observed during the last week of the experiment. Conducting a 56-day lifecycle bioassay with *L. stagnalis* enabled assessment of reproductive and developmental endpoints (Munley et al., 2013). The reproductive phase started at day 32 and continued till the end of the study. For the number of egg masses and time until first egg mass, the NOEC < 1.0 μg Pb/L and LOEC = 1.0 μg Pb/L. No effects of Pb on the number of embryos per egg mass were observed at any concentration tested. Individuals exposed to the highest concentration (8.4 μg Pb/L) did not reproduce during the lifecycle test. Egg capsule and embryo diameters after 7 days of development were significantly reduced at 2.7 μg Pb/L (the highest concentration in which snails reproduced in the study). Although growth exhibited some recovery in *L. stagnalis* in the longer 56-day lifecycle tests, growth effects observed at 28 days were predictive of the reproductive effects observed in the longer exposure (Munley et al., 2013). Additional growth studies conducted by Brix et al. (2012) reported an EC_{20} (biomass) at 8 days of exposure of 3.2 μg l^{-1} Pb and 3.5 μg l^{-1} Pb after 16 days of exposure. Under similar experimental conditions. Crémazy et al. (2018) reported a 14-day EC_{10} of 4 μg Pb/L, an EC_{20} of 7.67 μg Pb/L and an EC_{50} of 23.4 μg Pb/L for juvenile growth from compiled results of multiple toxicity tests. The corresponding chronic growth effect concentrations based on free-ion activity were EC_{10} = 0.157 μg Pb/L, EC_{20} = 0.320 μg Pb/L and EC_{50} = 1.08 μg Pb/L.

New acute data for cladocerans include a 48-hour EC_{50} = 280 μg Pb/L for immobilization in *D. magna* (Okamoto et al., 2015). Among the studies reviewed in the 2013 Pb ISA was a series of 48-hour acute toxicity tests using a variety of natural waters across North America. The cladoceran *C. dubia*. LC_{50} values in that study ranged from 29 to 180 μg Pb/L, and DOC was well correlated with protection against the toxicity of Pb (Esbaugh et al., 2011). In this same species, increasing DOC led to an increase in the mean EC_{50} for reproduction, ranging from approximately 25 μg Pb/L to >500 μg Pb/L in 7-day chronic toxicity bioassays (Mager et al., 2011a). In a study published after the 2013 Pb ISA in this same species, a series of 7-day reproductive toxicity tests to assess the effects of metal mixtures reported an EC_{50} range of 111 to 302 μg Pb/L in the Pb-only treatments (Nys et al., 2016a). In another study with *C. dubia*, the EC_{50} for reproduction ranged from 99.8 μg Pb/L at pH 6.4 to 320 μg Pb/L, at pH 8.2, and 81.2 μg Pb/L at 0.25 mM Ca to 130 μg Pb/L at 1.75 mM Ca (Nys et al., 2014). In comparison, in a series of chronic Pb toxicity tests conducted in a variety of natural waters across the United States with different water chemistries which expanded upon the findings of Esbaugh et al. (2011), 7-day EC_{20} for reproduction in *C. dubia* ranged from 12.1 to 223.3 μg Pb/L, and 7-day-EC_{50} ranged from 20.1 to 573.4 μg Pb/L (Esbaugh et al., 2012).

Using the same set of waters from across the United States, reproduction (as population growth) was also assessed in rotifer *P. rapida* over a 4-day exposure period (Esbaugh et al., 2012). Chronic EC_{20} and EC_{50} in this species based on dissolved Pb concentration ranged from 3.2 to 103.3 and 10.6 to 154.9 μg Pb/L, respectively. The variability in toxic response to Pb was linked to water chemistry; DOC had a protective effect for *C. dubia* and snail *L. stagnalis*, while rotifer response was most closely associated with Ca and pH, not DOC. In comparison, another species of rotifer, *B. calyciflorus*, was less sensitive to Pb; 4-day chronic reproductive toxicity EC_{20} ranged from 75 μg Pb/L to 336 μg Pb/L and EC_{50} ranged from 138 to 634 μg Pb/L in natural waters of varying chemistry (Nys et al., 2016b).

In response to a lack of chronic toxicity data in freshwater isopods based on measured concentrations Van Ginneken et al. (2017) conducted a series of exposure-response studies with trace metals including Pb in adult *A. aquaticus*. The authors determined LC_{10}, LC_{20} and LC_{50} effect values for this species (14-day LC_{10} = 49.7 μg Pb/L, LC_{20} = 130 μg Pb/L, LC_{50} = 677 μg Pb/L) and also calculated lethal concentrations based on free-ion activity using the Windermere Humic Aqueous Model (LC_{10} = 0.04 μg/L, LC_{20} = 0.31 μg/L and LC_{50} = 9.13 μg/L). In a separate study with *A. aquaticus,* the 10-day LC_{50} was 443 μg Pb/L (Van Ginneken et al., 2015). In another crustacean, juvenile prawns (*M. nipponense*)*,* no statistically significant effects on mortality were reported at 12 or 25 μg Pb/L concentration in chronic 60-day exposure trials; however, reductions in weight gain and specific growth rate were observed in the prawns exposed to 25 μg Pb/L (Ding et al., 2019).

In freshwater mussels, sensitivity to Pb has been demonstrated to vary with lifestage. In a study from the 2013 Pb ISA, newly transformed juvenile freshwater mussels (*Lampsilis siliquoidea*) were more sensitive than older juveniles in acute exposures. A chronic value (geometric mean of NOEC and the LOEC) of 10 μg Pb/L was reported in 28-day exposures of 2-month-old juveniles (Wang et al., 2010). The lowest median effect concentration for glochidia (larvae) of *L. siliquoidea* at 24 and 48 hours was >299 μg/L. A more recent study in glochidia of six different freshwater mussel species found in southeastern Australia (*Hyridella australis, Hyridella depressa, Velesunio ambiguus, Alathyria profuga, Cucumerunio novaehollandiae, Hyridella drapeta*) indicated these species were more sensitive in acute tests than glochidia of *L. siliquoidea* (native to the United States). The 24-hour EC_{50} values for valve closure ranged from 176 to 274 μg Pb/L (Markich, 2017). Following 72-hour Pb exposure in the same species, the EC_{50} values ranged from 65 to 110 μg Pb/L. Calculated no-effect concentrations (NECs) at 72 hours ranged from 11 to 21 μg Pb/L.

Other recent tests with freshwater invertebrates have illustrated the range in the sensitivity of North American species to Pb. In a battery of acute toxicity tests using resident invertebrates collected from the South Fork Coeur d'Alene River watershed, Idaho, U.S. and tested in the river water, the lowest EC_{50} concentration for Pb (96-hour EC_{50} = 253 μg Pb/L) was obtained with the stonefly Sweltsa sp., however, in other tests with Sweltsa sp., mortalities occurred at Pb concentrations up to three times greater, indicating a high degree of variability in repeated tests with the same species (Mebane et al., 2012). Additional invertebrates were tested in waters from the South Fork Coeur d'Alene River watershed, Idaho, U.S., and their lowest corresponding 96-hour EC_{50} values (some invertebrate species were tested multiple times) were: four mayfly species (*Baetis tricaudatus* [96-hour LC_{50} = 322 to <1,250 μg Pb/L tested at varying water hardness], Rhithrogena sp. [96-hour LC_{50} = >166 μg Pb/L], Drunella sp. [96-hour LC_{50} = >267 μg Pb/L], Epeorus sp. [96-hour LC_{50} = >346 μg Pb/L] and

Leptophlebiidae [96-hour LC_{50} = >346 μg Pb/L]), a caddisfly (Arctopsyche sp. 96-hour LC_{50} = >1,255 μg Pb/L), a Simuliidae black fly (96-hour LC_{50} = 415 μg Pb/L), Chironomidae midge (96-hour LC_{50} = 1,955 μg Pb/L), a Tipula sp. Crane fly (96-hour LC_{50} = >1,035 μg Pb/L), a Dytiscidae beetle (96-hour LC_{50} = >1,035 μg Pb/L) and two snail species (Physa sp. [96-hour LC_{50}0 = 1,159 μg Pb/L] and Gyraulus sp [96-hour LC_{50} = 380->1,035 μg Pb/L] tested at varying water hardness).

Since the 2013 Pb ISA, additional exposure-response information has been obtained from sediment bioassays for freshwater invertebrates. In 21-day whole sediment chronic toxicity bioassays, no negative effect was noted for larvae of the North American mayfly species, *Hexagenia limbata,* exposed up to 2,903 mg Pb/kg sediment (highest concentration tested); for survival, the porewater LOEC = >130 μg/L and overlying water LOEC = >53.6 μg/L (Nguyen et al., 2012). In the same study, for a European species *Ephoron virgo*, 21-day EC_{50} and LOEC of 2,201 and 2,071 mg Pb/kg were found, respectively, with a porewater LOEC = 105 μg Pb/L and overlying water LOEC = 19 μg Pb/L. In long-term whole-sediment toxicity tests with three benthic organisms exposed to various concentrations of Pb; *L. variegatus* (16 to 5,746 mg Pb/kg), *G. pulex* (21 to 2,734 mg Pb/kg) and mayfly *Ephoron virgo* (15 to 2,972 mgPb/kg), in which Pb-spiked sediments were allowed to fully equilibrate 35 or 40 days prior to testing and metal concentrations were monitored throughout, the survival of *E. virgo* (21-day EC_{10} = 1,455 mg Pb/kg dry weight) and the biomass of *L. variegatus* (28-day EC_{10} = 1,870 mg Pb/kg dry weight) were more sensitive endpoints compared with the growth of *G. pulex* (35-day EC_{10} = 2,541 mg Pb/kg dry weight) (Vandegehuchte et al., 2013).

For freshwater vertebrates, the majority of available exposure-response data are for fish. In the studies reviewed for the 2006 Pb AQCD, freshwater fish demonstrated negative effects at concentrations ranging from 10 to >5,400 μg Pb/L, generally depending on exposure duration and water quality parameters (e.g.,pH, hardness, salinity) as summarized in Table AX7 2.4.2 of the 2006 AQCD (U.S. EPA, 2006). In the 2013 Pb ISA, several acute and chronic bioassay studies with fish further elucidated the role of water chemistry in toxicity (Esbaugh et al., 2011; Grosell et al., 2006b; Grosell et al., 2006a). In a series of 96-hour acute toxicity tests with fathead minnow (*P. promelas*) conducted in a variety of natural waters across North America, LC_{50} values ranged from 41 to 3,598 μg Pb/L in this species (Esbaugh et al., 2011). Chronic assays with rainbow trout reported in the 2013 Pb ISA provided additional exposure-response data for this species. In a 69-day test with rainbow trout, the following chronic values were observed for survival: NOEC = 24 μg Pb/L, maximum acceptable toxicant concentration (MATC) = 36 μg Pb/L, EC_{10} = 26 μg Pb/L, EC_{20} = 34 μg Pb/L and LC_{50} = 55 μg Pb/L (Mebane et al., 2008). Results from a 62-day test, with fish length as the endpoint, were NOEC = 8 μg Pb/L, MATC = 12 μg Pb/L, EC_{10} = 7 μg Pb/L, EC_{20} = 102 μg Pb/L and LC_{50} = 120 μg Pb/L (Mebane et al., 2008).

New evidence since the 2013 Pb ISA includes additional studies on fish species native to North America. In 96-hour acute toxicity tests with white sturgeon (*A. transmontanus)*, which is experiencing population declines in the United States and Canada, two early lifestages (8 and 40 dph) were tested in lab water and in water from the Columbia River upstream of the Teck Trail smelter facility, British Columbia, Canada (Vardy et al., 2014). For 8 dph larvae, 96-hour LC_{50} = 177 µg/L (lab water) and 96-hour LC_{50} > 410 µg/L (river water); for 40 dph, 96-hour LC_{50} = 528 µg/L (lab water) and 96-hour LC_{50} = 1,556 µg/L (river water) (Vardy et al., 2014). In 27 dph juvenile white sturgeon exposed to Pb concentrations in water ranging from 0.03 to 60 µg Pb/L for 28 days, there was an EC_{20} > 60 µg Pb/L for survival, length, and biomass (Wang et al., 2014a). Considering that the early lifestages of white sturgeon are in close contact with sediment and porewater Balistrieri et al. (2018) reported an EC_{20} = 0.9 nM Pb^{2+} (0.18 µg Pb^{2+}/L) developed from predictive response modeling using in situ measurements of Pb in Columbia River sediment and porewater, free-ion concentrations from equilibrium speciation calculations and the laboratory toxicity testing results of Wang et al. (2014a) of Pb to the early lifestages of sturgeon. Similar dose-response curves based on free metal ion concentration were observed for effective mortality and for reduction in biomass at Pb^{2+} concentrations higher than quantified in sediment porewater, indicating young sturgeon at the sediment-water interface are unlikely to be affected by toxic concentrations of Pb in the upper reaches of the Columbia River. Mebane et al. (2012) tested westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) a native subspecies of conservation concern, in a series of bioassays using water from various locations within the South Fork Coeur d'Alene River watershed, Idaho. EC_{50} values for the effective mortality for this species ranged from 47 to 487 µg Pb/L.

In native rainbow trout (*O. mykiss*), 7-week waterborne-only exposure (4, 11, 21, 82, 251 and 907 µg Pb/L) conducted as part of a larger study to assess the toxicity of different dietary pathways in juvenile rainbow trout, survival was assessed daily, and fish were weighed weekly (Alsop et al., 2016). At 96-h, toxicity values were LC_{10} = 304.3 µg Pb/L, LC_{20} = 357.7 µg Pb/L and LC_{50} = 487.3 µg Pb/L. At 7 weeks, LC_{10} = 55.6 µg Pb/L, LC_{20} = 96.9 µg Pb/L and LC_{50} = 280.2 µg Pb/L. All fish exposed at the highest concentration did not survive, and no significant effects on growth were reported for any concentration for the duration of the experiment. In 27 dph juvenile rainbow trout, EC_{20} > 128 µg Pb/L for survival, length and biomass following 28 days of Pb exposure (Wang et al., 2014a). In tests with larval trout, EC_{20} values were the same as observed in the juveniles. In addition to studies on native fish species, other studies in fish support previous understanding of the role of water chemistry in Pb toxicity. For larval zebrafish (*D. rerio*) acute toxicity, 96-hhour LC_{50} values varied with water hardness; in soft water LC_{50}= 52.9 µg Pb/L and in hard water LC_{50} = >590 µg Pb/L (Alsop and Wood, 2011). µg Pb/L (Alsop and Wood, 2011).

As discussed in Section 11.1.7.3, the existing U.S. EPA AWQC for Pb for the protection of aquatic life are CMC of 65 µg Pb/L (for acute exposure) and CCC of 2.5 µg Pb/L (for chronic exposure) at a hardness of 100 mg/L (U.S. EPA, 1985a). Since these criteria were developed in 1984, there have been additional acute and chronic toxicity data and improved characterization of modifying factors that affect Pb bioavailability and toxicity. Taking these advances into consideration Deforest et al. (2017) proposed updated acute BLM-based aquatic life criteria, ranging from 18.9 to 998 µg Pb/L and chronic BLM-based Pb freshwater criteria ranging from 0.37 to 41 µg Pb/L (Table 11-5). The lowest criteria were for water with low DOC (1.2 mg/L), pH (6.7) and hardness (4.3 mg/L as $CaCO_3$), and the highest criteria were for water with high DOC (9.8 mg/L), pH (8.2) and hardness (288 mg/L as $CaCO_3$), which encompasses varying water quality conditions of North American surface waters and the importance of DOC and pH as modifying factors compared with hardness. The updated data sets in Deforest et al. (2017) incorporated toxicity information for *L. stagnalis, C. dubia, H. azteca* and *P. rapida*, freshwater invertebrates that are relatively sensitive to Pb exposure. The number of genera with acute toxicity data for Pb increased from 10 to 32, and for chronic toxicity, from 4 to 13, which enabled the proposed chronic criteria to be based on bioassay data rather than an acute-to-chronic ratio that was used in 1984 for derivation of the CCC.

Table 11-5 Studies in freshwater biota with analytically verified Pb concentrations and that report an effect on growth, reproduction or survival comparable to, or lower than, the lowest effect concentrations reported in previous Pb AQCDs or the 2013 Pb ISA

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Algae/Plants						
Green algae *(Pseudokirchneriella subcapitata),* Green algae *(Chlorella kessleri)* Green algae *(Chlamydomonas reinhardtii)*	*P. subcapitata* Total Pb: <1, 19, 42, 85, 228.5, 412 Pb µg/L Filtered Pb: <1, 16, 37, 77, 201, 418 Pb µg/L *C. kesslerii* Filtered Pb: <1, 7, 18, 39, 80, 164, 417 µg Pb/L *C. reinhardtii* Filtered Pb: <0.8, 9.5, 19.8, 43.3, 89.4, 194, 452, 783, 1,613 µg Pb/L	Standard 3-d toxicity tests conducted in OECD standard test medium with addition of 4 mg/L of Suwannee River Fulvic Acid. Cell densities were measured after 24, 48 and 72 h of exposure using a particle counter. The growth rates of *C. vulgaris* and *C. reinhardtii* were not considered exponential during the third day of exposure, so the 2-d ECx values were calculated for these two species. Additional tests were conducted with *P. subcapitata* with varying pH and fulvic acid	Temperature: 24°C pH = 6	**Growth:** Interspecies comparison of algal growth rate indicated that *P. subcapitata* is the most sensitive and *C. kesslerii* the least sensitive. In *P. subcapitata*, as pH increased from 6.0 to 7.6, the 72-hr EC_{50} decreased from 72.0 to 20.5 µg filtered Pb/L	*P. subcapitata* 2-d EC_{50} = 89.9 µg Pb/L 2-d EC_{20} = 44.7 µg Pb/L 2-d EC_{10} = 29.7 µg Pb/L 3-d EC_{50} = 83.9 µg Pb/L 3-d EC_{20} = 45.7 µg Pb/L 3-d EC_{10} = 32.0 µg Pb/L *C. kesslerii* 2-d EC_{50} = 388 µg Pb/L 2-d EC_{20} = 185 µg Pb/L 2-d EC_{10} = 120 µg Pb/L *C. reinhardtii* 2-d EC_{50} = 172 µg Pb/L 2-d EC_{20} = 108 µg Pb/L 2-d EC_{10} = 82.3 µg Pb/L	De Schamphelaere et al. (2014)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Green algae *(Raphidocelis subcapitata* formerly known as *Pseudokirchneriella subcapitata*)	(1.20; 2.41; 4.82 and 12.06 µM) Nominal, stock solution analytically verified	72-hr toxicity test with triplicates, maintained in a temperature-controlled room. Cell density assessed every 24 h	Temperature: 25 ± 2°C	**Growth:** Pb significantly inhibited algal growth. All treatments differed significantly ($p < 0.05$) from the control group at 72 h of exposure. Pb completely inhibited algal growth at 12.06 µM	72-hr IC10 = 0.15 µM, (31 µg Pb/L) 72-hr IC25 = 0.39 µM (81 µg Pb/L) 72-hr IC50 = 0.78 µM (161 µg Pb/L)	Alho et al. (2019)
Duckweed (*Lemna minor*)	A range of concentrations as low as 10 µg Pb/L to as high as 9,740 µg Pb/L. Total Pb added to each water was varied because waters differed in hardness, DOC, and pH. All waters were equilibrated for 24 h prior to bioassays	A series of 7-d static renewal tests with *L. minor* were conducted with seven different surface waters collected from across the United States with varied chemistries and spiked with a concentration series of $Pb(NO_3)_2$. Plants were held in a growth chamber and growth was assessed as % net root elongation	Temperature: 25 ± 2°C pH: 5.4–8.3 depending on surface water DOC: 0.5–12.5 mg/L depending on surface water Hardness: 8–266 mg/L $CaCO_3$ depending on surface water	**Growth:** The inhibition of net root elongation varied widely depending upon the chemistry of the assayed waters and its effects on Pb speciation	20% inhibitory concentration in 7-d static renewal tests with the waters ranged from 306 nM (63 µg Pb/L) to >6920 nM to (>1,433 µg Pb/L) total dissolved Pb	Antunes and Kreager (2014)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Invertebrates						
Amphipod *(Hyalella azteca)*	Control 5, 10, 20, 40, 80 µg Pb/L. Pb aqueous concentrations varied among diet treatments and over time, suggesting that food inputs modified Pb concentration and bioavailability	7-d-old amphipods in flow-through water-only exposure to Pb as Pb-nitrate in 42-d chronic bioassays. Amphipods were fed one of two experimental diets: a suspension of YCT or a DT fish food diet. Assays conducted concurrently in test water from the same diluter system	Hardness 100 mg/L as $CaCO_3$ pH about 8.2 Alkalinity 95 mg/L	**Survival:** Survival was similar with aqueous Pb exposure in amphipods fed two different diets **Growth:** Biomass significantly reduced in amphipods fed YCT, not significantly reduced in amphipods fed DT up to 63 µg Pb/L **Reproduction**: Fecundity significantly reduced in amphipods fed YCT, not significantly reduced in amphipods fed DT up to 63 µg Pb/L. (Note: fecundity and total young endpoints did not meet test acceptability criteria for YCT diet).	Lowest reliable toxicity value for each endpoint in µg/L filtered Pb: DT diet: 42-d EC_{20} = 13 µg Pb/L 42-d NOEC = 5.9 µg Pb/L 42-d LOEC = 13 µg Pb/L YCT diet: 42-d EC_{20} = 15 µg Pb/L 42-d NOEC = 6.1 µg Pb/L 42-d LOEC = 14 µg Pb/L Lowest biotic ligand model-normalized effect concentrations: EC_{20} = 8.2 µg Pb/L (total young for the DT test) EC_{50} = 6.6 µg Pb/L (biomass for the YCT test)	Besser et al. (2016)
Isopod *(Asellus aquaticus)*	15.1, 31.1, 74.7, 203, 443 µg Pb/L	Various metal mixtures and single metals were tested in a 10-d exposure with individuals of equal length (9.43 ± 0.17 mm) in a climate chamber. The Pb-only treatment was Pb as $PbCl_2$	Temperature: 20 ± 1°C Hardness: 117 mg L^{-1} $CaCO_3$	**Survival:** Focus of study was on mixture toxicity. Only LC_{50} was calculated for Pb-only treatment	10-d LC_{50} = 443 µg Pb/L	Van Ginneken et al. (2015)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Isopod *(Asellus aquaticus)*	0.71 (control), 25.6, 110, 358 and 37,616 µg Pb/L (measured values for effective concentration) <0.1, <0.1, 0.36, 4.67 and 18,982 µg Pb/L (free-ion activities of the measured effective concentrations calculated using the Windermere Humic Aqueous Model with 100% of DOC as fulvic acids)	Chronic 14-day exposure to $Pb(NO_3)_2$ with adult *A. aquaticus*. Assay water sampled on days 0,1,4, 7 and 14, isopods were removed from exposure containers for 4 h on day 7 for feeding	Temperature: 15 ± 1°C pH: 7.72 ± 0.03 DOC: 5.94 ± 0.13 mg/L Dissolved oxygen: 8.68 ± 0.03 mg/L	**Survival:** Severe mortality was only observed at the highest concentration tested after 14-d exposure. Low mortality was observed in the other concentrations. During the exposure period, LC values declined until day 4, then continued to slowly decrease. The free-ion activities produced the lowest LC values	14-d survival LC_{10} = 49.7 µg Pb/L LC_{10} for FIA = 0.04 µg Pb/L LC_{20} = 130 µg Pb/L LC_{20} for FIA = 0.31 µg Pb/L LC_{50} = 677 µg Pb/L LC_{20} for FIA = 9.13 µg Pb/L 7-d survival LC_{10} = 97.4 µg Pb/L LC_{20} = 602 µg Pb/L LC_{50} = 13,562 µg Pb/L (LC_{10}, 20 and 50 values were also calculated for day 1 and day 4).	Van Ginneken et al. (2017)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Cladoceran (*Ceriodaphnia dubia*)	pH 6.4, 7, 7.6 series: (nominal concentration 80, 110, 140, 170, 220, 320 µg Pb/L) pH 8.2 test: (nominal concentration 100, 160 220 280, 340, 400 µg Pb/L) Ca test series: (nominal concentration 50, 100, 150, 220, 320, 400 µg Pb/L) Total and filtered Pb in each series quantified but not reported for individual assays	Reproductive effects of Pb ($PbCl_2$) assessed in 7-d chronic assays. Juveniles (<24 h old) exposed to Pb and varying Ca or pH in static renewal assays. Mortality and number of juveniles noted daily	pH 4 series: 6.4; 7; 7.6; 8.2 Hardness 4 series: Ca = 0.25 mM; 1.0 mM; 1.75 mM; 2.5 mM DOC 3.2–3.3 mg/L in pH series 3.8–4.0 in hardness series	**Reproduction** Total reproduction (number of juveniles per female) relative to the mean control reproduction varied with Ca or pH over 7-d chronic exposure to Pb. High pH was protective of Pb toxicity and water hardness had less effect on chronic toxicity than pH	7-d EC_{50} for reproduction ranged from 99.8 µg Pb/L at pH 6.4 to 320 µg Pb/L at pH 8.2 7-d EC_{50} for reproduction ranged from 81.2 µg Pb/L at 10 mg/L (0.25 mM) Ca to 130 µg Pb/L at 70 mg/L (1.75 mM) Ca	Nys et al. (2014)

