

# **Integrated Science Assessment for Lead**

## **Appendix 11: Effects of Lead in Terrestrial and Aquatic Ecosystems**

### **External Review Draft**

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Health and Environmental Effects Assessment Division  
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://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282>.

Front Matter

Executive Summary

Integrative Synthesis

Appendix 1. Lead Source to Concentration

Appendix 2. Exposure, Toxicokinetics, and Biomarkers

Appendix 3. Nervous System Effects

Appendix 4. Cardiovascular Effects

Appendix 5. Renal Effects

Appendix 6. Immune System Effects

Appendix 7. Hematological Effects

Appendix 8. Reproductive and Developmental Effects

Appendix 9. Effects on Other Organ Systems and Mortality

Appendix 10. Cancer

**Appendix 11. Effects of Lead in Terrestrial and Aquatic Ecosystems**

Appendix 12. Process for Developing the Pb Integrated Science Assessment

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# CONTENTS

<b>LIST OF TABLES</b>	<b>v</b>
<b>LIST OF FIGURES</b>	<b>vi</b>
<b>ACRONYMS AND ABBREVIATIONS</b>	<b>vii</b>
<b>APPENDIX 11 EFFECTS OF LEAD IN TERRESTRIAL AND AQUATIC ECOSYSTEMS</b>	<b>1</b>
11.1 Introduction, Scope, Concepts, and Tools	2
11.1.1. Scoping and Criteria for Study Inclusion	2
11.1.2. Introduction to Ecosystem Connections and Pb Transfers	6
11.1.3. Concentrations of Pb in Non-Air Media	7
11.1.4. Concepts Related to Ecosystem Effects of Pb	16
11.1.5. Ecosystem Services	17
11.1.6. Bioavailability	18
11.1.7. Risk Screening Tools	21
11.2 Terrestrial Ecosystems	25
11.2.1. Summary of New Information on Effects of Pb in Terrestrial Ecosystems and Causality Determination Update Since the 2013 Pb ISA	25
11.2.2. Factors Affecting Bioavailability, Uptake and Bioaccumulation and Toxicity in Terrestrial Biota	29
11.2.3. Environmental Concentrations of Pb in Terrestrial Biota and Ecosystems in the United States at Different Locations and Over Time	52
11.2.4. Effects of Pb in Terrestrial Systems	55
11.2.5. Exposure and Response of Terrestrial Species	77
11.2.6. Terrestrial-Community and Ecosystem Effects	95
11.3 Freshwater Ecosystems	98
11.3.1. Summary of New Information on Effects of Pb in Freshwater Ecosystems and Causality Determination Update Since the 2013 Pb ISA	98
11.3.2. Factors Affecting Bioavailability, Uptake and Bioaccumulation and Toxicity in Freshwater Biota	104
11.3.3. Environmental Concentrations of Pb in Freshwater Biota and Ecosystems in the United States at Different Locations and Over Time	123
11.3.4. Effects of Pb in Freshwater Systems	124
11.3.5. Exposure and Response of Freshwater Species	140
11.3.6. Freshwater-Community and Ecosystem Effects	166
11.4 Saltwater Ecosystems	169
11.4.1. Summary of New Information on Effects of Pb in Saltwater Ecosystems and Causality Determination Update Since the 2013 Pb ISA	169
11.4.2. Factors Affecting Bioavailability, Uptake and Bioaccumulation, and Toxicity in Saltwater Biota	173
11.4.3. Environmental Concentrations of Pb in Saltwater Biota in the United States at Different Locations and Over Time	189
11.4.4. Effects of Pb in Saltwater Systems	191
11.4.5. Exposure and Response of Saltwater Species	199
11.4.6. Saltwater Community and Ecosystem Effects	211
11.5 References	213

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## LIST OF TABLES

Table 11-1	Pb concentration in non-air media and biota. _____	8
Table 11-2	Summary of Pb causality determinations for terrestrial plants, invertebrates, and vertebrates _____	28
Table 11-3	Studies of factors that affect the interpretability of exposure-response experiments in terrestrial biota, since the 2013 Pb ISA. _____	83
Table 11-4	Summary of Pb causality determinations for freshwater plants, invertebrates, and vertebrates _____	102
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. _____	147
Table 11-6	Updated causality determinations for Pb in saltwater organisms and ecosystems. _____	172
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. _____	204

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## LIST OF FIGURES

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. _____	13
Figure 11-2	Conceptual diagram for evaluating bioavailability processes and bioaccessibility for metals in soil, sediment, or aquatic systems. _____	19
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. _____	34
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.). _____	54
Figure 11-5	Main forms of Pb in seawater as a function of pH at 25°C and salinity of 35 ppt. _____	176
Figure 11-6	Acute genus sensitivity distribution for saltwater biota from Church et al., (2017). _____	202
Figure 11-7	Comparison of chronic sensitivity distributions in saltwater biota for dissolved Pb following the U.S. EPA and European Union methods. _____	203

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## ACRONYMS AND ABBREVIATIONS

ACE	abundance-based coverage estimator	FDA	fluorescein diacetate hydrolysis activity
AChE	acetylcholinesterase	GABA	gamma-aminobutyric acid
Ag	silver	GPx	glutathione peroxidase
ALAD	aminolevulinic acid dehydratase	GSH	glutathione
AMF	arbuscular mycorrhizal fungi	GST	glutathione-s-transferase
AQCD	Air Quality Criteria Documents	HAB	harmful algal blooms
As	arsenic	hpf	hours postfertilization
ASTM	American Society for Testing and Materials	IC	inhibitory concentration
AVS	acid volatile sulfides	ISA	integrated science assessment
AWCD	average cell wall color development	LECES	Level of Biological Organization, Exposure, Comparison, Endpoint and Study Design
AWQC	ambient water quality criteria		
BAF	bioaccumulation factor	LH	luteinizing hormone
BCF	bioconcentration factor	LOEC	lowest observed effect concentration
BEST	Biomonitoring of Environmental Status and Trends	LOAEL	lowest observed adverse effect level
BLM	biotic ligand model	LRMN	Large River Monitoring Network
BMF	biomagnification factors	MATC	maximum acceptable toxicant concentration
BRT	boosted regression tree	MBC	microbial biomass carbon
BSAF	biota-sediment accumulation factors	MDA	malondialdehyde
Ca	calcium	ME	mining ecotype
CAT	catalase	Mg	magnesium
CCA	canonical correspondence analysis	MIC	minimum inhibitory concentration
CCC	criterion continuous concentration	MLR	multiple linear regression
Cd	cadmium	mo	month, months
CEC	cation exchange capacity	MRG	metal-rich granules
CF	conversion factor	MTC	maximum tolerable concentration
CMC	criteria maximum concentration	MW	molecular weight
CORT	corticosterone	NAAQS	national ambient air quality standards
CRADA	Cooperative Research and Development Agreement	NASGLP	North American Soil Geochemical Landscapes Project
CSMW	California State Mussel Watch	NAWQA	national water quality assessment
Cu	copper	NEC	no-effect concentration
d	day, days	NME	nonmining ecotype
DOC	dissolved organic carbon	NOAA	National Oceanic and Atmospheric Administration
dpf	days postfertilization	NOEC	no observed effect concentration
dph	days posthatch	NOM	natural organic matter
DOM	dissolved organic matter	OC	organic carbon
DT	diatom + tetramin	OM	organic matter
eCEC	effective cation exchange capacity	OP	omnivores-predators
Eco-SSL	ecological soil screening levels	OTU	operational taxonomic unit
EDTA	ethylenediaminetetraacetic acid	Pb	lead
FCORT	fecal corticosterone	PEC	probable effects concentrations
FCV	final chronic value	PECOS	Population, Exposure, Comparison, Outcome and Study

PMF	Picher mine field
PNEC	predicted no-effect concentrations
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
ROS	reactive oxygen species
SEM	simultaneously extracted metal
SOD	superoxide dismutase
SSD	species sensitivity distribution
T3	triiodothyronine
T4	thyroxine
TBMF	trophic biomagnification factor
TEC	threshold effect concentrations
TRF	terminal restriction fragments
TTF	trophic transfer factor
USGS	United States Geological Survey
WACAP	Western Airborne Contaminants Assessment Project
WEOC	water-extractable organic carbon
wk	week; weeks
WQC	water quality criteria
YCT	yeast, cereal leaves and trout pellets
yr	year, years
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](https://www.epa.gov/isa)). 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 <sup>a</sup>	Freshwater <sup>a</sup>	Saltwater <sup>a</sup>
<b>Community- and Ecosystem</b>		Community and Ecosystem Effects	Likely Causal	Likely Causal	<b>Suggestive</b>
<b>Population–Level Endpoints</b>	<b>Organism–Level Responses</b>	Reproductive and Developmental Effects - Plants	Inadequate	Inadequate	Inadequate
		Reproductive and Developmental Effects - Invertebrates	Causal	Causal	<b>Likely Causal</b>
		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	<b>Suggestive</b>
		Neurobehavioral Effects - Invertebrates	Likely Causal	Likely Causal	Inadequate
		Neurobehavioral Effects - Vertebrates	Likely Causal	Likely Causal	Inadequate
	<b>Suborganismal Responses</b>	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

<sup>a</sup>Based on the weight of evidence for causal determination in Table II of the ISA Preamble ([U.S. EPA, 2015](https://www.epa.gov/isa)).

The Executive Summary, Integrated Synthesis, and all other appendices of this Pb ISA can be found at <https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282>.

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## 11.1 Introduction, Scope, Concepts, and Tools

1 This appendix synthesizes and evaluates the most policy-relevant scientific information on Pb  
2 welfare effects to help form the foundation for the review of the secondary (welfare<sup>1</sup>-based) National  
3 Ambient Air Quality Standards (NAAQS) for lead (Pb). The focus of this appendix is on studies  
4 published since the 2013 Integrated Science Assessment (ISA) for Pb (2013 Pb ISA) [U.S. EPA \(2013\)](https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282)  
5 that examine Pb interactions with the biotic components of terrestrial and aquatic ecosystems, including  
6 effects on vegetation and wildlife. Pb transport through abiotic compartments (air, soil, water, and  
7 sediment) is covered in Appendix 1: Lead Source to Concentration:  
8 <https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282>. Section 11.1 of this appendix includes key concepts  
9 and tools useful for characterizing the effects of Pb on biota. Section 11.2 examines the bioavailability,  
10 bioaccumulation, and effects of Pb in terrestrial ecosystems. The effects of Pb in terrestrial environments  
11 are followed by information on the bioavailability, bioaccumulation and effects of Pb in freshwater  
12 (Section 11.3) and saltwater (Section 11.4) ecosystems.

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### 11.1.1. Scoping and Criteria for Study Inclusion

13 This appendix builds upon the assessment of effects of Pb on ecosystems reported in the 2013 Pb  
14 ISA ([U.S. EPA, 2013](https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282)) and in prior Air Quality Criteria Documents (AQCDs) from 1977 ([U.S. EPA,](https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282)  
15 [1977](https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282)), 1986 ([U.S. EPA, 1986](https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282)), and 2006 ([U.S. EPA, 2006a](https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282)). The framework used to define the scope of  
16 the ecological effects portion of the current ISA is modeled after the Population, Exposure, Comparison,  
17 Outcome, and Study Design (PECOS) used for human health effects (Appendix 12  
18 <https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282>). For the health  
19 appendices, the PECOS statement defines the objectives of the review and establishes study inclusion  
20 criteria, thereby facilitating identification of the most relevant literature to inform the ISA for each health  
21 discipline. Similarly, the Level of Biological Organization, Exposure, Comparison, Endpoint, and Study  
22 Design (LECES) statement aids in identifying the relevant evidence in the literature for the ecological  
23 effects of Pb (Table 12-4; Appendix 12). Studies that reported the effects of Pb on biota were evaluated,  
24 included, and discussed in this appendix if they satisfied the following LECES criteria:

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<sup>1</sup> 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.”

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#### 11.1.1.1. Level of Biological Organization

1 Studies considered for this appendix included those that reported Pb effects on species,  
2 subspecies or populations of vegetation, microbes, invertebrates, or vertebrates at any lifestage on  
3 biological communities or on ecosystems in terrestrial, freshwater, or saltwater environments and  
4 transition zones present in the United States or similar to those in the United States. In the 2013 Pb ISA,  
5 ecological effects were generally organized in order of increasing biological complexity (i.e., from the  
6 subcellular and cellular levels through the individual organism and up to ecosystem-level effects) ([U.S.  
7 EPA, 2013](#)). This appendix follows the same organizing principle. For effects that occur at the  
8 suborganism scale such as perturbation of biomarkers of physiological stress or changes in hematological  
9 parameters, emphasis was placed on studies that concurrently reported effects experimentally linked to  
10 higher levels of biological organization. Organism-level endpoints such as growth, survival, and  
11 reproductive output have been definitively linked to effects at the population level and above. Examples  
12 of organism-level endpoints with direct links to population-level effects include mortality, gross  
13 abnormalities, survival, fecundity, and growth ([Suter et al., 2004](#)). Because of the complexity of processes  
14 than can affect an ecosystem and considering that Pb rarely occurs as the only contaminant in natural  
15 systems, it is difficult to attribute effects observed at higher levels of biological organization solely to Pb.

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#### 11.1.1.2. Exposure

16 The deleterious effects of any given concentration of Pb can vary greatly under different  
17 environmental and experimental conditions, as well as the duration and pathway of exposure. Relevant  
18 concentrations for this assessment take into consideration the range of Pb concentrations in environmental  
19 media from U.S. locations (Table 11-1) and the available evidence for concentrations at which effects are  
20 observed in microbes, plants, invertebrates, and vertebrates. Effects observed at or near environmental  
21 concentrations of Pb measured in soil, sediment, and water are emphasized. For the studies included in  
22 the 2013 Pb ISA, evidence from exposures or doses generally ranged “from current levels to one or two  
23 orders of magnitude above current levels” ([U.S. EPA, 2013](#)). Concentration cutoffs for literature inclusion  
24 were not applied in earlier EPA reviews of this metal. To focus on studies that are the most policy-  
25 relevant with regard to current environmental concentrations of Pb in the United States, concentration  
26 cutoff values were applied when evaluating the literature published since the 2013 Pb ISA (Appendix 12:  
27 <https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282>). For soil, the cutoff  
28 value for screening of terrestrial studies of Pb exposure and effects was set at a concentration of  
29 approximately 230 mg Pb/kg of soil, although higher concentrations were considered if the study added  
30 new information on a mechanism of action, or if the higher concentration was part of a series that  
31 contributed exposure-response information and included other concentrations below 230 mg Pb/kg. For  
32 aqueous exposures, the cutoff value for study screening was approximately 10 µg Pb/L, although higher  
33 concentrations were considered if the study added new information on a mechanism of action or if the

1 higher concentration was part of a series that contributed exposure-response information. For sediments,  
2 the literature cutoff value for study screening was approximately 300 mg Pb/kg dry weight or lower.  
3 Studies at very high concentrations of Pb were excluded unless they were part of a series in an  
4 experimental exposure-response study and at least one concentration in the test series was in the ranges  
5 stated above. Justification for selection of these Pb concentration cutoff values and additional information  
6 on scoping for the literature for ecological effects of Pb is provided in Appendix 12. Initial literature  
7 search and screening steps for this review identified many studies conducted at higher concentrations of  
8 Pb that were ultimately excluded from the draft ISA  
9 ([https://hero.epa.gov/hero/index.cfm/project/page/project\\_id/4081](https://hero.epa.gov/hero/index.cfm/project/page/project_id/4081)).

10 All reported values for biological effects in this appendix are from exposures in which  
11 concentrations of Pb were analytically verified unless they are stated to be nominal concentrations. For  
12 consistency, concentrations of Pb in soil and sediment are reported in mg Pb/kg dry weight (unless  
13 otherwise specified) and aqueous concentrations of Pb are reported as µg Pb/L. For study concentrations  
14 originally in other units such as µM or ppb, the values are converted to mg Pb/kg or µg Pb/L, and original  
15 reported units are retained in parentheses. Only a subset of the studies reporting Pb effects on biota  
16 analytically verified the concentration of Pb in media and the test organisms investigated.

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#### 11.1.1.3. Comparison

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

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#### 11.1.1.4. Endpoint

27 The biological endpoints considered in this appendix are relevant to the levels of biological  
28 organization discussed above. The endpoints encompass species or population-level effects including but  
29 not limited to effects on growth, reproduction or development, neurobehavioral effects, reduced survival,  
30 or fitness, and photosynthesis. At higher levels of biological organization, endpoints include but are not

1 limited to changes in community composition, altered ecosystem processes and functions, shifts in  
2 genotypes or species, species extirpation, declines in the total number of species or biomass, and  
3 decreased species richness.

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#### 11.1.1.5. Study Design

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

---

#### 11.1.1.6. Additional Scoping

15 Topics within scope also include effects of Pb biogeochemistry on bioavailability in terrestrial,  
16 freshwater, and saltwater environments as well as subsequent vulnerability of particular organisms,  
17 populations, communities or ecosystems and studies that address key uncertainties and limitations in the  
18 evidence identified in the previous review. Topics outside of the scope of this appendix included mixture  
19 studies that did not assess Pb effects independently and site-specific studies in non-U.S. locations that do  
20 not contribute novel insights on Pb biogeochemistry or effects. As in the 2013 Pb ISA, generally, studies  
21 on mine tailings, industrial effluent, sewage, bioremediation of highly contaminated sites and ingestion of  
22 Pb shot, pellets or fishing gear are not within the scope of this ISA due to the high concentration of Pb  
23 and lack of a connection to air-related sources or processes. This is consistent with the 2006 AQCD,  
24 which typically did not include “effects from irrelevant exposure conditions relative to airborne emissions  
25 of Pb (e.g., Pb shot, Pb paint, injection studies, studies conducted on mine tailings and studies conducted  
26 with hydroponic solutions)” (Section AX 7.1.3 of ([U.S. EPA, 2006a](#))).

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### 11.1.2. Introduction to Ecosystem Connections and Pb Transfers

1 Metals, including Pb, occur naturally in the geosphere, and anthropogenic enrichment of these  
2 elements can lead to elevated concentrations in terrestrial and aquatic ecosystems. Pb is a persistent metal  
3 that, once emitted, may cycle through multiple environmental media compartments (e.g., air, soil, water,  
4 sediment) prior to exposure to plants and animals, as discussed in Appendix 1:  
5 <https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282> (Section 1.3). In  
6 terrestrial ecosystems, non-air media can receive Pb from atmospheric deposition or other sources. Once  
7 deposited, Pb can be resuspended into the air or transferred among other environmental media (Section  
8 1.3). Exposure of freshwater and estuarine organisms to Pb, and associated effects, are tied to terrestrial  
9 systems via watershed processes. Atmospherically derived Pb can enter aquatic systems through erosional  
10 transport of soil particles in runoff from terrestrial systems (Section 1.3.3) or via direct wet or dry  
11 deposition over a water surface (Section 1.3.1.2). Once in the aquatic environment, Pb partitions between  
12 various compartments (water column, sediment, biota; Section 1.3.3). Saltwater ecosystems include  
13 habitats that encompass a range of salinities from just above that of freshwater to that of seawater. These  
14 ecosystems may receive Pb contributions from atmospheric deposition (Section 1.3.1.2), riverine  
15 transport (Section 1.3.3) and runoff (Section 1.3.3) from terrestrial systems. The contribution of  
16 atmospheric Pb differs by location. Ecosystems in more urban areas are also influenced by non-air  
17 sources of Pb such as paint, automobiles, wastewater, and industrial activities.

18 Although Pb is present in the natural environment, it has no biological function in plants or  
19 animals. Terrestrial, freshwater, and marine/estuarine organisms have developed adaptive physiological  
20 responses for living with metals. These adaptations may include intracellular sequestration (e.g., synthesis  
21 of metallothioneins or metal-rich granules [MRG]), induction of enzymes involved in oxidative stress  
22 response, and modification of metal uptake or elimination rates ([Gismondi et al., 2017](#)). Anthropogenic  
23 enrichment can result in concentrations that exceed the capacity of organisms to regulate internal  
24 concentrations, causing a toxic response and potentially death. Across taxa, effects of Pb exposure are  
25 likely mediated through common biological mechanisms. In the case of Pb, ecological receptors and  
26 humans are linked via shared pathways of exposure and commonalities in biological response to this  
27 metal ([Lassiter et al., 2015](#)). Connections between the atmosphere, the abiotic and biotic compartments of  
28 terrestrial and aquatic ecosystems, and humans are acknowledged for Pb. However, for the purposes of  
29 this ISA, these topics are divided into different appendices. Within this Ecological Effects appendix,  
30 terrestrial, freshwater, and saltwater ecosystems are considered separately because of different  
31 environmental and physiological factors that influence Pb toxicity, such as bioavailability of the metal,  
32 form of Pb, other water and soil chemistry factors, and organism adaptations.

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### 11.1.3. Concentrations of Pb in Non-Air Media

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

7           To provide sufficient information to support development of air quality criteria for Pb that are  
8   protective of terrestrial and aquatic systems, it is important to gain a general understanding of current  
9   distribution and the concentrations of Pb in the environment. Information on environmental  
10   concentrations of Pb at U.S. locations is tabulated in Table 11-1. This table updates Table 6-2 in the 2013  
11   Pb ISA [U.S. EPA \(2013\)](#) on Pb concentration in non-air media and biota. Sources of environmental  
12   concentration data in Table 11-1 were limited to regional or national-scale studies. Studies that reported  
13   concentrations in environmental media for one or a very small number of locations would be considered  
14   anecdotal for the purpose of this review. Measured concentrations of Pb in soils, sediment and water are  
15   not necessarily representative of the amount of Pb available to elicit a toxic effect. For Pb to interact with  
16   a biological membrane and be taken up into an organism, it must be in a bioavailable form (Section  
17   11.1.6), which is dependent upon the physical, chemical, and biological conditions under which an  
18   organism is exposed at a particular geographic location. In addition, caution must be taken while  
19   comparing Pb concentrations in different studies of environmental media because reported concentrations  
20   of Pb may not be directly comparable across studies, in part due to differences in sampling, collection and  
21   measurement methods. For example, soil Pb measurements may vary between studies that used partial  
22   and complete acid digestion. Furthermore, complete acid digestion is likely to overestimate the amount of  
23   bioavailable Pb in many cases. In aquatic systems, measurements of dissolved Pb may vary among  
24   collection methods, notably due to different sample filtration sizes, while the composition of sediment  
25   samples of Pb is often influenced by sieving size. These are given as illustrative examples of how Pb  
26   observations may be affected by methods, but a comprehensive discussion of Pb sampling, collecting, and  
27   measuring methods is beyond the scope of this ISA.