Cladoceran (*Ceriodaphnia dubia*) Rotifer (*Philodina rapida*) Snail *(Lymnaea stagnalis)*	Each species was tested in a range of concentrations starting at low µg Pb/L. Actual concentrations were measured but not reported for the individual assays	All three species exposed to Pb as $Pb(NO_3)_2$, in a range of representative surface waters across North America *C. dubia*: (<24-hr-old neonates) 7-d chronic reproductive bioassays conducted in a temperature-controlled chamber with a combination of dietary and aqueous exposure and monitored daily for survival and reproduction *P. rapida*: 4-d chronic Pb toxicity with adults was assessed using a population growth rate endpoint which conformed to classical concentration-dependent responses. *L. stagnalis*: 14-d chronic toxicity test for growth starting with 7 to 10 dph snails. Water changes and food replacement every 48 h	Representative surface waters for the bioassays had varying pH, DOC, and water hardness *C dubia:* pH: 6.51–8.47 DOC: 114–1443 Temperature: 26°C *P. rapida:* pH: 7.23–8.44 DOC: 79–1405 Temperature: 26°C *L. stagnalis:* pH: 5.79–8.61 DOC: 36–1314 Temperature: 26°C	**Reproduction:** Highest reproductive toxicity in *C. dubia* was observed in soft water, most protective water had high DOC. For *P. rapida* population growth, DOC was not predictive of chronic toxicity **Growth:** Effects on growth occurred at low µg/L concentration in *L. stagnalis* in some of the tested waters. For the snails, the greatest effects on growth occurred with low-DOC waters	*C. dubia:* 7-d-EC_{50}s for reproduction ranged from 20.1 to 573.4 µg/L in representative surface waters of varying chemistries. EC_{20}s ranged from 12.1 to 223.3 µg/L. *P. rapida:* EC_{20} and EC_{50} ranged from 3.2 to 103.3 and 10.6 to 154.9 µg/L dissolved Pb, respectively *L. stagnalis:* EC_{20}s and EC_{50}s for growth ranged from 1.5 to 49.5 and 3.6 to 244.6 µg/L dissolved Pb, respectively, in the natural waters	Esbaugh et al. (2012)
Rotifer (*Brachionus calyciflorus*)	For the Ca and pH series: (nominal	Reproductive effects of Pb ($PbCl_2$) assessed in recently	pH:	**Reproduction:** The EC_{50} (based on filtered Pb) for population size differed by up	For population size in natural waters:	Nys et al. (2016b)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
	concentration range 46–2,200 µg Pb/L) For DOC test series: (nominal concentration range 100–10,000 µg Pb/L). Total and filtered Pb in each series was quantified	hatched rotifers exposed to Pb for 48-hr (three generations). Tests were performed in four series (varying Ca, varying pH, varying DOC, and natural waters collected from five unpolluted waterbodies in different locations in Europe)	ranged from 6.8 to 8.2 in natural waters DOC: ranged from 3.2 to 31.5 in natural waters Temperature: 25°C	to 4.6-fold in the natural waters. The highest toxicity was observed in the synthetic reference water. For the modifying factor bioassays, both population growth rate and population size generally decreased with increasing pH. For DOC, toxicity expressed as filtered Pb decreased significantly with increasing DOC. Ca was not protective	EC_{10} ranged from 52 (synthetic reference water) to 231 µg Pb/L EC_{20} ranged from 75 (synthetic reference water) to 336 µg Pb/L EC_{50} ranged from 138 (synthetic reference water) to 634 µg Pb/L (based on filtered Pb concentration)	
Snail *(Lymnaea stagnalis)*	6, 12.5, 25, 100 µg Pb/L (analytically verified)	Juvenile snail growth was assessed in a static renewal assay over a 16-d period. Primary focus of the study was to investigate possible mechanisms of Pb toxicity	Temperature: 23°C–25°C pH = 7.8	**Growth:** After 4 d, a moderate effect of Pb on juvenile snail growth was observed, severity of growth inhibition increased after 8 d, effects on growth occurred prior to net Ca^{2+} flux in the snails, inhibition of carbonic anhydrase activity in the snail mantle also showed no effect with Pb	EC_{20} (biomass) at 8 d of exposure was 3.2 µg L^{-1} Pb EC_{20} (biomass) was 3.5 µg L^{-1} Pb after 16 d of exposure	Brix et al. (2012)

Snail *(Lymnaea stagnalis)*	0.18 (control), 1, 2.7 and 8.4 µg Pb/L (measured)	Newly hatched juvenile snails were exposed to Pb (as $Pb(NO_3)_2$ in Milli-Q water) for 56-d in a full lifecycle assessment toxicity test in a flow-through system to assess effects on survival, growth and reproduction (number of egg masses, time until first egg mass, number of embryos per egg mass). The reproductive phase started at day 32 (egg masses appeared in the control) and continued till the end of the study	Temperature: 24.8 ± 0.2°C pH: 6.89 ± 0.06 DOC: 330 ± 7.02 µM C	**Survival:** Survival was significantly decreased at the highest concentration (8.4 µg Pb/L) after 21-d exposure to the end of the experiment **Growth:** Growth was significantly decreased, even at the lowest tested concentration (1 µg Pb/L) on day 28. By day 56, growth remained significantly lower than the controls in the 2.7 and 8.4 µg Pb/L concentration; however, snails exposed to 1.0 µg Pb/L surpassed the growth rates of the unexposed snails. Inhibition of specific growth rate at the 2.7 µg Pb/L exposure was observed during the last week of the experiment. **Reproduction:** For the number of egg masses and time until first egg mass, the NOEC <1.0 µg Pb/L and LOEC = 1.0 µg Pb/L. No effects on the number of embryos per egg mass were observed at any concentration tested. Individuals exposed to the highest concentration (8.4 µg Pb/L) did not reproduce during the lifecycle test. Egg capsule and embryo diameter after 7 d of development were significantly reduced at 2.7 µg Pb/L (the highest concentration in which snails reproduced in the study)	Survival: 56-d chronic toxicity NOEC = 2.7 µg Pb/L LOEC = 8.4 µg Pb/L Growth: 28-d NOEC <1.0 µg Pb/L LOEC = 1.0 µg Pb/L Reproduction: NOEC <1.0 µg Pb/L LOEC = 1.0 µg Pb/L	Munley et al. (2013)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Snail *(Lymnaea stagnalis)*	Low µg Pb/L concentrations (Pb was measured in each assay) EC values are from combined results of Pb data from multiple toxicity tests	Series of 14-d chronic toxicity assays with single metals (Pb as $Pb(NO_3)_2$) and binary metal mixtures with juvenile *L. stagnalis* to assess effects on relative growth rate. Concentration-response curves were obtained by compiling all the single-metal toxicity tests performed at different times over a 2-yr period	Temperature: 25 ± 1°C pH = 7.81 ± 0.20 DOC = 0.76 ± 0.0 8 mg L^{-1} Alkalinity = 0.80 ± 0.05 mEq·L−1	**Growth:** Inhibition of relative growth rate was observed at low µg Pb/L concentrations, consistent with other bioassays with *L. stagnalis*	14-d chronic toxicity: EC_{10} = 4.0 µg Pb/L EC_{20} = 7.67 µg Pb/L EC_{50} = 23.4 µg Pb/L Corresponding chronic effect concentrations based on free-ion activity: EC_{10} = 0.157 µg Pb/L EC_{20} = 0.320 µg Pb/L EC_{50} = 1.08 µg Pb/L	Crémazy et al. (2018)
Mussel *(Hyridella australis)* *(Hyridella depressa)* *(Velesunio ambiguus)* *(Alathyria profuga)* *(Cucumerunio novaehollandiae)* *(Hyridella drapeta)*	Each acute toxicity test consisted of a control and 10 concentrations, which were based on preliminary range-finding tests. Individual test concentrations were not reported. Concentrations were measured	Glochidia (larvae) from gravid females collected from two different river catchments in southeastern Australia were used in the bioassays. Four static tests were conducted for each mussel species and exposure time (24, 48 or 72 hr) with PbCl in reconstituted freshwater. Viability (as assessed by valve closure) was determined at the end of the exposure period	Temperature: 22 ± 1°C pH 7.0 ± 0.2 Hardness 42 ± 4 mg $CaCO_3$ L^{-1} Alkalinity 22 ± 2 mg $CaCO_3$ L^{-1}	**Survival:** Pb sensitivity significantly increased with each exposure time and varied by species, with greatest toxicity observed in *C. novaehollandiae*	24-hr EC_{50} (for valve closure as a proxy for viability) ranged from 176 to 274 µg Pb 48-hr EC_{50} ranged from 102 to 165 µg Pb/L 72-hr EC_{50} ranged from 65 to 110 µg Pb/L 72-hr calculated NEC ranged from 11 to 21 µg Pb/L	Markich (2017)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Prawn *(Macrobrachium nipponense)*	0, 5, 10, 20, 40, 80, 160, 320 and 640 µg Pb/L (nominal values) Acute toxicity bioassay 12 µg Pb/L, 25 µg Pb/L (measured) Chronic growth bioassay	For the 96-hr acute toxicity assay, juveniles were exposed to Pb as Pb acetate in semistatic renewal (every 24 h) conditions, survival was assessed every 24 h. For the chronic growth assay, prawns were exposed for 60 days under the conditions described for the acute bioassay. Prawns fed a commercial diet twice daily	Temperature: 26 ± 1°C pH 7.0–7.3 dissolved oxygen >6.5 mg/L DOC: 190 µmol/L	**Survival:** LC_{50} values decreased over time in the acute bioassay from 24 to 96 h. Mortality was not significantly affected by Pb (12 µg Pb/L or 25 µg Pb/L) in the 60-day chronic bioassay. **Growth:** reductions in weight gain and specific growth rate in prawns exposed to 25 µg Pb/L, but not in prawns exposed to 12 µg Pb/L	Acute toxicity test: 24-hr LC_{50} = 646 µg Pb/L 48-hr LC_{50} = 250.6 µg Pb/L 72-hr LC_{50} = 175.6 µg Pb/L 96-hr LC_{50} = 131.3 µg Pb/L 60-d chronic bioassay: Reduction in weight gain observed at 25 µg Pb/L (approx. 20% of the 96-hr LC_{50})	Ding et al. (2019)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Vertebrates						
Zebrafish (*Danio rerio*)	There was low solubility of Pb in the hard water; the highest concentration of dissolved Pb measured in hard water was 590 µg Pb/L at a total Pb concentration of 630 µg Pb/L . Highest concentration tested was 3,830 µg Pb/L (measured) in the hard water while the dissolved fraction was 200 µg Pb/L.	Newly hatched larvae were tested in either soft water or hard water with Pb as Pb-nitrate for 96-h. Experiments were conducted in six-well culture plates with 10 mL water and 10 larvae per well. Water was changed every 24 h	Temperature: 28°C Soft water Hardness: 11.7 mg $CaCO_3$/L pH: 7.48 Na^+ = 220 M, K^+ = 14 M Ca^{2+} = 75 M Mg^{2+} = 42 M DOC = 0.9 mg/L. Hard water: hardness = 141 m g $CaCO_3$/L pH = 7.8 Na^+ = 700 M K^+ = 38 M Ca^{2+} = 1,350 M Mg^{2+} = 336 M, DOC = 3.5 mg/L	**Survival:** Pb was more toxic to larvae in soft water than hard water**.** No mortalities were observed in the bioassays with hard water even at the highest tested concentration	Soft water: 96-hr LC_{50} = 52.9 µg Pb/L Hard water: 96-hr LC_{50} = >590 µg Pb/L	Alsop and Wood (2011)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Zebrafish (*Danio rerio*)	2, 5, 10, 15, 20, 30 µg Pb/L; analytically verified concentration	Embryos/larvae were exposed to Pb acetate trihydrate from 2 h postfertilization (hpf) embryos to 144 hpf 50% of the exposure solution was renewed daily	Temperature: 28 ± 0.5°C	**Reproduction** No significant effect on percentage of hatched larvae at any of the tested concentrations **Growth** Significant increase in prevalence of malformations at 30 µg Pb/L compared with the control **Survival** Significant decrease in survival at 30 µg Pb/L compared with the control		Zhu et al. (2014)
Zebrafish (*Danio rerio*)	5, 9.7, 19.2 µg Pb/L; measured	6-hpf embryos exposed to Pb acetate trihydrate until 144-hpf. 50% of exposure solution was renewed daily	Temperature: 28 ± 0.5 °C	**Reproduction** No significant difference on hatching success rate at any of the tested concentrations **Growth** No significant differences were found for body length or body weight at tested concentrations compared with control **Survival** No significant effect on survival		Zhu et al. (2016)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Zebrafish (*Danio rerio*)	19.3 µg Pb/L	6-hpf embryos exposed to Pb acetate trihydrate until 144-hpf. 50% of exposure solution was renewed daily. Mortality rate, malformation rate (e.g., pericardial edema and axial spinal curvature) and hatching success recorded each day. After exposure, body length and body weight of each zebrafish larva was measured	Temperature: 28 ± 0.5°C	**Reproduction** No significant difference on hatching success rate at 19.3 µg Pb/L compared with control. **Growth** No significant differences were found for body length or body weight at 19.3 µg Pb/L compared with control **Survival** No significant effect on survival at 19.3 µg Pb/L		Chen et al. (2016b)
Zebrafish (*Danio rerio*)	4.5, 9.6, 18.6 µg Pb/L analytically verified concentration	6-hpf embryos exposed to Pb acetate trihydrate until 144-hpf. 50% of exposure solution was renewed daily. For each treatment, malformation, survival rate and hatching rate were recorded at 24, 48, 72 and 96 hpf. Additional behavioral assays were conducted at 144 hpf	Temperature: 28.5°C	**Reproduction:** Hatching success rate significantly decreased in all concentrations at 72 hpf compared with control; this delay in hatching rate also observed at 96 hpf. **Survival** Survival rate of Pb-exposed embryos at all tested concentrations significantly lower than controls at 96 hpf.		Zhao et al. (2020)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
rainbow trout *(Oncorhynchus mykiss)*	Waterborne-only study: 4, 10, 20, 80, 240 and 800 μg Pb/L (nominal concentration reported, concentrations analytically verified) Waterborne, diet and combined exposure study: 0, 8.5, 20, 60 and 110 μg Pb/L (measured)	In waterborne exposure to establish LC/EC values, juveniles (average size = 2–4 g) were exposed for 7 wk to Pb as Pb-nitrate; growth (weighed weekly) and survival were assessed at various timepoints including 96-h. In the second study, juvenile fish were exposed for 7 wk via waterborne Pb only, dietary Pb only in the form of live prey (worms Lumbriculus variegatus pre-exposed for 28-d to the same concentration of Pb as the fish) or simultaneously to waterborne and dietary Pb	Temperature: 13°C pH: 7.8–8.0 Hardness: 140 mg/L as $CaCO_3$ DOC: 2.5 mg/L	**Survival:** In the waterborne-only study to establish LC/EC values, all fish in the highest concentration tested (800 μg Pb/L) did not survive. In the second study, survival in all treatments (waterborne only, dietborne only or combination) and tested concentrations were comparable to the control (≥90%). **Growth:** Waterborne Pb exposure had no significant effects on specific growth rate or biomass in either experiment. In the dietary combination experiment, marginal (nonsignificant) reductions were observed in the dietborne and combined exposures only at 110 μg Pb/L	96-hr: LC_{10} = 304.3 μg Pb/L LC_{20} = 357.7 μg Pb/L LC_{50} = 487.3 μg Pb/L 7-w: LC_{10} = 55.6 μg Pb/L LC_{20} = 96.9 μg Pb/L LC_{50} = 280.2 μg Pb/L	Alsop et al. (2016)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
rainbow trout *(Oncorhynchus mykiss)* white sturgeon *(Acipenser transmontanus)*	Trout: 0, 10, 20, 40, 80, 160 µg Pb/L (nominal values) Sturgeon: 0, 5.0, 10, 20, 40, 80 µg Pb/L (nominal values) Measured concentrations of metals (not provided) were used for calculation of effect concentration	A series of chronic tests with two lifestages (newly hatched larvae and approximately 1-mo-old juveniles) of trout and sturgeon were conducted in aqueous-only exposure with Pb as Pb-nitrate. For trout: C1: 1-dph larval trout in a 21-d exposure; C2: 26-dph juvenile trout in a 28-d exposure; CC: 1-dph larval trout in a 52-d exposure. For sturgeon: C1: 2-dph larval sturgeon in a 25-d exposure C2: 27-dph juvenile sturgeon in a 28-d exposure; CC: 2-dph larval sturgeon in a 53-d exposure. An additional (C1-R) test was conducted with 1-dph larval sturgeon in a 24-d exposure	Trout: Temperature: 12 ± 1°C Hardness: Approximately 100 mg/L as $CaCO_3$, Alkalinity: approximately 90 mg/L as $CaCO_3$ pH: approximately 8.0 Sturgeon: Temperature: 15 ± 1°C Hardness: Approximately 100 mg/L as $CaCO_3$ Alkalinity: approximately 90 mg/L as $CaCO_3$ pH: approximately 8.0	**Growth/Survival** Note: Effect concentrations reported in this study are based on the most sensitive endpoint (mortality, immobility, fish length or biomass). Trout: No acute effects observed in larval or juvenile fish after 4-d. Generally, trout were tolerant to Pb concentration used in the study Sturgeon: No mortality or immobilization of newly hatched sturgeon was observed by 4-d. The 53-d exposures did not meet the test acceptability criteria (due to control mortalities); therefore, there are no 53-d EC_{20}s for the survival. However, the EC_{20}s based on the length and weight of surviving fish throughout the 53-d exposures were reported	Trout: Acute 4-d EC_{50} C1 (larvae): >136 µg Pb/L C2 (juvenile): >143 µg Pb/L CC (larvae) >136 µg Pb/L Chronic EC_{20} C1 (larvae 21-d) >128 µg Pb/L C2 (juvenile 28-d) >128 µg Pb/L CC (larvae 52-d) >126 µg Pb/L Sturgeon: Acute 4-d EC_{50} C1 (larvae): >55 µg Pb/L C2 (juvenile): >61 µg Pb/L CC (larvae) >55 µg Pb/L Chronic EC_{20} C1 (larvae 14-d) >56 µg Pb/L C2 (juvenile 28-d) >60 µg Pb/L CC (larvae 53-d) >27 µg Pb/L (note: low control survival in this experiment)	Wang et al. (2014a)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
white sturgeon *(Acipenser transmontanus)*	8 dph Lab: 0.1, 0.8, 2.3, 6.4, 19, 65, 210, 414 µg Pb/L Columbia River: 0.2, 0.4, 1.4, 6.1, 17, 60, 191, 410 µg Pb/L 40 dph Lab: 0.1, 21, 46, 97, 208, 396, 809, 1610 µg Pb/L Columbia River: 0.3, 20, 37, 95, 192, 325, 799, 1685 µg Pb/L	96-hr acute toxicity assays conducted with two lifestages (8 and 40 dph) under static renewal conditions with Pb as Pb-nitrate in laboratory water and field-based tests with Columbia River water. The laboratory- and field-based tests were conducted in parallel, under the same exposure conditions and following the same experimental protocols. Water from the Columbia River was pumped into a trailer retrofitted for toxicity testing	Laboratory water Temperature: 16 ± 0.9°C pH 7.5 ± 0.2 Ca^{2+} to Mg^{2+} Ratio: ~1.3:1 Columbia River Water Temperature: 16 ± 0.7°C pH 7.7 ± 0.1 Ca^{2+} to Mg^{2+} Ratio: ~4:1	**Survival** Fish exposed at 8 dph were more sensitive than fish exposed at 40 dph. Fish exposed in lab water were more sensitive than fish exposed to Columbia River water. There was a lack of mortality observed in 8 dph fish exposed to river water even at the highest concentration tested.	8 dph 96-hr LC_{50} = 177 µg Pb/L (lab water) 96-hr LC_{50} = >410 µg Pb/L (Columbia River water) 40 dph 96-hr LC_{50} = 528 µg Pb/L (lab water) 96-hr LC_{50} = 1,556 µg Pb/L (Columbia River water)	Vardy et al. (2014)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Asiatic toad (*Bufo gargarizans*)	0, 9.85, 48.73, 97.69, 497.34 and 998.27 µg Pb/L, (measured) corresponding to 0, 10, 50, 100, 500 and 1,000 µg Pb/L, nominal.	First larval stage (Gosner stage 26) tadpoles exposed to Pb acetate in static renewal (every 48 h) solutions up to Gosner stage 42 (forelimb emergence starting at 31 to 35 d depending on Pb treatment group). Tadpole growth and developmental stage assessed at day 10 and day 20. Exposure continued until day 60 to determine mean percent metamorphosis	Temperature: ~20°C	**Growth** On days 10 and 20, significant increase reported in total tadpole length and body mass at 50 µg Pb/L. At Gosner developmental stage 42 (metamorphic climax), snout-vent length was significantly longer than control in the 10 µg Pb/L treatment group. Snout-vent length and total length were significantly longer in tadpoles exposed to 50 µg Pb/L compared with control. No statistically significant difference in body mass or tail length in any treatment. **Survival** No mortality observed in control, 10, 50 or 100 µg Pb/L during 60-d exposure.		Yang et al. (2019)
Dark-spotted frog (*Pelophylax nigromaculata*)	38.2, 79.3, 158.4, 318.7, 638.1, 1278.9 µg Pb/L analytically verified concentration corresponding to 40, 80, 160, 320, 640, 1280 µg Pb/L nominal;	Embryos exposed to Pb-nitrate in static renewal assays from heartbeat (Gosner stage 19) to full metamorphosis (Gosner stage 46). Chronic exposure duration was up to 70 d	Temperature 19°C–25°C (room temperature) pH 7.04–7.69, DO 6.8–7.3 mg/L Hardness 249–258 mg $CaCO_3$/L	**Growth** Growth was inhibited at higher Pb concentrations; total malformation rate increased linearly with Pb concentration. **Survival** No significant effect on survival at 40, 80, 160 or 320 µg Pb/L	Lowest threshold concentration = 160 µg P b/L for effects on metamorphosis time, body mass, snout-vent length, and forelimb length	Huang et al. (2014)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Multiple						
37 species and 32 genera of invertebrates and fish (acute toxicity data included in derivation of proposed updated acute freshwater quality criterion for Pb) 15 species and 13 genera of invertebrates and fish (chronic toxicity data included in derivation of proposed updated chronic freshwater quality criterion for Pb)	Pb was analytically verified in all studies	U.S. EPA guidelines (U.S. EPA, 1985b) were followed to identify acceptable studies. Water chemistries over a wide range of conditions were predicted from the biotic ligand model. Acute: All included assays were waterborne Pb exposures reporting 48 to 96-hr EC_{50}s. The four lowest genus mean acute values (*Hyalella, Ceriodaphnia, Gammarus and Daphnia*) and a total of 32 genus mean values were used to determine a 50th percentile critical accumulation concentration to derive the proposed acute criterion based on U.S. EPA methods Chronic: Based on EC_{20} values from lifecycle tests in freshwater invertebrates as well		**Acute toxicity endpoints included survival, immobilization, and loss of equilibrium** The proposed updated acute criterion is based on expanded toxicity data sets and BLM predictions that demonstrate the influence of water hardness, used in the calculation of the current water quality criteria, is less important as a modifying factor relative to DOC. **Chronic toxicity endpoints included survival, growth, and reproduction** There is sufficient new chronic toxicity data for Pb since the 1984 water quality criteria to allow for direct determination of criteria from toxicity data, rather than the use of an acute-to-chronic ratio.	Proposed Freshwater Acute Water Quality Criterion based on BLM of North American surface water chemistry conditions ranged from 18.9 to 998 µg Pb/L. Proposed Freshwater Chronic Water Quality Criterion based on BLM of North American surface water chemistry conditions ranged from 0.37 to 41 µg Pb/L	Deforest et al. (2017)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
		as partial lifecycle or early lifestage tests in fish. The four lowest genus mean chronic values (*Lymnaea, Philodina, Hyalella, Ceriodaphnia*) and a total of 13 genus mean values were used to identify a chronic 5th percentile waterborne Pb concentration following U.S. EPA guidelines				

Ca^{2+} = calcium ion; CaCO3 = calcium carbonate; d = day; DOC = dissolved organic carbon; dph = days posthatch; DT = diatom + Tetramin; EC_x = X% effect concentration; hpf = hours postfertilization; K^+ = potassium ion; LC_X = X% lethal concentration; Mg^{2+} = magnesium ion; mo = month(s); Na^+ = sodium ion; Pb = lead; $Pb(NO_3)_2$ = lead nitrate; wk = week(s); YCT = yeast, cereal leaves, and trout; yr = year(s).

11.3.6 Freshwater-Community and Ecosystem Effects

Field studies in the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013) report reductions of species abundance, richness, or diversity, particularly in benthic macroinvertebrate communities coexisting with multiple metals where the sources of Pb were from mining or urban effluents. Changes to aquatic plant community composition have been observed in the presence of elevated surface water Pb concentrations. Additionally, field studies have linked Pb contamination to reduced primary productivity and respiration, and to altered energy flow and nutrient cycling. In the 2013 Pb ISA (U.S. EPA, 2013), the body of evidence was sufficient to conclude there is a likely to be causal relationship between Pb exposure and freshwater-community and ecosystem effects. Studies reviewed in that document noted ecological effects on invertebrate communities can occur at environmental Pb concentrations lower than those required to affect plant communities. High sediment Pb concentrations were linked to shifts in amphipod communities inhabiting plant structures, and potentially to alterations in ecosystem nutrient processing. Although the presence of Pb is associated with shifts in biological communities, this metal rarely occurs as a sole contaminant in natural systems, making the contribution of Pb to the observed effects difficult to ascertain. New information on the effects of Pb at the population, community, and ecosystem levels is reviewed below.

Several studies reviewed here reported negative associations between sediment Pb concentration and invertebrate community composition. A series of studies conducted in Caddo Lake, Texas has further elucidated the effects of Pb on benthic macroinvertebrate communities and Pb as a modifying factor in leaf-litter decomposition. Caddo Lake is a shallow, eutrophic lake which neighbors a superfund site (Longhorn Army Ammunition Plant, Texas). Oguma and Klerks (2015) found evidence that Pb contamination may affect leaf-litter decomposition in the lake. Litter decomposition (relative change in dry weight of American lotus [*Nelumbo lutea*] leaves deployed in litter bags) was determined after 30 days at sites spanning a gradient of sediment Pb concentration. Sediment Pb concentration in Caddo Lake ranged from 4.3 to 148.9 mg Pb/kg, with some sites exceeding the Probable Effects Concentration for sediment (128 mg Pb/kg). In a principal component analysis, total sediment Pb and sediment porewater Pb were positively correlated, and benthic macroinvertebrate abundance was negatively correlated with sediment Pb concentration and porewater Pb concentration. The authors suggested that the combination of sediment Pb content and decreased macroinvertebrate abundance, among other untested factors, may lead to reduced leaf-litter decomposition in Caddo Lake.

In a study on sediment macroinvertebrates in Caddo Lake, sediment Pb concentration was negatively correlated with the diversity and abundance of benthic macroinvertebrates although amphipod sensitivity to Pb and Cu was unrelated to sediment Pb and Cu concentrations (Oguma and Klerks, 2020). Using a univariate approach between benthic community metrics and heavy-metal concentrations, the benthic macroinvertebrate abundance, family richness, and Shannon H’ Index were negatively correlated with sediment Pb concentrations. Although this study provides correlational evidence that Pb sediment concentration affects benthic macroinvertebrate community structure, % sand/clay content, % OM, and Cu sediment concentration among other principal components are correlated with benthic macroinvertebrate community metrics. A sensitive amphipod (*H. azteca)* was exposed to sediment, and reproduction, survival and growth were assessed at 28, 35, and 42 days. The survival (28, 35, and 42 days), reproduction (35 and 42 days) and growth (42 days) of *H. azteca* were not affected by Pb sediment concentration.

Crayfish density was negatively correlated with sediment Pb concentration in the Old Lead Belt mining district in Missouri where Pb-Zn mining occurred from the 1700s to the 1970s (Allert et al., 2013). Parts of the district were designated as U.S. EPA Superfund sites. To test whether benthic macroinvertebrate, fish, and crayfish communities differed along Pb and other heavy-metal gradients in the Big River, benthic fish, crayfish, macroinvertebrates, sediment, and surface waters were sampled from riffles from eight sites (two reference sites where no mining activities occurred, two mining sites with high contamination, and four sites downstream of the mining sites with slightly lower contamination). The density of fish including sculpins (*Cottus* spp.), darters (*Etheostoma* spp. And *Percina* spp.), and madtoms (*Noturus*), and crayfish (*Orconectes* spp.) was estimated in situ. Individuals of the Missouri saddled darter (*Etheostoma tetrazonum*) and golden crayfish (*O. luteus*) were collected and used for metal analyses. Additionally, an in situ toxicity test on juvenile *O. luteus* and *O. hylas* was conducted at the two reference sites and two mining sites over 56 days, and the growth and survival of crayfish were assessed at the end of the test. Surface water Pb concentrations were lowest at the reference sites (0.06 ± 0.01 μg Pb/L, mean ± S.D.) and highest at the mining sites (7.85 ± 1.63 μg Pb/L). Sediment Pb concentrations followed the same pattern, with the lowest concentrations at the reference site (12.5 ± 2.1 mg Pb/kg dry weight), followed by the downstream sites (710 ± 530 mg Pb/kg dry weight) and the highest concentrations at the mining sites (1170 ± 467 mg Pb/kg dry weight). Pb in the sediment at the mining and downstream sites was significantly higher than the Probable Effects Concentration for sediment derived by (MacDonald et al., 2000) (128 mg Pb/kg dry weight). Pb concentration in detritus was significantly lower in reference sites compared with mining sites. Moving up the food web, Pb concentration in macroinvertebrates was lower in reference sites than in mining sites (12.7 ± 4.4 mg Pb/kg dry weight for reference sites and 720 ± 276 mg Pb/kg dry weight for mining sites, respectively). Similarly, in two different larval species of caged crayfish (*O. luteus* and *O. hylas*), Pb concentration was lower in reference sites compared with the mining site. Field-collected adult *O. luteus* Pb concentration followed the same pattern, reference Pb < downstream Pb < mining sites Pb. Pb concentration in *E. tetrazonum* was highest in the mining sites (mean ± S.D., 66.8 ± 7.3 mg Pb/kg dry weight), followed by the downstream sites (44.7 ± 14.4 mg Pb/kg dry weight) and the reference sites (0.55 ± 0.14 mg Pb/kg dry weight). *Orconectes luteus* carapace length (mm) was significantly negatively correlated with sediment Pb concentration, surface water Pb concentration, and *Orconectes luteus* Pb concentration. Sediment Pb concentration was significantly negatively correlated with crayfish density (number of crayfish × m^{-2}). Surface water Pb concentration was significantly negatively correlated with fish density and crayfish density. Although whole-body *E. tetrazonum* Pb concentration was not significantly correlated with fish density or crayfish density, *O. luteus* whole-body Pb concentration was significantly negatively correlated with crayfish density. Benthic fish density (number of benthic fish × m^{-2}) and crayfish density (number of crayfish× m^{-2}) were significantly reduced under high Pb Probable Effects Quotient values, defined as the Probable Effects Concentration divided by the total recoverable metals in the sediment.

In a field study, bioaccumulation of Pb and Cd in the common reed (*Phragmites australis*) was correlated with the density of periphyton in aquatic ecosystems in Greece (Obolewski et al., 2011). Forty-five reed sampling sites around Greece included saltwater lagoons, bays, freshwater lakes, dam reservoirs, irrigation and wastewater canals, and a river representing a gradient of hydrological parameters, salinity, water movement and contaminants. The concentrations of Pb in *P. australis* shoots varied among ecosystems and seasons, but most concentrations were between 19 and 21 mg Pb/kg for all sites and seasons. Using a redundancy analysis, biplot scores indicated that Pb was negatively correlated with Oligochaeta. Cyanophyta was found in sites with higher concentrations of Pb, Cd and Cu (and correlated metals Zn, Ni, Co, and Fe). *Scendesmus* were found in sites with lower concentrations of Pb and Mn (and correlated with Zn, Ni, Co, Fe).

The Pb gradient was not strongly correlated with shifts in aquatic insect diversity in Swedish lakes and ponds near an abandoned Zn-Pb mine (Lidman et al., 2020). The most important variables associated with larval insect community composition were bioavailable Zn, sediment Zn, bioavailable Pb, Ca, NO_3, and NH_4. For adult macroinvertebrate communities, bioavailable Pb, and sediment Pb were not statistically significant. In the analyses of larval and adult aquatic insect communities, sediment Pb was negatively correlated with community structure, while bioavailable Pb was positively correlated with community structure.

In summary, new observational and experimental studies published since the 2013 Pb ISA (U.S. EPA, 2013) reported either negative, positive, or null associations between sediment or porewater Pb concentration and community and ecosystem effects. Specifically, benthic macroinvertebrate abundance and leaf-litter decomposition were negatively correlated to sediment Pb concentrations in freshwater lakes (Oguma and Klerks, 2015). Macroinvertebrate community composition was found to be sensitive to mild Pb contamination in a freshwater lake (Oguma and Klerks, 2020). Crayfish and fish density was negatively correlated to surface water Pb concentrations and sediment concentrations for crayfish in a river system (Allert et al., 2013). Pb accumulated in reeds were found to be negatively, positively, or not correlated with abundance of some periphyton families (Obolewski et al., 2011) Finally, larval and adult insect community structures were affected by natural gradients of Pb in a lake system (Lidman et al., 2020).

11.4 Saltwater Ecosystems

11.4.1 Summary of New Information on Effects of Pb in Saltwater Ecosystems and Causality Determination Update Since the 2013 Pb ISA

Historically, the effects of Pb were less well characterized in saltwater biota compared with freshwater biota. In field studies of coastal and marine saltwater ecosystems it is difficult to attribute observed effects solely to Pb due to the presence of other stressors and highly variable conditions which influence Pb speciation and toxicity in these environments. Furthermore, the portion of Pb from atmospheric sources is usually not known. Most of the information on Pb effects on saltwater organisms are from laboratory-based studies. Fewer toxicity bioassays have been conducted on saltwater plant and algal species compared to freshwater species, and the observed effects generally occurred at concentrations that greatly exceeded reported concentrations of Pb from coastal waters (Table 11-1). Evidence in the 2013 Pb ISA was inadequate to infer causality relationships between Pb exposure and effects on physiological stress, growth, survival, and reproduction in saltwater plants and algae (U.S. EPA, 2013). In the 1977 Pb AQCD and the 1986 Pb AQCD, there were no studies that reported the effects of Pb in saltwater invertebrates. In the 2006 AQCD, few effects were noted in saltwater invertebrates including gender differences in sensitivity to Pb in copepods, increasing toxicity of Pb with decreasing salinity in mysids and effects on embryogenesis in bivalves (U.S. EPA, 2006). In the 2013 Pb ISA, available evidence was sufficient to be suggestive of a causal relationship between Pb exposure and the endpoints of physiological stress, hematological effects, and reproduction for saltwater invertebrates (U.S. EPA, 2013). Evidence for effects on neurobehavior, growth and survival in saltwater invertebrates and vertebrates, as well as effects on ecological populations and communities, was concluded to be inadequate to infer a causality relationship.

For many of the endpoints for saltwater biota (Table 11-7), evidence remains inadequate to assess causality. For other endpoints, new evidence continues to support, or expands somewhat, the basis for the causality determination in the 2013 Pb ISA. For suborganism-level endpoints, evidence was suggestive of a causal relationship between Pb exposure and physiological stress in saltwater invertebrates in the 2013 Pb ISA, and this remains the case. There is very little new evidence for hematological effects of Pb in saltwater invertebrates, which, at the time of the 2013 Pb ISA, was suggestive of, but not sufficient to infer, a causal relationship (U.S. EPA, 2013). Evidence for hematological effects in previous AQCDs and the 2013 Pb ISA were primarily from field monitoring studies of marine bivalves using ALAD as a biomarker for Pb exposure and correlated ALAD inhibition to increased Pb tissue content. For the organism-level endpoints of neurobehavior and growth effects associated with Pb exposure, there is inadequate experimental evidence to assess causality for saltwater species.

Since the 2013 Pb ISA, there is additional research for saltwater organisms that supports a change in causality determinations for some endpoints. Several newer studies quantify Pb in exposure media and report effects on endpoints at lower concentration than previously observed for saltwater biota. The increased availability of studies that report analytically verified concentrations have enabled updated estimates of effects criteria. For example, an increase in toxicological data for saltwater organisms over the last several years and the availability of studies that analytically verified Pb exposure concentration has led to a study proposing updates to the acute and chronic AWQC for Pb (Church et al., 2017). For the acute criterion, the proposed update of 100 µg Pb/L is less than the current acute criterion of 210 µg Pb/L due to more recent toxicity data from relatively sensitive early lifestages of Echinodermata and Mollusca.

In the 2013 Pb ISA, the evidence at that time for Pb effects on the survival of saltwater vertebrates was inadequate to infer a causal relationship with Pb exposure (U.S. EPA, 2013). New evidence (Section 11.4.5) is limited to laboratory-based bioassays in a few fish species. Toxicity data for other saltwater vertebrates remains lacking. Several recent chronic bioassays conducted with early lifestages of three saltwater fish species reported NOEC in the range of 11–14 µg Pb/L (Table 11-7). Furthermore, Pb in these bioassays was analytically verified. In the larval fish topsmelt (*Atherinops affinis*), LC_{50} = 15.1 µg Pb/L and NOEC <13.8 µg Pb/L were obtained at a salinity of 14 ppt (Reynolds et al., 2018). Calculated chronic values for additional saltwater fish species that are consistent with the range reported above include grey mullet (*Mugil cephalus*) fingerling survival and Tiger perch (*Terapon jarbua*) fingerling survival (Hariharan et al., 2016). Based on these new chronic studies in saltwater fish, **the evidence is suggestive of, but not sufficient to infer, a causal relationship between Pb exposure and saltwater vertebrate survival**.

In the 2013 Pb ISA the evidence was concluded to be suggestive of, but not sufficient to infer, a causal relationship between Pb exposure and reproduction and developmental effects in saltwater invertebrates (U.S. EPA, 2013). Endpoints reported in the previously available studies included a delay in the onset to reproduction (amphipod *Elasmopus laevis*) (Ringenary et al., 2007), impaired larval development (Wang et al., 2009) and embryogenesis inhibition (Wang et al., 2009; Beiras and Albentosa, 2004) in bivalves and a decrease in the fertilization rate of eggs (marine polycheate annelid *Hydroides elegans*) (Gopalakrishnan et al., 2008). Since the 2013 Pb ISA, the evidence base for Pb effects on reproductive and developmental endpoints in saltwater invertebrates has expanded, primarily due to multiple new embryo-larval developmental assays in Mollusca and Echinodermata (Section 11.4.5 and Table 11-7). Several of these acute exposure bioassays analytically verified the concentration of Pb at which effects were observed (Markich, 2021; Romero-Murillo et al., 2018; Nadella et al., 2013) and reported effects at lower concentrations than those reported in the 2013 Pb ISA. The 48-hour EC_{10} larval development in the mussels *Mytilus trossulus* and *Mytilus galloprovincialis*, was 9 and 10 µg Pb/L respectively, and 72-hour EC_{10} was 19 µg Pb/L in the sea urchin *Strongylocentrotus purpuratus* (Nadella et al., 2013). In the scallop *Argopecten purpuratus,* there was a 48-hour EC_{50} = 44 µg Pb/L for abnormal larval development (Romero-Murillo et al., 2018). These effects concentrations are comparable to those reported for larval developmental assays from two species of oysters *Magallana gigas* (48-hour EC_{50} = 49.5 µg Pb/L, 48-hour NEC = 9.9 µg Pb/L) and *Saccostrea glomerata* (48-hour EC_{50} = 52.1 µg Pb/L, 48-hour NEC = 10.1 µg Pb/L) (Markich, 2021). Considering the coherence of reproductive and developmental effects of Pb across species, observations in saltwater invertebrates are consistent with terrestrial and freshwater invertebrates (both "causal" in the 2013 Pb ISA) As a result of the newly available evidence since the 2013 Pb ISA, **the evidence is sufficient to conclude there is likely to be a causal relationship between Pb exposure and reproductive and developmental effects in saltwater invertebrates**.

For community and ecosystem effects, evidence was inadequate in the 2013 Pb ISA to assess causality between Pb exposures and the alteration of species richness, species composition and biodiversity in saltwater ecosystems. Reduced species abundance and the biodiversity of protozoan and meiofauna communities were observed in laboratory microcosm studies with marine water and marine sediments reviewed in the 2006 Pb AQCD, as summarized in Table AX7 2.5.2 (U.S. EPA, 2006). In the 2013 Pb ISA, there were a few additional studies including effects on community structure and nematode diversity that were altered in a microcosm study with marine sediments (Mahmoudi et al., 2007). Since then, new experimental and observational studies have examined the relationship between Pb in sediment and microbial abundance and/or diversity (Section 11.4.4.1), as well as Pb associations with saltwater foraminiferal communities (Section 11.4.6). Several of the benthic foraminifera studies reported effects on community richness, diversity, and abundance. In other studies with foraminifera, there were changes in the abundance of certain taxa associated with Pb, but not diversity metrics. Considering the new evidence, Pb quantified in sediment is a factor that explains variations in microbial diversity and foraminiferal species distributions and abundance in a variety of distinct geographic locations. In these studies, Pb was often correlated with other heavy metals.

These effects observed in saltwater biota are coherent with the observed community and ecosystem-level effects of Pb in terrestrial and freshwater environments, which were reported as "likely causal" in the 2013 Pb ISA (U.S. EPA, 2013). In addition to the available studies assessing Pb effects on saltwater communities, primarily foraminifera, the effects of Pb on reproduction in sensitive saltwater invertebrates and possible effects on survival in early lifestages of some saltwater vertebrates, especially when considered cumulatively, could affect populations as well as community and ecosystem structure and function. Population, community, or ecosystem-level studies are typically conducted at sites that have been affected by multiple stressors (several chemicals alone or combined with physical or biological stressors), which increase the uncertainty of attributing the observed effects to Pb. Therefore, for saltwater **the evidence is suggestive of, but not sufficient to infer, a causal relationship between Pb exposure and community and ecosystem effects.**

Table 11-6 Updated causality determinations for Pb in saltwater organisms and ecosystems

Level		Effect	Saltwater[a]	
			2013 Pb ISA[b]	2024 Pb ISA[c]
Community and Ecosystem		Community and Ecosystem Effects	Inadequate	**Suggestive**
Population-level Endpoints	**Organism-level Responses**	Reproductive and Developmental Effects – Plants	Inadequate	Inadequate
		Reproductive and Developmental Effects – Invertebrates	Suggestive	**Likely Causal**
		Reproductive and Developmental Effects – Vertebrates	Inadequate	Inadequate
		Growth – Plants	Inadequate	Inadequate
		Growth – Invertebrates	Inadequate	Inadequate
		Growth – Vertebrates	Inadequate	Inadequate
		Survival – Plants	Inadequate	Inadequate
		Survival – Invertebrates	Inadequate	Inadequate
		Survival – Vertebrates	Inadequate	**Suggestive**
		Neurobehavioral Effects – Invertebrates	Inadequate	Inadequate
		Neurobehavioral Effects – Vertebrates	Inadequate	Inadequate
	Suborganismal Responses	Hematological Effects – Invertebrates	Suggestive	Suggestive
		Hematological Effects – Vertebrates	Inadequate	Inadequate
		Physiological Stress – Plants	Inadequate	Inadequate
		Physiological Stress – Invertebrates	Suggestive	Suggestive
		Physiological Stress – Vertebrates	Inadequate	Inadequate

[a]Conclusions were based on the weight of evidence framework for causal determination in Table II of the ISA Preamble (U.S. EPA, 2015).
[b]Ecological effects observed at or near Pb concentrations measured in sediment and water in Table 6-2 of the 2013 Pb ISA were emphasized, and studies generally within one to two orders of magnitude above the reported range of these values were considered in the body of evidence for saltwater (Section 6.4.21) (U.S. EPA, 2013).
[c]Changes from the 2013 Pb ISA are indicated as bolded text.