28           Some surveys of Pb in environmental media in Table 11-1 predate the 2013 Pb ISA ([U.S. EPA,](#)  
29   [2013](#)) and 2006 Pb AQCD ([U.S. EPA, 2006a](#)). Although they may have used less optimal methods than  
30   more recent studies, these data are not excluded from the ISA in cases wherein they remain the best  
31   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)	2007–2010	<a href="#">Smith et al. (2013a)</a>
	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)		
	Northeastern U.S. forest floor soil mean: 151 ± 29 mg Pb/kg (dry weight)	1980	<a href="#">Richardson et al. (2014b)</a>
	Northeastern U.S. forest floor soil mean: 68 ± 13 mg Pb/kg (dry weight) (resurvey of 16 of 25 1980 sites)	2011	<a href="#">Richardson et al. (2014b)</a>
	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	<a href="#">Mackowiak et al. (2021)</a>
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	<a href="#">Nahlik et al. (2019)</a>
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	<a href="#">Mahler et al. (2006)</a>
	National Water Quality Assessment of lotic systems Median: 28 mg Pb/kg (dry weight)	1991–2003	<a href="#">U.S. EPA (2006a)</a>
	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	<a href="#">Horowitz and Stephens (2008)</a>
	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)	2010–2011	<a href="#">Horowitz et al. (2012)</a>



Media	Pb Concentration	Years Data Obtained	References
	Gulf rivers: mean: 32 mg Pb/kg; median: 24 mg Pb/kg (dry weight) Pacific rivers: mean: 19 mg Pb/kg; median: 13 mg Pb/kg (dry weight)		
	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	<a href="#">Sadiq (1992)</a>
Saltwater Sediment	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	<a href="#">Cantillo and O'connor (1992)</a>
	Median: 0.50 µg Pb/L Max: 30 µg Pb/L, 95th percentile 1.1 µg Pb/L	1991–2003	<a href="#">U.S. EPA (2006a)</a>
	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	<a href="#">Jiann et al. (2013)</a>
Fresh Surface Water (Dissolved Pb)	Range: 0.0003–0.075 µg Pb/L (Set of National Parks in western U.S.)	2002–2007	<a href="#">Field and Sherrell (2003)</a> <a href="#">NPS (2011)</a>
	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	<a href="#">Olson et al. (2019)</a>
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	1997–1998	<a href="#">Jiann et al. (2013)</a>

Media	Pb Concentration	Years Data Obtained	References
	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 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	<a href="#">Sadiq (1992)</a>
	Lichens: 0.3–5 mg Pb/kg (dry weight) (Set of National Parks in western U.S.)	2002–2007	<a href="#">NPS (2011)</a>
Vegetation	Leaves from woody shrubs and trees from 54 sites in Los Angeles, Orange, San Bernardino and Riverside counties in California <i>Adenostoma fasciculatum</i> Mean: 0.17 ± 0.08 (SE) mg Pb/kg <i>Artemisia californica</i> Mean: 0.16 ± 0.01 (SE) mg Pb/kg <i>Baccharis salicifolia</i> Mean: 0.22 ± 0.03 (SE) mg Pb/kg <i>Encelia farinosa</i> Mean: 0.20 ± 0.02 (SE) mg Pb/kg <i>Eriogonum</i> spp. Mean: 0.23 ± 0.03 (SE) mg Pb/kg <i>Heteromeles arbutifolia</i> Mean: 0.42 ± 0.17 (SE) mg Pb/kg <i>Malosma luarina</i> Mean: 0.38 ± 0.06 (SE) mg Pb/kg <i>Quercus agrifolia</i> Mean: 0.29 ± 0.04 (SE) mg Pb/kg	2019	<a href="#">Mackowiak et al. (2021)</a>
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	<a href="#">Hinck et al. (2009)</a>
	Fish (96 sites in large U.S. rivers): Female bass ( <i>Micropterus</i> spp.): median: 0.04 mg Pb/kg; mean: 0.06 ± 0.02 mg Pb/kg (wet weight) (whole fish) Male bass ( <i>Micropterus</i> spp.): median: 0.03 mg Pb/kg; mean: 0.05 ± 0.01 mg Pb/kg (wet weight) (whole fish)	1995–2004	<a href="#">Hinck et al. (2008)</a>

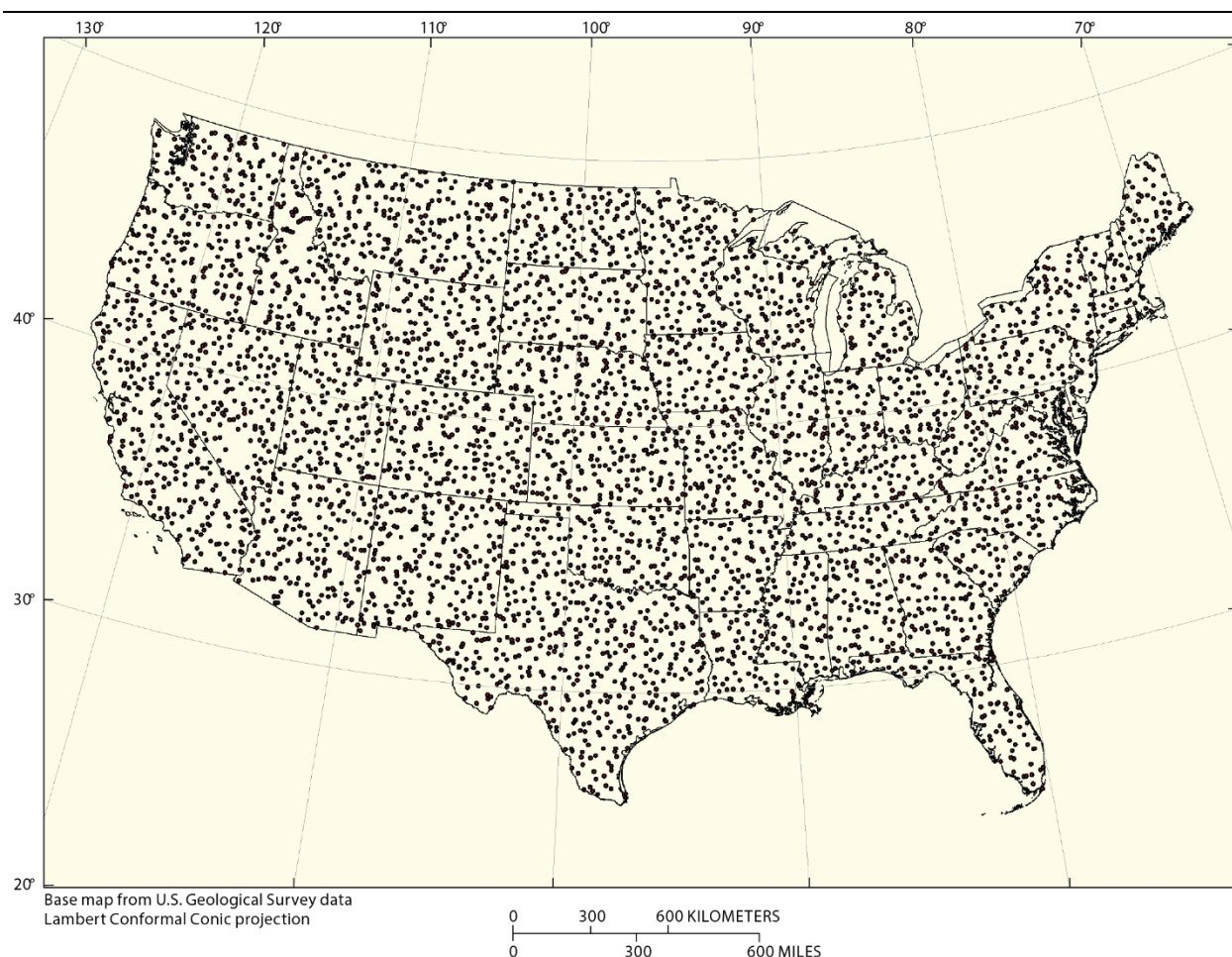
Media	Pb Concentration	Years Data Obtained	References
	Female carp ( <i>Cyprinus carpio</i> ): median: 0.10 mg Pb/kg; mean: $0.11 \pm 0.01$ mg Pb/kg (wet weight) (whole fish) Male carp ( <i>Cyprinus carpio</i> ): median: 0.09 mg Pb/kg; mean: $0.12 \pm 0.01$ mg Pb/kg (wet weight) (whole fish)		
	Dolphinfish ( <i>Coryphaena hippurus</i> ) in southern Gulf of California (wet weight) (muscle tissue): Mean: 0.059 mg Pb/kg	2006–2015	<a href="#">Gil-Manrique et al. (2022)</a>
	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	<a href="#">NPS (2011)</a>
	Anna's hummingbirds ( <i>Calypte anna</i> ) surveyed in coastal, valley and Sierra Nevada foothills regions of northern California Mean: $0.23 \pm 0.25$ mg Pb/kg; range: 0.00–1.35 mg Pb/kg (body feathers; live) (dry weight) Mean: $3.00 \pm 7.64$ mg Pb/kg; range: 0.28–46.0 mg Pb/kg (body feathers; carcasses) (dry weight) Mean: $1.01 \pm 3.10$ mg Pb/kg; range: 0.01–16.9 mg Pb/kg (liver) (dry weight) Mean: $0.94 \pm 2.07$ mg Pb/kg; range: 0.03–12.43 mg Pb/kg (kidney) (dry weight) Mean: $8.17 \pm 36.27$ mg Pb/kg (combined feathers) (dry weight)	2015	<a href="#">Mikoni et al. (2017)</a>
	Neotropical Cormorants ( <i>Phalacrocorax brasilianus</i> ) surveyed in Lake Livingston, Texas: Female mean: $4.92 \pm 4.11$ (SE) mg Pb/kg (breast feathers) (dry weight) Male mean: $1.68 \pm 0.822$ (SE) mg Pb/kg (breast feathers) (dry weight) In Richland Creek Wildlife Management Area, Texas: Female mean: $0.191 \pm 0.044$ (SE) mg Pb/kg (breast feathers) (dry weight) Male mean: $0.115 \pm 0.015$ (SE) mg Pb/kg (breast feathers) (dry weight)	2014	<a href="#">Mora et al. (2021)</a>
Invertebrates	7 earthworm species in northeastern U.S. Overall mean: $29 \pm 6$ (SE) mg Pb/kg (dry weight) <i>Amyntas agrestis</i> mean: $21 \pm 11$ (SE) mg Pb/kg (dry weight) <i>Aporrectodea rosea</i> mean: $43 \pm 5$ (SE) mg Pb/kg (dry weight) <i>Aporrectodea tuberculata</i> mean: $30 \pm 7$ (SE) mg Pb/kg (dry weight) <i>Dendrobaena octaedra</i> mean: $43 \pm 20$ (SE) mg Pb/kg (dry weight) <i>Lumbricus rubellus</i> mean: $24 \pm 5$ (SE) mg Pb/kg (dry weight) <i>Lumbricus terrestris</i> mean: $14 \pm 4$ (SE) mg Pb/kg (dry weight)	2013	<a href="#">Richardson et al. (2015)</a>

Media	Pb Concentration	Years Data Obtained	References
	<i>Octolasion cyaneum</i> mean: 20 ± 8 (SE) mg Pb/kg (dry weight)		
	Oysters ( <i>Crassostrea virginica</i> ) and mussels ( <i>Mytilus edulis</i> ) in east coast U.S. Range: 0.11–2.2 mg Pb/kg Pb (dry weight)	2003–2006	<a href="#">Shiel et al. (2012)</a>
	Oysters ( <i>Crassostrea gigas</i> ) in west coast Canada Range: 0.05–0.22 mg Pb/kg Pb (dry weight)	2002–2004	<a href="#">Shiel et al. (2012)</a>

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. \(2014b\)](#), 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.**

1 The 2006 Pb AQCD and 2013 Pb ISA reported representative Pb concentrations in fresh surface  
 2 water (median 0.50  $\mu\text{g Pb/L}$ , range 0.04 to 30  $\mu\text{g Pb/L}$ ) and freshwater sediments (median 28 mg Pb/kg  
 3 dry weight, range 0.5 to 12,000 mg Pb/kg dry weight) in lotic systems in the United States based on a  
 4 synthesis of National Water Quality Assessment (NAWQA) data ([U.S. EPA, 2013, 2006b](#)). Another  
 5 analysis of the NAWQA data set provides additional detail to the prior 2006 Pb AQCD analysis by  
 6 stratifying the summary of Pb concentrations in freshwater sediment by land use within river basins  
 7 ([Horowitz and Stephens, 2008](#)). The baseline freshwater sediment concentration, comprising  
 8 measurements taken in low-population areas only, is reported to have a median of 20 mg Pb/kg with a  
 9 range of 2 to 200 mg Pb/kg. Land-use categories for agricultural, cropland, pasture, forested and  
 10 rangeland sites are reported in Table 11-1. A more recent survey of Pb concentrations in freshwater  
 11 sediment found higher concentrations in Atlantic rivers (mean 110 mg Pb/kg) compared with Pacific and

1 Gulf of Mexico rivers (means of 19 and 32 mg Pb/kg, respectively) ([Horowitz et al., 2012](#)). This observed  
2 spatial variation in freshwater sediment Pb concentrations is likely driven by higher historical population  
3 density and industrial activity associated with Pb emissions in the eastern United States compared with  
4 the central and western regions of the country. [Mahler et al. \(2006\)](#) dated sediment cores and reported a  
5 decline in Pb concentrations in sediment deposited between the 1970s and the 1990s, which corresponds  
6 to the phasing out of widespread use of leaded gasoline. One additional regional survey of dissolved and  
7 particulate Pb in fresh surface water was identified for inclusion in this ISA. In a study of water quality in  
8 eight Texas rivers, [Jiann et al. \(2013\)](#) identified elevated particulate and dissolved Pb near areas with  
9 greater anthropogenic influence and noted that Pb concentrations were decreased downstream of dams  
10 and reservoirs, where slow-moving water causes suspended Pb to settle into sediment. Summary statistics  
11 of the rivers included in [Jiann et al. \(2013\)](#) are included in Table 11-1. Additional information on  
12 temporal trends observed in aquatic ecosystems is summarized in Sections 11.3.3 and 11.4.3.

13 No new surveys in coastal areas of the United States measuring dissolved Pb in saltwater or Pb in  
14 saltwater sediment were identified for inclusion in this Pb ISA, although concentrations measured from  
15 1984 to 1987 are included in Table 11-1 to provide additional information on Pb concentrations in  
16 saltwater sediment ([Cantillo and O'connor, 1992](#)). The 2013 Pb ISA ([U.S. EPA, 2013](#)) reported saltwater  
17 dissolved and sediment Pb concentrations from studies dated 1977 to 1990 summarized in [Sadiq \(1992\)](#),  
18 which reports a global range of 0.6 to 1,050 mg Pb/kg in saltwater sediment, although the authors noted  
19 that the maximum value reported was observed in an Australian inland saltwater lake. Observations from  
20 only one U.S. saltwater sediment study were reported in [Sadiq \(1992\)](#), in which Pb concentrations  
21 ranging from 13.4 to 52.8 mg Pb/kg from Puget Sound were recorded. [Sadiq \(1992\)](#) remains the only  
22 study identified for inclusion in the ISA in which global dissolved saltwater Pb concentrations are  
23 reported. Excluding observations from inland seas, open-ocean concentrations of dissolved Pb ranged  
24 from 0.01 to 4.8 µg Pb/L. Pb measurement methods have developed substantially in the last few decades,  
25 and measurements of dissolved Pb from older studies may be less accurate than those measured using  
26 modern methods. Table 11-1 summarizes the information available on concentrations of dissolved and  
27 sediment Pb observed in U.S. saltwater aquatic ecosystems.

28 Information on Pb concentrations observed in regional surveys of U.S. biota at sites located far  
29 from significant modern point sources of Pb have been collated in Table 11-1. The included surveys  
30 provide a range of reference values which may provide context for Pb concentrations observed in similar  
31 species and ecosystems. The Western Airborne Contaminants Assessment Project (WACAP) is the most  
32 comprehensive database on contaminant transport and depositional effects in U.S. sensitive ecosystems  
33 ([U.S. EPA, 2013](#); [NPS, 2011](#); [Landers et al., 2010](#)), although it only covers locations in the western part  
34 of the country. The project aimed to assess the locations where atmospheric pollutants were accumulating  
35 due to deposition in remote ecosystems in the western United States and identify the most likely sources  
36 of the identified pollutants. Pb (and other pollutants) was measured in sediment, snow, water, lichen, and  
37 fish at eight western U.S. national parks. For species sampled across multiple national parks, Pb



1 concentrations in biota in terrestrial and aquatic ecosystems surveyed in this project were reported in the  
2 2013 Pb ISA and are included in Table 11-1.

3       Recent regional surveys of Pb in terrestrial ecosystems published in the peer reviewed literature  
4 include Anna's hummingbirds (*Calypte anna*) surveyed in the coastal, valley and Sierra Nevada foothills  
5 regions of northern California ([Mikoni et al., 2017](#)) and cormorants (*Phalacrocorax brasilianus*) sampled  
6 from two colonies in Lake Livingston and Richland Creek, Texas ([Mora et al., 2021](#)). A summary of  
7 feather Pb concentrations observed in each of these studies is included in Table 11-1. The study of Anna's  
8 hummingbirds is unique in its investigation of bioaccumulation of metals in a nectar-feeding bird species.  
9 The sources of Pb measured in hummingbird organs and feathers were not determined in this study, but  
10 the authors listed absorption from food sources including plant and insect species, particularly those  
11 living in urban environments, as the most likely routes of exposure ([Mikoni et al., 2017](#)). [Mora et al.](#)  
12 [\(2021\)](#) investigated the interaction between location and sex on Pb concentrations in cormorant feathers in  
13 the Trinity River watershed in Texas and found no statistically significant effect for either variable.

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

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

32       The Large River Monitoring Network of the Biomonitoring of Environmental Status and Trends  
33 (BEST-LRMN) surveyed fish from nine U.S. river basins from 1995–2004. This survey is the most recent  
34 national-scale survey of Pb concentrations observed in biota in freshwater aquatic ecosystems, with  
35 results summarized in two studies. [Hinck et al. \(2008\)](#) measured species-dependent Pb concentrations in  
36 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.

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#### 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<sup>2+</sup> 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 EPA’s Framework for Metals Risk Assessment ([U.S. EPA, 2007](#)).

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#### **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

1 publications that specifically link an ecological effect attributed to Pb to a change in an ecosystem  
2 service. No new studies were identified that explicitly address Pb effects on ecosystem services associated  
3 with terrestrial, freshwater, or saltwater systems.

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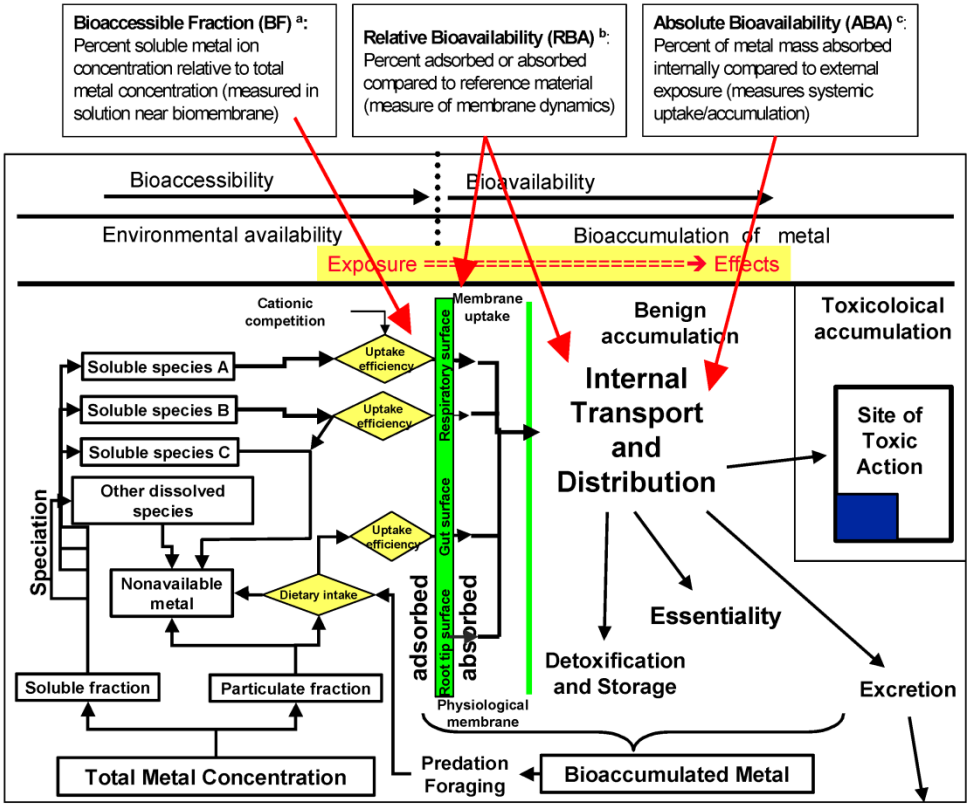
#### 11.1.6. Bioavailability

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

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

25 The bioavailability of a metal is also dependent upon the fraction of metal that is bioaccessible.  
26 As stated in the Framework for Metals Risk Assessment ([U.S. EPA, 2007](#)), the bioaccessible fraction of a  
27 metal is the portion (fraction or percentage) of environmentally available metal that interacts at the  
28 organism’s contact surface and is potentially available for absorption or adsorption by the organism. The  
29 framework states that “the bioaccessibility, bioavailability, and bioaccumulation properties of inorganic  
30 metals in soil, sediments, and aquatic systems are interrelated and abiotic (e.g., OC) and biotic  
31 (e.g., uptake and metabolism) modifying factors determine the amount of an inorganic metal that interacts  
32 at biological surfaces (e.g., at the gill, gut, or root tip epithelium) and that binds to and is absorbed across  
33 these membranes. A major challenge is to consistently and accurately measure quantitative differences in

1 bioavailability between multiple forms of inorganic metals in the environment.” A conceptual diagram  
2 presented in the Framework for Metals Risk Assessment ([U.S. EPA, 2007](#)) summarizes metals  
3 bioavailability and bioaccumulation in aquatic, sediment, and soil media (Figure 11-2).



<sup>a</sup>BF is most often measured using in vitro methods (e.g., artificial stomach), but should be validated by in vivo methods.

<sup>b</sup>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).

<sup>a</sup>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

1 large range of water chemistry conditions that considers complexations with inorganic ligands and  
2 competition of active free metal ions with other cations, such as calcium (Ca) and magnesium (Mg), for  
3 the site of action (i.e., biotic ligand) ([Niyogi and Wood, 2004](#); [Paquin et al., 2002](#); [Di Toro et al., 2001](#)). It  
4 predicts both the bioaccessible and bioavailable fraction of Pb in the aquatic environment and can be used  
5 to estimate the importance of environmental variables such as DOC in limiting uptake by aquatic  
6 organisms ([Alonso-Castro et al., 2009](#)). The U.S. EPA-recommended freshwater ambient water quality  
7 criteria (AWQC) for copper (Cu) are based on the BLM. [Deforest et al. \(2017\)](#) proposed a BLM-based  
8 freshwater aquatic life criteria for Pb (Section 11.3.5).