The 2013 Pb ISA concluded that the body of evidence was suggestive of a causal relationship between Pb exposure and physiological stress, hematological effects, and reproductive and developmental effects in saltwater invertebrates (Table 11-6). Evidence was inadequate at the time to assess causality for additional effects in saltwater invertebrates and for marine algae and vertebrates. Key uncertainties from the last review for saltwater ecosystems included the uncertainties associated with generalization of effects observed in controlled laboratory studies to conditions in coastal environments where many modifying factors affect Pb bioavailability and toxicity. In general, Pb toxicity to marine or estuarine plants, invertebrates and vertebrates was less well characterized than toxicity to Pb in freshwater systems in the 2013 Pb ISA due to an insufficient quantity of studies on saltwater organisms. Specifically, there was a lack of chronic toxicity data, and relatively few studies reported analytically verified Pb concentration in the experimental media. Information regarding the contribution of atmospheric Pb to total Pb in coastal environments was sparse. This was attributed to multiple sources of Pb, confounding effects of transport from terrestrial and freshwater systems and the lack of studies connecting the air concentration of Pb and saltwater ecosystem exposure.

Studies published since the 2013 Pb ISA (literature cutoff for inclusion in the 2013 Pb ISA was September 2011) that characterized bioavailability, uptake, bioaccumulation, and effects of Pb in saltwater ecosystems or that decreased uncertainties identified in the prior NAAQS review of this criteria air pollutant are presented throughout the following sections. Saltwater ecosystems considered encompass a range of salinities from just above that of freshwater (<1 ppt) to that of seawater (generally described as 35 ppt). Coastal ecosystems may receive Pb from multiple sources such as contributions from atmospheric deposition and via inputs from terrestrial systems including runoff and riverine transport (Appendix 1: https://assessments.epa.gov/isa/document/&deid=359536). Habitats associated with coastal areas include salt marshes, estuaries, shallow open waters, sandy beaches, mud and sand flats, rocky shores, oyster beds, coral reefs, mangrove forests, river deltas, tidal pools, and seagrass beds (U.S. EPA, 2016). Estuaries, where freshwater inflows gradually mix with salt water, are dynamic, heterogeneous environments characterized by gradients of salinity. Salinity is one of the modifying factors affecting Pb speciation in coastal systems, and changes in salinity affect the ionic strength of the water (Section 11.4.2). The Pb^{2+} ion, which is the most bioavailable form of Pb, is not common in seawater; rather, Pb primarily exists as a carbonate complex and to a lesser extent as a chloride complex (Church et al., 2017; Millero et al., 2009).

Brief summaries of conclusions from the 1977 Pb AQCD (U.S. EPA, 1977), the 1986 Pb AQCD (U.S. EPA, 1986), the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013) are included where appropriate. Recent research on the bioavailability and uptake of Pb into saltwater organisms including plants, invertebrates and vertebrates is presented in Section 11.4.2. Section 11.4.3 covers environmental concentrations of Pb in saltwater biota and ecosystems in the United States at different locations and over time. The toxicity of Pb to marine flora and fauna including growth, reproductive and developmental effects (Section 11.4.4) is followed with data on exposure and the response of saltwater organisms (Section 11.4.5). Responses at the community and ecosystem levels of biological organization are reviewed in Section 11.4.6.

11.4.2 Factors Affecting Bioavailability, Uptake and Bioaccumulation, and Toxicity in Saltwater Biota

The environmental fate processes affecting Pb in the marine environment are distinct from freshwater environments. Pb speciation in seawater is a function of chloride concentration and the primary species are $PbCl_3$, $PbCO_3$, $PbCl_2$, and $PbCl^+$ while in freshwater, Pb^{2+} is the predominant species (U.S. EPA, 2013). PbCl is poorly soluble and will tend to precipitate and be found in bottom sediments.

The generally high pH and salinity of marine systems, and in some cases high DOC and particulate matter as well in estuarine waters, create conditions in which the percentage of free Pb^{2+} tends to be very low (Appendix 1, Section 1.3.3.1.2).

Factors affecting bioavailability of Pb to saltwater organisms are many of the same factors affecting bioavailability to freshwater biota (Section 11.3.2), notably OM, particulate matter, other minerals, DO, and pH. Other factors, such as salinity, play a greater role in Pb fate, transport, and bioavailability in saltwater systems, especially in dynamic estuarine environments characterized by gradients of salinity. Marine environments are characterized by higher levels of ions, such as Na^{+}, Ca^{2+}, and Mg^{2+}, which compete for potential binding sites on biotic ligands such as gills, thereby generally reducing the effective toxicity of metal ions as compared to freshwater environments (U.S. EPA, 2013) (See also Appendix 1, Section 1.3.3.1.2). Since the 2013 Pb ISA, there is additional information (summarized below) on these chemical factors which can be quantified and directly related to toxicity. Studies have further explored the effects of varying DOM composition and changing pH on Pb uptake and bioaccumulation in saltwater biota. Other factors that affect the uptake and toxicity of Pb, such as biological adaptations by organisms, are more difficult to link quantitatively to toxicity. As discussed in previous U.S. EPA reviews of Pb, species differences in metabolism, sequestration, and elimination rates have been shown to control the relative sensitivity and vulnerability of exposed organisms and influence the potential for effects on survival, reproduction, growth, metabolism, and development. Diet and lifestage at the time of exposure also contribute significantly to sensitivity and vulnerability in populations and communities. The 2006 Pb AQCD (U.S. EPA, 2006) reviewed the effects of genetics, age, and body size on Pb toxicity. While genetics appears to be a significant determinant of Pb sensitivity, the effects of age and body size are complicated by environmental factors that alter the metabolic rates of saltwater organisms. Literature reviewed in the 2013 Pb ISA corroborated these findings and discussed seasonal physiological changes and lifestage as important determinants of differential sensitivity to Pb.

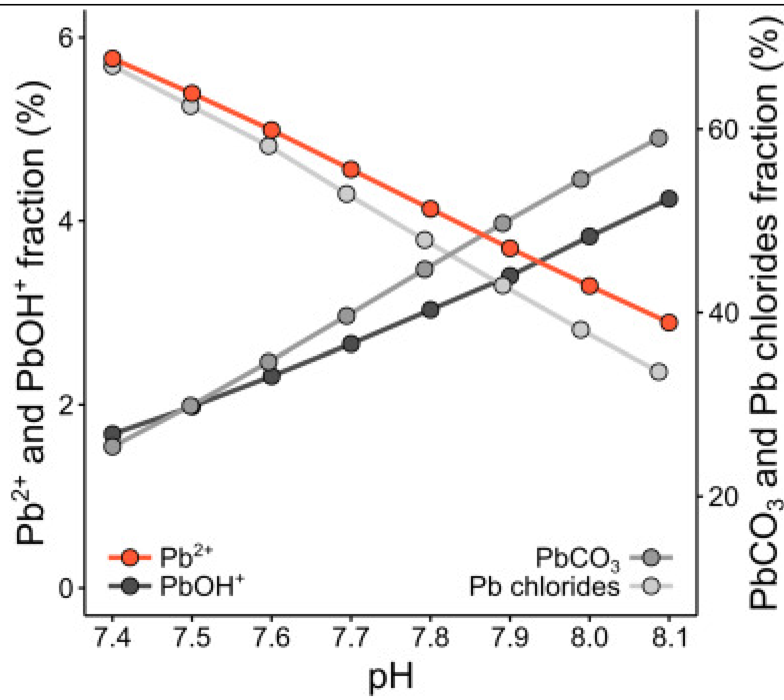
11.4.2.1 Dissolved Organic Matter

In seawater, DOM is a major factor controlling bioavailability of Pb (U.S. EPA, 2013). Studies reviewed in the 2013 Pb ISA showed that different components of DOM have different effects on Pb bioavailability in marine systems. Increasing humic acid concentrations increased Pb uptake by mussel gills and increased toxicity to sea urchin (*Paracentrotus lividus*) larvae (Sánchez-Marín et al., 2007), while in contrast, fulvic acid reduced Pb bioavailability (Sánchez-Marín et al., 2011). Continuing their research in a study published after the 2013 Pb ISA Sánchez-Marín and Beiras (2012) observed that more soluble DOM (fulvic acids and DOM extracted from the Suwannee River) also increased the bioavailability and toxicity of Pb to sea urchin embryos, although not to the same extent as humic acid. Furthermore, the experimental evidence suggests that the mechanisms by which DOM enhances Pb uptake and toxicity implies direct contact of the organic compounds with the plasma membrane. In another study examining the effects of different forms of DOM, Tang et al. (2020) observed that the bioaccumulation of Pb in saltwater shrimp was likely affected by the quality of OM; with more autochthonous OM present, there was less bioaccumulation compared with the levels in winter months when more allochthonous OM is present. Additionally, because the ingestion of DOM bound to metals is the major route of entry for metals, this suggests that the allochthonous OM may have a greater percentage of functional groups that bind Pb (e.g., fluorophores).

Several studies published since the 2013 Pb ISA have explored the protective effects of different types of OM by quantifying enzymatic activity and oxidative response in saltwater invertebrates. Nogueira et al. (2018) examined the toxicity of Pb alone and in combination with natural OM (NOM) from different sources (allochthonous, autochthonous, and mixed) on larvae of the Canadian native bay mussel (*Mytilus trossulus*). With 48-hour exposure to Pb alone (20 µg Pb/L) there was an increase in carbonic anhydrase activity and lipid peroxidation. Various NOMs did not protect against Pb toxicity, and lipid peroxidation increased significantly with some types of NOM. A parallel study conducted on the invasive Mediterranean mussel (*Mytilus galloprovincialis*) (Nogueira et al., 2017) also showed that various sources of NOM differentially induced increases of enzyme activities and oxidative stress to a greater extent than Pb alone; however, *M. galloprovincialis* was less sensitive than native *M. trossulus* overall. In these studies, no protective effects of NOM were observed. The interaction of NOM with metals is influenced by the source and composition of NOM, and some forms of NOM may exert a sublethal response independently. In a series of bioassays, Nadella et al. (2013) assessed the influence of DOM on the embryo development of two mussels, *M. galloprovincialis* and *M. trossolus,* and the pacific purple sea urchin (*S. purpuratus*). Addition of DOM from a freshwater source and a seawater source decreased the toxicity of Pb to embryos of the mussels compared with toxicity tests in 100% seawater. However, there was no concentration-dependent relationship with increasing addition of DOM. Unexpectedly, DOM exacerbated Pb toxicity in 48-hour embryo toxicity tests with *S. purpuratus*. In the absence of Pb, one of the DOM sources resulted in 100% mortality of *S. purpuratus* embryos. The authors speculated that this is a species-dependent response, attributable to DOM interaction with the epithelial interface.

11.4.2.2 pH

The importance of pH in the speciation of Pb in saltwater environments and as a modifying factor of Pb toxicity was previously reported (U.S. EPA, 2013, 2006). Several additional studies published since the 2013 Pb ISA further describe pH effects on Pb uptake and toxicity in saltwater organisms. A decrease in pH under the scenario of increasing ocean acidification may lead to additional bioavailable Pb (Pb^{2+}) in marine environments (Figure 11-5) and associated toxic effects on biota as reviewed in Ivanina and Sokolova (2015). Belivermiş et al. (2020) demonstrated that a decrease in pH (from 7.94 to 7.16) resulted in a significant increase in ^{210}Pb in the soft tissues, but not the shells, of blue mussels (*M. edulis*) after a 9-day exposure. Pb uptake in mussels was highly variable, likely due to the variability of the physiological status of individual mussels. The lower Ca^{2+} in acidified seawater can make Pb2+ more available to mussels due to decreased competition, and the lower pH means a higher partial pressure of CO_2, which can result in decreased biomineralization that may facilitate the uptake of Pb.



Source: Belivermiş et al. (2020) adapted from (Millero et al., 2009).

Figure 11-5 Main forms of Pb in seawater as a function of pH at 25°C and salinity of 35 ppt.

11.4.2.3 Salinity

In marine and estuarine systems, salinity is an important factor influencing the speciation of metals and subsequent bioavailability (de Sousa Machado et al., 2016; Wright, 1995). Generally, an increase in salinity reduces the bioavailability of metals by increasing complexing with chloride and carbonate ions and decreasing the amount of Pb^{2+} (U.S. EPA, 2006; Wright, 1995). New information published since the 2013 Pb ISA further characterizes the bioavailability of Pb under different salinity levels. For coastal sediments, Liu et al. (2019a) observed that the bioavailability of Pb at 35 ppt salinity was sequentially higher than that at salinity levels of 25 ppt and 15 ppt. When salinity was 35 ppt, the bioavailable fractions of Pb in surface sediments increased by 20.38% compared with Pb at a salinity of 15 ppt. However, it was found that excess dissolved phosphate resulted in the precipitation of $Pb_3(PO_4)_2$, which was spurred on by the increased bioavailability of Pb. In tropical estuary wetlands, Chu et al. (2015) found that increased salinity can increase Pb mobility. This is due to Pb being transformed primarily into exchangeable and reducible fractions at higher salinity, making Pb more bioavailable. The exchangeable Pb fraction increased and the oxidizable fraction of Pb and carbonate bound fraction decreased with increasing salinity.

In a study reviewed in the 2006 AQCD, 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_{50} 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_{50} = 4,274 μg/L [95% CL = 3,540, 5710 μg/L]). The reduction in toxicity was attributed to increased complexation of Pb^{2+} with Cl^{-} ions. Studies published since the 2013 Pb ISA have further considered salinity as a modifier of Pb uptake and toxicity in saltwater invertebrates. The relationships between tissue concentration of Pb and inorganic cations (Na^{+}, Mg^{2+}, K^{+}, and Ca^{2+}) were assessed in the Hong Kong oyster (*Crassostrea hongkongensis*) at four different salinities at a single Pb concentration (3 μg Pb/L, nominal) under laboratory conditions (Yin and Wang, 2017). All four cations were negatively correlated with trace metal uptake by oysters; the tissue concentration of Pb was lower at higher salinities during the 6-week exposure (due to decreasing free-ion concentration of Pb at higher salinity). For the rotifer *Proales similis,* exposed nominally to Pb (13, 25, 50, 100 μg Pb/L) in 5-day chronic reproductive toxicity tests conducted at four salinity conditions (5, 15, 25 and 35 ppt), population density was highest at the lowest salinity, and toxicity increased with increasing Pb concentration (Rebolledo et al., 2021). As salinity increased, population density decreased in all treatments and the control; however, across all salinities, the population growth rate was lowest at 100 μg Pb/L (the highest tested concentration). In contrast, embryo development assays in larval mussels (bay and Mediterranean) and pacific purple sea urchins conducted at two salinities (33 ppt and 21 ppt) reported no effect of salinity on Pb toxicity (Nadella et al., 2013).

Recent studies in saltwater fish have examined the modifying effect of salinity. In chronic exposure with larval topsmelt fish (*A. affinis)*, Pb was consistently more toxic at lower salinity (14 ppt) than at higher values (28 ppt) (Reynolds et al., 2018). Free Pb^{2+} ion concentrations, the most bioavailable form of Pb, were higher in the lower-salinity water, determined based on Pb speciation calculations in the study. Lower-salinity water contains fewer cations, leading to decreased competition of free ionic Pb with binding sites. Differential responses to salinity have also been reported in other studies in fish including juvenile yellowfin seabream (*Acanthopagrus latus)*; the LC_{50} was significantly higher in fish acclimated to 17 ppt salinity compared with fish acclimated to 0 ppt, 9 ppt, 25 ppt and 34 ppt salinity (Tsui et al., 2016).

11.4.2.4 Association with Sediments

Habitat type is a factor in the bioaccumulation of trace metals, as invertebrates closely associated with benthic environments have greater contact with porewater and sediments, where metal concentrations are higher than those in seawater. Several new studies published since the 2013 Pb ISA reported differences in the biouptake of Pb associated with sediment characteristics. Belzunce-Segarra et al. (2015) compared bioaccumulation in the benthic bivalve *Tellina deltoidalis* with two sediment types (silty, sandy) in the lab and deployed in the field. During the 31-day exposure period, Pb bioaccumulation from sediments generally increased in a linear fashion with increasing sediment Pb concentration and was greater in sandy sediments. For the silty sediments, there was more bioaccumulation in field-deployed bivalves compared with bivalves in a parallel laboratory exposure, whereas the opposite was observed with sandy sediments. Bioaccumulation in bivalves was attributed primarily to dietary exposure via ingestion of particles due to the poor relationship between dissolved Pb in overlying waters (1 to 2.2 µg Pb/L) and bioaccumulation. The authors noted that under laboratory exposure conditions, the absence of processes occurring in the natural environment such as sediment resuspension, dilution of surface sediments by deposition, and avoidance behaviors by organisms, likely lead to overestimation of bioavailability. Battuello et al. (2018) quantified trace metals in two predaceous marine invertebrates native to coastal waters of Italy: *Eurydice spinigera* (Isopoda), which burrows in sediments during the day and rises to feed in the pelagic zone at night, and *Flaccisagitta enflata* (Chaetognatha), a zooplanktonic species. Although the invertebrates have a similar feeding behavior and occupy the highest invertebrate trophic level, Pb was an order of magnitude higher in *E. spinigera* (3.1 mg Pb/kg wet weight) compared with *F. enflata.*

Fan et al. (2014) observed that the accumulation of Pb in polychaetes (marine annelid worms) was significantly related to the total metal concentrations in sediment; however, metal concentrations in polychaetes were less strongly correlated with metal concentrations in sediments if normalized for OC concentration. The correlation improved when the metal concentrations in sediments were normalized for Mn content, whereas normalization for Fe did not affect the correlation between Pb in sediment and Pb accumulation in polychaetes. This suggested that Mn content in the sediment may be the driving factor affecting bioaccumulation, while OM content in the sediment played little role in controlling the bioaccumulation of Pb in polychaetes. Additionally, Pb accumulation in polychaetes was highly positively correlated with its concentrations in FeMn oxides and organic fractions, and Pb bioaccumulation in polychaetes was not related to its partitioning in different geochemical fractions.

11.4.2.5 Seasonality

Seasonal differences in Pb uptake and concentration in bivalves were noted in several European field monitoring studies included in the 2013 Pb ISA (Carvalho et al., 2011; Couture et al., 2010; Pearce and Mann, 2006). These differences could be due to seasonal changes in anthropogenic inputs or to altered organism physiological condition in warmer versus colder months. Newer studies also reported seasonal fluctuations in Pb uptake in saltwater invertebrates. Seasonal and spatial variation of trace metal accumulation was observed in *M. galloprovincialis* mussels collected from sites around Port Phillip Bay, Australia in the summer and winter (Shen et al., 2020). In mussels collected from locations identified as high risk for contamination, Pb body burden was higher in summer than in winter. In mussels collected from less affected sites, there was no significant difference in Pb burden with season. This suggests that the increase in trace metals detected in mussels at more affected sites was due to greater anthropogenic influence in summer. Metal bioaccumulation in red cherry shrimp (*Neocaridina denticulata,* now *N. davidi*) sampled from a brackish wetland in Taiwan showed a seasonal variation in body residues, with the highest accumulation of Pb in winter (Tang et al., 2020). The saltwater shrimp could accumulate more metal when wetlands shifted to a more heterotrophic system, as observed by the negative correlation between net ecosystem production and Pb accumulation in shrimp. The highest ratios of Pb in shrimp to waterborne Pb levels were found in winter (February), during the wetland's highest season of heterotrophy. Hernández-Almaraz et al. (2016) measured heavy-metal content including Pb of white sea urchins (*Tripneustes depressus*) and slate pencil sea urchins (*Eucidaris thouarsii*) collected in the southwestern Gulf of California, Baja Sur California, Mexico in summer and winter and reported that Pb concentrations were higher in *E. thouarsii* in the summer compared with the winter, likely due to diet.

11.4.2.6 Diet Composition

Few studies in saltwater biota have examined the role of diet composition on Pb uptake and toxicity. Several studies in the 2013 Pb ISA reported tissue distribution patterns of Pb or assessed toxicity to biota following dietary exposure (U.S. EPA, 2013). A study published since the 2013 Pb ISA comparing the gut contents and Pb concentration of field-collected white sea urchins (*T. depressus*) and slate pencil urchins (*E. thouarsii*) suggested different diets may influence Pb concentrations in these organisms (Hernández-Almaraz et al., 2016). Specifically, Pb concentrations in the gonads of *T. depressus* were below the detectable limit at all sites (<0.07 mg Pb/kg dry weight), while Pb concentrations in the gonads of *E. thouarsii* ranged from 12.8 ± 1.7 mg Pb/kg dry weight (mean ± SE) to 38.6 ± 4.2 mg Pb/kg dry weight. The diet for *T. depressus* varied with season and site and included both brown and red macroalgae (mainly *Sargassum*, *Gracilaria* and *Laurencia*). The main food source for *E. thouarsii* was red macroalgae, although they are considered a generalist omnivore that also fed on some invertebrates, which was confirmed by higher $\delta^{15}N$ than *T. depressus*. Given Pb was only detected in *E. thouarsii*, the authors suggested that these urchins might be exposed to Pb via macroalgae, specifically, crustose macroalgae (*Lithophyllum)* or articulated coralline macroalgae (*Amphiroa)*, as well as invertebrates including mollusks, and/or barnacles.

In another dietary study Guo et al. (2013) examined whether the burned nassa sea snail (*Nassarius siquijorensis*) showed differences in bioaccumulation patterns after being fed either Japanese carpet shell clams (*Ruditapes philippinarum*), Asian green mussels (*Perna viridis*), *Fistulobalanus albicostatus* (barnacles) or Portuguese oysters (*Crassostrea angulata*) for 8 weeks. The prey items were collected from an intertidal zone in Xiamen, southeastern China. *N. siquijorensis* were sampled every 2 weeks and muscle and viscera metal concentrations, including Pb, were determined. In addition to the body burden of metals in the snails, metal concentrations were also determined for the subcellular fractions of the snails (heat-sensitive protein fraction, metallothionein-like protein fraction, MRG, cellular debris and organelles). Pb concentrations differed between the four prey items (*P. viridis*:

0.66 ± 0.19 mg Pb/kg dry weight, mean + S.D., n = 8; *R. philippinarum*:1.1 ± 0.3 mg Pb/kg dry weight; *C. angulata*: 2.4 ± 0.3 mg Pb/kg dry weight; *F. albicostatus*: 5.9 ± 1.1 mg Pb/kg dry weight). Subcellular metal distribution in *N. siquijorensis* viscera and muscle at the beginning of dietary exposure was concentrated in the cellular debris (44.3%). After exposure to four prey items over 8 weeks, the dominant pool for Pb in the muscle was the cellular debris, while MRG became the dominant storage pool for viscera across most prey items. Throughout feeding, MRG became a more important storage pool for Pb relative to cellular debris. Pb was largely accumulated in the cellular debris and MRG for all prey items.