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

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

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### 11.1.7. Risk Screening Tools

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

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#### 11.1.7.1. Critical Loads for Atmospheric Deposition

5 The critical load concept is widely used as an organizing principle to relate atmospheric  
6 deposition to ecological endpoints that indicate impairment ([Pardo et al., 2011](#); [Bobbink et al., 2010](#);  
7 [Porter and Johnson, 2007](#)). The definition of a critical load is “a quantitative estimate of an exposure to  
8 one or more pollutants below which significant harmful effects on specified sensitive elements of the  
9 environment do not occur according to present knowledge” ([Nilsson and Grennfelt, 1988](#)). No recently  
10 published critical loads for Pb from terrestrial ecosystems in the United States were identified for this  
11 ISA. Several critical load studies from Europe reviewed in the 2013 Pb ISA ([de Vries and Groenenberg,](#)  
12 [2009](#); [Hall et al., 2006](#); [Morselli et al., 2006](#)) and a recent review study ([Koptsik and Koptsik, 2022](#)) noted  
13 uncertainties inherent in a critical load approach to Pb risk assessment, such as soil type, critical  
14 concentration of dissolved metal, adsorption coefficients of exposed soils, combined effects of different  
15 metals in multimetal mixtures and the influences of a changing climate. Since the 2013 Pb ISA, critical  
16 load studies for atmospheric deposition for aquatic systems have largely focused on eutrophication and  
17 acidification associated with nitrogen (N) deposition, with no detailed assessments for Pb in freshwater or  
18 coastal areas in Europe ([RoTAP, 2012](#)) or the United States. In the literature search for the current  
19 assessment, no published critical loads for atmospheric deposition of Pb were identified for U.S. inland or  
20 coastal waters.

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#### 11.1.7.2. Soil Screening Levels

21 Developed by EPA, ecological soil screening levels (Eco-SSLs) are maximum contaminant  
22 concentrations in soils that are predicted to result in little or no quantifiable effect on terrestrial receptors.  
23 The Pb Eco-SSL was completed in March 2005 and has not been updated since. Values for terrestrial  
24 birds, mammals, plants, and soil invertebrates are 11, 56, 120 and 1,700 mg Pb/kg soil (dry weight),  
25 respectively. These conservative values were developed so that contaminants that potentially present an  
26 unacceptable hazard to terrestrial ecological receptors are reviewed during the risk evaluation process  
27 while removing from consideration those that are highly unlikely to cause substantive effects. The studies  
28 considered for the Eco-SSLs for Pb and detailed consideration of the criteria for developing the Eco-SSLs  
29 are provided in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)). Preference is given to studies using the most

1 bioavailable form of Pb to derive values. Soil concentrations protective with respect to avian and  
2 mammalian exposure through diet are calculated by first converting dietary concentration to dose (mg/kg  
3 body weight per day) for a critical study, then using food (and soil) ingestion rates and conservatively  
4 derived uptake factors to calculate a soil concentration that would result in unacceptable dietary doses.  
5 This approach frequently results in Eco-SSL values below the average background soil concentration  
6 ([U.S. EPA, 2005a, 2003](#)), as is the case with Pb for the birds Eco-SSL. [Sample et al. \(2019\)](#) used a re-  
7 analysis of some of the early studies included in the 2005 derivation of the avian Eco-SSL to propose a  
8 new value.

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#### 11.1.7.3. Ambient Water and Sediment Quality Criteria

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

19 The current EPA AWQC for Pb in freshwater, published in 1984, are hardness-based and the  
20 chronic criteria were developed based on the acute-to-chronic ratio due to the lack of chronic toxicity tests  
21 in freshwater biota at that time. Since the AWQC for Pb were first published, additional acute and chronic  
22 toxicity data has become available and better characterization of factors that influence Pb bioavailability  
23 including development of a BLM for Pb. In view of this information, several researchers have proposed  
24 updated approaches for WQC derivation for this metal. Taking into account the range of surface water  
25 chemistry across the United States and the inclusion of newer toxicity data, [Deforest et al. \(2017\)](#)  
26 proposed a BLM-based acute Pb criteria range from 18.9 to 998 µg Pb/L and chronic BLM-based Pb  
27 criteria range from 0.37 to 41 µg Pb/L for freshwater (Section 11.3.5). The lowest criteria were for water  
28 with low DOC (1.2 mg/L), pH (6.7) and hardness (4.3 mg/L as calcium carbonate [CaCO<sub>3</sub>]), and the  
29 highest criteria were for water with high DOC (9.8 mg/L), pH (8.2) and hardness (288 mg/L as CaCO<sub>3</sub>).  
30 Compared to the current EPA AWQC for freshwater, the number of genera with acute toxicity data  
31 increased from 10 to 32, and the number with chronic toxicity increased from 4 to 13, which enabled the  
32 proposed chronic criteria to be based on bioassay data rather than an acute-to-chronic ratio. Furthermore,  
33 DOC and pH are represented in BLM; these water quality factors have a significant influence on Pb  
34 bioavailability and toxicity along with hardness and other water characteristics ([Adams et al., 2020](#)).



1 In comparison to the freshwater chronic criteria proposed by [Deforest et al. \(2017\)](#), Pb effect  
2 thresholds to protect 95% of freshwater species calculated by [Van Sprang et al. \(2016\)](#) for seven selected  
3 European freshwater scenarios were between 6.3 µg Pb/L and 31.1 µg Pb/L, based on chronic toxicity  
4 datasets. There were several differences in development of the European thresholds for chronic Pb  
5 toxicity compared with EPA guidelines, including the use of the 10% effect concentration (EC<sub>10</sub>) rather  
6 than EC<sub>20</sub> chronic toxicity data, selection of species mean values rather than genus mean values and  
7 consideration of toxicity data for plants and algae in combination with bioavailability models to derive  
8 effect thresholds. Furthermore, the range of water chemistries considered did not include the high  
9 bioavailability conditions evaluated in ([Deforest et al., 2017](#)).

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

$$\frac{\text{SEM} - \text{AVS}}{f_{\text{oc}}} = K_{\text{oc}}\text{FCV}$$

(Equation 1)

32  
33 The final chronic value (FCV) (µg/L) in the equation is calculated with the following formula  
34 (Equation 2) using a conversion factor (CF) for Pb in freshwater. The FCV for Pb in saltwater is 8.1 µg/L.

$$CF[e^{1.273[\ln(\text{hardness})]-4.705}]^c \quad (\text{Equation 2})$$

The most recent aquatic life AWQC for Pb in saltwater were released in 1984 ([U.S. EPA, 1985a](#)) by 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 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 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 (USEPA, 1985). The minimum diversity required to develop SSDs has historically precluded this method for saltwater biota due to lack of toxicity data. Using EC<sub>10</sub> 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).

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](#)).



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## 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

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

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

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

27 In terrestrial invertebrates, the evidence reviewed in the 2013 Pb ISA ([U.S. EPA, 2013](#)) was  
28 sufficient to conclude that there is a causal relationship between Pb exposure and decreased survival and  
29 between Pb exposure and reproductive and developmental effects, a likely causal relationship between Pb  
30 exposure and decreased growth, neurobehavior effects and physiological stress, and the evidence is  
31 inadequate to conclude that there is a causal relationship between Pb exposure and hematological effects.

1 Evidence collected since then provides additional support for the effects of Pb exposure on organismal  
2 and suborganismal responses including a decrease in survival, and decreased growth and fecundity.  
3 Recently published studies on physiological responses to Pb include decreases in protein and lipid content  
4 and increases in malondialdehyde (MDA) in earthworms. Acetylcholinesterase (AChE) activity decreased  
5 in response to Pb in snails and honeybees while the effects on protein, glycogen, other enzymes, and  
6 glutathione-s-transferase (GST) responses were variable depending on the site or species examined.  
7 Several new studies quantified behavioral changes to Pb exposure in bees. Evidence also suggests that in  
8 earthworms, Pb exposure can have lasting effects on growth even postexposure on earthworms and slow  
9 the time to maturation. Pb exposure delayed onset of the breeding season and shortened duration in  
10 isopods, as well as influenced mate selection in fruit flies. Evidence published after the 2013 Pb ISA  
11 ([U.S. EPA, 2013](#)) includes new organisms as well as modifying factors of organism response such as  
12 habitat, exposure history, and seasonality.

13 Effects of Pb commonly observed in terrestrial vertebrates include decreased survival, and  
14 reproduction, as well as effects on development and behavior ([U.S. EPA, 2006a](#)). The 2013 Pb ISA ([U.S.  
15 EPA, 2013](#)) also provided evidence for Pb effects on hormones and other biochemical variables. In the  
16 2013 Pb ISA ([U.S. EPA, 2013](#)) the body of evidence was sufficient to conclude that there is a causal  
17 relationship between Pb exposure and reproductive and developmental effects, and between Pb exposure  
18 and hematological effects, and a likely causal relationship between Pb exposure and decreased survival,  
19 physiological stress, and neurobehavioral effects for terrestrial vertebrates. The evidence was inadequate  
20 to conclude that the relationship between Pb exposure and growth is causal for terrestrial vertebrates.  
21 Studies published since the 2013 Pb ISA provide additional evidence for effects on suborganism- and  
22 organism-level endpoints, and specifically on hematological and physiological endpoints, but they do not  
23 affect determinations of causality. New studies have expanded upon the relationship between Pb exposure  
24 and ALAD activity by adding more species of birds, amphibians, and mammals to the evidence base.  
25 More evidence of oxidative stress has been gathered, as well as evidence of effects on corticosterone  
26 levels and immunity in birds. Literature since the 2013 Pb ISA continues to add to evidence relating to  
27 reproductive effects at both the organism and suborganism levels including effects on lifetime breeding  
28 success and some specific secondary sexual traits. New studies of behavioral effects included increased  
29 aggression in mockingbirds.

30 Systematic studies of the validity of using results of Pb salt-addition experiments for estimating  
31 effects of Pb exposure under field conditions have continued since the 2013 Pb ISA. As previously,  
32 experiments showed that the form of Pb, pH, CEC, OC, Fe and Mn oxides, percolation, aging, and soil  
33 composition are all strong modifiers of toxicity. Recent studies demonstrated additional interactions  
34 among those variables and showed that their effects are at times mediated by additional variables such as  
35 salinity. Those studies continue to support the conclusion that data from exposure-response experiments  
36 in terrestrial environments conducted using spiking of soils with soluble salts of Pb, are unlikely to  
37 generate accurate estimates of effects in contaminated natural environments. However, [Oorts et al. \(2021\)](#)

1 suggested that two corrections to the results of exposure-response experiments conducted with additions  
2 of soluble salts of Pb to soil may be sufficient to derive predicted no-effect concentrations (PNEC)  
3 according to the European REACH Regulation [Parliament and Council \(2006\)](#).

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

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

Level		Effect	Terrestrial	
			2013 Pb ISA <sup>a</sup>	2023 Pb ISA
<b>Community and Ecosystem</b>		Community and Ecosystem Effects	Likely Causal	Likely Causal
<b>Population-level Endpoints</b>	<b>Organism-level Responses</b>	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
	<b>Suborganismal Responses</b>	Neurobehavioral Effects – Invertebrates	Likely Causal	Likely Causal
		Neurobehavioral Effects – Vertebrates	Likely Causal	Likely Causal
		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

<sup>a</sup>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 regards 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. In particular, 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, 2006a) 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.

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## **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://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282>). 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, 2006a). 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.

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#### 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<sub>2</sub>)-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 multiple linear regression 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<sub>3</sub> 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 EC<sub>50</sub> 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<sub>d</sub>) 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<sub>d</sub> 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<sub>d</sub> 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<sub>50</sub> values varied considerably amongst the different soil types, ranging from 35–5,080 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<sub>10</sub> and EC<sub>50</sub> 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<sup>2+</sup> 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<sup>2+</sup> in porewater or as total dissolved Pb in porewater) rather than total Pb in soil. The results showed greater variation in EC<sub>50</sub> values based on environmentally available fractions compared with EC<sub>50</sub> 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<sup>2+</sup> 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<sup>+</sup>, 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<sup>2+</sup> and soil porewater as well as the physiological mechanisms of earthworm absorption and metabolism. Similar



1 results were reported in a study that examined Pb and Cd bioavailability in soils located in the vicinity of  
2 a smelter; uptake rate constants of Pb in earthworms were significantly greater at higher pH. [Giska et al.](#)  
3 [\(2014\)](#) suggested that higher pH may be associated with a decrease in competition between heavy-metal  
4 ions and H<sup>+</sup> ions for binding sites on biotic ligands.

#### 11.2.2.1.2. Organic Matter and Inorganic Compounds

5 The 2013 Pb ISA described the significant roles that both organic and inorganic soil constituents  
6 play in immobilizing Pb and decreasing bioavailability. Surfaces of both OM and inorganic materials  
7 (clays and sesquioxide minerals) contain negatively charged functional groups, which serve as sites of Pb  
8 adsorption. In addition, Pb can form immobile precipitates with CO<sub>3</sub>, phosphate and sulfate that may also  
9 be present in soil porewater. [Shaheen and Tsadilas \(2009\)](#) noted that soils with higher clay content, OM,  
10 total CaCO<sub>3</sub> equivalent and total free sesquioxides also exhibited higher total Pb concentration, indicating  
11 that less Pb had been taken up by resident plant species.

12 While recent studies confirm findings from the 2013 Pb ISA regarding the roles of OM and  
13 inorganic surfaces in Pb immobilization, they also suggest that OM is capable of increasing or decreasing  
14 Pb mobility. [Shahid et al. \(2012\)](#) reviewed the role of humic substances on Pb phytoavailability and  
15 toxicity and concluded that the overall role of humic substances in Pb bioavailability is complex due to  
16 the heterogenous nature of humic substances and varying soil physicochemical properties. Depending on  
17 both of these factors, humic substances may exist as dissolved OM (DOM) capable of binding free Pb<sup>2+</sup> in  
18 soil porewater, as solid constituents with high adsorption affinity for Pb or as DOM capable of increasing  
19 the extractable and bioavailable fractions of Pb. [de Santiago-Martin et al. \(2014\)](#) used bioassays with  
20 romaine and iceberg lettuce grown in calcareous Mediterranean soils with low levels of OM that were  
21 spiked with Pb, Cu, Cd and Zn to assess the contribution of soil physicochemical properties toward  
22 bioavailability. CO<sub>3</sub>, OM and fine mineral fractions accounted for 85% of the variance in bioavailability,  
23 and OM was the most important variable explaining Pb and Cd bioavailability patterns. However, OM  
24 seemed to exert contrary effects on Pb and Cu bioavailability. At lower concentrations of the metals, OM  
25 and bioavailability were negatively correlated, but a positive correlation was observed at higher  
26 concentrations. The authors suggested that differences in the role OM had at different concentrations may  
27 be attributed to competitive binding between Pb and Cu onto humic acids, resulting in a larger  
28 bioavailable fraction at higher concentrations due to saturation of binding sites on humic acids ([de](#)  
29 [Santiago-Martin et al., 2014](#)). Similar results of the contradictory role that OM may have on  
30 bioavailability were reported by [Zeng et al. \(2011\)](#), whereby OM was observed to have a positive  
31 correlation with ethylenediaminetetraacetic acid (EDTA)-extractable chromium (Cr), Cu, Fe, Mn, Pb and  
32 Zn, and both positive and negative correlations with concentrations in rice straw grown in the  
33 contaminated soils. [Pauget et al. \(2011\)](#) evaluated the influence of pH, OM and clay content on chemical  
34 availability and bioavailability of Pb to land snails (*Cantareus aspersus*) exposed to nine contaminated

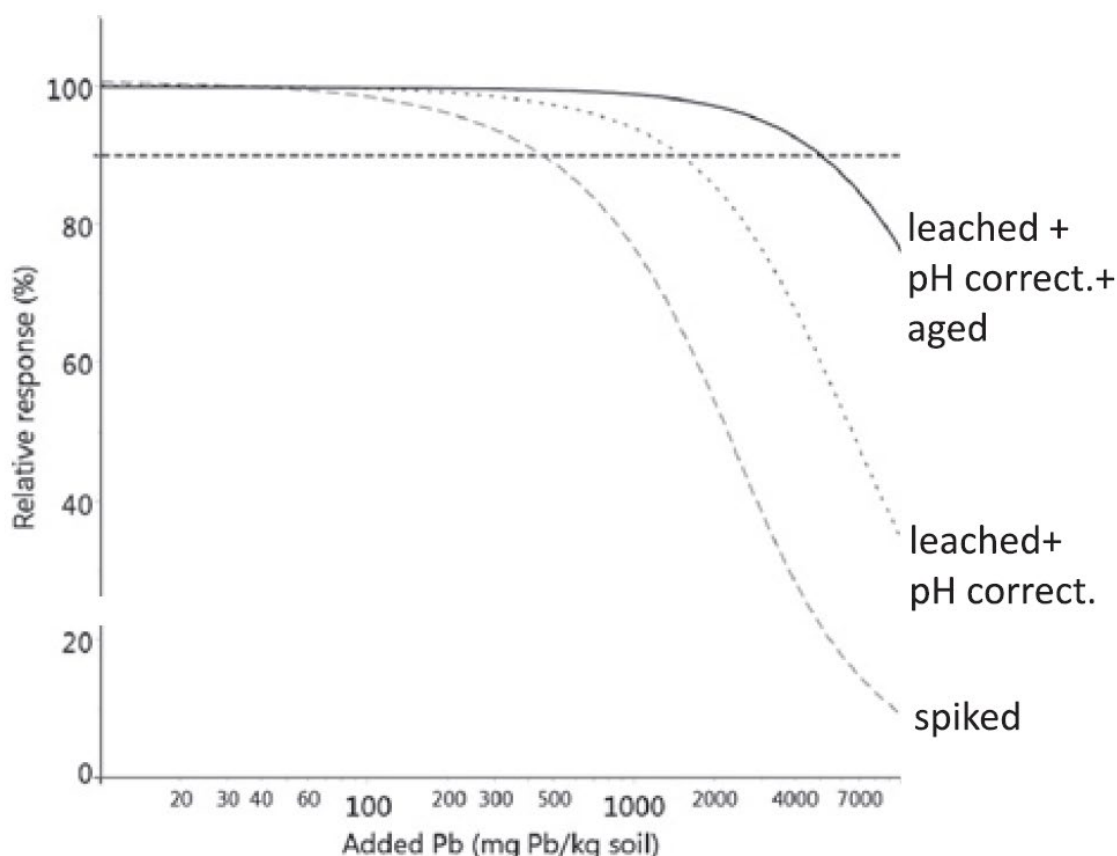


soils, each differing by a single characteristic (pH, OM, or clay content). The results demonstrated that an increase in both pH and OM decreased Pb bioavailability to snails. However, clay did not have a significant influence. It is worth noting that the clay mineral used for this assessment was kaolinite. 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<sub>2</sub>, 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<sub>2</sub> 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<sup>2+</sup> 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<sup>2+</sup> 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<sub>10</sub> values. EC<sub>10</sub> values were often near background levels for freshly spiked soils, but after leaching, pH correction and 5 years of aging, the majority of EC<sub>10</sub> 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<sub>2</sub> 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/kg Pb as lead nitrate ( $\text{Pb}(\text{NO}_3)_2$ ), 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  $\text{CO}_3$  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

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

10 To disentangle the effects of salinity, acidification, and aging on the sensitivity of microbial communities,  
11 plants, and invertebrates to Pb, [Smolders et al. \(2015\)](#) conducted an experiment in which toxicity to these  
12 groups was tested in soils spiked with  $\text{Pb}^{2+}$  salts, leached and aged. Uncontaminated soils were collected  
13 from grasslands and agricultural lands in Spain, the United Kingdom and Belgium and were exposed to 0,  
14 250, 500, 1,000, 2,000, 4,000 or 8,000 mg Pb/kg using  $\text{PbCl}_2$ . Some of the soil was set aside (treatment:  
15 freshly spiked), while the rest was incubated for a week, leached using artificial rainwater and pH-  
16 corrected to maintain soil pH within 0.2 pH units within each Pb concentration using CaO (treatment:  
17 leached and pH-corrected). Five years prior to spiking soils with  $\text{PbCl}_2$ , additional soils were exposed to  
18 the same Pb gradient using  $\text{Pb}(\text{NO}_3)_2$  and stored in perforated pots which were left outdoors to age. After  
19 5 years, pH was corrected using CaO (treatment: aged, leached and pH-corrected). Soil solution Pb  
20 concentration, i.e., porewater Pb concentration, increased in a dose-dependent manner with spiked soils,  
21 followed by leached soils and finally aged soils containing the least soil solution Pb (except in aged soils  
22 from Spain). Toxicity was then tested in microbial communities, earthworms (*E. fetida*), Collembola (*F.*  
23 *candida*), tomato (*Lycopersicon esculentum*) and barley (*Hordeum vulgare*). Toxicity was highest in  
24 freshly spiked soils (mean  $\pm$  S.E.,  $\text{EC}_{50}$  for all organisms tested:  $2,300 \pm 145$  mg/kg Pb), followed by  
25 leached and pH-corrected soils ( $6,500 \pm 750$  mg Pb/kg) and then aged soils ( $>10,000$  mg Pb/kg);  
26 however, the effects of leaching with pH correction and aging with pH correction were inconsistent  
27 among organisms and toxicity tests. Depending on the origin of the soil, leaching and pH correction  
28 reduced toxicity based on  $\text{EC}_{10}$  values by a factor of 1.9–2.3 compared with freshly spiked soils, while  
29 aging and pH correction reduced toxicity by a factor of 2.7–13. Microbial activity (potential nitrification  
30 rate, substrate-induced nitrification, and respiration rate), invertebrate reproduction and plant growth were  
31 negatively correlated with total soil Pb concentration, porewater Pb concentration,  $\text{Pb}^{2+}$  ion activity and  
32 porewater ionic strength. With the exception of *E. fetida* reproduction, these factors were positively  
33 correlated with soil pH. Given porewater ionic strength had the strongest influence on toxicity across all  
34 tested organisms, the authors suggest that salt stress may modify the toxicity of Pb, as acidification and  
35 aging were unable to explain variation in toxicity.

#### 11.2.2.1.5. Summary

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

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#### 11.2.2.2. Uptake and Bioaccumulation in Terrestrial Plants

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

1 have been found to better correlate with bioavailable Pb soil concentration, defined as diethylenetriamine  
2 pentaacetate-extractable Pb, than with total Pb concentration ([U.S. EPA, 2006b](#)).