11.4.2.7 Lifestage

Additional studies on Pb effects in saltwater biota published since the 2013 Pb ISA provide further evidence for variance in response to Pb at different lifestages. Embryo and juvenile lifestages are commonly tested in bioassays due to their increased sensitivity to pollutant exposure. Many studies in saltwater invertebrates discussed in the following sections continue to support findings in prior AQCDs and the 2013 Pb ISA of differential toxicity with organism lifestage and increased sensitivity of larval or other early lifestages compared with adults. In saltwater vertebrates, chronic toxicity bioassays with topsmelt (*A. affinis*) at two lifestages (larvae and 2.5-month-old juveniles) lend further support to greater sensitivity of earlier lifestages to Pb in saltwater fish (Reynolds et al., 2018).

11.4.2.8 Historical Exposure

In the 2013 Pb ISA, the few studies that reported the development of tolerance to prolonged Pb exposure were limited to freshwater invertebrates and fish: information was lacking for saltwater. A recent study with the mangrove crab (*Ucides cordatus*) collected from two locations in Brazil suggests that a crab population inhabiting an historically polluted area may have developed mechanisms to cope with elevated metals, resulting in differences in Pb accumulation compared with individuals from a relatively pristine mangrove (Duarte et al., 2020). After 28 days of laboratory exposure to low concentration of Pb (10.6 μg Pb/L), crabs collected from the protected site accumulated statistically significantly more Pb in four of the six quantified tissues (gills, carapace, gonads, and muscle) and almost double the total concentration of Pb compared with the crabs from the historically contaminated location. The population from the protected site also took up more Pb in the biologically active form and exhibited greater genotoxic effects (assessed by frequency of micronucleated cells and DNA strand breaks). Furthermore, metallothionein induction in crabs from the historically contaminated location was more than twice as high as that from the clean site.

11.4.2.9 Species Sensitivity

As is the case for terrestrial and freshwater organisms, there are considerable differences in response to Pb among saltwater biota. This information serves as the basis for the SSDs (Section 11.4.5) for saltwater invertebrates and fish reported by (Church et al., 2017). Both inter and intraspecific differences in Pb uptake and bioaccumulation may occur in macroinvertebrates of the same functional feeding group (U.S. EPA, 2013). For example, in the 2013 Pb ISA, data from 20 years of monitoring of contaminant levels in filter-feeding mussels of the *Mytilus* genus and eastern oysters (*C. virginica*) sampled along the U.S. coast, as part of the NOAA Mussel Watch program, indicate that Pb is on average three times higher in mussels than in oysters (Kimbrough et al., 2008). Wang et al. (2014b) compared acute toxicity data (hazard toxicity ratios based on LC_{50} values; EC_{50} values for algal responses) for temperate and tropical saltwater SSDs across five broad taxonomic groups (algae, crustaceans, fish, mollusks, worms). Based on the hazardous concentration for 10% of the species (HC_{10}) ratios, temperate saltwater species are more sensitive to Pb than tropical saltwater biota. In the meta-analysis, algae were the most sensitive taxa to Pb (HC_{10} = 29 µg Pb/L, [95% CI 9.5, 86], n = 8) followed by fish (HC_{10} = 166 µg Pb/L [95% CI 49], n = 10), crustaceans (HC_{10} = 428 µg Pb/L [95% CI, 263, 696], n = 22), mollusks (HC_{10} = 1230 µg Pb/L [95% CI 412, 3,660], n = 7), and worms (HC_{10} = 2,430 µg Pb/L [95% CI 1,200, 4,610], n = 9).

11.4.2.10 Uptake and Bioaccumulation in Saltwater Plants and Algae

In the 1977 Pb AQCD, the cordgrass *Spartina alterniflora* was found to reduce the quantity of Pb in sediments by a small amount (U.S. EPA, 1977). Limited data on marine algae and saltwater plants reviewed in the 1986 Pb AQCD, 2006 Pb AQCD, the 2013 Pb ISA and this appendix provide evidence for species differences in Pb uptake and bioaccumulation rates.

One study examined element concentrations in pelagic *Sargassum* that washed up along the coast of the Yucatan peninsula in Mexico from the Caribbean (Rodríguez-Martínez et al., 2020). Of 63 different samples collected across eight sites from August 2018 to June 2019, only five samples had Pb levels at 2–3 mg Pb/kg dry weight (as measured by X-ray fluorescence, which has a detection limit of 2 ppm). Other metals such as As were detected in much higher amounts. Though Pb is not present in high amounts in *Sargassum*, the study showed that pelagic seaweed may be an avenue of transport across large distances and contribute to Pb levels in coastal environments where it washes ashore.

An additional area of new research is the uptake of Pb by mangroves and the mechanisms that may limit or confer tolerance. Mangrove swamps are coastal wetlands found in tropical and subtropical regions. They are characterized by halophytic woody plants growing in brackish to saline tidal waters. One greenhouse experiment aimed to investigate the possible function of root lignification/suberization on Pb uptake and tolerance in two pacific mangrove species with different degrees of root lignification and suberization: holly mangrove (*Acanthus ilicifolius*) and red mangrove (*Rhizophora stylosa*) (Cheng et al., 2015). Plants were grown in pots with three nominal Pb treatments applied to the sediment—low (250 mg Pb/kg), medium (500 mg Pb/kg) and high (1,000 mg Pb/kg)—and one control with no Pb; Pb exposure was a period of 3 months. In the species with little lignification and suberization, *A. ilicifolius*, biomass yield decreased significantly as plants were exposed to increasing concentrations of Pb; about 20, 35 and 50% reductions were observed in low, medium, and high Pb treatments when compared with the respective controls. *R. stylosa*, however, was not affected by low and medium Pb exposure. A significant decrease in relative Pb was observed within the outer cortex cell layers, indicating that lignified/suberized exodermis acts as a barrier to the movement of Pb. A further study with six pacific mangrove species subjected to different levels of a metal mixture (Pb with Zn and Cu) corroborates these findings and suggests that mangrove species, which possess more extensive lignification and suberization within their root exodermis, exhibit higher tolerance for heavy metals (Cheng et al., 2014).

The U.S. EPA Framework for Metals Risk Assessment states that the latest scientific data on bioaccumulation do not currently support the use of BCFs and BAFs when applied as generic threshold criteria for the hazard potential of metals (U.S. EPA, 2007); however, such metrics are useful to provide information about the amount of uptake of metals into plants, compartmentalization into different plant tissues, and differences between species. In a field study conducted in four marine and four inland wetlands in Sicily with differing levels of anthropogenic impacts, Pb concentrations were quantified in soil, water, and plant tissues of two Mediterranean seagrasses, *Posidonia oceania* and *Cymodocea nodosa*, and five freshwater species were quantified (Bonanno et al., 2017). Sediment Pb levels ranged from 2.56 ± 0.33 mg Pb/kg at the lowest impacted site to 11.5 ± 1.57 mg Pb/kg at the most impacted site for the marine sites and 1.05 ± 0.21 to 17.2 ± 4.58 mg Pb/kg for the freshwater sites. BCFs ($C_{root}/C_{sediment}$) were higher for the two marine seagrasses than those for any of the freshwater species, more than twice as high as the values for the highest freshwater species (0.71 and 0.84 for *P. oceania* and *C. nodosa,* respectively, compared with 0.03–0.30 for the freshwater species). For both marine species, Pb was concentrated in root tissue, but translocation factors into different tissues differed between species. An additional study Bonanno et al. (2020) examining the seagrass *C. nodosa* and marine green algae *Ulva lactuca* showed that both species are comparable in their ability to sequester high levels of trace elements including Pb.

11.4.2.11 Uptake and Bioaccumulation in Saltwater Invertebrates

At the time of the 1977 AQCD, it was understood that shellfish concentrate Pb in their tissues and shells (U.S. EPA, 1977). Uptake and subsequent bioaccumulation of Pb in marine invertebrates varies greatly between species and across taxa as previously characterized in the 2006 AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013). In the case of invertebrates, Pb can be bioaccumulated from multiple sources, including the water column, sediment, porewater and dietary exposures, and factors such as the proportion of bioavailable Pb, lifestage, age and metabolism can alter the accumulation rate. Since the 2013 Pb ISA additional information on uptake rates, Pb sequestration patterns and Pb accumulation from aqueous and dietary exposures has been published for saltwater invertebrates.

As reported in studies in previous reviews, major sites for Pb accumulation following aqueous exposure include the gill and digestive gland or hepatopancreas; current studies continue to support these findings. In pacific oyster (*C. gigas)* exposed nominally to 5 µg Pb/L for 9 days, Pb concentration in the gill and digestive glands were 19- and 24-fold higher, respectively, than Pb measured at the beginning of the experiment (Meng et al., 2018). Following 28-day exposure to a low concentration of Pb (10.6 µg Pb/L), the highest concentration of Pb was accumulated in the gill, followed by the carapace in the mangrove crab (*U. cordatus*) (Duarte et al., 2020). The crabs sequestered Pb in detoxified forms, with differences in Pb accumulation and storage observed in two distinct populations (crabs collected from a protected mangrove area and those collected from a historically contaminated site).

Adult female Atlantic Horseshoe crabs *(Limulus polyphemus)* collected from several different beaches in Long Island, NY, had higher Pb concentration in gills than legs or eggs; Pb in leg tissue was significantly and positively correlated with egg Pb burden, suggesting maternal transfer of the internalized metal to eggs (Bakker et al., 2017b). Pb quantified in field-collected horseshoe crab embryos (range 0.05–0.43 mg Pb/kg dry weight) and developing larvae (range 0.07–0.59 mg Pb/kg dry weight) was compared with Pb concentration in eggs, sediment, porewater and overlaying water (Bakker et al., 2017a). Although Pb measured in environmental media varied between sites, the concentration of Pb significantly increased from egg to embryo at four out of five sampling locations, indicating uptake of Pb from the surrounding substrate following hatching since the embryonic lifestage develops in the sediments. There was no significant change in Pb concentration when comparing embryos to larvae; however, the authors noted that it is possible some trace metals are lost at the larval stage during molting.

Embryos of the sea urchin *S. purpuratus* exposed to an analytically verified Pb concentration of 55 µg Pb/L during 96-hour embryo toxicity assays showed significant Pb accumulation after 12 hours through 96 hours of development, with a peak at 84 hours (Tellis et al., 2014). Pb disrupted Ca uptake during initial development stages, especially during gastrulation, and there was a corresponding increase in Ca^{2+}ATPase activity in the embryos; however, Ca levels in Pb-exposed embryos returned to control amounts by 72 hours.

A few dietary exposure studies in marine invertebrates have been conducted since the 2013 Pb ISA. In sea hare (*Aplysia californica*) exposed to Pb solely through diet (green seaweed *U. lactuca* previously exposed to an analytically verified concentration of either 10 µg Pb/L or 100 µg Pb/L for 48 hours), the Pb accumulation pattern in the mollusk was greatest in the hepatopancreas followed by the gill and crop (Jarvis et al., 2015). In sea cucumbers (*Apostichopus japonicus*) fed a Pb-supplemented diet (100, 500, or 1,000 mg Pb/kg dry weight) for 30 days, the profile of tissue Pb accumulation was body wall>intestine>respiratory tree (Wang et al., 2015). The bioavailability of Pb from food and subsequent trophic transfer is affected by how Pb is stored in the prey. In a feeding study, the common prawn *Palaemon serratus* was fed for 28 days with either tissues from the mussel *(M. galloprovincialis*) exposed to 100 µg Pb/L for 48 hours or tissues from the field-collected clam *Dosinia exolete,* wherein Pb is stored primarily in nonbioavailable MRG in the kidney (Sánchez-Marín and Beiras, 2017). Although the Pb concentration in both food items was similar (15 and 17 mg Pb/kg wet weight, respectively), Pb accumulation in prawns was 10× higher when fed tissue from the mussels, in which Pb was in a more soluble subcellular faction, compared with the prawns consuming *D. exolete*, in which Pb was in a less bioavailable form.

Pb uptake is influenced by feeding strategy. In the filter-feeding bivalve *Andara trapezia,* uptake, and bioaccumulation from Pb-spiked sediments (analytically verified concentration of 100 and 300 mg Pb/kg) to the gill and mantle, hemolymph and hepatopancreas were quantified on days 0, 14, 28, 42 and 56 of a 56-day exposure (Taylor and Maher, 2012). At the end of the experiment, total Pb concentration in the mollusk was 1 mg Pb/kg at the low concentration and 12 mg Pb/kg at the high concentration. In the highest Pb treatment, an increase in Pb in hemolymph was observed from day 42 to day 56, resulting in a doubling of Pb tissue concentration. The authors speculated this could be related to greater availability of dissolved Pb in porewater over time due to oxidation of the sediments. Generally, the order of tissue accumulation was hemolymph ≥ gill and mantle > hepatopancreas over the 56-day exposure. In contrast, the deposit-feeding bivalve *Tellina deltoidalis* exhibited a distinct pattern of Pb uptake under similar experimental conditions and exposure to spiked sediments (28-day exposure to analytically verified concentrations of 100 and 300 mg Pb/kg) (Taylor and Maher, 2014). Individuals in the 100 mg/kb Pb-spiked sediment rapidly accumulated Pb early in the exposure period (day 3) followed by continued uptake over the remainder of the experiment, to reach a final tissue concentration (96 mg Pb/kg) equal to that of the spiked sediment. In the 300 mg Pb/kg microcosm, the bivalves seemed to exhibit a pattern of uptake and loss over the 28-day period, with the highest Pb concentration at day 21 and a final total Pb concentration of 430 mg Pb/kg.

Aquatic invertebrate strategies for detoxifying Pb reviewed in the 2006 Pb AQCD and 2013 Pb ISA included sequestration of Pb in lysosomal-vacuolar systems, excretion of Pb by some organisms and deposition of Pb to molted exoskeleton. Pb can be stored in two forms: biologically detoxified metal (which includes MRG) and biologically available metal. Following the biouptake experiments described above, subcellular partitioning of Pb was determined in the bivalves (Taylor and Maher, 2014, 2012). Of the recovered Pb in *A. trapezia* tissues, Pb was associated to the greatest extent with the biologically detoxified metal fraction (ranging from 66% to 69% in the gill and mantle to 56% in the hepatopancreas), distributed fairly evenly between the metallothionein-like proteins and MRG fractions (Taylor and Maher, 2012). In *T. deltoidalis,* Pb was also primarily found in the biologically detoxified metal fraction (approximately 70%), with 74% of the total detoxified Pb converted to MRG and the remainder in the metallothionein-like protein fraction (Taylor and Maher, 2014).

In a 96-hour exposure to analytically verified concentrations of Pb (0–1,800 µg Pb/L), intracellular partitioning data in adult clams *Venerupis decussata* showed that most Pb accumulated in the insoluble fraction (>80%), a form not readily bioavailable to consumers at higher trophic levels (Freitas et al., 2014). Total Pb in clams increased with increasing water concentration up to 230 µg Pb/L, then decreased at higher concentrations. The clams bioconcentrated Pb in the soluble fraction more efficiently at low water concentrations (BCF > 26) compared with higher concentrations (>450 µg Pb/L; BCF <16).

11.4.2.12 Uptake and Bioaccumulation in Saltwater Vertebrates

Studies reviewed in prior AQCDs and ISAs report Pb accumulation in tissues sampled from seabirds, saltwater fish, and marine mammals (U.S. EPA, 2013, 2006, 1977); however, there are fewer biouptake studies of Pb in saltwater than in freshwater. Because marine fish drink seawater to maintain osmotic homeostasis, Pb can be taken up from the water column via both the gills and intestine (Lee et al., 2019; Wang and Rainbow, 2008). In the 2013 Pb ISA, storage of Pb in metal granules was reported as a detoxifying mechanism in mummichogs (*Fundulus heteroclitu*s). Fish at more polluted sites stored a higher amount of Pb in MRG as compared with other detoxifying cellular components such as heat-stable proteins, heat-denaturable proteins and organelles (Goto and Wallace, 2010). Since the 2013 Pb ISA, additional studies have further elucidated the role of subcellular fractions in metal detoxification in saltwater fish. Metal binding to subcellular fractions in the livers of wild-caught yelloweye rockfish (*Sebastes ruberrimus)* collected from the southeast coast of Alaska was assessed to gain a better understanding of the degree to which this long-lived endangered fish species can detoxify nonessential metals including Pb (Barst et al., 2018). Combining data from the rockfish, Pb was detected to a greater extent in the detoxified compartment (46%); however, detoxification was incomplete given that Pb was also present in metal-sensitive fractions (a total of 35%, divided between heat denatured proteins [12.2%], mitochondria [11.4%], microsomes and lysosomes [10.8%]). Metals associated with sensitive subcellular fractions indicate a risk of disruption to cellular processes; however, the concentrations of Pb in rockfish were low compared with other detected metals. These patterns were consistent with results from subcellular partitioning in livers of yellow eels native to North America (*Anguilla rostrata*) and Europe (*Anguilla anguilla*) (Rosabal et al., 2015). In both eel species, the granule-like detoxification fraction showed the strongest increase in Pb concentrations among all subcellular fractions, with the metal-sensitive mitochondrial fraction representing a significant binding compartment for Pb.

A novel study exploring the use of fish eyes as an organ for monitoring Pb exposure compared Pb concentration in mullet (*Liza aurata)* eyes, water column and sediment in a metal-contaminated location and reference area within the same estuary (Pereira et al., 2013). Eyes from individuals collected from the contaminated site (0.81 µg Pb/L water column, 417 mg Pb/kg sediment) had significantly higher Pb accumulation (10×) than the less affected site (0.032 µg Pb/L water column, 61 mg Pb/kg sediment), suggesting the eye is a target organ for Pb. It is not known if the accumulation of metals in the eye is from direct contact with water or redistribution of Pb taken up by the fish via other routes of exposure.

Studies that considered uptake of Pb in saltwater birds and mammals are limited to surveys of field-collected individuals that reported Pb concentration in tissue or trace-element patterns of tissue distribution.

11.4.2.13 Uptake and Bioaccumulation Through Marine Food Web

Trophic transfer of Pb in marine food webs was found to be negligible in the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013). In many studies reported in previous assessments and those reviewed here, Pb was found to decrease with increasing trophic levels, although some studies found evidence of bioaccumulation. Whether Pb is biodiluted or bioaccumulated in marine food webs depends on the sediment and porewater Pb, the type of marine ecosystem, the organisms examined, and other contaminants. In a review published in 2013, Cardwell et al. (2013) compiled laboratory and field studies to examine the transfer of Pb and other heavy metals through marine food webs. In most of the field studies reviewed, no evidence was found for biomagnification of Pb across trophic levels. Specifically, nine studies examined trophic transfer of heavy metals through marine food webs in the field. Eight of these studies found no evidence of biomagnification of Pb, and one did not examine Pb or did not present data on Pb. More recent studies are presented below.

Biodilution of Pb in marine food webs was supported by an environmental gradient study on a green sea turtle food web in San Diego Bay, California, U.S. (Komoroske et al., 2012). Green sea turtles (*Chelonia mydas*) largely forage on eelgrass (*Zostera marina*) and invertebrates, and exposure to heavy metals occurs primarily through foraging, as these organisms breathe air and do not feed during migration. At each of eight eelgrass sites, sediment samples, eelgrass, red algae (*Gracilaria* spp.), green algae (*Ulva* spp.), soft-bodied invertebrates (i.e., *Zoobotryon* spp.), sponges, and green sea turtle carapace tissues were collected and analyzed for trace metals. Mean Pb concentrations in sediments and organisms varied across season and site in San Diego Bay. Pb did not bioaccumulate in eelgrass or algae: Pb in the sediment was significantly higher than Pb in eelgrass and red algae, but not higher in green algae.

Biodilution of Pb was also reported across six intertidal sites in New England (four in the Gulf of Maine and two in Narragansett Bay, Rhode Island) spanning a gradient of watershed land use and urbanization (Chen et al., 2016a). Sediments, invertebrates, and benthic and pelagic fish were sampled and analyzed for heavy metals. Trophic position was characterized using stable-isotope analysis on biotic tissues. Specifically, $\delta^{13}C$ is correlated with the relative proportion of pelagic diet sources, while $\delta^{15}N$ is related to trophic position. Invertebrate and fish samples were categorized into five taxonomic groups, as the same species were not collected at all sites. Biota-sediment accumulation factors (BSAFs) were calculated for each taxonomic group (amphipod, crab, *Fundulus*, mussel, and shrimp) as the metal concentration in the organisms divided by the metal concentration in the sediment. Positive $\log_{10}$ BSAF values indicate bioaccumulation, while negative values indicate biodilution. Pb concentrations in the sediment across six sites in the Gulf of Maine and Narragansett Bay ranged from 4.7 mg Pb/kg to

79.6 mg Pb/kg, and these concentrations increased linearly with the percent of total OC. All $\log_{10}$ BSAF values were negative for Pb, indicating that organisms in higher trophic levels contained less Pb than organisms occupying lower trophic levels. Pb concentration across five taxonomic groups (mussel, shrimp, crab, *Fundulus,* and amphipod) showed considerable variation across taxa and sites. Simultaneously extracted metal-AVS in the sediment were marginally predictive of biota Pb content, while trophic level and pelagic feeding were not predictive of biota Pb.

Trophic level positions of a marine invertebrate community and body Pb concentrations of a marine invertebrate community were not correlated in the Bay of Fundy, Nova Scotia, Canada suggesting Pb does not bioaccumulate in this system (English et al., 2015). The invertebrate community included barnacles (*Balanus balanus*), worms (*Cerebratulus lacteus, Clymenella torquata, Glycera dibranchiate, Hediste diversicolor*), amphipods (*Corophium volutator, Gammarus oceanicus*), clams (*Ensis directus, Mya arenaria, Macoma balthica*), snails (*Ilyanassa obsoleta, Littorina littorea*), mussels (*Mytilus edulis*), and crabs (*Pagurus pubescens*). Stable isotopes were used to characterize the relative trophic position as organisms in higher trophic levels contain higher levels of $\delta^{15}N$, while $\delta^{13}C$ is often associated with lower trophic levels. Although the species sampled likely did not belong to the same food web, they occupy similar trophic levels in different food webs and are all important prey items for species in higher trophic levels. In this study, $\delta^{15}N$ was negatively correlated with $\delta^{13}C$ for most species. Pearson correlation coefficients were calculated between stable-isotope and trace-element content for each species to test for bioaccumulation or biodilution through the food web. Pb concentration varied among invertebrate species in the community; however, no single species had higher Pb concentrations than the others. Pb ranged from 0.07 ± 0.01 mg Pb/kg (mean ± S.D.) in *Glycera dibranchiate* (Polychaeta) to 1.25 ± 1.40 mg Pb/kg in *I. obsoleta* (Gastropoda). There were no significant correlations between trophic level position ($\delta^{15}N$ or $\delta^{13}C$) and $\log_{10}$ Pb concentration, suggesting Pb does not show considerable bioaccumulation in the food web.