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

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

18 Although exposures are often high, field studies carried out in the vicinity of Pb smelters and  
19 other industrial point sources have determined the relative importance of direct foliar uptake and root  
20 uptake of atmospheric Pb deposited in soils, with greater overall uptake corresponding to closer proximity  
21 to the source ([Angelova et al., 2010](#); [Hu and Ding, 2009](#); [Cui et al., 2007](#)). [Hu and Ding \(2009\)](#) concluded  
22 that metal accumulation in some leafy greens grown in the vicinity of a smelter were greater in shoot than  
23 in root tissue, which suggested both high atmospheric Pb concentration and direct stomatal uptake into  
24 the shoot tissue. Similarly, evidence since the last review shows substantial accumulation of Pb in needles  
25 in areas with high contributions of atmospheric Pb ([Kandziora-Ciupa et al., 2016](#); [Gandois and Probst,](#)  
26 [2012](#)). Studies also noted a significant difference between Pb concentrations in washed and unwashed  
27 leaves, indicating that aerial deposition and surface dust is likely a significant source of plant-associated  
28 Pb ([Ugolini et al., 2013](#); [El-Rjoob et al., 2008](#)). Foliar Pb may include both incorporated Pb (i.e., from  
29 atmospheric gases or particles) and surficial particulate Pb deposition. The plant may eventually absorb  
30 the surficial component; however, the main importance of surficial Pb is its likely contribution to the  
31 exposure of plant consumers or to leaf litter. The consideration of these Pb exposures to humans via  
32 consumption of food crops is briefly discussed in Section 2.1.3 of Appendix 2.

33 Because of their long life spans, certain trees can provide essential information regarding the  
34 sources of bioavailable Pb. A Scots pine (*Pinus sylvestris*) forest in northern Sweden was found to  
35 incorporate atmospherically derived Pb pollution directly from ambient air, accumulating this Pb in the  
36 bark, needles, and shoots ([Klaminder et al., 2005](#)). More recent studies have also shown that accumulation

1 in the bark of some species is a useful bioindicator of exposure to atmospheric Pb ([Janta and Chantara,](#)  
2 [2017](#); [Palowski et al., 2016](#)). Metal content can also vary in relation to altitude as a result of long-range  
3 transport. [Korzeniowska et al. \(2021\)](#) found that metal content in the moss (*Pleurozium schreberi* (Willd.)  
4 Mitten) and in Norway spruce (*Picea abies* (L.) H. Karst) in the Tatra National Park in the Carpathian  
5 Mountains of Poland was greater with increasing altitude.

6 Dendrochronology (tree-ring analysis) is an important tool for measuring the exposure of trees to  
7 environmental Pb ([Watmough, 1999](#)). While effectiveness may vary by species investigated, tree-ring  
8 studies reviewed in the previous AQCDs and ISAs showed that trees could be used as indicators of  
9 increasing environmental Pb concentrations with time ([U.S. EPA, 2013, 2006a, 1977](#)). Trees accumulate  
10 and sequester atmospheric Pb in close correlation with the rate of point-source emissions ([Guyette et al.,](#)  
11 [1991](#)). Studies published since the 2013 Pb ISA continue to demonstrate dendrochronology is a useful  
12 tool for monitoring historical uptake of Pb into trees exposed to atmospheric or soil Pb ([Sensula et al.,](#)  
13 [2017](#); [Dinis et al., 2016](#); [Beramendi-Orosco et al., 2013](#); [Doucet et al., 2012](#)) (Section 11.2.3).

14 In the 2013 Pb ISA ([U.S. EPA, 2013](#)), plant-associated arbuscular mycorrhizal fungi (AMF) were  
15 found to protect the host plant from Pb uptake. Additional evidence indicates that the presence of AMF or  
16 bacteria hosts can influence Pb accumulation in and alleviate Pb stress on plants. Inoculation of David's  
17 mountain laurel (*Sophora davidii*, previously *Sophora viciifolia*) with the AMF *Funneliformis mosseae*  
18 resulted in lower concentrations of Pb in belowground and aboveground biomass ([Xu et al., 2016a](#)). *S.*  
19 *davidii* seeds collected from around the Qiandongshan Pb and Zn mine in northwest China were grown in  
20 pots receiving 0, 50, 500, or 1000 mg Pb/kg (aqueous Pb(NO<sub>3</sub>)<sub>2</sub>). Half of the pots with *S. davidii* plants  
21 were inoculated with *F. mosseae*. After 4 months, mycorrhizal colonization, Pb accumulation, plant  
22 height, diameter, aboveground and belowground biomass, and root characteristics were recorded (Section  
23 11.2.4.2). Vesicular, arbuscular, hyphal, and total root colonization of *S. davidii* decreased with increasing  
24 Pb treatment. Both mycorrhizal and nonmycorrhizal plants showed increasing Pb content in their roots  
25 and aboveground tissue in a dose-dependent manner, but belowground and aboveground Pb  
26 concentrations were lower for mycorrhizal plants. Pb concentration in aboveground tissue of mycorrhizal  
27 plants was 54–66 % less Pb than that in nonmycorrhizal plants, while roots contained 15–85 % less,  
28 depending on Pb exposure. The root-to-shoot Pb concentration of mycorrhizal plants increased with Pb  
29 exposure while nonmycorrhizal plant root-to-shoot concentration decreased with increasing Pb exposure,  
30 suggesting that Pb was sequestered in the root following inoculation with *F. mosseae*. Furthermore,  
31 transmission electron micrographs and X-ray microanalysis of *S. davidii* roots under different Pb and  
32 mycorrhizal treatments suggested Pb in the cytoplasm was sequestered in the cell walls and vacuoles of *F.*  
33 *mosseae*, while Pb was transported into the root cells and intracellular space of nonmycorrhizal plants.

34 Pot marigolds (*Calendula officinalis*) inoculated with *Glomus mossea* and *G. intradices*  
35 accumulated more Pb relative to nonmycorrhizal plants, yet experienced greater fitness-([Tabrizi et al.,](#)  
36 [2015](#)). *Calendula officinalis* were grown in pots and received 0, 150, or 300 mg Pb/kg (aqueous  
37 Pb(NO<sub>3</sub>)<sub>2</sub>). 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 1000 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 glutinosa*) by an actinobacteria, *Frankia*, affected Pb uptake in roots and shoots ([Belanger et al., 2015](#)). *Alnus glutinosa* 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<sub>3</sub>)<sub>2</sub> (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. glutinosa* 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.

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### 11.2.2.3. Uptake and Bioaccumulation in Terrestrial Invertebrates

At the time of publication of the 2006 Pb AQCD ([U.S. EPA, 2006a](#)), 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



1 110 mg Pb/kg at the high pollution site. Foot muscle and hepatopancreas tissue concentration increased  
2 with increasing exposure levels. More Pb was stored in the hepatopancreas than the foot tissue, and  
3 hepatopancreas levels were generally higher than soil contamination. BAF values were less than 1 for foot  
4 muscle (0.47, 0.9, and 0.42) and greater than 1 for hepatopancreas tissue at the low and medium pollution  
5 sites (1.61, 1.72, 0.76). The greater concentration found in the hepatopancreas indicates greater uptake via  
6 food. Concentrations reported within snail tissues in this study were higher than those reported in studies  
7 examining other snail species, suggesting uptake and accumulation are partly species-specific; see also  
8 ([Mleiki et al., 2017](#)).

9 New literature further supports that Pb uptake by snails is influenced by soil characteristics as  
10 well as being dose- and duration-dependent. The concentration in the digestive gland of the green garden  
11 snail (*Cantareus apertus*) increased with increasing exposure level after 1 week of exposure for low  
12 (25 mg Pb/kg), medium (100 mg Pb/kg) and high (2500 mg Pb/kg, nominal values reported) exposure  
13 levels ([Mleiki et al., 2016](#)). However, tissue concentration was not significantly greater in the 2500  
14 mg Pb/kg treatment compared with the 100 mg Pb/kg treatment. Similarly, after eight weeks of exposure,  
15 digestive gland tissue concentration was higher under Pb exposure compared with the control, but the  
16 highest concentrations were found under the 100 mg Pb/kg exposure. An observational field study  
17 examining the uptake and elimination kinetics of Pb by the common garden snail (*Cantareus aspersus*)  
18 found soil Pb concentration (positive), CEC (positive) and soil OC content (negative) have a multivariate  
19 effect on Pb bioavailability. Similarly, soil silt (positive), sand (positive) and OC content (negative)  
20 modulate Pb uptake by snails ([Pauget et al., 2013b](#)). In another study, soil Pb concentration was correlated  
21 with Pb concentration in juvenile *C. aspersus* but when OC content and Al and Fe oxides were included  
22 in the model, R<sup>2</sup> increased from 0.37 to 0.56. The most polluted plots (i.e., plots with the highest Pb  
23 concentration) did not have the highest Pb transfer to snails. OC content is known to influence metal  
24 mobility and bioavailability for soil organisms ([Pauget et al., 2013a](#)).

#### 11.2.2.3.2. Earthworms

25 In the 2013 Pb ISA, studies of bioaccumulation of Pb in earthworms reported that many soil  
26 physicochemical properties, including pH, OM and CEC, affect metal bioavailability for these organisms;  
27 recent studies confirm these observations. Following 4 weeks in soil spiked with a solution of Pb (NO<sub>3</sub>)<sub>2</sub>  
28 (40, 250, 500, 1000, 2500 mg Pb/kg, nominal values reported), juvenile *E. fetida* body Pb concentration  
29 increased with exposure concentration. BCFs ranged from 0.14 to 0.3, indicating either low  
30 bioavailability of Pb in the soil or low ability to accumulate Pb within tissues. After 4 weeks of recovery  
31 (no Pb exposure), earthworm body Pb was significantly lower than the value at the end of the exposure  
32 period but was still higher than the control and positively correlated with exposure values ([Zaltauskaite et](#)  
33 [al., 2020](#)). A study on native *Eisenoides lonnbergi* earthworms in Maryland found *E. lonnbergi* can  
34 accumulate extraordinarily high levels of Pb, with a BAF of 83 recorded ([Beyer et al., 2018](#)).

1 Accumulation was driven by soil Ca levels and indirectly by pH and clay content, not by soil Pb content  
2 or availability. In acidic, low Ca soils, Pb uptake and accumulation is greater. Over soil Ca concentrations  
3 ranging from 49 to 1695 mg Pb/kg, *E. lonnbergi* can maintain body Ca concentrations between 4000 and  
4 8000 mg Pb/kg. Thus, even in Ca-poor soils, *E. lonnbergi* can uptake enough needed Ca to maintain  
5 necessary body concentrations. The Ca BCF was 3.3 in high Ca soils and 117 in low Ca soils. The Pb  
6 concentration factor was 1.02 in high Ca soils and 83 in low Ca soils, suggesting Pb is absorbed by the Ca  
7 transport system, which is known to occur in vertebrates ([Beyer et al., 2018](#)).

8 In *E. fetida* earthworms exposed to a range of soil Pb values from 125–350 mg Pb/kg across a  
9 range of pH, Pb concentration in the worms was higher in low pH (<5.5) soils than in neutral or alkaline  
10 soils with similar Pb concentrations ([Tang et al., 2018](#)). Following 4 weeks in soil spiked with a solution  
11 of Pb (NO<sub>3</sub>)<sub>2</sub> (40, 250, 500, 1000, 2500 mg Pb/kg, nominal values reported), juvenile *E. fetida* body Pb  
12 concentration increased with exposure concentration. BCF varied from 0.14 to 0.3 indicating either low  
13 bioavailability of Pb in the soil or low ability to accumulate Pb within tissues ([Zaltauskaite et al., 2020](#)).  
14 After 4 weeks of recovery (no Pb exposure), earthworm body Pb was significantly lower than at the end  
15 of the exposure period but was still higher than the control and positively correlated with exposure values.  
16 In another study examining earthworm Pb concentrations, BAFs in low pH soils were also higher than  
17 those in other soils but all BAFs were less than one ([Richardson et al., 2015](#)). Soil Pb, OC, and pH  
18 together gave the best predictive model outcome on earthworm Pb concentration. Earthworm ecotype can  
19 influence Pb tissue concentrations as well. Endogeic and epigeic species were found to have higher Pb  
20 tissue concentration than epi-endogeic and anecic earthworms ([Richardson et al., 2015](#)). A recent meta-  
21 analysis by [Richardson et al. \(2020\)](#) examined the influence of soil concentration, soil characteristics,  
22 earthworm genus and ecotype on trace metal uptake. They found soil concentration did not predict  
23 earthworm tissue concentration but ecophysiological group, earthworm genus, metal source, exposure  
24 duration, and soil OM were important predictors.

25 In studies cited in the 2013 Pb ISA, earthworm feeding and burrowing behavior altered the  
26 bioavailability, mobility, and uptake of Pb by earthworms and other soil biota. Recent studies further  
27 elucidate the effects of earthworms on soil Pb processes. One study examined the decomposition of  
28 *Amyntas agrestis* and *Lumbricus rubellus* earthworms and the subsequent release of Pb in different  
29 fractions within the soil column over 60 days ([Richardson et al., 2016b](#)). Both species had similar Pb  
30 tissue concentrations but due to the greater mass of *A. agrestis* added to experimental soils on a dry  
31 weight basis, *A. agrestis* contributed a larger pool of Pb to the soil column. Leachate from both  
32 earthworm treatments was significantly higher in Pb than leachate from control (no earthworm) soils.  
33 Exchangeable Pb pools were greater under both earthworm treatments but only at days 7 and 21. By  
34 day 60, there was only slightly more exchangeable Pb under the *A. agrestis* treatment compared with the  
35 control. The stable Pb pool was greater under earthworm treatments across all sampling dates and the  
36 majority of Pb under earthworm treatments was in the stable fraction. In a lab experiment using field-  
37 collected polluted soils, *Lumbricus terrestris* earthworms were exposed to a high Pb-contaminated soil

(4550 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<sup>2+</sup> and inorganic Pb, but less organic Pb compared with control soils. In low pollution earthworm soils, there was less Pb<sup>2+</sup> 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 (*Amyntas agrestis* 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. agrestis* after 80 days and 2.39 for *L. rubellus*. The BAF for the exchangeable fraction only was 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<sup>2+</sup> 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 3730 mg Pb/kg concentration) (Leveque et al., 2014). Earthworms also increased lettuce Pb concentration but only at exposure concentrations of 2822 and 3730 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 (nominal values reported) 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, nominal 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, 1000 mg Pb/L, nominal 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 value) 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 1000 µM Pb nominal values), 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.

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#### 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 man-made 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<sup>2+</sup> 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 ( $0.393 \pm 0.131$  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*)



1 and turkey vultures (*Cathartes aura*) collected in Virginia. Bone Pb was significantly higher than Pb in  
2 liver in both species indicating that Pb in the birds was primarily associated with long-term exposure.  
3 Possible sources of Pb in these long-lived birds based on comparison of Pb isotope ratios in femur bones  
4 and Pb isotope ratios associated with Pb sources included ammunition, coal-fired power plants, leaded  
5 gasoline, and zinc smelting operations.

6 In summary, literature since the 2013 Pb ISA ([U.S. EPA, 2013](#)) adds support to existing evidence  
7 of Pb accumulation in blood, bones, and a variety of different tissues in terrestrial vertebrates. Pine snakes  
8 accumulated Pb in liver, kidney, muscle, skin, and heart tissue, with the highest concentrations found in  
9 the muscles. In toads, Pb was found to accumulate in blood and increased with increasing soil Pb levels.  
10 New evidence continues to support findings of the accumulation of Pb in tissues from a wide range of  
11 mammalian species. Pb ammunition continues to be a prevalent source of Pb contamination in both  
12 mammals and birds. Consumption of prey species has also been found to be an important route of Pb  
13 exposure especially in species that consume earthworms such as woodcocks and robins.  
14

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#### 11.2.2.5. Uptake and Bioaccumulation Through Food Web

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

24 Pb was transferred through a soil, nettle, snail food web in Antwerp, Belgium ([Boshoff et al.,](#)  
25 [2015](#)). In a microcosm field experiment, adult European land snails (*Cepaea nemoralis*) from an  
26 uncontaminated site were exposed to sites varying in distance from the Umicore Precious Metal Refinery,  
27 a nonferrous smelter in Antwerp, Belgium. The snails were sampled along with nettle (*Urtica dioica*), one  
28 of their food sources. *Cepaea nemoralis* were placed in microcosms at each site and allowed to feed on  
29 soil, litter, and vegetation for 16 weeks. A subset of snails was collected at weeks 0, 1, 2, 4, 8, and 16 for  
30 metal analysis (Pb, arsenic [As], Cd, Cu, Zn, nickel [Ni]) and morphological and physiological biomarker  
31 response (Section 11.2.4.4). Nettle (*U. dioica*) samples were collected three times throughout the  
32 experiment for trace metal analysis. Pb concentration in the soil was the only significant factor explaining  
33 Pb concentration in *U. dioica*. Pb concentrations in the digestive glands of the *C. nemoralis* varied

1 spatially and temporally, as there was a statistically significant interaction between site and time. Pb  
2 concentration in the soil was higher than that in *U. diocia*, while the concentrations of Pb in the digestive  
3 glands of *C. nemoralis* were similar to or higher than Pb concentrations in *U. diocia*.

4 Detoxification may be an important mechanism behind biodilution of Pb with trophic level in the  
5 food web. Silkworms (*Bombyx mori*) were shown to excrete Pb when fed Pb-exposed mulberry (*Morus*  
6 *alba*) ([Zhou et al., 2015](#)). Soils collected from an agricultural field in China were exposed to nominal  
7 concentrations of 0, 200, 400, or 800 mg Pb/kg via Pb (NO<sub>3</sub>)<sub>2</sub>. *Morus alba* was planted in the Pb-spiked  
8 soils for 3 months, and the leaves were collected and fed to fifth instar larvae of *B. mori*. The available  
9 fraction of Pb in the soils, the total concentration in mulberry leaves, shoots and roots, and *B. mori* larvae,  
10 silk, feces, and adult moth increased with increasing soil Pb addition in a dose-dependent manner. Roots  
11 sequestered the most Pb, followed by stems, and leaves. The translocation factor was highest for the  
12 transfer of Pb from the soil to the root in the 400 mg Pb/kg treatment, followed by 1.60 in the  
13 800 mg Pb/kg treatment, and 1.13 in the 200 mg Pb/kg treatment. All other translocation factors between  
14 the soil and plant (root–soil, stem–soil, leaves–stem, stem–root, leaf–root, leaves–stem) were below 1.0 or  
15 near 1.0 for the control (0 mg Pb/kg). Across all treatments, the subcellular distribution of Pb in the leaves  
16 was greatest in the cell wall, followed by the soluble fraction, and organelles. Pb treatment did not affect  
17 silkworm survival or mean weight, but, increasing Pb treatment negatively affected the silkworm growth  
18 rate. Specifically, the body weight of silkworms was significantly lower at the end of the experiment in  
19 the 800 mg Pb/kg treatment compared to the control and the 200 mg Pb/kg treatment. Pb concentration in  
20 the silkworm increased with increasing treatment. Pb concentration in the feces was the greatest, followed  
21 by the concentration in the peel, the larvae, the silk moths and finally the silk. Metallothionein synthesis  
22 increased in *B. mori* when fed with Pb-treated leaves. Metallothionein content in the midgut was more  
23 sensitive to lower Pb exposure (200 mg Pb/kg) than metallothionein in the posterior of the silk gland and  
24 in the fat body, both of which increased in the high Pb exposures (400 and 800 mg Pb/kg). These results  
25 suggest that *B. mori* can detoxify Pb through excretion and homeostasis.

26 Field studies published since the 2013 Pb ISA ([U.S. EPA, 2013](#)) provide additional evidence for  
27 biodilution in terrestrial food webs. Oil rapeseed (*Brassica napus*) and insects were collected from 35  
28 agricultural sites in Southwest Poland ([Orlowski et al., 2019](#)). These agricultural sites varied in size,  
29 habitat fragmentation, and percent cover by forests and were characterized by percent arable land,  
30 permanent vegetation, linear woody features, dirt or unpaved roads, and wooded areas. *Brassica napus*  
31 and the insect community (grouped into guilds: pollinators, consumer/herbivores, saprovores, predators,  
32 and parasitoids) were analyzed for Pb and other trace elements. The concentration of Pb in *Brassica*  
33 *napus* (mean: approximately 2 mg Pb/kg) was higher than those in all insects examined (range: 0.77 to  
34 2.31 mg Pb/ kg), and Pb concentration generally decreased with increasing trophic level, suggesting Pb is  
35 diluted in this food web. As the size of the field area increased, the Pb concentration in pollinators  
36 decreased, suggesting that even under low Pb levels, larger areas with more diversified landscapes could  
37 reduce Pb body burden for pollinators.

The presence of other heavy metals in the soil, specifically Cd in the soil, 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/kg Pb + 4 mg/kg Cd and 400 mg/kg Pb + 8 mg/kg Cd). Soils were treated with Pb(NO<sub>3</sub>)<sub>2</sub> and Cd(NO<sub>3</sub>)<sub>2</sub> salts, resulting in 1.45 ± 0.23 mg/kg 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 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/kg 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.

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### **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.

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#### **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.

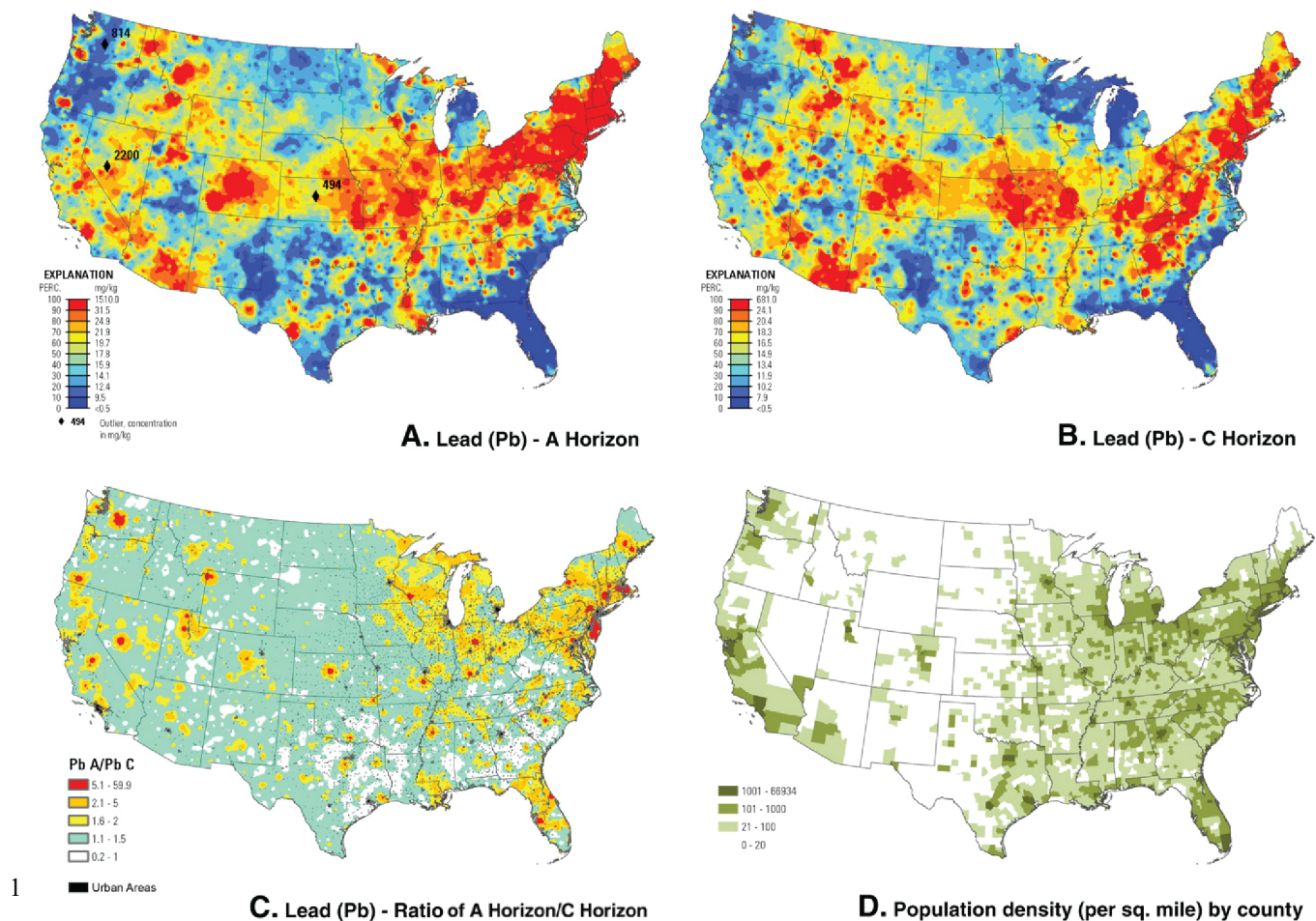
A regional survey of forest floor soils sampled in the northeastern United States provides a time series of Pb concentrations from 1980 to 2011. The region has a large amount of urban and industrial development associated with high historical anthropogenic Pb emissions. Soils were sampled at 25 sites 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  $151 \pm 29$  (SE) mg Pb/kg in 1980 to  $68 \pm 13$  (SE) mg Pb/kg in 2011 ([Richardson et al., 2014b](#)) (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  $2.0 \pm 0.3\%$  per year ([Richardson et al., 2014a](#)).