In another example, trophic level position determined using stable isotopes of white sea urchins (*T. depressus*), slate pencil sea urchins (*E. thouarsii*), and nine types of macroalgae food sources in four *Sargassum* beds in the southwestern Gulf of California in Baja California Sur, Mexico were not correlated (Hernández-Almaraz et al., 2016). Out of the macroalgae and two sea urchins studied, *E. thouarsii* had the highest Pb concentrations (ranging from 12.8 ± 1.7 mg Pb/kg dry weight [mean ± SE] to 38.6 ± 4.2 mg Pb/kg dry weight) and stable-isotope content.

Pb accumulation in a tropical estuarine lagoon in Mexico decreased with increasing trophic level (Mendoza-Carranza et al., 2016). Sediment Pb concentration was 20.86 ± 5.80 mg Pb/kg (mean ± S.D.), and the suspended load Pb concentration was 16.59 ± 2.79 mg Pb/kg in the San Pedrito Lagoon, which is impacted by wastewater discharge and petroleum extraction. Pb concentration was only above the limit of detection in two plant species, spider lily (*Hymenocallis littoralis*) and mangrove fern (*Acrostichum aureum*), and fish samples (2.9 mg Pb/kg) from the lagoon. In general, BCFs of Pb were low, and BCFs were higher in plants than in fish, suggesting trophic dilution.

Bioaccumulation, but not biomagnification, was found in a semiarid coastal lagoon in Sonora, Mexico along the Gulf of California (Jara-Marini et al., 2020). The community consists of primary producers (phytoplankton, algae, mangrove), primary consumers (zooplankton, barnacles, oysters, clams, snails, shrimp, crab, snapper, and juveniles of flathead mullet), secondary consumers (adults of flathead mullets, crab, snapper, mojarra, and grunt), and tertiary consumers (night herons, great blue herons, magnificent frigate, and cormorants). BMF was corrected for the trophic position, and the trophic BMF (TBMF) was estimated from the antilogarithm of the slopes of the linear correlation between the trophic level and the metal concentration. BMF and TBMF values above 1.0 indicate that a metal is being transferred upward through the trophic levels, while values below 1.0 indicate biodilution along the food web. Pb values in suspended particulate matter and sediment varied between seasons, ranging from 0.70 mg Pb/kg in autumn to 1.03 mg Pb/kg in winter. Pb concentrations among primary producers (range: 0.63 to 1.03 mg Pb/kg), secondary consumers (range: 0.80 to 1.53 mg Pb/kg), and most tertiary consumers did not vary seasonally. Only two tertiary consumers, neotropic cormorant (*Phalacrocorax brasilanus*) and magnificent frigatebird (*Fregata magnificens*), showed the highest Pb concentrations in the summer. Pb only showed a positive relationship between log-transformed Pb concentrations and trophic level in the summer. Pb concentrations generally decreased through the food web, depending on the season. The BMF ranged from 0.50 to 3.57 for Pb across organisms, and TBMF ranged from 1.02 to 1.15. Although TBMF values were above 1.0, biomagnification was unlikely because the relationship between trophic level and Pb concentration was only significantly positively correlated in the summer.

In addition to evidence from field studies, laboratory findings also suggest a decrease in the concentration of Pb with trophic transfer. In an 8-week feeding study, TTFs were calculated for sea snail (*Nassarius siquijorensis*) fed either venerid clams (*Ruditapes philippinarum*), mussels (*Perna viridis*), barnacles (*Fistulobalanus albicostatus*), or oysters (*Crassostrea. angulata*) collected from an intertidal zone in Xiamen, southeastern China (Guo et al., 2013). The net trophic transfer factor, which is the ratio of net accumulated metal concentrations over the experiment to metal concentrations in the prey was well below 1 for barnacles and 0 for oysters, clams, and mussels, suggesting biodilution in this system. Although not tested statistically, the variation in trophic transfer factors across prey species demonstrated prey-specific bioavailability.

Although most observational studies suggest biodilution of Pb occurs through marine food webs, Pb was found to bioaccumulate in mummichog (*Fundulus. heteroclitus*) in the Goose Pond estuary in Brooksville, Maine (Broadley et al., 2013). The Goose Pond estuary was impacted by the former Callahan Mine, which is one of the few documented open-pit hard-rock mining sites in an intertidal zone. The sediment concentration of Pb was above the probable effects level in some of the sample sites. BAFs ranged from 3.3 to 3.65 across Goose Pond sites and from 2.62 to 3.77 at the reference sites. The reference site values bioaccumulation factors were conservative as they included water and tissue Pb concentrations below the instrument detection limit. The sediment-to-*F. heteroclitus* and water-to-*F. heteroclitus* ratios were high for Pb. The ratio of metal enrichment to background levels (concentrations at the Goose Pond site adjacent to the tailings pile / the mean concentrations at a reference site) were 34.2 for sediment, 32.3 for water, and 45.6 for *F. heteroclitus*.

Environmental gradient field studies outside of North America provide additional evidence to support the biodilution of Pb in marine food webs. For example, trophic transfer of Pb was low in a seagrass food web in an estuarine lake in Australia, as the trophic level was negatively correlated with Pb concentration (Schneider et al., 2018). In another example, Pb concentrations decreased with increasing trophic level in a Mediterranean coastal lagoon (Vizzini et al., 2013) and in a small pelagic fish marine food web along a Mediterranean coast (Chouvelon et al., 2019). Finally, Pb accumulation was higher in invertebrates compared with higher trophic level species (fish) in an aquatic food web in Liaodong Bay, China (Radomyski et al., 2018).

In summary, studies published since the 2013 Pb ISA support findings in the ISA that Pb generally decreased with increasing trophic level in coastal and marine food webs, although some studies found evidence of bioaccumulation.

11.4.3 Environmental Concentrations of Pb in Saltwater Biota in the United States at Different Locations and Over Time

Studies of aquatic bivalves in coastal ecosystems can be used to reconstruct historical records of Pb concentrations. The NOAA Mussel Watch program has monitored pollutant trends since 1986 via periodic sampling of bivalve tissue (Mytilus species and *C. virginica* oysters) and sediment along the U.S. coastline (Kimbrough et al., 2008). In general, the highest concentrations of Pb are in bivalves in the vicinity of urban and industrial areas, and Pb is, on average, three times higher in coastal mussels than in oysters. Metal concentrations in *Mytilus californianus* were sampled at long-term biomonitoring sites off the coast of California from 1977 to 2010 (specific years vary by site) as part of the National Mussel Watch (NSW) (n = 35 sites) and California State Mussel Watch (CSMW) (n = 21 sites) (Melwani et al., 2014). Decreasing trends were observed at 11 NMW sites and 8 of the CSMW sites; no significant trends were found at the remaining sites. These observations show that Pb inputs to coastal aquatic ecosystems from runoff have decreased significantly, especially at sites off the coast of southern California near large municipal wastewater treatment facilities.

Quantification of chemical variation in relative presence of Pb and of other elements taken up and deposited in shells of marine organisms (sclerochronology) provides a temporal record of Pb deposition inputs to coastal environments. In a 2005 study of *Mercenaria* shells collected off the coast of Cape Lookout, North Carolina in 1980, 1982, 2002 and 2003, annual average Pb/Ca ratios were estimated from 1949 to 2002 using concentration measurements milled between the mollusk shell growth lines, which provide corresponding chronological measurements (Gillikin et al., 2005). Although high variability between samples was observed, overall Pb/Ca ratios in shells peaked near 1980 and decreased until the conclusion of the sampling in 2003. This study provides an indicator that reductions in Pb pollution resulted in decreased Pb inputs to aquatic ecosystems through runoff on the east coast. Elemental analysis of shell carbonate of the long-lived bivalve *Arctica islandica* collected off the coast of Virginia revealed a pattern of continuous increase in Pb concentration after 1910, reaching a peak in 1979 and declining after that date to pre-1930 values after 2000 (Krause-Nehring et al., 2012). The elevated shell Pb corresponded to the period of peak leaded gasoline use in the United States, with Pb deposition to the offshore site including atmospheric transport by easterly winds. Cariou et al. (2017) synthesized data from 15 studies from different geographic locations that quantified Pb in marine bivalve shells. They found that shell concentration had a strong relationship with the environmental level of local contamination; values in the shells, which ranged from 0.08 mg Pb/kg to 2 mg Pb/kg, were associated with environments with distant Pb sources including atmospheric deposition.

In addition to bivalve tissue and shell, heavy metals in horseshoe crab (*L. polyphemus*) eggs collected from breeding grounds on beaches along Delaware Bay provide some historical data for trend analysis. Horseshoe crab eggs collected in 1993, 1994, 1995, 1999, 2000, and 2012 showed a decline in Pb over time in a comparison of compiled data from the earlier surveys (1993, 1994, 1995) ($\bar{x}$ = 0.289 ± 0.068 mg Pb/kg) and to the data from 1999 to 2000 ($\bar{x}$ = 0.0353 ± 0.00496 mg Pb/kg) (Burger and Tsipoura, 2014). Some of the individual resampled sites showed a clear temporal decrease in Pb from 1993 to 2012, while at other locations, the temporal Pb concentration trend was more variable.

A study of migratory shorebird species in Delaware Bay compared feather Pb concentrations from the 1990s with samples from 2011 and 2012 (Burger et al., 2015). The decline of shorebirds migrating through Delaware Bay over the study period has been widely attributed to the reduced size of horseshoe crab populations, whose eggs the migratory birds feed on. Declining populations have been observed elsewhere in the shorebirds' ranges, and the authors investigated heavy metals as a driver of those declines. Across the time period studied, Pb concentrations increased in red knots (*Calidrus canutus*), decreased in semipalmated sandpipers (*Calidrus pusilla*), and did not change significantly in sanderlings (*Calidris alba*) (Burger et al., 2015). The authors noted that Pb concentrations observed in this study were below the known adverse effect risk levels for similar species.

In a decade-long biomonitoring study of metals in the muscle tissue of dolphinfish (*Coryphaena hippurus*) in the southern Gulf of California from 2006 to 2015, Gil-Manrique et al. (2022) found no temporal trend in Pb concentrations. However, a negative correlation was identified between sea surface temperature and Pb concentrations in dolphinfish during the decade-long biomonitoring study. Summary statistics of dolphinfish sampled in Gil-Manrique et al. (2022) are included in Table 11-1.

In long-term biomonitoring studies of saltwater ecosystems, there is some evidence of declining Pb concentrations, particularly in studies which began sampling before the 1990s. However, other studies document mixed results, with some observations of insignificant change or even increases in Pb concentrations.

11.4.4 Effects of Pb in Saltwater Systems

Saltwater taxa included in this section are broadly grouped into vegetation, microbes, invertebrates, and vertebrates. The biological effects of Pb in the 2013 Pb ISA and in this appendix are generally presented from suborganismal responses (i.e., enzymatic activities, changes in blood parameters) to endpoints relevant to the population level and higher (growth, reproduction, and survival) up to effects on ecological communities and ecosystems. Exposure-response studies that report toxicological dose descriptors (e.g., LC_{50}, EC_{50}, LOAEL) for effects on growth, reproduction or survival endpoints are reported in Section 11.4.5.

11.4.4.1 Effects on Saltwater Microbes

Microbial communities in saltwater ecosystems were not reviewed in detail in the 2006 Pb AQCD (U.S. EPA, 2006) or the 2013 Pb ISA (U.S. EPA, 2013). More recent experimental and observational studies reviewed here examine the relationship between Pb concentration in the sediment and saltwater and the effects on marine microbial communities. Pb was largely negatively or not associated with microbial community structure and abundance, although a few studies found positive associations between sediment Pb concentrations and microbial abundance.

Pb negatively affected microbial diversity and structure in rhizosediments of sea rush (*Juncus maritimus*) and the common reed (*P. australis*) collected from the Lima estuary, Portugal and exposed to a Pb gradient (Mucha et al., 2013). *Juncus maritimus* rhizosediment exhibited lower OTU number, diversity, evenness, and dominance in Pb-exposed sediments relative to the control. Similarly, *P. australis* rhizosediments exposed to Pb exhibited lower OTU number, diversity, and evenness than the control, whereas dominance was unaffected by Pb exposure. Both rhizosediment microbial community structures under 218 mg Pb/kg and 2180 mg Pb/kg were dissimilar to the controls and to one another.

Sediment Pb concentration was correlated with bacterial richness and evenness along a gradient of metal pollution in estuaries on the southeast Australian coast (Sun et al., 2012). The relationships between sediment heavy-metal content (Pb, Cr, Cu, Fe, Mn, Ni, Pb, and Zn), organic contaminants (polycyclic aromatic hydrocarbons), physicochemical variables (silt content and OM), water column environmental parameters (temperature, pH, and salinity) and bacterial community structure were explored using Automated Ribosomal Intergenic Spacer Analysis profiles across six sites with different degrees of anthropogenic disturbance. High collinearity existed between silt content and Cr, Ni, Zn, and Pb; therefore, only latitude, salinity, temperature, pH, %silt, Cu, and Zn were included in the analysis of bacterial community structure. Silt, which was highly correlated with Pb concentration, was the main driver of bacterial community structure, followed by temperature. Pb explained the highest relative proportion of variance in bacterial diversity (16.1% explained by Pb followed by 14.5% by Cu and 7.5% by silt), and bacterial diversity decreased with increasing Pb and Cu sediment concentration.

The relative abundances of certain bacterial groups were negatively correlated with the Pb sediment concentration of mangroves in southern China (Meng et al., 2021). Sediment Pb concentrations ranged from 0.142 ± 0.094 mg Pb/kg to 3.257 ± 0.094 mg Pb/kg (mean ± S.D.) across seven sites, and the mean Pb concentrations in surface sediments (0–5 cm) were higher than those in deep sediments (25–30 cm). Pb was significantly correlated with Zn sediment concentration. The abundance of the genus *Fusibacter* was negatively correlated to Pb, Zn, Cu, Co, Ni, Cd, and Ag with statistical significance, while *Syntrophorhabdus* was positively correlated with Pb. Among the >200 genera and functional genes involved in heavy-metal transportation, most bacteria associated with Pb elimination and transport demonstrated lower abundances compared with other genera.

Although some studies report negative correlations between Pb sediment concentration and bacterial community structure, other studies found no such relationship. For example, Pb sediment concentration was not correlated with bacterial community structure in the Jiaozhou Bay, China (Yao et al., 2017). Sediment samples were collected from inside Jiaozhou Bay and a site outside of the bay to achieve an environmental gradient of water quality.. The concentration of Pb varied among the three sites (mean ± S.D. site Shilaoren Beach: 19.09 ± 1.86 mg Pb/kg, site Haibohe estuaries: 38.65 ± 9.26 mg Pb/kg and site Licunhe estuaries: 72.87 ± 17.56 mg Pb/kg). The concentrations of Co, Zn, Hg, As, and Se explained more of the variation in bacterial community composition in this system, and Pb concentration was not one of the top three strongest predictors of bacterial diversity at any site.

In eastern Guangdong, China, marine microbial community diversity was not correlated with Pb content; however, Pb was significantly correlated with the abundance of a few dominant taxa (Zhuang et al., 2019). Pb in the sediment from the Shantou coastline ranged from 4.9 mg Pb/kg to 95.7 mg Pb/kg, with a mean value of 37.04 mg Pb/kg. The only significant correlations between bacterial diversity and abundance and environmental variables were total OC and Cr, and the correlation between Pb and microbial abundance and diversity was not significant. Although bacterial diversity and abundance were not correlated with Pb, the metal was significantly negatively correlated with the abundance of Nitrospirae and positively correlated with candidate phylum OD1. Additionally, sediment Pb concentration was significantly negatively correlated with a few dominant classes, including Epsilon-proteobacteria, Nitrospira, and Sva0725. Given there was a significant positive correlation between OD1 and all metals and a negative relationship between Nitrospirae and all metals and Pb was highly correlated with other heavy metals (As, Hg, Cu, Zn, and total OC), it is difficult to disentangle the sole effects of Pb on marine microbial communities.

Finally, Pb concentration in the water column did not affect bacterioplankton community composition in the Toulon Bay, France (Coclet et al., 2019). Mn, DOC, salinity, Cu, and Cd explained the most variation in the bacterioplankton community composition (range in variance: 1.01%–1.22%), while Pb concentration only explained 0.51% of the variance in bacterioplankton community composition. *Rhodobacteraceae*, SAR11 (Alphaproteobacteria), *Balneola* (Bacteroidetes), and *Synechococcus*

(Cyanobacteria) were negatively correlated with either Cd, Cu, Pb or Zn, while *Candidatus aquilina* (Actinobacteria) was positively correlated with Pb.

In summary, several experimental and observational studies since the 2013 Pb ISA (U.S. EPA, 2013) reported negative relationships between sediment or saltwater Pb concentration and microbial abundance and diversity (Meng et al., 2021; Mucha et al., 2013), while other studies found no relationship (Coclet et al., 2019; Zhuang et al., 2019; Yao et al., 2017).

11.4.4.2 Effects on Saltwater Plants and Algae

In the 2013 Pb ISA, evidence was inadequate to infer a causal relationship between Pb exposure and endpoints relevant to saltwater plants and algae (growth, survival, physiological stress) (Table 11-6). Key studies in the 2013 Pb ISA included a 72-hour EC_{50} for growth inhibition reported in the marine algae *Chaetoceros* sp. at 105 µg Pb/L (Debelius et al., 2009). A study with the microalga *Tetraselmis suecica* reported a statistically significant decrease in growth rate, total dry biomass and final cell concentration between control cultures and algae cultured in 20 µg Pb/L (Soto-Jiménez et al., 2011). Few data are available in prior Pb reviews for saltwater plant and algal species. Effects in plants, in general, are observed at concentrations of Pb that greatly exceed concentrations of this metal typically measured in soils, water and sediment (Table 11-1).

No new information is available on the effects of Pb in saltwater algae at levels that are within the concentrations of interest for this ISA (Section 11.1.1). There was, however, one new endpoint of note. In a novel assay designed to assess the effects of toxicants on algal swimming behavior, Pb was shown to inhibit motility in four saltwater algal motile species (Feng et al., 2016). The lowest EC_{10} for 2-hour algal swimming inhibition was 2.36 µM (488 µg Pb/L) in *Platymonas subcordiformis*; effects on the three other algal species tested were found at higher exposures. All exposures at which effects on swimming behavior were observed support previous findings of Pb toxicity to algae at concentrations that greatly exceed concentrations of Pb encountered in the natural environment.

There are a few studies on the effects of seepweeds (plants in the genus Suaeda found in saltmarshes) which support previous findings of Pb toxicity at higher exposures. For instance, significant negative effects on the growth of *S. heteroptera* were observed at concentrations of Pb higher than 400 mg Pb/kg (He et al., 2016). A study of metabolic biomarkers in *S. salsa* revealed that Pb exposure at 20 µg Pb/L could induce osmotic stress and disturbances in energy metabolism after long-term exposure for 1 month, whereas no effects were seen in the short term (1 week) (Wu et al., 2012b). Growth effects were not seen in a congeneric species, *S. fruitcosa*, even at exposures of 600 µM (125 mg Pb/L), though the study affirmed the plant nutrient content and activity of antioxidant enzymes were affected by metal stress at high levels of exposure (Bankaji et al., 2016). As in freshwater plants, Pb is concentrated in root tissue, but sensitivity is species-specific. In general, effects in saltwater plants are observed at much higher Pb exposures than are found in the natural environment.

11.4.4.3 Effects on Saltwater Invertebrates

No studies reporting effects of Pb in saltwater invertebrates were reviewed in the 1977 Pb AQCD or the 1986 Pb AQCD. In the 2006 AQCD, a few effects were noted in saltwater invertebrates including gender differences in sensitivity to Pb in copepods, increasing toxicity of Pb with decreasing salinity in mysids and effects on embryogenesis in bivalves (U.S. EPA, 2006). In the 2013 Pb ISA, available evidence was sufficient to be suggestive of a causal relationship between Pb exposure and the endpoints of physiological stress, hematological effects, reproduction, and development in saltwater invertebrates (U.S. EPA, 2013). For all other effects, the evidence was inadequate to assess causality (Table 11-6). New information for saltwater invertebrates since the 2013 Pb ISA includes additional studies that report physiological perturbations associated with Pb exposure, including a few observations in previously untested taxa. Only a few of the many studies identified in the literature search on suborganism-level responses to Pb exposure in saltwater invertebrates were conducted in the low µg Pb/L range and hence met the criteria for inclusion in the ISA (Section 11.1.1).

11.4.4.3.1 Suborganism-Level Response

The majority of studies in saltwater invertebrates do not link the effects reported at the molecular and cellular levels to effects at the organism level of biological organization (e.g., survival, growth, reproduction). One study in Tiger prawn (*Penaeus monodon)* exposed to a range of Pb concentrations (14 to 232 µg Pb/L) in seawater for 30 days reported an increase in lipid peroxidation starting at the 56 µg Pb/L exposure concentration. Chronic exposure yielded NOEC = 14 µg Pb/L and LOEC = 29 µg Pb/L for survival in this species (Hariharan et al., 2012).

For the endpoint of physiological stress, many studies from the 2013 Pb ISA, especially those that considered enzymatic responses to Pb exposure, were conducted with nominal Pb concentrations in mollusks. Since the 2013 Pb ISA, additional studies have explored the mechanisms of Pb-induced physiological stress in saltwater invertebrates by linking observed responses to changes in gene expression. Over the course of a 4-week exposure of the mussel *M. edulis* to 111.68 µg Pb/L (0.54 µM), transcripts involved in the unfolding protein response were differentially expressed with Pb, which correlated with the bioaccumulation of Pb in gill tissue (Poynton et al., 2014). In addition, a sequence of unknown function showed a statistically significant relationship with Pb concentration in gill tissue, and the authors proposed the sequence may be identified in the future as a dose-dependent Pb-specific biomarker in this species.

Physiological stress responses associated with Pb exposure were also observed in sediment bioassays with saltwater invertebrates. Biomarkers of oxidative stress, cellular damage and genotoxicity were measured in the benthic bivalve *A. trapezia* following 56-day exposure to Pb-spiked sediments (analytically verified concentration of 100 and 300 mg Pb/kg) (Taylor and Maher, 2012). Pb concentration in bivalves (1 mg Pb/kg for the low concentration and 12 mg Pb/kg for the high concentration) suggested low bioavailability from sediment, especially at the lower concentration. The total antioxidant capacity was statistically significantly reduced in both Pb treatments compared with the control. Lysosomal stability in hepatopancreas of Pb-exposed bivalves was significantly decreased, suggesting effects on cellular membrane integrity and function. In gill tissue, which is an important site for Pb uptake in bivalves, there was a statistically significant increase in both treatment groups in micronuclei frequency, a biomarker of genotoxicity. A similar suite of biomarkers was assessed in the deposit-feeding bivalve *T. deltoidalis,* also exposed to Pb-spiked sediments (100 and 300 mg Pb/Kg) (Taylor and Maher, 2014). In contrast to the filter-feeding *A. trapezia*, *T. deltoidalis* accumulated Pb to a concentration equal to that of the spiked sediment over the course of the experiment (28 days). Exposed *T. deltoidalis* individuals had significantly reduced total antioxidant capacity and significantly higher lysosomal destabilization and micronuclei frequency compared with control organisms.