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  $23.9 \pm 13.8$  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. \(2014b\)](#) 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 11-4 C. 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 11-4 C. 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., 2014b](#)) demonstrate the persistence of historical Pb contamination in U.S. soils.

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### 11.2.3.2. Pb in Tree Rings

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

7 Several studies conducted after the 2013 Pb ISA report temporal trends in Pb as observed in tree  
8 rings, three from Canada and one from Mexico. A study of white spruce trees (*Picea glauca*) located in  
9 the Northern Athabasca Oil Sands Region of western Canada near oil sands mining operations  
10 reconstructed Pb concentrations from 1878 to 2009. Tree-ring records of Pb concentrations increased  
11 beginning in 1922, peaked in 1968–1973, then decreased until 2009 ([Dinis et al., 2016](#)). In eastern  
12 Canada, a study reconstructed Pb trends from 1880 to 2007 in red spruce (*Picea rubens*), beech (*Fagus*  
13 *grandifolia*), white pine (*Pinus strobus*), and white cedar (*Thuja occidentalis*). The beech trees located in  
14 both Montreal and Georgian Bay exhibited a decline in concentrations after a 1970–1985 peak. The  
15 authors attribute the lack of an observed temporal trend in Pb concentrations in white pine to the radial  
16 mobility of Pb within the tree ([Doucet et al., 2012](#)). Another study of tree-ring Pb concentrations in white  
17 cedar in Québec dated concentrations from 1850–2010 and recorded increased concentrations from 1950–  
18 2000 near a Pb smelter. The increasing trend at a control site further from the smelter was delayed to  
19 1990–2010. Concentrations across most sites in this study decreased from 2000–2010 ([Arteau et al.,](#)  
20 [2020](#)). In contrast to the trends observed in the Canadian studies, a study of *Prosopis juliflora* tree rings  
21 dated from 1903 to 2007 located near a copper smelter in San Luis Potosi, Mexico found increasing Pb  
22 concentrations from 1990–2007 ([Beramendi-Orosco et al., 2013](#)).

23 Although trends in reconstructed Pb concentrations varied across tree species and regions, studies  
24 identified a temporal pattern of Pb that increased after 1850–1900 and, in some cases, peaked in 1970–  
25 1985, then decreased afterward. Tree-ring studies with temporal patterns in exception to this pattern were  
26 conducted near persisting industrial point sources of Pb pollution.

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### 11.2.4. Effects of Pb in Terrestrial Systems

27 This section focuses on studies of the biological effects of Pb on terrestrial biota published since  
28 the 2013 Pb ISA. First, new information on factors that affect biological sensitivity to Pb is discussed,  
29 followed by subsections on effects on vegetation, microbes, invertebrates, and vertebrates. The biological  
30 effects of Pb in the 2013 Pb ISA and in this appendix are generally presented in increasing order of the  
31 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.

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#### 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  $\text{Pb}(\text{NO}_3)_2$  and permitted to age for 3 months while maintaining soil moisture. After 3 months, soil physicochemical variables and bacterial community structure were analyzed. Available Pb and total Pb concentration in the soil varied with Pb treatment level (available Pb in the control (mean  $\pm$  S.D.):  $3.97 \pm 0.08$  mg Pb/kg, 150 mg Pb/kg treatment:  $126.6 \pm 4.98$  and 350 mg Pb/kg treatment:  $254.46 \pm 7.13$ ). 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 glutinosa*) was unaffected by Pb treatment, while *Frankia* microbial respiration was significantly affected by Pb treatment ([Belanger 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  $76.39 \pm 1.37$  mg Pb/kg (mean  $\pm$  S. E.) and ranged from 3.0 mg/kg 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<sub>3</sub>)<sub>2</sub>). 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, [Vetrovsky 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 1373 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  $67.4 \pm 1.6$  mg Pb/kg (mean  $\pm$  S.D.,  $n = 3$ ) to  $759.3 \pm 11.4$  mg Pb/kg, while bioavailable Pb, measured as 0.1 M HCl-extractable Pb (HCl-Pb) ranged from  $33.0 \pm 1.9$  mg Pb/kg to  $681.0 \pm 33.9$  mg/kg Pb. In this study, neither total soil Pb nor HCl-Pb was correlated with microbial enzyme including fluorescein diacetate hydrolysis activity (FDA), 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 *Acidimicrobiales* 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  $\text{Pb}(\text{NO}_3)_2$ ). 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  $9.74 \pm 0.11$ ,  $210.27 \pm 0.41$ ,  $412.24 \pm 0.60$ ,  $607.17 \pm 0.65$  and  $811.74 \pm 0.44$  mg Pb/kg (mean  $\pm$  S.D.), and available Pb was  $2.15 \pm 0.24$ ,  $72.23 \pm 0.28$ ,  $166.30 \pm 0.38$ ,  $242.94 \pm 0.19$  and  $382.17 \pm 0.60$  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<sub>2</sub> increased with increasing Pb exposure; however, plant ecotype did not affect soil qCO<sub>2</sub>. 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](#); [Golebiewski 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.

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#### 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, 2006a, 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

1 to alteration of chlorophyll structure, as well as decreases in chlorophyll content in plants, lichens, and  
2 mosses. Recent studies have continued to demonstrate decreases in photosynthetic performance due to Pb  
3 exposure ([Alkhatib et al., 2019](#); [Silva et al., 2017a](#); [Rodriguez et al., 2015](#)) as well as documented damage  
4 to chlorophyll structure caused by Pb ([Tokarz et al., 2020](#); [Alkhatib et al., 2019](#); [Rodriguez et al., 2015](#)).  
5 A substantial amount of evidence of oxidative stress in response to Pb exposure has also been produced  
6 and documented in the 2013 Pb ISA and previous AQCDs. Monocot, dicot, and bryophytic taxa grown in  
7 Pb-contaminated soil or in experimentally spiked soil all responded to increasing exposure with increased  
8 antioxidant activity. Recent studies continue to confirm increased antioxidant activity in plants in  
9 response to Pb stress ([Kaur et al., 2015](#); [Reis et al., 2015](#); [Rossato et al., 2012](#)), as well as the genotoxic  
10 effects of Pb exposure ([Silva et al., 2017b](#)), albeit at concentrations that greatly exceed Pb measured in  
11 soils (Table 11-1). Finally, studies of the effects of Pb on terrestrial plants published since the last ISA  
12 continue to support the previous known findings of declines in plant growth under controlled exposures of  
13 Pb ([Muradoğlu et al., 2016](#); [Kaur et al., 2012](#); [Rossato et al., 2012](#)).

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

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

1 lower orders and suppress transportation of Pb to high-order roots, which may partially explain the greater  
2 Pb tolerance in males when evaluating tree physiological variables.

3         Recent studies have also examined the protective effects of certain plant nutrients as well as the  
4 influence of mycorrhizal inoculation on the effects of Pb in terrestrial plants. In a hydroponic experiment  
5 with two different ecotypes of *Elsholtzia argyi* (one from an agricultural site and one from an abandoned  
6 Pb mine in China), plants were exposed to 50  $\mu\text{M}$  Pb with normal Zn levels (0.5  $\mu\text{M}$ ) and high Zn  
7 (20  $\mu\text{M}$ ) for 12 days ([Islam et al., 2011](#)). Application of Pb with normal Zn had negative effects on the  
8 overall growth and antioxidant capacity of both ecotypes; however, the effects were more pronounced in  
9 agriculturally sourced plants. The addition of high Zn improved the growth and antioxidant capacity of  
10 both ecotypes under Pb stress. Finally, a study using Pb exposures on *Torreya grandis* seedlings (0, 700  
11 and 1400 mg  $\text{Pb}^{2+}/\text{kg}$ ) examined the possible protective effects of the addition of 1040 mg/kg  $\text{Mg}^{2+}$  ([Shen  
12 et al., 2016](#)). The addition of  $\text{Mg}^{2+}$  improved the growth of the Pb-stressed seedlings, increased  
13 chlorophyll content, enhanced chloroplast development and improved both the photosynthetic rate and  
14 maximum quantum efficiency in Pb-stressed plants. In addition,  $\text{Mg}^{2+}$  addition increased root growth and  
15 oxidative activity and protected the root ultrastructure. These studies showed that some mineral nutrients,  
16 when added beyond the minimal plant requirements, can increase plant tolerance of Pb stress. This is  
17 particularly true of Mg addition. [Ling and Hong \(2009\)](#) hypothesized that  $\text{Pb}^{2+}$  may replace either  $\text{Mg}^{2+}$  or  
18  $\text{Ca}^{2+}$  in chlorophyll or the oxygen-evolving center, inhibiting photosystem II function through an  
19 alteration of chlorophyll structure.

20         Mycorrhizal inoculation also appears to protect terrestrial plants from the effects of Pb. One study  
21 examined the effects of AMF (*Funneliformis mosseae*) on the growth and Pb uptake of *Sophora viciifolia*  
22 ([Xu et al., 2016a](#)). As expected, the AMF altered root growth and architecture (increasing root length,  
23 fork number, tip number, surface area and volume), and these effects are also present under high Pb stress  
24 (1000  $\mu\text{g}/\text{g}$ ). Examining roots under transmission electron microscope and X-ray spectroscopy revealed  
25 that Pb was deposited not only in plant cells but also the cell walls and vacuoles of the AMF intracellular  
26 hyphae, meaning that AMF uptake some of the Pb, alleviating the effects on the plant. Whether the  
27 protective effect of mycorrhizae is species-dependent or not is unknown.

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



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#### 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 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, 2006a](#)). 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 cutoffs 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<sub>2</sub> 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, 1000, 2500 mg Pb/kg,

nominal values) for 4-weeks followed by a 4-week recovery period, MDA was higher in Pb-exposed earthworms during both the exposure and recovery periods ([Zaltauskaite 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, 1000, 2500 mg Pb/kg, nominal values) ([Zaltauskaite and Sodiene, 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.

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 values reported) ([Mleiki et al., 2015](#)). Activity was 200  $\mu\text{mol/nm/mg}$  in control snails, approximately 75  $\mu\text{mol/nm/mg}$  at 25 mg Pb/kg exposure and about 25  $\mu\text{mol/nm/mg}$  at 2500 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).



1 Metal concentration in plants was positively correlated with soil concentrations, and concentrations in the  
2 snail digestive gland were positively correlated with plant concentrations. Protein, glycogen, GST, and  
3 total energy levels measured within the digestive gland showed no clear pattern in relation to Pb and  
4 instead depended on interactions between the specific site, exposure time, and different heavy metals.  
5 There were also no correlational changes in shell morphology.

6 Physiological stress response linked to Pb exposure was reported in a few additional terrestrial  
7 invertebrates. Overall, gut enzyme activities, with the exception of alpha-glucosidase, were higher in  
8 honeybees (*A. mellifera*) within urban-located hives in Nigeria compared with wild beehives.  
9 Carbohydrases (amylase and cellulase) were higher than lipase and proteinase across both nesting  
10 habitats. However, there was no difference in Pb concentration in bees between habitats, and differences  
11 in enzyme activities showed no direct correlation to Pb specifically ([Lawal et al., 2014](#)). In another study,  
12 honeybees in a laboratory setting were fed a sucrose solution with Pb concentrations of 10, 1, 0.1, and  
13 0 mg Pb/L over a 48-hour period. GST enzyme activity and gene expression were examined, along with  
14 AChE activity. No effect of Pb was observed at any exposure concentration on GST activity or gene  
15 expression after 48 hours. AChE activity was lower at 0.1 mg Pb/L and higher at 10 mg Pb/L  
16 concentrations ([Nikolić et al., 2019](#)). In a trophic study examining Pb uptake by mulberry trees (*M. alba*)  
17 and subsequent transfer to silkworms (*B. mori*), Pb content in silkworms and silkworm excretions (feces  
18 and silk) increased with increasing Pb treatment (0, 200, 400, and 800 mg Pb/kg soil treatments, nominal  
19 values) across lifestages (larvae and moth). Additionally, metallothionein was higher in the midgut in all  
20 Pb treatments compared with control larvae and was higher in the 800 mg/kg treatment compared with the  
21 200 and 400 mg Pb/kg treatments. Metallothionein was also higher in silk-glands and body fat in the 400  
22 and 800 mg Pb/kg treatments ([Zhou et al., 2015](#)).

#### 11.2.4.3.2. Organism-Level Response

23 In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a likely causal  
24 relationship between Pb exposure and neurobehavioral responses in terrestrial invertebrates ([U.S. EPA,](#)  
25 [2013](#)) (and see Table 11-2 of this appendix). Evidence was primarily from feeding studies in snails and  
26 altered behaviors in nematodes (*Caenorhabditis elegans*). Several new studies have assessed behavior  
27 modification following Pb exposure in soil organisms and flying insects; most were conducted at nominal  
28 Pb concentrations.

29 Additional studies in nematodes lend further support to Pb neurotoxicity in these organisms. In a  
30 behavioral food preference and food-finding lab study using agar plates, nematodes (*C. elegans*) avoided  
31 contaminated food and chose uncontaminated food spots at 1 mg Pb/L, but at 129 mg Pb/L (50% lethal  
32 concentration; LC<sub>50</sub>), Pb contamination interfered with food-finding ability, and there was no difference  
33 in movement toward either contaminated or uncontaminated food ([Monteiro et al., 2014](#)). Another study  
34 using *C. elegans* found that feeding activity decreased as Pb concentration increased. EC<sub>50</sub> for feeding

behavior was approximately 15 mg Pb/L (54  $\mu$ M). 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, nominal 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, nominal 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 (nominal 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

1 suggested this species may already have adapted to low levels of Pb in their diets because brassicas  
2 (natural food source for larvae of *P. rapae*) mature quickly and are often found in disturbed locations  
3 where Pb may be present. Therefore, the 4 mg Pb/kg concentration may not have been high enough to  
4 induce a different response between treatments in the laboratory-exposed butterflies.

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

11 Additional studies in earthworms since the 2013 Pb ISA continue to support findings of Pb on  
12 growth. [Zaltauskaite et al. \(2020\)](#) examined the effects of Pb exposure on earthworm (*E. fetida*) weight,  
13 growth, and recovery postexposure. During 4 weeks of soil exposure (40, 250, 500, 1000, 2500 mg Pb/kg,  
14 nominal values), no effect on weight loss was found, but Pb decreased growth rate with a difference of  
15 15.8–40% lower fresh weight compared with control worms. Following exposure, earthworms were given  
16 a 4-week recovery period with no Pb exposure. While earthworms recovered some weight, they did not  
17 reach equal weights compared with non-exposure worms (11–17.6% lower than control at end of  
18 recovery period). Fresh weight was negatively correlated with increasing Pb soil concentration during  
19 both the exposure and recovery periods. Growth and recovery rate varied with concentration, with  
20 earthworms exposed to 40 mg Pb/kg having the greatest growth rate compared with other Pb  
21 concentrations. Earthworms grew slower during the recovery period compared with the exposure period  
22 except for those exposed to 2500 mg/kg, which showed equal growth rates during exposure and recovery.  
23 MDA was also positively correlated with Pb levels. During recovery, MDA concentrations were lower  
24 but did not reach the same levels as control worms. Weight response to Pb exposure and recovery  
25 suggests Pb inhibits earthworm growth and may have a short-term legacy or lag effect as recovery did not  
26 reach 100% within the same time frame. Increased MDA concentration is indicative of oxidative stress,  
27 which may explain the reduced growth since MDA concentrations were still comparatively high after the  
28 recovery period. Another earthworm study by [Zaltauskaite and Sodiene \(2014\)](#) examined juvenile  
29 earthworm growth and time to maturation across nominal soil Pb concentrations of 40, 250, 500, 1000,  
30 2500 mg Pb/kg. There was no overall effect on weight loss, but juveniles exposed to Pb were smaller than  
31 control worms. The EC<sub>50</sub> for juvenile growth increased with increasing time of exposure—at 3 weeks, the  
32 EC<sub>50</sub> was approximately 100 mg Pb/kg but after 14 weeks the EC<sub>50</sub> for reduced weight was 179 mg Pb/kg.  
33 The time of maximum growth in the 40 mg Pb/kg exposure group was during the 8–10-week period,  
34 while maximum growth was delayed in higher Pb treatments. Pb significantly lengthened the time to  
35 sexual maturation. The minimum time to maturation was 9 weeks for the control and Pb treatment groups,  
36 and the minimum weight at this development point was 0.182 g in the 40 mg Pb/kg treatment group.  
37 Since increasing Pb concentrations reduced the growth rate, the time needed to reach the minimum

1 maturation size would increase with increasing Pb; therefore, the time needed at 250 mg Pb/kg would be  
2 16 weeks. The total number of earthworms that reached maturity by the end of the experiment was  
3 negatively correlated with Pb concentrations, with only 5–7% of worms reaching maturity in the  
4 250 mg Pb/kg treatment group.

5 Adding to the evidence for growth effects in snails from the 2013 Pb ISA, studies on green  
6 garden snail (*Cantareus apertus*) bioaccumulation and growth in response to increasing Pb dietary  
7 concentrations (25, 100, and 2500 mg Pb/kg, nominal values) over 1 week and 8 weeks of exposure found  
8 the wet weight of snails increased with time across all Pb treatments, and the effect was dose-dependent  
9 in Pb-treated snails ([Mleiki et al., 2016](#)). The weight of snails was significantly lower than the weight of  
10 control snails by week 2 in the high Pb-treatment group, by week 3 for medium Pb-treatment snails and  
11 by week 7 for snails in the low Pb-treatment group. The cumulative growth rate followed a similar pattern  
12 but was lower by week 1 for the high Pb-treatment snails, by week 3 for medium treatment and by week 7  
13 for low Pb treatment. Overall, dietary Pb decreased growth in green garden snails, with a lowest observed  
14 effect concentration (LOEC) of approximately 25 mg Pb/kg food within several weeks. A trophic snail  
15 study found soil Pb levels varied from approximately 6 mg Pb/kg to 52 mg Pb/kg across a gradient of  
16 polluted sites in Romania ([Nica et al., 2012](#)). Shell height was negatively correlated with Pb in nettle  
17 leaves (food source), and relative shell height was positively correlated with snail hepatopancreas Pb  
18 levels. Pb in soil was also correlated with other metals (Zn and Cd). Heavy metals are known to  
19 accumulate in snail shells and can often lead to changes in shell size and geometry.

20 In a study reviewed in the 2013 Pb ISA, body size in nematodes decreased with increasing Pb  
21 concentration in growth medium, albeit at high concentrations (0.5, 16, and 41 mg Pb/L) ([Wang and](#)  
22 [Yang, 2007](#)). A more recent study conducted at lower concentrations of 0.05 and 0.1 mg Pb/L (50 and  
23 100 µg Pb/L, nominal values) in aqueous solution showed Pb had a stimulatory effect on growth at  
24 0.05 mg Pb/L and no stimulatory or inhibitory effect at 0.1 mg Pb/L ([Monteiro et al., 2014](#)).

25 The growth effects of Pb reported for earthworms, snails and nematodes are augmented by  
26 studies in a few additional terrestrial invertebrates. In a generational study with tobacco cutworms  
27 (*Spodoptera litura*) reared on artificial diets with increasing Pb concentration, both Pb and generation  
28 effects were observed on relative growth rate, pupation rate, and eclosion rate ([Shu et al., 2015](#)). First-  
29 generation pupae experienced no effects of Pb stress on pupation rate or relative growth rate. Eclosion  
30 rates did decrease in the 100 and 500 mg Pb/kg treatments groups (nominal values) (eclosion rates were  
31 51.48% and 28.89%, compared with approximately 70% for all other treatments). Fifth generation larvae  
32 showed significantly lower eclosion and pupation rates at 25 and 50 mg Pb/kg compared with  
33 12.5 mg Pb/kg and control treatments. The relative growth rate of fifth generation pupae declined as well  
34 for the 25 and 50 mg Pb/kg treatments. Differences between generations occurred at the 50 mg Pb/kg  
35 treatment, with 50 mg Pb/kg having stronger negative effects in the fifth generation compared with the  
36 first. There was no effect of Pb (4 mg Pb/kg) on cabbage white butterfly (*Pieris rapae*) development time  
37 or body size regardless of Pb concentration or butterfly sex ([Philips et al., 2017](#)). [Kenig et al. \(2013\)](#)

1 reared fruit flies (*Drosophila subobscura*) in the lab for eight generations at low and high Pb exposure  
2 (10 µg Pb/mL and 100 µg Pb/mL, nominal values) from two wild-caught populations with a difference in  
3 Pb exposure history (298.6 mg Pb/kg and 25.7 mg Pb/kg soil). Flies from the population originally  
4 collected from the site with high pollution levels exhibited a decrease in development time over  
5 generations reared at control (no Pb) lab conditions, a decrease in development time when reared at low  
6 Pb-exposure lab conditions and an increase when reared at high Pb-exposure conditions. Flies from the  
7 low historic contamination site exhibited an increase in development time at control conditions, a  
8 decrease at low Pb exposure, and a decrease at high exposure. Across all levels of Pb exposure in the lab,  
9 there were population, generation, and population × generation effects on fruit fly development time.  
10 Overall, the flies from the high Pb-exposure contamination group had faster development time across  
11 both lab exposure Pb concentrations compared with the low historic contamination population responses.  
12 The authors suggest this response in development time in the high historic exposure population may be an  
13 ancestral adaptation response to allow for growth and reproduction to occur before Pb toxicity occurs.