Evidence in the 2013 Pb ISA was suggestive of a causal relationship between Pb exposure and hematological effects, primarily based on field studies that correlated ALAD activity to measured Pb levels in bivalve tissue (Company et al., 2011; Kalman et al., 2008). Generally, these studies have noted that Pb content varies significantly among species and is related to habitat and feeding behavior. A few additional studies have reported hematological effects in Pb-exposed saltwater invertebrates; however, at concentrations higher than the concentration of Pb typically encountered in seawater (Table 11-1). Duarte et al. (2020) demonstrated various sublethal biomarkers were activated during 28-day exposure at a low concentration of Pb (10.6 µg Pb/L) in the crab *U. cordatus*. This concentration was too low to inhibit ALAD activity in the crabs; however, metallothioneins were induced and DNA damage occurred in exposed individuals. Various immunotoxic endpoints were assessed in hemolymph of the marine crab *Charybdis japonica* during a 30-day exposure and subsequent quantification of Pb in tissue (Xu et al., 2019). At the lowest concentration, 0.066 µM (13.6 µg Pb/L) immune responses were not significantly different from control responses. At the next lowest concentration (0.132 µM, 27.2 µg Pb/L), there was an initial increase followed by a decrease in the total hemocyte count. Total hemocyte count was significantly lower than control counts at the end of the exposure duration.

11.4.4.3.2 Organism-Level Response

Saltwater invertebrate studies that report effects on growth, reproduction and development, and survival are primarily reviewed in the exposure-response section (Section 11.4.5). In sea hare (*A. californica)* exposed to Pb solely through diet over 2 or 3 weeks (green seaweed *U. lactuca* previously exposed to analytically verified concentration of either 10 µg Pb/L or 100 µg Pb/L for 48 hours), growth was significantly lower in the treatment groups compared with the control (Jarvis et al., 2015).

11.4.4.4 Effects on Saltwater Vertebrates

In the 2013 Pb ISA, there was inadequate evidence to infer causality relationships between Pb exposure and effects in saltwater vertebrates (Table 11-6). Few studies on saltwater vertebrates were reviewed in the 2013 Pb ISA or in the previous Pb AQCDs, especially for reproduction, growth, and survival (endpoints that may have relevance to the population level of biological organization and higher). Studies reviewed in the exposure-response section (Section 11.4.5, Table 11-7) of this appendix include chronic toxicity data for growth and survival endpoints in saltwater fish species published since the 2013 Pb ISA. Summarized below are recent studies that report Pb perturbation on physiological endpoints in fish and other saltwater vertebrates.

11.4.4.4.1 Fish

Most of the available studies in saltwater fish seek to identify molecular and cellular responses to Pb exposure and do not report effects at the organism level of biological organization (e.g., survival, growth, reproduction). Furthermore, studies since the 2013 Pb ISA that quantify effects on biomarkers in saltwater and euryhaline fish are typically conducted at Pb concentrations considerably higher than conditions found in natural environments. Nunes et al. (2014b) assessed the response of anadromous European eel (*A. anguilla*) to Pb exposure down to 165 µg Pb/L in 28-day aqueous exposure studies and observed no statistically significant effects on the biomarkers of neurotoxicity or peroxidative membrane damage. Only gill tissue GST activity was significantly increased at 165 µg Pb/L, and further increased with higher Pb concentration. Similar 28-day chronic bioassays were performed in juvenile turbot (*Scophthalmus maximus*). Very few significant effects were reported at the lowest concentration tested (291 µg Pb/L). Hepatic CAT activity significantly decreased, liver GST significantly increased and no measurable changes in biomarkers of neurotoxicity were observed (Nunes et al., 2014a). Fernández et al. (2015) evaluated the suitability of ALAD as a biomarker for Pb exposure in wild-caught red mullet (*Mullus barbatus*) along several locations of the Spanish coast. Pb concentration in muscle tissue was low. However, there was a weak, but significant, inverse relationship with ALAD activity; ALAD activity showed no statistically significant relationship to the condition factor, gonadosomatic index and hepatosomatic index of the fish.

Since the 2013 Pb ISA, a series of studies have further elucidated the effects of Pb exposure via diet on multiple physiological endpoints in saltwater fish, and these perturbations were linked to a decrease in weight gain (growth) in one study. In juvenile Korean rockfish (*Sebastes schlegelii*), biomarkers of oxidative stress (SOD, GST) were significantly increased, AChE was significantly decreased in muscle (Kim et al., 2017), and physiological stress indicators (heat shock protein 70 mRNA gene expression and plasma cortisol) were significantly increased (Kim and Kang, 2016), as were hematological parameters (hemocrit, hemoglobin) (Kim and Kang, 2015) by 4-week dietary Pb > 60 mg/kg in experimental diet formulation. This is consistent with dietary exposure in starry flounder (*Platichthys stellatus*), in which the same hematological parameters as well as red blood cell count were significantly decreased at 4-week dietary Pb exposure over 60 mg Pb/kg (Hwang et al., 2016). In rockfish, immune response was elicited at a higher dietary concentration (>120 mg Pb/kg) at 4 weeks (Kim and Kang, 2016). A decrease in daily weight gain was observed in rockfish at >120 mg Pb/kg (Kim and Kang, 2015). A dietary intake above 60 mg Pb/kg daily after 4 weeks of exposure to Pb appeared to be the threshold for most effects.

11.4.4.4.2 Other Saltwater Vertebrates

There are a few new studies of nonfish saltwater vertebrates that report blood lead levels (BLLs) and associated effects. In a survey of blood Pb levels in common eider ducks (*Somateria mollissima*) at a breeding colony in the northern Hudson Bay, birds with higher BLLs had lower body condition indexes (body mass/head length) when they arrived at the breeding grounds (Provencher et al., 2016). Birds with higher BLLs arrived later at the breeding grounds. Birds that arrive later at the breeding grounds and with lower body condition indexes are more likely to have lower reproductive success. A study of loggerhead sea turtles *(Caretta caretta*) in Casey Key, Florida examined the connection between blood Pb concentrations and hematological effects (Perrault et al., 2017). Over a range of blood Pb levels (0.07–0.52 µg/g dry weight), there was a significant negative relationship between BLLs and albumin, α2-globulins, total solids, and Fe.

11.4.5 Exposure and Response of Saltwater Species

Evidence regarding exposure-response relationships and potential thresholds for Pb effects on saltwater biota can provide tools for quantitative analyses of risks for coastal saltwater ecosystems. No exposure-response studies in saltwater algae or vertebrates, and very few studies on saltwater invertebrates, were reported in the 1977, 1986 or 2006 Pb AQCDs. For saltwater invertebrates, available evidence at the time of the 2013 Pb ISA was suggestive of a causal relationship between Pb exposure and reproductive and developmental effects (U.S. EPA, 2013). Much of the evidence was from exposure-response bioassays.

Since the 2013 Pb ISA, new toxicity data for saltwater algae, invertebrates and fish have been reported based on analytically verified Pb concentration. This information reduces uncertainties identified in the previous review in terms of a lack of exposure-response data for saltwater biota, especially for chronic toxicity, and enables calculations of effect levels for saltwater biota based on experimental data (Church et al., 2017). The studies listed in Table 11-7 are those that report exposure-response values at concentrations comparable to, or lower than, the most sensitive saltwater biota identified in the 2013 Pb ISA or the 2006 AQCD (i.e., the most environmentally relevant studies). Exposure-response data from previously untested taxonomic groups are also discussed in this section. In general, marine organisms are tolerant of Pb at much higher concentrations than those encountered in uncontaminated natural environments.

In studies reviewed in the 2013 Pb ISA, marine algae exhibited a range of sensitivity to Pb, with a 72-hour EC_{50} of 105 µg Pb/L reported for *Chaetorceros* spp. Other tested species were considerably less sensitive (72-hour EC_{50} = 740 µg Pb/L or higher) (Debelius et al., 2009). Exposure-response data for marine algal species published since the 2013 Pb ISA greatly exceed environmental concentrations; for example, in the marine alga *Nannochloropsis oculata,* the 72-hour IC_{50} = 1,810 µg Pb/L for growth inhibition (Zamani-Ahmadmahmoodi et al., 2020). Longer-term exposure studies assessing the population growth rates of polar marine algal species have reported effects as low as 24-day EC_{10} = 152 µg Pb/L for *Cryothecomonas armigera* (Koppel et al., 2017) and a 10-day IC_{10} = 260 µg Pb/L for *Phaeocystis antarctica* (Gissi et al., 2015).

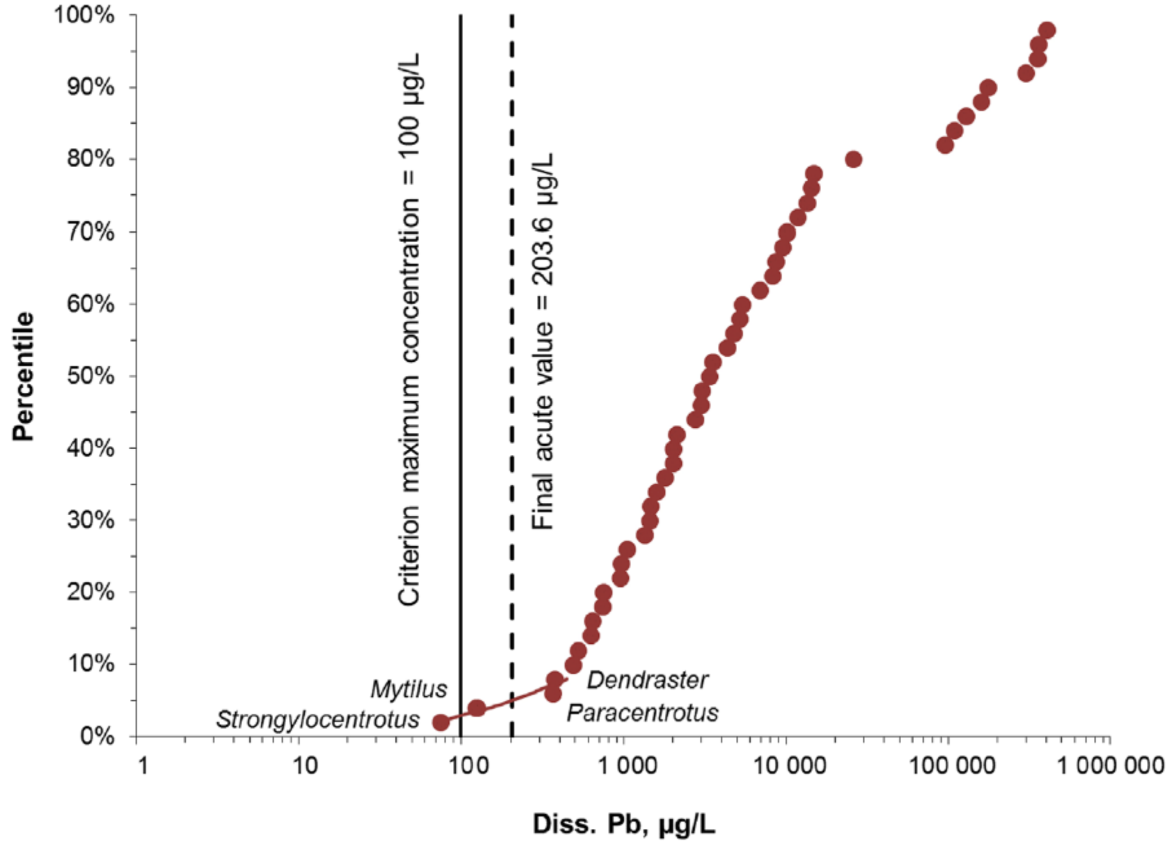
In the 2013 Pb ISA, studies that reported effect concentrations in saltwater invertebrates included a delay in reproduction onset in the marine amphipod, *E. laevis,* at 118 mg/Pb kg sediment, a concentration the authors indicated was below the current marine sediment regulatory guideline for Pb (218 mg Pb/kg sediment) (Ringenary et al., 2007; NOAA, 1999). A 96-hour EC_{50} = 197 µg Pb/L for the growth of larvae and EC_{50} = 297 µg Pb/L for embryogenesis inhibition was observed for the clam *Meretrix meretrix* (Wang et al., 2009). Another study reported a decrease in the fertilization rate of eggs of the marine polycheate *Hydroides elegans*; in eggs pretreated with 48 µg Pb/L, hatching decreased to 20% of control levels (Gopalakrishnan et al., 2008). The lifestages of *H. elegans* varied in their sensitivity to Pb, with the most sensitive period being larval settlement, with an EC_{50} of 100 µg Pb/L. In the 2013 Pb ISA, the most sensitive endpoint for growth in a saltwater invertebrate was LOAEL = 85 mg Pb/kg in sediment in the polychaete Capitella sp. (Horng et al., 2009). Other saltwater invertebrate exposure-response studies in the 2013 Pb ISA reported effects at higher Pb concentrations. In the 2006 AQCD, the most sensitive endpoint was a 48-hour EC_{50} = 221 µg Pb/L and LOEC = 50 µg Pb/L for embryogenesis in the mussel *M. galloprovincialis* (based on nominal Pb concentration only) (Beiras and Albentosa, 2004).

Recent exposure-response data for saltwater invertebrates include reproductive and developmental bioassay results based on analytically verified concentrations for mollusks and echinoderms, with effects reported at lower concentrations than in studies included in the 2013 Pb ISA (Table 11-7). Embryo development of the scallop *Argopecten purpuratus* was impaired with Pb exposure, with the 48-hour EC_{50} reported as = 44 µg Pb/L (Romero-Murillo et al., 2018). The order of sensitivity of 10 marine bivalve species (based on the percentage of normal D-veliger larvae assessed at 48 hours of Pb exposure) was oysters > mussels ≥ scallops ≥ cockles ≥ clams (Markich, 2021). The oysters *M. gigas* (48-hour EC_{50} = 49.5 µg Pb/L, 48-hour NEC = 9.9 µg Pb/L) and *S. glomerata* (48-hour EC_{50} = 52.1 µg Pb/L, 48-hour NEC = 10.1 µg Pb/L) were most sensitive while the clam *Irus crenatus* (48-hour EC_{50} = 196 µg Pb/L, 48-hour NEC = 39.8 µg Pb/L) was the least sensitive bivalve tested. In a series of bioassays, Nadella et al. (2013) assessed Pb effects on embryo development in two mussels, *M. galloprovincialis* and *M. trossolus,* and the sea urchin *S. purpuratus*. Both mussel species exhibited similar acute toxicity to Pb in 48-hour embryo-larval toxicity tests in 100% seawater (*M. galloprovincialis*-EC_{50} = 63 µg Pb/L, EC_{20} = 19 µg Pb/L; EC_{10} = 10 µg Pb/L, NOEC = 3.2 µg Pb/L and *M. trossolus*, EC_{50} = 45 µg Pb/L; EC_{20} = 16 µg Pb/L; EC_{10} = 9 µg Pb/L; NOEC = 3.4 µg Pb/L). In the 72-hour embryo-larval toxicity test in the sea urchin *S. purpuratus,* the EC_{50} = 74 µg Pb/L, EC_{20} = 31 µg Pb/L, EC_{10} = 19 µg Pb/L and NOEC = 2.7 µg Pb/L. In a similar 72-hour larval development toxicity test with the sea urchin *Evechinus chloroticus,* the EC_{50} = 52.2 µg Pb/L, with skeletal abnormalities observed in the lower range of concentrations (10 µg Pb/L and 20 µg Pb/L) (Rouchon and Phillips, 2017). However, Pb in the exposure water was not analytically verified in the study. Developmental endpoints in oyster *C. gigas* were less sensitive to Pb, with EC_{50} = 660.3 µg Pb/L for embryo toxicity, 96-hour LC_{50} = 699.5 µg Pb/L for larval mortality and LOEC = 96.7 µg Pb/L for significant increase of abnormal D-shaped larvae (Xie et al., 2017). In a series of fertilization bioassays with the marine polychaete broadcast spawner *Galeolaria caespitosa*, the EC_{10} for reproduction varied with the density of sperm used in the bioassays and ranged from 65 to 910 µg Pb/L. The toxicity of Pb was significantly decreased at higher sperm density (Lockyer et al., 2019). The EC_{10} was calculated to be 30 µg Pb/L at a sperm density required to achieve 50% of the maximum fertilization.

New exposure-response data on previously untested marine invertebrate taxa, including species of corals and sea anemones, generally show that these organisms are tolerant to Pb at relatively high concentrations. Hédouin et al. (2016) assessed survival in adult and larval stages of the Scleractinian coral *Pocillopora damicornis*. Results from 96-hour acute toxicity testing in adults collected during two seasons near Oahu, Hawaii (summer 96-hour LC_{50} = 742 µg Pb/L, winter 96-hour LC_{50} = 477 µg Pb/L) and coral larvae tested in the laboratory at two temperatures (96-hour LC_{50} = 681 µg Pb/L at 27°C, 96-hour LC_{50} = 462 µg Pb/L at 30°C) showed similar tolerance to Pb. In Cnidarian (sea anemone) *Aiptasia pulchella*, the 96-hour LC_{50} values were 8,060 µg Pb/L and 12,400 µg Pb/L in two separate tests. In the same species, the 6-hour EC_{50} = 2,610 µg Pb/L and 12-hour EC_{50} = 1,740 µg Pb/L for rapid tentacle retraction, suggesting that anemones are tolerant to Pb, even at concentrations that greatly exceed that of Pb in seawater (Howe et al., 2014). In contrast, a 30-day exposure to Pb in the marine Tiger prawn *P. monodon* yielded NOEC = 14 µg Pb/L and LOEC = 29 µg Pb/L for survival, suggesting that these crustaceans are relatively sensitive to Pb (Hariharan et al., 2012). A recent review of Pb effects on marine invertebrates by Botté et al. (2022) summarizes many of the effect concentrations and studies described above.

For vertebrates, several studies published since the 2013 Pb ISA provide chronic toxicity data for saltwater fish species, information that was previously lacking for evaluating the longer-term effects of Pb on these organisms. Reynolds et al. (2018) conducted 28-day chronic toxicity tests with larval topsmelt *A. affinis* (a fish species native to the coast of the western United States) at two salinities (14 ppt and 28 ppt) to represent conditions in estuarine and marine environments. In the larval fish, survival was affected to a greater extent at the lower salinity (LC_{50} = 15.1 µg Pb/L, NOEC <13.8 µg Pb/L) than at the higher salinity (LC_{50} = 79.8 µg Pb/L, NOEC = 45.5 µg Pb/L) due to the higher fraction of Pb in the form of Pb^{2+} at lower salinity. Growth effects (assessed as standard length) were reported in the same study, with greater response observed at the lower salinity (EC_{10} = 16.4 µg Pb/L) compared with the higher salinity (EC_{10} = 82.4 µg/L). Tests conducted with juvenile topsmelt at 28 ppt (28-day LC_{50} = 167.6 µg Pb/L) showed that this lifestage was less sensitive to Pb than the larval stage (28-day LC_{50} = 79.8 µg Pb/L). The authors observed abnormal swimming and morphology, but these endpoints were not quantified. Calculated chronic values for additional saltwater fish species include NOEC = 14 µg Pb/L and LOEC = 29 µg Pb/L for grey mullet (*M. cephalus*) fingerling survival and NOEC = 11 µg Pb/L and LOEC = 22 µg Pb/L for Tiger perch (*T. jarbua*) fingerling survival following 30-day exposure to Pb (Hariharan et al., 2016). The 96-hour LC_{50} values in these species were 2,570 and 2,990 µg Pb/L, respectively.

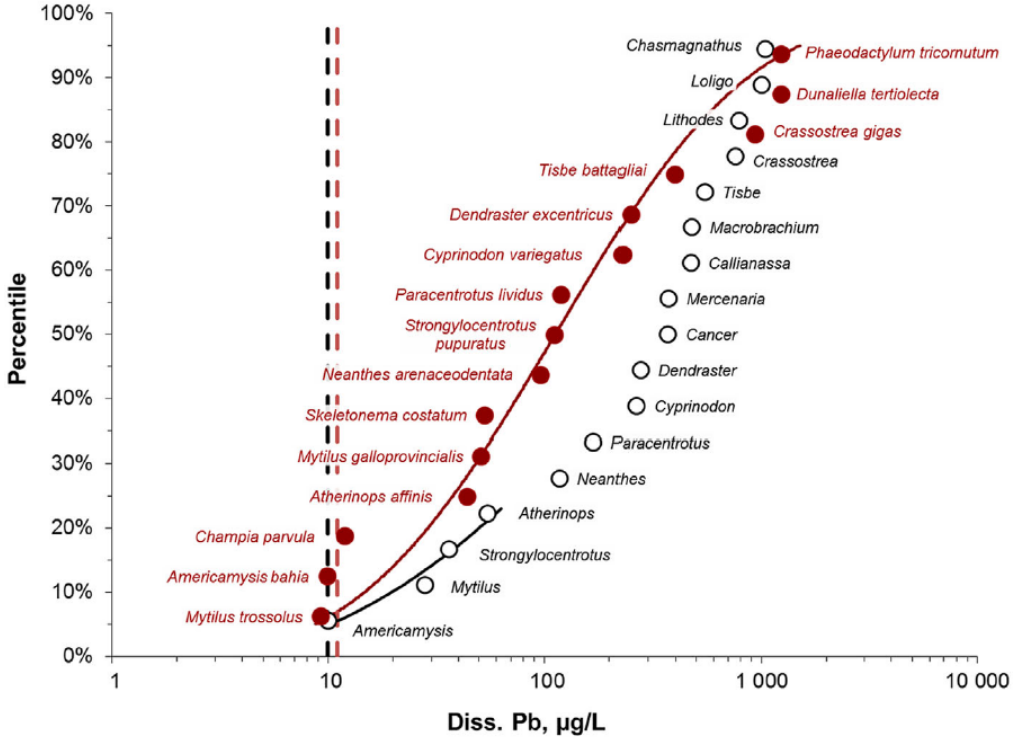
Given the increased availability of toxicity data for saltwater biota since development of the AWQC for Pb by the U.S. EPA Office of Water in 1984 (U.S. EPA, 1985a) (Section 11.1.7.3), Church et al. (2017) recently proposed updated U.S. saltwater acute AWQC of 100 µg Pb/L (acute) and chronic AWQC of 10 µg Pb/L (chronic) based on genus mean toxicity values following U.S. EPA methodology (U.S. EPA, 1985b). For the acute genus sensitivity distribution (Figure 11-6), data from 54 species and 49 genera were included. The proposed value of 100 µg Pb/L is less than the current acute criterion of 210 µg Pb/L due to toxicity data from relatively sensitive early lifestages of Echinodermata and Mollusca. Although Church et al. (2017) derive regulatory values using SSD approaches, it is noted that some of the toxicity values used in their analyses are from data sources not included in the ISAs (i.e., unpublished reports, university theses, memoranda).



Dissolved Pb and triangular distribution fit to the four lowest genus mean acute values following U.S. EPA guidelines. Genus mean acute values (red circles); solid curved line = triangular distribution; dashed vertical line = final acute value; solid vertical line = criterion maximum concentration (proposed acute criterion); black text = genera associated with genus mean acute values. Source: Church et al. (2017).

Figure 11-6 Acute genus sensitivity distribution for saltwater biota from Church et al. (2017).

The proposed Church et al. (2017) chronic value of 10 µg Pb/L for saltwater (based on EC_{20} or, in some cases, EC_{50} data divided by a factor of two when EC_{20} data could not be calculated from available data) is based on data for 21 species and 17 genera. The four lowest genus mean chronic values were 10 µg Pb/L for a mysid, 28 µg Pb/L for blue mussel (Mytilis spp.), 36 µg Pb/L for purple sea urchin (*S. purpuratus*), and 55 µg Pb/L for topsmelt (*A. affinis)*. In their derivations of acute and chronic values, Church et al. (2017) included some non-North American species. If the analysis was limited to North American biota, the proposed acute and chronic values would be 110 µg Pb/L and 8.8 µg Pb/L, respectively. Comparison of chronic sensitivity distributions in saltwater biota for dissolved Pb following U.S. EPA and European Union methods is shown in Figure 11-7. Following the publication of these proposed values, Reynolds et al. (2018) conducted additional testing with topsmelt larvae (LC_{20} = 10.7 µg Pb/L at 14 ppt salinity).



Species mean chronic values (European Union method) are shown in red circles; genus mean chronic values (U.S. EPA method) are shown in open circles; solid red curve = Weibull distribution fitted to species mean chronic values; solid black curve = triangular distribution fit to the four most sensitive genus mean chronic values; dashed red vertical line = median 5th percentile hazardous concentration based on Weibull distribution; dashed black vertical line = criterion continuous concentration (proposed chronic criterion); black text = genera associated with genus mean chronic values; red text = species associated with species mean chronic values.
Source: Church et al. (2017).

Figure 11-7 Comparison of chronic sensitivity distributions in saltwater biota for dissolved Pb following the U.S. EPA and European Union methods.

Table 11-7 Studies in saltwater biota with analytically verified Pb concentration that report an effect on growth, reproduction, or survival comparable to, or lower than, the lowest effect concentrations reported in previous Pb AQCDs or the 2013 Pb ISA

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
Invertebrates						
Mussel *(Mytilus galloprovincialis)* Mussel *(Mytilus trossulus)* *Sea urchin (Strongylocentrotus purpuratus)*	Nominally 3.2, 10, 32, 100, 320,1,000 µg Pb/L (concentrations were measured for each individual assay)	Standard embryo development acute toxicity tests for larvae of mussel (to 48-hr postfertilization) and sea urchin (to 72-hr postfertilization) conducted using ASTM protocols in 100% sea water	DOC: 1.79 ± 0.02 mg/L Additional toxicity tests were conducted with added DOC Salinity: 33 ppt Developmental assays conducted over a range of salinities from 15 to 33 ppt Temperature: 20°C ± 1°C for mussels 15°C ± 1°C for sea urchin	**Reproduction:** Development of larvae: The percentage of embryos exhibiting normal development was assessed after 48-hr (mussels) or 72-hr (sea urchin) exposure to Pb at varying concentration in seawater. The acute toxicity of Pb was similar between the two species of mussel and sea urchin	**M. galloprovincialis** 48-hr EC_{50} = 63 µg Pb/L 48-hr EC_{20} = 19 µg Pb/L 48-hr EC_{10} = 10 µg Pb/L 48-hr NOEC = 3.2 µg Pb/L **M. trossolus** 48-hr EC_{50} = 45 µg Pb/L 48-hr EC_{20} = 16 µg Pb/L 48-hr EC_{10} = 9 µg Pb/L 48-hr NOEC = 3.4 µg Pb/L **S. purpuratus** 72-hr EC_{50} = 74 µg Pb/L 72-hr EC_{20} = 31 µg Pb/L 72-hr EC_{10} = 19 µg Pb/L 72-hr NOEC = 2.7 µg Pb/L	Nadella et al. (2013)
Scallop *(Argopecten purpuratus)*	7 (control), 25, 50, 100, 140, 570, 730,1,000, 1,590 µg Pb/L (measured)	48-hr embryo-larval development assay with Pb-nitrate conducted in 100% sea water. In addition, a 96-hr acute toxicity test was	Salinity: 35 ppt pH:	**Reproduction:** Embryos exhibited abnormal development (impaired D-larvae	Embryo: 48-hr EC_{50} = 44 µg Pb/L Juvenile:	Romero-Murillo et al. (2018)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
		conducted with juveniles (21 mm in shell length)	8.0 Temperature (embryo exposure) 19°C ± 1°C	development) following Pb exposure. **Survival:** Assessed in juvenile lifestage only	96-hr LC_{50} = 1,420 µg Pb/L	
Oyster (*Magallana gigas*) Oyster (*Saccostrea glomerata*) Mussel (*Xenostrobus securis*) Scallop (*Scaeochlamys livida*) Cockle (*Anadara trapezia*) Cockle (*Fulvia tenuicostata*) Clam (*Hiatula alba*) Clam	Each test with 1.5 to 2-hr-old embryos (8-cell stage) consisted of a control and 12 metal concentrations (based on preliminary range-finding tests). Concentrations were analytically verified but not reported	48-hr embryo-larval development assay with Pb-nitrate conducted in 100% sea water. Test waters were not renewed, and embryos were not fed. Percentage of normal D-veliger larvae was determined by direct observation of 100 larvae (per replicate)	Salinity: 30 ppt ± 0.5% pH: 7.85 ± 0.05 Temperature: 21°C ± 1°C DO: 80% to 95% saturation	**Reproduction:** Embryos exhibited abnormal development (impaired D-larvae development) following Pb exposure. The order of sensitivity of the bivalves to Pb was oysters > mussels ≥ scallops ≥ cockles ≥ clams	***M. gigas*** 48-hr EC_{50} = 49.5 µg Pb/L 48-hr NEC = 9.9 µg Pb/L ***S. glomerata*** 48-hr EC_{50} = 52.1 µg Pb/L 48-hr NEC = 10.1 µg Pb/L ***X. securis*** 48-hr EC_{50} = 59.9 µg Pb/L 48-hr NEC = 12 µg Pb/L ***S. livida*** 48-hr EC_{50} = 67.2 µg Pb/L 48-hr NEC = 13.7 µg Pb/L ***A. trapezia*** 48-hr EC_{50} = 84.9 µg Pb/L	Markich (2021)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
(*Barnea australasiae*)					48-hr NEC = 16.8 µg Pb/L	
Clam (*Spisula trigonella*)					***F. tenuicostata*** 48-hr EC_{50} = 108 µg Pb/L	
Clam *(Irus crenatus*)					48-hr NEC = 22.3 µg Pb/L	
					H. alba 48-hr EC_{50} = 129 µg Pb/L 48-hr NEC = 24.8 µg Pb/L	
					B. australasiae 48-hr EC_{50} = 140 µg Pb/L 48-hr NEC = 28 µg Pb/L	
					S. trigonella 48-hr EC_{50} = 177 µg Pb/L 48-hr NEC = 36.7 µg Pb/L	
					I. crenatus 48-hr EC_{50} = 196 µg Pb/L 48-hr NEC = 39.8 µg Pb/L	

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	**Reference** (Published since the 2013 Pb ISA)
Prawn *(Penaeus monodon)*	1.7 (control-lab seawater used in bioassays), 14, 29, 56, 108, 230 µg Pb/L (measured)	Post larvae were exposed to Pb acetate for 30 days in a continuous flow-through system. Prawns fed twice daily and Pb concentrations measured every 10 days	Salinity: 27.7 ± 0.5 ppt Temperature: 25.4 ± 0.7°C DO: 6.3 ± 0.6 mg/L pH: 7.1 ± 0.5	**Survival:** Survival of *P. monodon* was significantly decreased at the higher exposure concentrations	30-d NOEC = 14 µg Pb/L 30-d LOEC = 29 µg Pb/L	Hariharan et al. (2012)
Vertebrates						
Topsmelt *(Atherinops affinis)*	**Measured: Mean ± SD** Low salinity, larval fish: Total Pb BDL, 17 ± 1 µg Pb/L, 34 ± 1 µg Pb/L, 69 ± 4 µg Pb/L, 85 ± 15 µg Pb/L, 127 ± 16 µg Pb/L Dissolved Pb BDL, 14 ± 1 µg Pb/L, 27 ± 2 µg Pb/L, 51 ± 3 µg Pb/L, 80 ± 7 µg Pb/L, 117 ± 19 µg Pb/L High salinity, larval fish: Total Pb BDL, 58 ± 9 µg Pb/L, 107 ± 20 µg Pb/L, 200 ± 14 µg Pb/L,	Larval fish (≤ 3 day old) were tested in two different salinities (14 ppt and 28 ppt) in 28-day exposures to Pb nitrate administered in a flow-through test system set to replace the total volume of synthetic seawater in each 2-L exposure chamber replicate once every 12 h. In addition, a 28-d exposure was conducted with juvenile fish (2.5 mo old) at 28ppt at higher Pb concentration (control, 100 and 200 µg Pb/L)	**Low Salinity larval fish:** Salinity 14.1 ± 0.1 ppt Temperature: 18.2 ± 0.3°C Alkalinity: 58 ± 5 mg/L as $CaCO_3$ pH: 7.96 ± 0.17 DO: 7.58 ± 0.39 **High Salinity larval fish:** Salinity 28.1 ± 0.6 ppt Temperature: 18.1 ± 0.2°C	**Survival:** Pb was consistently more toxic to larva fish at the lower salinity (14 ppt) compared with the higher salinity and larvae were more sensitive than juvenile fish at 28 ppt. Free Pb^{2+} ion concentrations, the most bioavailable form of Pb, were higher in the lower salinity water based on Pb speciation calculations. **Growth:** Growth effects in larval fish (assessed as	28-d survival of larval fish at 14 ppt salinity LC5 = 7.7 µg Pb/L LC_{10} = 8.3 µg Pb/L LC15 = 9.9 µg Pb/L LC_{20} = 10.7 µg Pb/L LC25 = 11.5 µg Pb/L LC40 = 13.6 µg Pb/L LC_{50} = 15.1 µg Pb/L NOEC = <13.8 µg Pb/L LOEC = 13.8 µg Pb/L 28-day survival of larval fish at 28 ppt salinity LC5 = 36.6 µg Pb/L LC_{10} = 43.4 µg Pb/L LC15 = 48.8 µg Pb/L LC_{20} = 53.5 µg Pb/L LC25 = 58.0 µg Pb/L	Reynolds et al. (2018)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
	386 ± 43 µg Pb/L, 563 ± 45 µg Pb/L Dissolved Pb BDL, 46 ± 10 µg Pb/L, 90 ± 20 µg Pb/L, 171 ± 22 µg Pb/L, 259 ± 24 µg Pb/L, 435 ± 48 µg Pb/L High salinity juvenile fish: Total Pb BDL, 154 ± 67 µg Pb/L, 239 ± 98 µg Pb/L Dissolved Pb BDL, 100 ± 21 µg Pb/L, 190 ± 30 µg Pb/L		Alkalinity: 105 ± 8 mg/L as $CaCO_3$ pH: 7.92 ± 0.07 DO: 6.88 ± 0.60	standard length) were more pronounced at the lower salinity (EC_{10} = 16.4 µg Pb/L) compared with the higher salinity (EC_{10} = 82.4 µg Pb/L)	LC40 = 70.8 µg Pb/L LC_{50} = 79.8 µg Pb/L NOEC = 45.5 µg Pb/L LOEC = 89.9 µg Pb/L 28-day survival of juvenile fish at 28 ppt salinity LC5 = 105.3 µg Pb/L LC_{10} = 110.9 µg Pb/L LC15 = 116.8 µg Pb/L LC_{20} = 123 µg Pb/L LC25 = 129.5 µg Pb/L LC40 = 151.2 µg Pb/L LC_{50} = 167.6 µg Pb/L 28-d EC_{10} for larval growth (standard length) at 14ppt salinity = 16.4 µg Pb/L 28-d EC for larval growth (standard length) at 28 ppt salinity = 82.4 µg Pb/L	
Grey mullet *(Mugil cephalus)* Tiger perch *(Terapon jarbua)*	7, 16, 34, 65, 136 µg Pb/L (*M. cephalus*) 7,15,29,60,118 µg Pb/L (*T. jarbua*)	Wild-caught fingerlings (3.0–4.5 cm in size) were acclimated to laboratory conditions then exposed to Pb as Pb acetate in a continuous flow-through system for 30d	Salinity: 33.5 ± 1.4 ppt Temperature: 23.5 ± 0.9°C pH: 7.8 ± 0.5	**Survival**: Survival of *M. cephalus* and *T. jarbua* decreased with the increase in exposure concentrations	Grey mullet: 30-d NOEC = 14 µg Pb/L 30-d LOEC = 29 µg Pb/L Tiger perch:	Hariharan et al. (2016)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	**Reference** (Published since the 2013 Pb ISA)
			DO: 6.5 ± 0.6		30-d NOEC = 11 µg Pb/L 30-d LOEC = 22 µg Pb/L	
54 species and 49 genera of invertebrates and fish (acute toxicity data included in derivation of proposed updated acute saltwater quality criterion for Pb) 21 species and 17 genera of invertebrates and fish (chronic toxicity data included in derivation of proposed updated chronic saltwater quality criterion for Pb)	Pb was analytically verified in all studies	U.S. EPA guidelines (U.S. EPA, 1985b) were used to identify acceptable studies. Acute: All included assays were embryo-larval toxicity studies reporting 48 to 96-hr EC_{50}s. The four lowest genus mean acute values (*Strongylocentrotus purpuratus* = 75 µg Pb/L; *Mytilus* spp = 123 µg Pb/L; *Paracentrotus lividus* = 363 µg Pb/L; and *Dendraster excentricus* = 371 µg Pb/L) and a total of 49 genus mean values were used to determine a final acute value of 203.6 µg Pb/L. This value was divided by 2 to derive the proposed acute criterion based on U.S. EPA methods. Chronic: Based on EC_{20} from lifecycle tests or EC_{50} data divided by a factor of 2 when EC_{20} data could not be calculated and augmented with 48-hr toxicity data in some cases. The four lowest genus mean chronic values (*Americamysis bahia* = 10 µg Pb/L; *Mytilus* spp. = 28 µg Pb/L;		**Acute toxicity endpoints included survival, immobilization, and embryo-larval development** The proposed updated acute criterion is lower than the current U.S. EPA acute saltwater criterion of 210 µg Pb/L due to embryo-larval toxicity tests with sensitive echinoderm and mussel species. **Chronic toxicity endpoints included survival, growth, development, and reproduction** The proposed updated chronic criterion is greater than the current U.S. EPA acute saltwater criterion of 8.1 µg Pb/L. Uncertainty in the derivation of the	Proposed Saltwater Acute Water Quality Criterion: 100 µg Pb/L. Limiting the derivation to North American species, the proposed criterion is 110 µg Pb/L. Proposed Saltwater Chronic Water Quality Criterion: 10 µg Pb/L. Limiting the derivation to North American species, the proposed criterion is 8.8 µg Pb/L	Church et al. (2017)

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
		Strongylocentrotus purpuratus = 36 µg Pb/L; *Atherinops affinis* = 55 µg Pb/L) and a total of 17 genus mean values were used to identify a chronic 5th percentile of 10 µg Pb/L following U.S. EPA guidelines		chronic criterion has decreased due to increased availability of studies; an acute-to-chronic ratio was not used.		

ASTM = American Society for Testing and Materials; BDL = below the method detection limit; $CaCO_3$ = calcium carbonate; DO = dissolved oxygen; DOC = dissolved organic carbon; EC = effect concentration; LC = lethal concentration; LOEC = lowest observed effect concentration; mo = month(s); NEC = no-effect concentration; NOEC = no-observed-effect concentration; Pb = lead.

11.4.6 Saltwater Community and Ecosystem Effects

As discussed in the 1986 Pb AQCD (U.S. EPA, 1986), the 2006 Pb AQCD (U.S. EPA, 2006) and the 2013 Pb ISA (U.S. EPA, 2013), the body of evidence was inadequate to infer a causal relationship between Pb exposure and saltwater community- and ecosystem-level effects. Observations from field studies in the 2006 Pb AQCD and the 2013 Pb ISA found either negative or null relationships between Pb and species abundance, richness, and diversity in saltwater macroinvertebrates; however, Pb was not the only contaminant in most observational studies, making it difficult to separate the effects of Pb from those of other metal pollutants. New studies published since the 2013 Pb ISA examined the relationship between Pb in sediment and saltwater as well as the community effects. Several reported negative or null relationships between sediment Pb concentrations and foraminiferal abundance and community structure, while others reported positive associations.

Foraminiferal diversity and community structure via changes in the abundance of certain taxa have been found to vary with sediment Pb along environmental gradients in various locations including in the Pearl River estuary, China (Li et al., 2013), the Ria de Aveiro lagoon, Portugal (Martins et al., 2011), the San Jose Bay estuary, Puerto Rico (Martínez-Colón et al., 2018), the Gulf of Milazzo, Sicily, Italy (Cosentino et al., 2013), the Strait of Malacca, Malaysia (Minhat et al., 2020) and Chilika lagoon in India (Barik et al., 2022).

In the Pearl River estuary, surface sediment OC, grain size and benthic foraminifera communities were assessed (Li et al., 2013). Mean ± S.D. sediment Pb concentrations in the study area were 36.98 ± 11.18 mg Pb/kg (range: 13.5–62.9 mg Pb/kg). Trace metal concentrations in the sediment of Pb, Cu, Co, Cr, Ni, V, and Zn were negatively correlated with the Shannon-Weaver index, Fisher α index, species richness, and abundance of certain foraminiferal species. The CCA demonstrated that Pb explained 7.5% of variation in the foraminiferal community.

Some foraminifera taxa were found to positively correlate with bioavailable Pb in the channels of Ria de Aveiro, Portugal, but diversity was unaffected by bioavailable Pb (Martins et al., 2011). The concentrations of Pb in the sediment in the resistant mineralogical phase, adsorbed by clay minerals, and associated with OM ranged from about 20 mg Pb/kg to 180 mg Pb/kg. There was a positive correlation between total bioavailable concentrations of Pb in the sediment (the fraction absorbed by clay and OM and coprecipitated with carbonates) and the abundance of miliolids, and bioavailable Pb was not significantly correlated with the abundance of *Ammonia tepida, Bulimina* spp., *Bolivina* spp., *Haynesina germanica, Elphidium* spp., agglutinated spp., and Shannon diversity index. CCA indicated that miliolids and agglutinated species were correlated with Pb and Al. Principal components analysis suggested that higher bioavailable concentrations of Pb in addition to As, Cd, Cu, Ni, and Zn generally lead to less diverse foraminifera communities and that the agglutinated foraminifera and miliolids were more tolerant to Pb than other taxa examined. The authors noted that agglutinated foraminifera and miliolids were typically concentrated near the lagoon mouth where Pb concentrations were higher.

In another example, Pb was negatively correlated with the abundance of certain foraminiferal taxa, but not to diversity metrics in the San Jose Bay estuary, Puerto Rico (Martínez-Colón et al., 2018). Sediment Pb concentration ranged from 2 to 38 mg Pb/kg in the lagoon. Pb was significantly negatively correlated with the relative abundance of *Amphistegina gibbosa*, *Archaias angulatus*, *Asterigerina carinata*, *Discorbis*, *Elphidium crispum*, *Heterostegina depressa*, *Miliolinella*, *Quinqueloculina agglutinans,* and *Triloculina bicarinata* and positively correlated with the relative abundance of *Triloculina* and *Quinqueloculina agglutinans.* Pb sediment concentration was not significantly correlated with any of the other foraminiferal abundances or diversity indices such as species diversity, Shannon's index, Equitability Index, foraminiferal density, or the relative abundances of *Ammonia*.

Pb enrichment factors were slightly positively correlated with *Ammonia* spp. (*Ammonia beccarii, A. gaimardii, A. tepida,* and *A. parkinsoniana*) and low-oxygen foraminiferal assemblages in the Gulf of Milazzo, Italy, but not to total deformed foraminifera*,* foraminiferal density, or the abundance of other foraminiferal taxa (Cosentino et al., 2013). Pb concentrations in the sediment ranged from 4.75 to 49.19 mg Pb/kg. Finally, Pb and Al were negatively correlated with foraminiferal abundance across a gradient of sites in the Strait of Malacca, Malaysia (Minhat et al., 2020), with Pb showing the greatest enrichment among all metals, with values ranging from 8.8 to 29.2 mg Pb/kg. Overall, dissolved oxygen, depth, Al, and Pb concentrations explained the most variation in foraminiferal species distributions. The abundance of *Ammonia tepida*, which was the highest, was not significantly correlated with Pb sediment concentration, while those of *Bulimina marginata, Pararotalia ozawai,* and *Nonion subturgidum* were negatively correlated with Pb.

Foraminiferal abundance and diversity were correlated with certain bioavailable Pb sediment concentrations in Chilika, which is the largest brackish water lagoon in Asia (Barik et al., 2022). The concentrations of Pb in the sediment were 68.27 ± 22.14 mg Pb/kg (mean ± S.D.) across 22 sampling sites (range: 22.14–107.57 mg Pb/kg). Pb was statistically significantly positively correlated to the concentrations of Co. In addition to Pb concentrations in the sediment, bioavailable fractions of Pb and other heavy metals were determined. Specifically, Pb in the first fraction is the Pb bound to carbonates, the second fraction includes Pb bound to FeMn oxides, the third is bound to OM, and the fourth is bound to silicate. Pb concentration was significantly negatively correlated to the percentage of Pb in the second (reducible) and third (oxidizable) fractions and positively correlated to the percentage of Pb in the fourth (residual) fraction. Pb concentration alone was not correlated to the total number of live and dead abundance, diversity, or species richness, while the percentage of Pb in the first fraction was positively correlated to the abundance of dead foraminifera per gram sediment and negatively correlated to the diversity of dead foraminifera. The diversity, measured by the Shannon diversity index, of live and dead foraminifera was negatively correlated to the percentages of Pb in the second and third fractions and positively correlated to the percentage of Pb in the fourth fraction. Finally, live and dead foraminiferal species richness was significantly negatively correlated to the percentage of Pb in the third fraction.

In summary, some mesocosm and observational studies published since the 2013 Pb ISA found reductions in foraminiferal and meiofaunal community richness, diversity or abundance associated with higher concentrations of Pb in sediment and water (Barik et al., 2022; Minhat et al., 2020; Martínez-Colón et al., 2018). Other studies found positive or null correlations (Barik et al., 2022; Martínez-Colón et al., 2018; Cosentino et al., 2013; Martins et al., 2011).

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