14 In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship  
15 between Pb exposure and reproduction in terrestrial invertebrates ([U.S. EPA, 2013](#)) (see Table 11-2 of  
16 this appendix). Reproduction endpoints examined in the 2013 Pb ISA included brood size and hatching  
17 success. Additional studies in soil invertebrates published since the 2013 Pb ISA continue to report Pb  
18 effects on reproduction and development, adding to the evidence base for this endpoint. [Monteiro et al.](#)  
19 [\(2014\)](#) found Pb had a variable effect on number of nematode *C. elegans* offspring depending on the  
20 concentration tested. An increase in offspring was observed at 0.01, 0.05, and 0.1 mg Pb/L (10, 50, and  
21 100 µg Pb/L, nominal values), a decrease at 1 mg Pb/L (1000 µg Pb/L) and no difference from the control  
22 at 0.5 mg Pb/L (500 µg Pb/L).

23 Pb exposure (40, 250, 500, 1000, 2500 mg Pb/kg, nominal values) significantly lengthened time  
24 to sexual maturation for juvenile *E. fetida* earthworms ([Zaltauskaite and Sodiene, 2014](#)). The minimum  
25 time to maturation was 9 weeks for the control and Pb treatment groups, and the minimum weight at this  
26 development point was 0.182 g in the 40 mg Pb/kg treatment group. Since increasing Pb concentrations  
27 reduced growth rate, the time needed to reach the minimum maturation size would increase with  
28 increasing Pb; therefore, the time needed at 250 mg/kg would be 16 weeks. The total number of  
29 earthworms that reached maturity by the end of the experiment was negatively correlated with Pb  
30 concentrations, with only 5–7% of worms reaching maturity in the 250 mg/kg treatment group. In  
31 addition, cocoons were only found at the lowest treatment of 40 mg Pb/kg, and the number of cocoons  
32 was less than half of the number of cocoons produced in control soils.

33 In a multigeneration vinegar fruit fly (*D. melanogaster*) study, females that were reared under no  
34 Pb conditions preferentially mated with control males (60% of the time) over males reared in Pb  
35 conditions (108 mg Pb/kg) ([Peterson et al., 2017](#)). In the same study, Pb-reared females preferentially  
36 mated with Pb-reared males over control males (65% of the time). Second-generation females did not  
37 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  $\mu$ M; nominal values, PbAc). However, females reared on the highest Pb treatment of approximately 434 mg Pb/kg (1000  $\mu$ M) 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  $\mu$ g Pb/mL, nominal values) and a higher Pb contamination substrate (100  $\mu$ g Pb/mL, nominal 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

1 decrease to F2 viability levels by generation F8. Under low exposure conditions, both populations  
2 followed the same pattern they showed under control conditions. Under high Pb lab conditions, neither  
3 population showed a change in egg viability across generations but the egg viability of the population  
4 from low historic exposure conditions had overall lower egg viability than the population that experienced  
5 historically high exposure. Individuals from the historic high exposure showed higher viability and  
6 fecundity when exposed to higher Pb concentrations in all generations compared with those from the  
7 historically low exposure population, exhibiting higher tolerance to heavy-metal exposure.

8 [Mazzei et al. \(2013\)](#) examined isopod *Armadillidium granulatum* reproductive response to metal  
9 contamination of food. According to the authors, isopod heavy-metal concentration factors vary widely  
10 across species as does their breeding patterns. In this study, Pb concentration (100, 500, 1000 mg Pb/kg,  
11 nominal values) in food led to an alteration of reproductive patterns in *A. granulatum*. Increasing  
12 concentrations led to a delayed onset in breeding season while also reducing the duration of the season.  
13 Breeding season onset did not differ between control and 100 mg Pb/kg treatments. Breeding season  
14 occurred 1 week later in the 500 mg/kg treatment group and 6 weeks later in the 1000 mg Pb/kg treatment  
15 group. The length of the breeding season decreased from 79 days (control) to 59 (500 mg Pb/kg) and 46  
16 (1000 mg Pb/kg) days. There was no effect of Pb on incubation period (approximately 23 days), and the  
17 percent gravid rate of females increased from 97.2% (control) and 95.8% (100 mg Pb/kg) to 100% for  
18 higher Pb treatments. However, while gravid rate increased, brood number declined (from 1.22 to 1).  
19 Lastly, the number of juveniles for each brood increased with 500 mg Pb/kg treatment. Overall,  
20 contamination at 100 mg Pb/kg did not influence any reproductive endpoint examined for *A. granulatum*  
21 but higher levels led to changes in breeding seasonality and the number of juveniles.

22 In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship  
23 between Pb exposure and survival in terrestrial invertebrates ([U.S. EPA, 2013](#)) (see Table 11-2 of this  
24 appendix). Additional evidence continues to show Pb effects on mortality in some terrestrial  
25 invertebrates, while others appear to be unaffected. In a laboratory study examining green garden snail  
26 (*Cantareus apertus*) response to increasing Pb dietary concentrations (25, 100, 2500 mg Pb/kg, nominal  
27 values) over a period of 1 to 8 weeks, cumulative mortality was greater in all Pb treatments than the  
28 control after 6 weeks of exposure, with the high treatment having significantly greater mortality after  
29 1 week ([Mleiki et al., 2016](#)). At the end of the experiment, cumulative mortality was below 30% for all  
30 treatments. An observational study of *C. apertus* exposed to multiple metal-polluted soils with Pb  
31 concentrations ranging from 28.1 to 4574 mg Pb/kg found only 6.5% of snails died after 28 days of  
32 exposure ([Pauget et al., 2013b](#)). Studies in the 2006 Pb AQCD found earthworm LC<sub>50</sub> for 14 and 28-day  
33 exposure fell within a range of 2400–5800 mg Pb/kg. A study reported in the 2013 Pb ISA evaluated *E.*  
34 *fetida* earthworms exposed to field-collected soils with Pb concentrations up to 390 mg Pb/kg and found  
35 no effect on earthworm survival ([Delistraty and Yokel, 2011](#)). In support, juvenile *E. fetida* earthworms  
36 exposed to a range of Pb concentrations (40, 250, 500, 1000, 2500 mg/kg, nominal values) over 14 weeks  
37 found mortality increased, but only in the 500-2500 mg Pb/kg treatments, with mortality reaching 90% in



1 the highest treatment ([Zaltauskaite and Sodiene, 2014](#)). Juvenile mortality increased with the time of  
2 exposure in these treatment groups, with an LC<sub>50</sub> of 911 mg Pb/kg for 14 weeks of Pb exposure. Juvenile  
3 mortality did reach 10% by week 3 for the 40 and 250 mg Pb/kg treatments but did not increase any  
4 further over time. However, in another earthworm exposure experiment using adults of *E. fetida*, across  
5 only 4 weeks of exposure (40, 250, 500, 1000, 2500 mg Pb/kg, nominal values), there was no significant  
6 effect on survival ([Zaltauskaite et al., 2020](#)). In cabbage white butterflies (*P. rapae*) raised from eggs  
7 from wild-caught females, no effect on survival was observed in a laboratory study with a diet of  
8 4 mg Pb/kg ([Philips et al., 2017](#)).

9 In terrestrial invertebrates, literature since the 2013 Pb ISA provides additional support on the  
10 effects of Pb exposure on organismal and suborganismal responses including a decrease in survival and  
11 reduced growth and fecundity. Recently published studies on physiological responses to Pb included  
12 decreases in protein and lipid content and increases in MDA in earthworms. AChE activity decreased in  
13 response to Pb in snails and honeybees while protein, glycogen, other enzymes, and GST responses were  
14 variable depending on modifying site factors or species examined. There are several new studies  
15 quantifying behavioral changes to Pb exposure in bees. Soil Pb contamination altered foraging behavior,  
16 and at high levels (above 600 mg Pb/kg), also altered sucrose intake. However, at low concentrations  
17 (0.66 mg Pb/kg), honeybees showed lower flexibility in response to changing flower rewards, suggesting  
18 Pb may lead to lower nectar and pollen supply and subsequently slower colony development or winter  
19 survival. New literature on growth endpoints suggests Pb can have lasting effects even postexposure on  
20 earthworms. Growth, eclosion, and pupation rates of the common cutworm were all lower under Pb  
21 exposure, and fruit fly development time increased within eight generations in populations with historic  
22 Pb pollution exposure. In addition to previously assessed endpoints of Pb on brood size and hatching  
23 success, new literature shows Pb exposure slows time to maturation in earthworms, delays onset to and  
24 duration of breeding season in isopods and influences mate selection in fruit flies. While the literature  
25 since the 2013 Pb ISA has primarily provided additional support on previously examined organisms and  
26 endpoints, there has been new information on new organisms as well as on modifying factors on organism  
27 response including habitat, exposure history, seasonality, and duration of effects.

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#### 11.2.4.4. Effects on Terrestrial Vertebrates

28 In observational and experimental studies, commonly observed effects of Pb on terrestrial  
29 vertebrates include decreased survival, reproduction, and growth, as well as effects on development and  
30 behavior ([U.S. EPA, 2006a](#)). The 2013 Pb ISA ([U.S. EPA, 2013](#)) also provided evidence for Pb effects on  
31 hormones and other biochemical variables ([U.S. EPA, 2013](#)). Recent studies provide additional support to  
32 suborganism-level and organism-level endpoints and expand on the effects on hematological and  
33 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 ([Rodriguez-Estival et al., 2012](#)), and sheep ([Rodriguez-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 =  $5.9 \pm 1.4$  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 =  $9.9 \pm 0.6$  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. [Rodriguez-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  $1.00 \pm 0.76$  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 (*Ichthyaelus 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 Audouin'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 1000 ppm) for 49 days. Pb exposure of 250, 500, and 1000 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. [Farsang et al. \(2017\)](#) 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, 2006a](#)) 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<sub>4</sub> 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

1 blood and feathers than those from the neighborhood with low soil Pb concentrations. This study used  
2 simulated territory intrusions to examine the level of aggression displayed by individuals from different  
3 neighborhoods. Birds from the high Pb neighborhoods exhibited a more aggressive response to simulated  
4 intrusions than birds from the low Pb neighborhood.

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

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

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### 11.2.5. Exposure and Response of Terrestrial Species

22 As previously reported in the ([U.S. EPA, 1977](#)), the 1986 Pb AQCD ([U.S. EPA, 1986](#)), the 2006  
23 Pb AQCD ([U.S. EPA, 2006a](#)) and the 2013 Pb ISA ([U.S. EPA, 2013](#)), a large number of experimental  
24 studies have exposed a wide variety of terrestrial organisms to gradients of Pb exposures and reported a  
25 broad assortment of responses, including growth, reproduction, survival, antioxidant levels and markers  
26 of oxidative stress. More than 80 such additional experimental studies conducted since the 2013 Pb ISA  
27 were identified. Organisms subjected to these exposure-response experiments have included various wild  
28 plants including reeds and ferns, cultivated crops, microbes, lichens, fungi including mycorrhizae,  
29 bacteria, nematodes, worms, collembolans, beetles, spiders, rodents, and birds. The 2006 AQCD and  
30 2013 Pb ISA ([U.S. EPA, 2013, 2006a](#)) reported that variation in exposure is generally associated with  
31 commensurate variation in growth, reproduction, survival, antioxidant activity and more. Such coupling  
32 of exposure and response is considered a strong indicator of causality ([U.S. EPA, 2015](#)), and exposure-  
33 response studies with Pb thus continue to provide evidence supporting the causality of Pb for the effects



1 they investigate, as highlighted in the sections of this appendix dedicated to specific groups of terrestrial  
2 organisms.

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

15 Section 11.2.2.1 discussed environmental variables that have a strong impact on bioavailability in  
16 soils. They include pH, CEC, salinity, aging, OM, soil type and the presence of other metals. The use of  
17 soluble salts of Pb brings pH, CEC, salinity, and aging into ranges far removed from those found in  
18 natural environments following exposure to Pb emissions. Predicted effects derived from those  
19 experiments cannot be expected to be accurate in environmental conditions, not only because the  
20 experimental conditions of pH, CEC, salinity and aging diverge too far from those present in the  
21 environment, but, more intractably, because in both the experiments themselves and in the environments  
22 in which a prediction is attempted, the measurement of Pb concentration may sharply diverge from the  
23 concentration actually affecting the organism. These difficulties were discussed in the 2006 Pb AQCD  
24 ([U.S. EPA, 2006a](#)) and the 2013 Pb ISA ([U.S. EPA, 2013](#)), as well as in studies explicitly designed to  
25 clarify these issues, such as [Smolders et al. \(2009\)](#), [Cheyns et al. \(2012\)](#) or [Dayton et al. \(2006\)](#). In 2009,  
26 following extensive toxicity testing with both spiked soils and contaminated field soils, [Smolders et al.](#)  
27 [\(2009\)](#) concluded that “despite all of the efforts made, a large proportion of the difference between the  
28 toxicity observed in field-contaminated soils and that in laboratory-amended soils remains unexplained.”  
29 [Smolders et al. \(2009, p. \)](#) further demonstrated that not only are the effects of pH, for example, more  
30 complex than previously thought, but pH, CEC, DOM, Fe and Mn oxides, aging and soil type are all  
31 powerful modifiers of Pb toxicity to soil-dwelling organisms. Underscoring the complexity of modifying  
32 effects, [Cheyns et al. \(2012\)](#), for instance, showed that in tomato and barley plants, soil type is a major  
33 modifier of toxicity, but that once pH is controlled, toxicity can be mediated by the nutrient deficiencies  
34 that stem from reactions of Pb with essential nutrients in the soil solution, whereby the apparent effects of  
35 Pb are caused by nutrient deficiencies from Pb robbing the plants of P, for example, by forming Pb  
36 phosphates.



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

9       Among other questions, [Zhang and Van Gestel \(2019b\)](#) investigated the effects of 18 months of  
10 aging, form of Pb and percolation (leaching) on the toxicity of Pb to the worm *Enchytraeus crypticus* in  
11 natural standard soil spiked with nine levels of Pb between 0 and 3200 mg Pb/kg dry soil using  $\text{Pb}(\text{NO}_3)_2$   
12 and between 0 and 1000 mg Pb/kg dry soil using PbO. Among the complex interactions between these  
13 variables, they found that while leaching dramatically decreased porewater concentration of Pb in fresh  
14 and aged soils, and more so for  $\text{Pb}(\text{NO}_3)_2$  than for PbO, it did not affect Pb uptake, which was greater for  
15 the more soluble form ( $\text{Pb}(\text{NO}_3)_2$ ).  $\text{LC}_{50}$  and  $\text{LC}_{10}$ , estimated from logistic regression on all nine levels  
16 was higher following leaching for  $\text{Pb}(\text{NO}_3)_2$  but not for PbO. The authors concluded that generally, the  
17 effect of percolation on the toxicity of Pb-spiked soils was dependent on the chemical form used for  
18 spiking as well as on aging, and porewater Pb concentration could not explain Pb toxicity. For survival,  
19 leaching decreased the toxicity of  $\text{Pb}(\text{NO}_3)_2$  but did not affect the toxicity of PbO. For effects on  
20 reproduction, leaching had a greater influence in freshly spiked soils than in aged soils. This suggests that  
21 manipulating or accounting for aging and form of Pb might be useful in generating effect predictions in  
22 natural environments from spiking experiments, but that manipulating leaching may not be.

23       The same authors also included variation in the length of the aging period in another exposure-  
24 response experiment ([Zhang and Van Gestel, 2019a](#)). Using the same materials and methods as in [Zhang](#)  
25 [and Van Gestel \(2019b\)](#), they incubated the soil samples for five periods from 0 to 18 months, after  
26 spiking and before exposure of the worms. Toxicity increased with aging when soils were spiked with  
27 PbO but not with  $\text{Pb}(\text{NO}_3)_2$ , as did availability when estimated via  $\text{CaCl}_2$  extraction. This may conflict  
28 with ([Smolders et al., 2015](#)), who found that lethality declined with five years of aging, but in outdoors  
29 conditions that included leaching by rain rather than laboratory incubation. Including aging in the  
30 translation from experiment to field thus appears warranted, but not without also including the form of Pb  
31 and leaching.

32       Finally, [Zhang et al. \(2019a\)](#) investigated the effects of soil properties toxicity to *Enchytraeus*  
33 *crypticus* using the same materials and methods as [Zhang and Van Gestel \(2019b\)](#) and six standard  
34 natural soils, using  $\text{Pb}(\text{NO}_3)_2$  but not PbO treatments, and not varying aging or percolation. The soils  
35 varied in OM content, pH, CEC, water-holding capacity, dissolved OC, and composition. Soil type had  
36 very large effects on survival of earthworms in the presence of Pb even though no effect was observed on  
37 the internal Pb concentration of worms, with effects ranging from no survival at the mid range of Pb

1 concentration, to complete survival even at the highest concentration. However, soil type had only weak  
2 effects on survival when exposure was measured as porewater Pb and no effect on survival when  
3 measured as CaCl<sub>2</sub>-extractable Pb. Similarly strong effects of soil type were seen on the exposure-  
4 response relationship of Pb concentration and earthworm reproduction. However, the same weak effects  
5 of Pb as for survival were observed for reproduction when using porewater Pb concentrations, and no  
6 effects were observed when using CaCl<sub>2</sub>-extractable Pb. Furthermore, measuring exposure as CaCl<sub>2</sub>-  
7 extractable Pb resulted in accurate and precise predictions of responses regardless of soil type. In  
8 contradiction with other studies cited above, such as [Cheyns et al. \(2012\)](#) or [Smolders et al. \(2009\)](#), the  
9 authors suggested that despite soil type having a strong effect on toxicity when exposure is measured as  
10 simple soil concentration using CaCl<sub>2</sub>-extractable Pb as a metric of exposure may be sufficient when  
11 estimating the effects of Pb on worms, since using that metric supported accurate and precise prediction  
12 of earthworm responses regardless of soil type, and the exposure-response relationship was then  
13 insensitive to soil type.

14 [Romero-Freire et al. \(2015\)](#) assessed the respective influence of soil properties in laboratory  
15 toxicological assays, with the same aim of making experimental exposure-response studies with spiked  
16 soils usable for environmental risk assessment. Seven natural soils of varying pH, conductivity, texture,  
17 OC, water-holding capacity, CEC, specific area, carbonate content and metal oxides were spiked with five  
18 levels of Pb(NO<sub>3</sub>)<sub>2</sub> and incubated for 4 weeks. The authors observed that pH and CaCO<sub>3</sub> content were the  
19 soil properties with the highest influence on Pb extractability and interacted strongly with total Pb  
20 concentration, with extractability most affected at higher concentrations of Pb. However, they also found  
21 that retention via organic complexation kept most of the Pb from being bioavailable and that texture  
22 (silt/sand/clay proportions) and Fe and Mn oxides also had major effects on extractability. In three tests of  
23 toxicity—one with lettuce seeds, one with a strain of marine bacterium, and one measuring microbial soil  
24 respiration—soil type strongly modified overall toxicity in all tested organisms and the relative effects of  
25 each concentration of Pb (in other words, the slope of the response curve). In addition, the magnitude of  
26 these modifying effects differed among the three tests. The authors did not attempt to partition the effects  
27 of every soil property beyond the most salient effects on extractability noted above. They concluded that  
28 soil properties in the particular locations and land use where risk is to be assessed must be taken into  
29 consideration when conducting risk assessment, including at minimum, pH, OM and carbonate.

30 Many variables distinguish natural soils from each other with regard to influence on Pb toxicity,  
31 as enumerated in the experiments cited here. As noted by [Romero-Freire et al. \(2015\)](#), [Zhang et al.](#)  
32 [\(2019a\)](#), [Smolders et al. \(2009\)](#) and others, given practical limitations on the number of soils that can be  
33 included in one experiment, it is not possible to definitively separate the effect of each of the variables  
34 that define soil type, let alone quantify their interactions. It is possible however to separate some variables  
35 that affect the exposure-response more strongly from those that have little or no influence, and it may be  
36 possible to identify measures of exposure under which the exposure-response relationship is insensitive to  
37 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  $\text{Pb}(\text{NO}_3)_2$  and as  $\text{PbCl}_2$ . 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  $\text{EC}_{10}$  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  $\text{EC}_{50}$  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  $\text{EC}_{50}$  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  $\text{EC}_{50}$  increased the least. [Smolders et al. \(2015\)](#) attempted to identify which variables among total Pb concentration, porewater Pb,  $\text{Pb}^{2+}$  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  $\text{Pb}^{2+}$  salts does not appear to be an appropriate model for environmental sources of Pb where Pb gradually enters soils via atmospheric deposition as  $\text{PbO}$ ,  $\text{PbS}$ , and  $\text{PbSO}_4$ ..."

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 [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<sub>3</sub>)<sub>2</sub> between 0 and 3200 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<sub>3</sub>)<sub>2</sub> 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 ( <i>Hordeum vulgare</i> )	<b>Form of Pb:</b> PbCl <sub>2</sub>	<b>pH:</b> Soils 7.4, 6.5, 6.7, 5.7, 5.2, 4.7 (pH CaCl <sub>2</sub> (0.01 M) Adjusted with CaO)	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	Soil (location of origin)	Tomato shoot dry weight NOEC for six soils: 4,400, 750, <250, 440, 260, 1,100 mg Pb/kg soil	Strong interaction effect of soil type and Pb concentration on growth	<a href="#">Cheyns et al. (2012)</a>
Tomato ( <i>Lycopersicon esculentum</i> )	<b>Medium:</b> Six topsoils from five European countries	<b>CEC:</b> Soils 14.7, 27.1, 8.7, 4.2, 7.6, 41.7 (cmol <sub>c</sub> /kg soil)	Hydroponics 6.1	Soil P content 44, 48, 67, 89, 90, 121 mg P/kg soil	Tomato growth: decreasing with increasing Pb in all soils	P content in plants was strongly influenced by Pb and explained the effect of Pb across soils and in hydroponic experiment	
	Hydroponic system	Hydroponics N/A	Hydroponics 1, 3.2, 10, 32, 100, 320 mM	Hydroponics Gradually increasing P supply for 17 days to maintain growth rate and avoid precipitation	Barley growth: no effect of Pb in three soils, decreasing with increasing Pb in three soils		
	<b>Exposure method:</b> Salt mixed with soil	<b>OC:</b> Soils 14, 31, 10, 15, 21, 310 (gC/kg soil)	Hydroponics N/A	AND 7 levels of P supply based on P content in plant tissue (0.10–0.32% P in plant tissue)	EC <sub>50</sub> : 6,000, 6,500, 2,200, 2,700, 1,600, 5,400 mg Pb/kg soil		
	Salt in hydroponic solution	<b>Aging/leaching:</b> 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			Barley shoot dry weight NOEC for six soils: >7,200, >5,000, 2,000, >3,400, 260, 1,100 mg Pb/kg soil		
					EC <sub>50</sub> : >7,200, >5,000, 4,900, >3,400, 1,900, 8,300 mg Pb/kg soil		

Organism	Experimental conditions		Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
Hydroponics N/A								
Potworm ( <i>Enchytraeus crypticus</i> )	<b>Form of Pb:</b> Pb(NO <sub>3</sub> ) <sub>2</sub>	<b>pH:</b> Nominal pH 5.49	Nominal concentrations of:	Aging and chemical form of Pb	<i>E. crypticus</i> mortality increased with increasing Pb soil concentration	Pb(NO <sub>3</sub> ) <sub>2</sub> : CaCl <sub>2</sub> extractable Pb 0, 3, 6, 12, 18-mo LC <sub>50</sub> = 2.18, 3.06, 2.49, 2.28, 1.72 mg Pb/kg EC <sub>50</sub> = 0.149, 0.125, 0.090, 0.103, 0.093 mg Pb/kg	The dose-response curves and toxicity values (LC <sub>50</sub> and EC <sub>50</sub> ) based on total Pb concentrations differed widely between the two forms of Pb	(Zhang and Van Gestel, 2019a)
	PbO	<b>CEC:</b> 9.10 cmolc/kg	Pb(NO <sub>3</sub> ) <sub>2</sub>					
	<b>Medium:</b> LUFA 2.2 standard soil	<b>OC:</b> not reported	0, 50, 100, 200, 400, 600, 800, 1600 and 3200 mg Pb/kg dry soil					
	<b>Exposure method:</b> Soil spiked with powdered salt	<b>Aging/leaching:</b> Spiked soils were aged for 0, 3, 6, 12 and 18 mo. No leaching	PbO			Porewater Pb 0, 3, 6, 12, 18-mo LC <sub>50</sub> = 0.247, 0.346, 0.328, 0.366, 0.583 mg Pb/L EC <sub>50</sub> = 0.020, 0.016, 0.019, 0.015, 0.046 mg Pb/L	Pb(NO <sub>3</sub> ) <sub>2</sub> was more toxic than PbO in freshly spiked soils, but the toxicity of PbO increased with aging, while the toxicity of Pb(NO <sub>3</sub> ) <sub>2</sub> remained constant	
						0, 78, 156, 312, 625, 1250, 2500, 5000 and 10000 mg Pb/kg dry soil	Internal Pb 0, 3, 6, 12, 18-mo LC <sub>50</sub> = 76.2, 76.4, 77.1, 73.4, 76.8 mg Pb/kg dry body weight EC <sub>50</sub> = 22.2, 24.7, 30.0, 31.5,	

Organism	Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
					20.1 mg Pb/kg dry body weight		
					PbO: CaCl <sub>2</sub> extractable Pb 0, 3, 6, 12, 18-mo		
					LC <sub>50</sub> = 3.02, 3.15, 2.36, 2.66, 2.45 mg Pb/kg		
					EC <sub>50</sub> = 0.170, 0.135, 0.098, 0.138, 0.101 mg Pb/kg		
					Porewater Pb 0, 3, 6, 12, 18-mo LC <sub>50</sub> = 0.262, 0.312, 0.286, 0.302, 0.391 mg Pb/L		
					EC <sub>50</sub> = 0.025, 0.048, 0.050, 0.023, 0.048 mg Pb/L		
					Internal Pb		



Organism	Experimental conditions		Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
						0, 3, 6, 12, 18-mo LC <sub>50</sub> = 78.0, 77.7, 74.4, 78.4, 78.7 mg Pb/kg dry body weight		
						EC <sub>50</sub> = 23.7, 18.1, 19.8, 16.9, 12.0 mg Pb/kg dry body weight		
Potworm ( <i>Enchytraeus crypticus</i> )	<p><b><u>Form of Pb:</u></b> Pb(NO<sub>3</sub>)<sub>2</sub></p> <p>PbO</p> <p><b><u>Medium:</u></b> LUFA 2.2 standard soil</p> <p><b><u>Exposure method:</u></b> Soil spiked with powdered salt.</p>	<p><b><u>pH:</u></b> Aged Soil:</p> <p>pH<sub>pw</sub>: 5.61</p> <p>pH<sub>CaCl2</sub>: 5.14</p> <p>Freshly Spiked:</p> <p>pH<sub>pw</sub>: 5.93</p> <p>pH<sub>CaCl2</sub>: 5.65</p> <p><b><u>CEC:</u></b> not reported</p> <p><b><u>OC:</u></b> not reported</p> <p><b><u>Aging/leaching:</u></b></p>	<p>Nominal concentrations of:</p> <p>Pb(NO<sub>3</sub>)</p> <p>0, 50, 100, 200, 400, 600, 800, 1600 and 3200 mg Pb/kg dry soil</p> <p>PbO</p> <p>0, 78, 156, 312, 625, 1250, 2500, 5000 and 1000 mg Pb/kg dry soil</p>	Percolation, chemical form of Pb and aging.	<i>E. Crypticus</i> mortality increased with increasing Pb soil concentration	<p>Pb(NO<sub>3</sub>)<sub>2</sub>:</p> <p>CaCl<sub>2</sub>-extractable Pb</p> <p>aged, aged+leached, freshly spiked and freshly spiked+leached.</p> <p>LC<sub>50</sub> = 1.72, 2.42, 2.07 and 2.78 mg Pb/kg</p> <p>EC<sub>50</sub> = 0.093, 0.173, 0.044 and 0.109 mg Pb/kg</p> <p>Porewater Pb</p> <p>aged, aged+leached, freshly spiked and freshly spiked+leached.</p> <p>LC<sub>50</sub> = 0.583,</p>	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 <sub>2</sub> -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
	One soil form was spiked and then aged for 18 mo, while the other soil form was used without aging as freshly spiked soil				0.201, 0.686 and 0.148 mg Pb/L		
	Half of each set of soils were leached with deionized water equal to two times the base moisture content				EC <sub>50</sub> = 0.046, 0.063, 0.012 and 0.033 mg Pb/L		
					Internal Pb aged, aged+leached, freshly spiked and freshly spiked+leached. LC <sub>50</sub> = 76.8, 84.4, 77.3 and 83.6 mg Pb/kg dry body weight		
					EC <sub>50</sub> = 20.1, 22.1, 25.5 and 32.7 mg Pb/kg dry body weight		
					<u>PbO:</u> CaCl <sub>2</sub> extractable Pb aged, aged+leached, freshly spiked and freshly spiked+leached. LC <sub>50</sub> = 2.45, 2.01, 2.79 and 2.16 mg Pb/kg		

Organism	Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
					EC <sub>50</sub> = 0.101, 0.160, 0.123 and 0.168 mg Pb/kg		
					Porewater Pb aged, aged+leached, freshly spiked and freshly spiked+leached. LC <sub>50</sub> = 0.391, 0.233, 0.197 and 0.097 mg Pb/L		
					EC <sub>50</sub> = 0.048, 0.043, 0.047 and 0.031 mg Pb/L		
					Internal Pb aged, aged+leached, freshly spiked and freshly spiked+leached. LC <sub>50</sub> = 78.7, 76.4, 83.3 and 84.5 mg Pb/kg dry body weight		
					EC <sub>50</sub> = 12.0, 14.5, 41.1 and 38.6 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
Potworm ( <i>Enchytraeus crypticus</i> )	<p><b>Form of Pb:</b> Pb(NO<sub>3</sub>)<sub>2</sub></p> <p><b>Medium:</b> LUFA 2.2 standard soil</p> <p><b>Exposure method:</b> Soil spiked with aqueous salt solution</p>	<p><b>pH:</b> Nominal pH of 5.49</p> <p><b>CEC:</b> 9.10 cmolc/kg</p> <p><b>OC:</b> not reported</p> <p><b>Aging/leaching:</b> After 14-d exposure in spiked soils, the surviving <i>E. crypticus</i> were transferred to clean soil for the 14-d elimination phase</p>	<p>Nominal concentrations of 0, 100, 200, 400, 800, 1600 and 3200 mg Pb/kg dry soil</p> <p>Measured Concentrations of 16, 114, 202, 391, 793, 1,601 and 3,585 mg Pb/kg dry soil</p>	Exposure duration	<p>Toxicity was dependent on both the concentration and duration of exposure.</p> <p>Pb toxicity developed more slowly than uptake, with final LC<sub>50</sub> not yet reached after 21 d</p>	<p>Days 4, 7, 10, 14 and 21</p> <p><b>Total concentration in soil:</b> LC<sub>50</sub> = 2,336, 2278, 1,220, 756 and 558 mg Pb/kg dry soil</p> <p><b>Internal concentration:</b> LC<sub>50</sub> = &gt;287, &gt;270, 161, 76.6 and 76.4 mg Pb/kg dry body weight</p>	Strong interaction effect of duration and Pb concentration on mortality	(Zhang and Van Gestel, 2017)
Potworm ( <i>Enchytraeus criticus</i> )	<p><b>Form of Pb:</b> Pb(NO<sub>3</sub>)<sub>2</sub></p> <p><b>Medium:</b> Five standard soils (LUFA standard soil 2.1, 2.2, 2.3, 2.4, 5 M) and one soil from a soccer field</p>	<p><b>pH:</b> 4.86, 5.66, 5.38, 6.87, 6.99, 6.85</p> <p><b>CEC:</b> 2.23, 7.59, 4.04, 20.1, 10.1 and 20.0 cmolc/kg</p> <p><b>OC:</b> DOC 45.7, 61.7, 34.4, 72.0, 51.2 and 189 mg/L</p> <p><b>Aging/leaching:</b></p>	<p>Nominal concentrations of 0, 100, 200, 400, 600, 800, 1200, 1600, 2400 and 3200 mg Pb/kg dry soil</p>	Soil type, soil properties: OM, DOC, pH, CEC, water-holding capacity, composition	Reproductive toxicity and mortality increased with Pb concentration in soil	<p>LUFA standard soil 2.1, 2.2, 2.3, 2.4, 5 M and soccer field, respectively.</p> <p><b>Total Pb:</b> LC<sub>50</sub> = 246, 1,192, 655, 3,125, 2,875 and &gt;3,092 mg Pb/kg dry soil</p> <p>EC<sub>50</sub> = 81.4, 238, 205, 948,</p>	Correlation of single soil properties with endpoints, followed by simple regression, followed by stepwise multiple regression suggested that pH <sub>CaCl2</sub> was the best explanatory factor for LC <sub>50</sub>	(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
in the Netherlands	Soil equilibrated for 14-d.				1,008 and 991 mg Pb/kg dry soil	values based on total Pb concentration	
	<b><u>Exposure method:</u></b> Soils spiked with aqueous solution				<u>CaCl<sub>2</sub> extractable Pb:</u> LC <sub>50</sub> = 2.35, 2.11, 1.86, 1.64, 2.11 and >1.39 mg Pb/kg dry soil  EC <sub>50</sub> = 0.329, 0.193, 0.107, 0.180, 0.241 and 0.115 mg Pb/kg dry soil  <u>Porewater Pb:</u> LC <sub>50</sub> = 0.308, 1.25, 0.335, 0.334, 0.933 and >0.754 mg Pb/L  EC <sub>50</sub> = 0.044, 0.127, 0.117, 0.169, 0.046 and 0.105 mg Pb/L  <u>Internal Pb:</u> LC <sub>50</sub> = 95.7, 83.0, 87.0, 84.3, 81.7 and	The differences between soil toxicity were not present when exposure was measured as CaCl <sub>2</sub> -extractable Pb concentration	

Organism	Experimental conditions		Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
						>47.7 mg Pb/kg dry body weight		
						EC <sub>50</sub> = 13.6, 34.1, 26.0, 39.9, 27.2 and 32.6 mg Pb/kg dry body weight		
Tomato ( <i>Lycopersicon esculentum</i> )	<b>Form of Pb:</b> PbCl <sub>2</sub>	<b>pH:</b> 6.1–7.4	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 leaching and pH correction	All effects increased with increasing Pb in freshly spiked (unaged, unleached) soils	EC <sub>50</sub> s calculated for tomato growth, barley growth, nitrification rate, nitrification 28-d, respiration, <i>E. fetida</i> reproduction and <i>F. candida</i> reproduction in each soil, respectively.	Strong interaction effect of leaching, aging and Pb concentration on all 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	<a href="#">Smolders et al. (2015)</a>
Barley ( <i>Hordeum vulgare</i> )	Pb(NO <sub>3</sub> ) <sub>2</sub>	<b>CEC:</b> 8.2–27.1 cmolc/kg soil						
Collembola ( <i>Folsomia candida</i> )	<b>Medium:</b> Soils gathered from topsoils in Spain, the United Kingdom and Belgium	<b>OC:</b> 10–43 g C/kg soil						
Earthworm ( <i>Eisenia fetida</i> )	<b>Exposure method:</b> Soil spiked with salt	<b>Aging/leaching:</b> 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				<b>Spain:</b> Freshly spiked EC <sub>50</sub> = 2,900, 2,380, 3,240, 7,190, 8,720, 480 and 712 mg Pb/kg soil  Leached and pH-corrected EC <sub>50</sub> = 6,370, 7,190, 2,200,		

Organism	Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
					7,120, 12,300, 1,182 and n.s. mg Pb/kg soil	decreasing toxicity	
					Aged 5 yr EC <sub>50</sub> = 12,600, n.s, n.s, n.s, 7,020, 1,270 and n.s. mg Pb/kg soil		
					<u>United Kingdom:</u> Freshly spiked EC <sub>50</sub> = 6,140, 6,750, 2,820, 1,750, 9,970, 2,400 and 4,530 mg Pb/kg soil		
					Leached and pH-corrected EC <sub>50</sub> = 6,420, 5,020, 4,920, n.s., 6,160, 1,700 and 5,020 mg Pb/kg soil		
					Aged 5 yr EC <sub>50</sub> = n.s., n.s., n.s., n.s., n.s., 3280 and n.s. mg Pb/kg soil		



Organism	Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
					Belgium: (no test for <i>E. fetida</i> )		
					Freshly spiked EC <sub>50</sub> = 1,240, 1,710, 1,470, 1,410, 1,680 and 1,710 mg Pb/kg soil		
					Leached and pH-corrected EC <sub>50</sub> = 1,430, 4,580, 1,640, 2,820, 8,150 and 2,700 mg Pb/kg soil		
					Aged 5 yr EC <sub>50</sub> = 4480, n.s., n.s., n.s., n.s. and n.s. mg Pb/kg soil		
Lettuce ( <i>Lactuca sativa</i> )	<b>Form of Pb:</b> Pb(NO <sub>3</sub> ) <sub>2</sub>	Reported for soils H1–H7 respectively	Nominal concentrations of 500, 1000, 2000, 4000 and 8000 mg Pb/kg soil	Soil type (location of origin)	All effects increased with increasing Pb in all soils	Reported for soils H1–H7, respectively	<a href="#">Romero-Freire et al. (2015)</a>
Bacterium ( <i>Vibrio fischeri</i> )	<b>Medium:</b> Seven soils representing the main	<b>pH:</b> 7.96, 8.67, 8.79, 6.74, 7.20, 5.87 and 7.03 <b>CEC:</b>				<b><i>L. sativa</i>:</b> EC <sub>10</sub> = 499, 1,363, 254, 1,097, 3,452, 498 and	Strong interaction effect of soil type and Pb concentration on all responses. Authors suggest that the main

Organism	Experimental conditions	Pb concentrations	Study factors other than Pb exposure	Effects of Pb	Effect concentration	Effects of additional study factors	Reference
soil groups in Spain	21.4, 9.83, 2.94, 9.91, 25.9, 3.83 and 15.5 cmolc/kg				344 mg Pb/kg soil	soil properties that affected toxicity were pH, carbonate content and OC	
<b><u>Exposure method:</u></b> Spiked with aqueous solution.	<b><u>OC:</u></b> 5.43, 0.42, 0.38, 0.61, 8.22, 0.49 and 0.66%  <b><u>Aging/leaching:</u></b> Soils were incubated for 4 wk after spiking				<b><u>V. fischeri:</u></b> EC <sub>10</sub> = >8,000, 5,337, 2,901, 386, 2,473, 8 and 744 mg Pb/kg  <b><u>Soil Respiration:</u></b> EC <sub>10</sub> = >8,000, 3,128, 5,951, 90, >8,000, 122 and 45 mg Pb/kg		

CaCl<sub>2</sub> = calcium chloride; CaO = calcium oxide; CEC = cation exchange capacity; DOC = dissolved organic carbon; EC<sub>50</sub> = 50% effect concentration; LC<sub>50</sub> = 50% lethal concentration; LUFA = Landwirtschaftliche Untersuchungs- und Forschungsanstalt; mo = months; N/A = not available; NOEC = no observed effect concentration; n.s. = nonsignificant. OC = organic carbon; OM = organic matter; P = lead; Pb = lead; Pb(NO<sub>3</sub>)<sub>2</sub> = lead nitrate; PbCl<sub>2</sub> = lead chloride; PbO = lead(II) oxide; pH<sub>pw</sub> = pH of porewater; pH<sub>CaCl2</sub> = pH via calcium chloride; wk = weeks; yr = years

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### 11.2.6. Terrestrial-Community and Ecosystem Effects

1 In the 2013 Pb ISA the body of evidence was sufficient to conclude there is a likely causal  
2 relationship between Pb exposure and terrestrial-community and ecosystem effects ([U.S. EPA, 2013](#)). In  
3 the 2006 Pb AQCD ([U.S. EPA, 2006a](#)), terrestrial ecosystems near stationary Pb sources exhibited  
4 decreased species diversity, changes in floral and faunal composition, and a reduction in vegetation  
5 fitness. In the 2013 Pb ISA ([U.S. EPA, 2013](#)), a study reported decreased population growth of  
6 earthworms. Additional studies in the 2013 Pb ISA examined how the presence of AMF or earthworms  
7 affect plant Pb uptake and fitness. Recent evidence of the effects of Pb at the community and ecosystem  
8 levels include several studies of the relationship between Pb soil concentration and species interactions  
9 and invertebrate community structure. Specifically, studies conducted since the 2013 Pb ISA have  
10 reported that Pb affects plant-insect interactions and is correlated with invertebrate community structure.

11 In an experimental study, [Jiang et al. \(2020\)](#) demonstrated trophic transfer of Pb can affect the  
12 chemical defenses of larch seedlings (*Larix olgensis*) against an economically important pest, the Asian  
13 gypsy moth (*Lymantria dispar*), in China. Larch seedlings were enriched with Pb at 0, 500, or  
14 1500 mg Pb/kg. Second instar *L. dispar* larvae raised from field-collected egg masses were placed on *L.*  
15 *olgensis* seedlings for 7 days. Pb content in *L. dispar* larvae were significantly higher than *L. olgensis*  
16 needles for the 500 mg Pb/kg and 1500 mg Pb/kg treatments, and Pb bioaccumulated in this experiment,  
17 as the transfer coefficients were 0.97 for the 0 mg Pb/kg treatment, 5.43 for the 500 mg Pb/kg treatment  
18 and 6.03 for the 1500 mg Pb/kg treatment. Pb treatment reduced *L. olgensis* total biomass (40.36%  
19 reduction in the 1500 mg Pb/kg compared with control) and *L. dispar* larval weights (by 34.44–52.05%)  
20 and survival rates (by 30.91–59.28%) in a dose-dependent manner compared with the control.  
21 Antioxidants (peroxidase and SOD) of *L. olgensis* increased under 500 mg Pb/kg treatment and were  
22 reduced under 1500 mg Pb/kg. Phytochemical defenses, protease inhibitors (trypsin inhibitor and  
23 chymotrypsin inhibitor) and the secondary metabolites (total phenolic acids) were significantly increased  
24 under the low dose of Pb (500 mg Pb/kg) compared with the control, while all phytochemical defense  
25 chemicals, including condensed tannins, decreased significantly under high Pb stress (1500 mg Pb/kg).  
26 *Lymantria. dispar* fed with *L. olgensis* seedlings had higher antioxidant activities in the fourth instar  
27 (SOD and CAT), while nonenzymatic antioxidants were significantly decreased (glutathione content and  
28 ascorbic acid content), suggesting that the reduction of antioxidants might lead to the oxidative stress  
29 experienced by *L. dispar* larvae. Finally, MDA content increased with Pb exposure.

30 Heavy-metal concentration along a pollution gradient in Romania affected soil mite (Acari:  
31 Mesostigmata) community structure ([Manu et al., 2019](#); [Manu et al., 2017](#)). [Manu et al. \(2017\)](#) examined  
32 soil mite communities in relation to soil metal content and physicochemical properties in 12 grasslands.  
33 Some heavy metals (Pb, As, Cu and Zn) influenced the soil mite community in highly polluted sites,  
34 while altitude and soil humidity played larger roles in less polluted sites. Pb soil concentration ranged

from  $28.21 \pm 4.62$  mg/kg Pb to  $421.12 \pm 71.62$  mg/kg 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  $300 \pm 300$  mg Pb/kg (mean  $\pm$  S.D.) to  $9,600 \pm 14,100$  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:  $0.103 \pm 0.068$  mg Pb/kg to  $0.477 \pm 0.212$  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  $\text{Pb}(\text{NO}_3)_2$  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

1 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,  
2 18.08 ± 0.67 mg Pb/kg and 34.98 ± 2.57 mg Pb/kg. Soil nematode communities were characterized before  
3 *L. esculentum* were planted and after 18 weeks of growth. Nematode community structure was analyzed  
4 using a variety of metrics, from trophic guilds to maturity indices to the abundance of colonizers and  
5 persister (cp-1 = colonizer to cp-5 = persister). Pearson's correlation coefficients between Pb and  
6 nematode community indices were largely nonsignificant, except for the negative relationship between Pb  
7 and the richness of cp-3 as well as the maturity index and the positive relationship between Pb and the  
8 abundance of fungivores as well as the abundance of cp-2. There was a significant decrease in nematode  
9 abundance in omnivores-predators (OP) and cp-4 at the highest concentrations of Pb. Nematode richness  
10 decreased at higher concentrations of Pb, particularly for OP, cp-4, and cp-5. The authors suggested that  
11 these groups are likely most sensitive to environmental stress, as they have longer-life cycles and lower  
12 reproduction rates.

13 In another nematode study, the diversity and abundance of nematode communities were  
14 correlated with soil Pb concentration near a ferroalloy manufacturer in North Slovakia ([Salamun et al.,](#)  
15 [2011](#)). Soil samples near the factory and downwind of the factory were analyzed for heavy metals,  
16 including Pb. The total Pb concentration ranged from 0.815 ± 0.471 mg Pb/kg to 1.766 ± 0.082 mg Pb/kg  
17 (mean ± S.D). Soil Pb concentration was positively correlated with the abundance of certain trophic  
18 guilds and ecological indices of nematodes, specifically, predators, root-fungal feeders, and maturity  
19 index (MI2-5). Maturity index (2-5) is used as a measure of functional diversity, which incorporates the  
20 abundance of *r* and *K*-strategists in a community. Pb was not significantly correlated with any other  
21 trophic group or ecological index (bacterial feeders, fungal feeders, omnivores, plant feeders, maturity  
22 index, plant-parasite index, genera richness, Shannon-Weaver index, Simpson index or abundance). In a  
23 follow-up study, [Šalamún et al. \(2012\)](#) examined nematode community structure in relation to the total  
24 element concentration of Pb, Zn, Cu Cr, Ca, and As, in another region of Slovakia using an HNO<sub>3</sub>  
25 extraction and mobilization fraction Na<sub>2</sub>EDTA extraction. Unlike [Salamun et al. \(2011\)](#), in which Pb was  
26 positively correlated with certain trophic groups, total soil Pb concentration was negatively correlated  
27 with the abundance of omnivorous nematodes, MI2-5, structure index and genera richness.

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

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## 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, 2006a, 1986, 1977](#)). 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 *P. 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



1 significantly induced in the freshwater snail *B. aeruginosa* during 28-day exposure to Pb-spiked sediment  
2 (29.7 mg Pb/kg dry weight) ([Liu et al., 2019b](#)).

3 The 2013 conclusion of a likely to be causal relationship between Pb exposure and  
4 neurobehavioral effects in freshwater vertebrates is bolstered in this current ISA by multiple studies with  
5 zebrafish (*D. rerio*) as an animal model for human health effects including developmental and  
6 neurological changes associated with Pb exposure (Section 11.3.4.4). Effects on behavioral endpoints  
7 such as locomotion and social interactions in larval zebrafish were reported at lower effect concentrations  
8 than studies in the 2013 Pb ISA, with some effects reported at  $\leq 20$   $\mu\text{g Pb/L}$ ; a subset of these studies  
9 analytically verified Pb in the exposure water ([Kataba et al., 2020](#); [Zhao et al., 2020](#); [Wang et al., 2018b](#);  
10 [Zhu et al., 2016](#)). Neurological responses of fish to Pb exposure were first reported in the 1986 Pb AQCD  
11 ([U.S. EPA, 1986](#)). The likely to be causal determination in the 2013 Pb ISA was based primarily on  
12 altered behaviors, such as reduced locomotion and prey capture ability, observed in fish following Pb  
13 exposure. These included a decrease in zebrafish larval startle response to mechanosensory and visual  
14 stimuli following nominal exposure to Pb (2.0 and 6.0  $\mu\text{g Pb/L}$ ) ([Rice et al., 2011](#)), and reduced prey  
15 capture in assays with 10-day old fathead minnows born from adult fish exposed to 120  $\mu\text{g Pb/L}$  for  
16 300 days then subsequently tested in a breeding assay for 21 days ([Mager et al., 2010](#)). In another study in  
17 the 2013 Pb ISA with fathead minnows, swimming performance measured as critical aerobic swim speed  
18 was significantly impaired in minnows in 24-hour acute (139  $\mu\text{g Pb/L}$ ) and chronic 33 to 57-day  
19 (143  $\mu\text{g Pb/L}$ ) exposures; however, no significant difference in swim speed was observed in chronic  
20 exposures to 33  $\mu\text{g Pb/L}$  ([Mager and Grosell, 2011](#)). The evidence in the 2013 Pb ISA and previous  
21 AQCDs also included effects on molecular targets; however, these experiments were typically conducted  
22 at Pb concentrations that greatly exceeds environmental concentrations.

23 In the 2013 Pb ISA, there was a conclusion of a causal relationship between Pb exposure and  
24 reduced survival in both freshwater invertebrates and vertebrates. Newly available evidence continues to  
25 support these causal determinations. For invertebrates, several studies provide further characterization for  
26 known effects on survival in a few sensitive species of freshwater invertebrates at  $<20$   $\mu\text{g Pb/L}$  (Section  
27 11.3.5). In the gastropod *L. stagnalis*, survival was significantly decreased at 8.4  $\mu\text{g Pb/L}$  after 21-day  
28 exposure to the end of a 56-day full life cycle assessment ([Munley et al., 2013](#)). In a chronic 42-day  
29 bioassay with the amphipod *H. azteca*, the  $\text{EC}_{20}$  for survival was similar under two different experimental  
30 diets administered concurrently ( $\text{LC}_{20} = 15$   $\mu\text{g Pb/L}$  and  $\text{LC}_{20} = 13$   $\mu\text{g Pb/L}$ ) ([Besser et al., 2016](#)). For  
31 freshwater vertebrates, studies in fish provided the basis for causality determination in the 2013 Pb ISA  
32 (Section 11.3.5). Additional fish bioassays conducted in varying water chemistry conditions report effects  
33 on survival at Pb concentrations similar to those reported in the 2013 Pb ISA. For larval zebrafish (*D.*  
34 *rerio*), 96-hour  $\text{LC}_{50}$  values varied with water hardness;  $\text{LC}_{50} = 52.9$   $\mu\text{g Pb/L}$  in soft water and  
35  $\text{LC}_{50} = >590$   $\mu\text{g Pb/L}$  in hard water ([Alsop and Wood, 2011](#)). Several studies considered the role of Pb  
36 and other trace metals on the decline of the white sturgeon in U.S. waters, and one study examined  
37 endpoints in westslope cutthroat trout. In 96-hour acute toxicity assays conducted with two lifestages of

1 white sturgeon (*A. transmontanus*), the lowest 96-hour LC<sub>50</sub> was 177 µg Pb/L for 8 dph larvae ([Vardy et](#)  
2 [al., 2014](#)).

3 For growth effects in freshwater organisms associated with Pb exposure, recent studies continue  
4 to support the findings in the 2013 Pb ISA. There was a likely to be causal relationship between Pb  
5 exposure and reduced plant growth concluded in the 2013 Pb ISA. Most primary producers experience  
6 EC<sub>50</sub> values for growth at concentrations that greatly exceed Pb concentrations typically found in U.S.  
7 surface waters. One new study reported growth rates in three commonly tested algal species (*P.*  
8 *subcapitata*, *C. kesslerii*, and *C. reinhardtii*) at lower effect concentrations than previously reported. *P.*  
9 *subcapitata* was the most sensitive in 72-hour bioassays, with an EC<sub>50</sub> = 83.9 µg Pb/L,  
10 EC<sub>20</sub> = 45.7 µg Pb/L and EC<sub>10</sub> = 32.0 µg Pb/L based on filtered Pb concentration. Varying the pH resulted  
11 in greater sensitivity ([De Schamphelaere et al., 2014](#)). In the 2013 Pb ISA, there was a causal relationship  
12 concluded to exist between Pb exposure and reduced growth in invertebrates. Since then, additional  
13 studies have supported previous findings of Pb effects on the growth of snails (*L. stagnalis*) in the  
14 low µg Pb/L range (Crémazy, 2018, 6708984} ([Munley et al., 2013](#); [Brix et al., 2012](#); [Esbaugh et al.,](#)  
15 [2012](#)). Reduction in weight gain and specific growth rate were observed in juvenile Oriental river prawn  
16 (*M. nipponense*) exposed to 25 µg Pb/L in chronic 60-day trials. No growth effects were observed in  
17 prawns at 12 µg Pb/L ([Ding et al., 2019](#)). The evidence remains inadequate to infer a causality  
18 relationship for Pb exposure and reduced growth in freshwater vertebrates. One study reported a threshold  
19 of 160 µg Pb/L for tadpole growth in dark-spotted frogs (*P. nigromaculata*) ([Huang et al., 2014](#)).

20 Reproductive and developmental effects were concluded to be causally related to Pb exposure for  
21 freshwater invertebrates in the 2013 Pb ISA. This remains the case in newer studies. Recent evidence  
22 further supports previous observations of Pb effects on reproductive endpoints at low µg/L concentrations  
23 in sensitive species of gastropods, cladocerans and rotifers, especially under chronic exposure scenarios  
24 (Section 11.3.5) (see Table 11-5). In *L. stagnalis*, a gastropod known to be sensitive to Pb at low µg Pb/L  
25 concentration, NOEC<1.0 µg Pb/L and LOEC = 1.0 µg Pb/L were determined for the number of egg  
26 masses and time until the first egg mass in a 56-day life cycle bioassay ([Munley et al., 2013](#)). In this  
27 species, the egg capsule and embryo diameter were significantly reduced after 7 days of development at  
28 2.7 µg Pb/L (the highest concentration in which reproduction was observed in the study). For the  
29 cladoceran *C. dubia*, 7-day EC<sub>20</sub> values for reproduction ranged from 12 to 223 µg Pb/L in assays  
30 conducted in a variety of natural waters across the United States with different water chemistries; 7-day-  
31 EC<sub>50</sub> values ranged from 20 to 573 µg Pb/L in the same test waters ([Esbaugh et al., 2012](#)). Using the same  
32 sampled waters from across the United States, reproduction (as population growth) was also assessed in  
33 rotifer *P. rapida* over a 4-day exposure period. Chronic EC<sub>20</sub> and EC<sub>50</sub> in this species ranged from 3 to  
34 103 µg Pb/L and from 10 to 154 µg Pb/L, respectively.

35 Several studies in fish in which Pb concentration was analytically verified further support the  
36 causal determination reported in the 2013 Pb ISA between Pb and reproductive and developmental effects  
37 for freshwater vertebrates (Section 11.3.4.4). For example, hatching success rates in zebrafish embryos

1 were reduced at 4.5, 9.6 and 18.6 µg Pb/L aqueous exposure; at 72 hpf, the hatching success rates at all  
2 three concentrations were significantly decreased compared with the control, indicating that Pb caused a  
3 hatching delay. This effect persisted until the end of the experiment at 96 hpf ([Zhao et al., 2020](#)).  
4 Endocrine disruption (significant reduction in thyroid hormones T3 and T4) was observed in zebrafish  
5 larvae following exposure to 30 µg Pb/L, although there was no effect on the hatching success rate ([Zhu](#)  
6 [et al., 2014](#)). These studies at analytically verified concentration of Pb are bolstered by additional fish  
7 studies conducted at nominal concentrations (Section 11.3.4.4.1) and several studies in amphibians  
8 (Section 11.3.4.4.3).

9 In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a likely to be causal  
10 relationship between Pb exposure and freshwater-community and ecosystem effects, and recent evidence  
11 continues to support this finding (Section 11.3.6). Reductions in species abundance, richness or diversity  
12 associated with the presence of Pb in freshwater habitats are reported in the literature, usually in heavily  
13 contaminated sites where Pb (and other metal) concentrations are higher than typically observed  
14 environmental concentrations. Most evidence is from sediment-associated macroinvertebrate  
15 communities. Observational and experimental studies published since the 2013 Pb ISA continue to show  
16 negative associations between sediment and/or porewater Pb concentration and macroinvertebrate  
17 communities. The evidence is expanded somewhat with studies reporting associations with Pb and  
18 periphyton abundance. Uptake of Pb into aquatic and terrestrial organisms and subsequent effects on  
19 mortality, growth, developmental and reproduction at the organism level can cascade up to ecological  
20 populations and communities and lead to ecosystem-level consequences, and thus provide consistency  
21 and plausibility for causality in ecosystem-level effects. Although the evidence is strong for the effects of  
22 Pb on growth, reproduction, and survival in certain species in experimental settings at or near the range of  
23 Pb concentrations reported in surveys of U.S. freshwater systems, considerable uncertainties exist in  
24 generalizing effects observed at a smaller scale, particular conditions up to predicted effects at the  
25 ecosystem level of biological organization. In many cases, it is difficult to characterize the nature and  
26 magnitude of effects and to quantify relationships between ambient freshwater concentrations of Pb and  
27 ecosystem response due to the presence of multiple stressors, variability in field conditions and  
28 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	
			2013 Pb ISA <sup>a</sup>	2023 Pb ISA
<b>Community and Ecosystem</b>		Community and Ecosystem Effects	Likely Causal	Likely Causal
<b>Population-level Endpoints</b>	<b>Organism-level Responses</b>	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
	<b>Suborganismal Responses</b>	Neurobehavioral Effects – Invertebrates	Likely Causal	Likely Causal
		Neurobehavioral Effects – Vertebrates	Likely Causal	Likely Causal
		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

<sup>a</sup>Conclusions were based on the weight of evidence framework for causal determination in Table II of the 2013 Pb ISA ([U.S. EPA, 2013](https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282)). 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](https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282)).

Inputs of Pb into freshwater ecosystems include air-related sources and non-air sources (Appendix 1: <https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282>). 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).

1           The following sections review the recent literature published since the 2013 Pb ISA on effects of  
2 Pb on freshwater ecosystems. The new evidence is considered along with the ecological findings of  
3 previous Pb assessments. The 2013 Pb ISA developed causality determinations for freshwater biota based  
4 on the weight of evidence for Pb effects on specific endpoints and taxonomic groups (Table 11-4). In the  
5 2013 Pb ISA, the body of evidence was sufficient to conclude that there was a causal relationship between  
6 Pb exposure and reproductive and developmental effects in freshwater invertebrates and vertebrates,  
7 reduced growth and survival of invertebrates, reduced survival of vertebrates, and hematological effects  
8 in vertebrates. Relevant concentrations for causality judgments for the welfare effects of Pb in the 2013  
9 Pb ISA were determined considering Pb concentrations “generally within one or two orders of magnitude  
10 above those which have been observed in the environment and the available evidence for concentrations  
11 at which effects were observed in plants, invertebrates, and vertebrates” ([U.S. EPA, 2013](#)). Of these  
12 causal relationships concluded for freshwater ecosystems, effects on reproduction, growth, and survival in  
13 sensitive freshwater invertebrates are well characterized from controlled studies at concentrations at or  
14 near Pb concentrations occasionally encountered in U.S. fresh surface waters. The 2013 Pb ISA  
15 concluded there is a likely to be causal relationship between Pb exposure and physiological stress in  
16 freshwater biota. For hematological effects there was a likely to be causal relationship for freshwater  
17 invertebrates. Effects on neurobehavioral endpoints were likely to be causal for freshwater invertebrates  
18 and vertebrates. Pb effects on plant growth were likely to be causal and were only reported at relatively  
19 high concentrations compared with effects on invertebrates. There was also a likely to be causal  
20 relationship between Pb exposure and community and ecosystem-level effects. For all effects in  
21 freshwater biota, the toxicity of Pb varied with species and lifestage, duration of exposure, form of Pb,  
22 and water quality characteristics. Key uncertainties from the last review for freshwater ecosystems  
23 included the uncertainties associated with generalization of effects observed in controlled laboratory  
24 studies to conditions in streams, rivers, and lakes where many modifying factors affect Pb bioavailability  
25 and toxicity. For example, there is a discrepancy between the sensitivity of aquatic insect taxa in  
26 laboratory studies compared with longer-term field studies. In a meta-analysis of study findings, longer-  
27 term studies suggest that aquatic insect taxa are more sensitive to metals than indicated in acute exposure  
28 scenarios ([Brix et al., 2011](#)). In aquatic ecosystems affected by Pb, exposures are most likely  
29 characterized as low-dose, chronic exposures, whereas the majority of available toxicological data for this  
30 metal is from acute laboratory exposures, typically conducted at higher concentrations. There are  
31 considerable uncertainties associated with generalizing effects observed in controlled studies to effects at  
32 higher levels of biological organization. Furthermore, available studies on community and ecosystem-  
33 level effects are usually from contaminated areas where Pb concentrations are much higher than typically  
34 encountered in the environment and multiple contaminants are present. At the time of the 2013 Pb ISA,  
35 the connection between air concentration of Pb and ecosystem exposure was poorly characterized for  
36 aquatic habitats ([U.S. EPA, 2013](#)). Furthermore, the previous review noted that the level at which Pb  
37 elicits a specific effect is difficult to establish in freshwater systems, due to the influence of other  
38 environmental variables (e.g., pH, OM) on both Pb bioavailability and toxicity, and due to substantial

1 species differences in Pb sensitivity. Evidence indicated that Pb is bioaccumulated in biota; however, the  
2 sources of Pb in freshwater organisms have only been identified in a few studies, and the relative  
3 contribution of Pb from all sources, including atmospheric deposition, is usually not known.

4 Studies published since the 2013 Pb ISA that characterize bioavailability and uptake of Pb, and its  
5 effects in freshwater organisms and ecosystems, that identify additional uncertainties, or decrease  
6 uncertainties identified in the prior NAAQS review of this criteria air pollutant are presented throughout  
7 the following sections. Brief summaries of conclusions from the 1977 Pb AQCD ([U.S. EPA, 1977](#)), the  
8 1986 Pb AQCD ([U.S. EPA, 1986](#)), the 2006 Pb AQCD ([U.S. EPA, 2006a](#)) and the 2013 Pb ISA ([U.S.  
9 EPA, 2013](#)) are included where appropriate. Recent research on the bioavailability and uptake of Pb into  
10 freshwater organisms including plants, invertebrates and vertebrates is presented in 11.3.2. Information on  
11 environmental concentrations in freshwater biota and ecosystems in the United States at different  
12 locations and over time is presented in Section 11.3.3. Toxicity of Pb to freshwater flora and fauna  
13 including growth, reproductive and developmental effects (Section 11.3.4) are followed with data on the  
14 exposure and response of freshwater organisms (Section 11.3.5). Responses at the community and  
15 ecosystem levels of biological organization are reviewed in Section 11.3.6.

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

16 Toxicity of Pb to aquatic life varies with the physicochemical properties of surface waters ([U.S.  
17 EPA, 2013](#)). Factors affecting the bioavailability and subsequent toxicity of Pb to biota include chemical  
18 factors (primarily water hardness, DOC, pH) and biological factors (e.g., lifestage, development of  
19 tolerance, organism interactions). Water hardness, DOC, and pH can be quantified, are directly related to  
20 the toxic effects and are used in bioavailability models to predict acute and chronic toxicity ([Adams et al.,  
21 2020](#)) (Section 11.1.6). Biological factors discussed in prior Pb AQCDs or the 2013 Pb ISA that may  
22 influence organism response to Pb exposure include the lifestage of an organism, genetics, and nutrition  
23 (see Section 7.2.3, 2006 AQCD ([U.S. EPA, 2006a](#)) and Section 6.4.9, 2013 Pb ISA ([U.S. EPA, 2013](#))).  
24 These factors are more difficult to link quantitatively to toxicity. Often, species differences in  
25 metabolism, sequestration and elimination rates control the relative sensitivity and vulnerability of  
26 exposed organisms. The organism route of exposure also influences Pb toxicity. Uptake of Pb by aquatic  
27 invertebrates and vertebrates may preferentially occur via exposure routes other than direct absorption  
28 from the water column, such as ingestion of contaminated food and water, uptake from sediment  
29 porewater, or incidental ingestion of sediment ([U.S. EPA, 2013, 2006a](#)). Fewer studies assess uptake,  
30 bioaccumulation, and subsequent toxicity of Pb via diet than via aqueous exposure. Of the available Pb  
31 feeding studies in freshwater biota, only a few pair the same concentration of waterborne exposure with  
32 dietary exposure to compare the relative importance of dietary versus aqueous uptake pathways ([Alsop et  
33 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  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions) in Pb uptake and subsequent toxicity was reported in previous Pb AQCDs and the 2013 Pb ISA. Furthermore, 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  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  for binding sites. Newer literature has continued to examine the role of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and other cations commonly present in surface waters (e.g.,  $\text{K}^+$ ,  $\text{Na}^+$ ) in modulating Pb bioaccumulation and toxicity. For example, in a study of the amphipod *Gammarus pulex* exposed 2 days to 10  $\mu\text{g Pb/L}$  and a range of environmentally relevant cation concentrations ( $\text{Na}^+$ ,  $\text{Mg}^{2+}$  or  $\text{Ca}^{2+}$ ), both  $\text{Na}^+$  and  $\text{Mg}^{2+}$  had no significant effect on Pb uptake while increasing  $\text{Ca}^{2+}$  concentrations inhibited Pb uptake ([Urien et al., 2015](#)). In a study reviewed in the 2013 Pb ISA,  $\text{Ca}^{2+}$  influenced Pb accumulation and toxicity in the fathead minnow (*Pimephales promelas*) during waterborne exposure ([Grosell et al., 2006a](#)). In a newer study in fish,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  or  $\text{H}^+$  significantly decreased Pb accumulation and toxicity in zebrafish larvae *Danio rerio*, while  $\text{K}^+$  and  $\text{Na}^+$  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  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  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  $\text{EC}_{50}$  was 81.2  $\mu\text{g Pb/L}$  at 10  $\text{mg/L Ca}$  (0.25 mM) and 130  $\mu\text{g Pb/L}$  at 70  $\text{mg/L Ca}$  (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-h) exposure ([Nys et al., 2016b](#)). In bioassays with zebrafish larvae, Pb was more toxic in soft water (11.7  $\text{mg CaCO}_3/\text{L}$ ) compared with hard water (141  $\text{mg CaCO}_3/\text{L}$ ) ([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



1 importance of water chemistry variables including DOC, Ca, and pH in the toxic response of freshwater  
2 cladoceran (*Ceriodaphnia dubia*), mollusk (*Lymnaea stagnalis*) and rotifer (*Philodina rapida*) to a range  
3 of Pb concentrations in bioassays conducted in a variety of natural waters from across North America.  
4 The greatest toxicity to the cladoceran and snail species was observed in low-DOC waters, and toxicity  
5 was found to be correlated with DOC using multilinear regression modeling analysis. This was not the  
6 case in rotifer *P. rapida*, where toxicity was most closely correlated with Ca and pH, not DOC. In  
7 contrast, in the rotifer *B. calyciflorus*, high DOC was protective against Pb chronic reproductive toxicity;  
8 however, when expressed as free-ion activity, toxicity increased with increasing fulvic acid concentration  
9 ([Nys et al., 2016b](#)). The authors suggest that fulvic acid-Pb complexes may also contribute to Pb  
10 bioavailability in *B. calyciflorus*. Taking metal speciation into consideration, [Dong et al. \(2014\)](#)  
11 calculated the Comparative Toxicity Potential of Pb (described as the ecotoxicological impact associated  
12 with a unit emission of substance to defined ecological receptors via different pathways of exposure). Pb  
13 had the highest Comparative Toxicity Potential in water with low DOC, moderate pH and hardness, and  
14 the lowest Comparative Toxicity Potential in water with moderate DOC, high pH, and hardness. Pb  
15 typically has high affinity to DOC, resulting in a low fate factor (residence time) and bioavailability factor  
16 (fraction of truly dissolved metal within total metal) ([Dong et al., 2014](#)). Additionally, [Zhang et al. \(2021\)](#)  
17 found that modeling Pb and other heavy metals was improved when incorporating total OC and AVS.

18 Since the 2013 Pb ISA, studies have further elucidated the relationship between the  
19 characteristics of humic substances and Pb bioavailability, such as molecular weight (MW) or other  
20 additional effects associated with solar irradiation. In lake sediments, Pb-humic acid complexes are more  
21 stable when the MW of the humic acid is lower. In particular, humic acids with MW lower than 10 kDa  
22 could increase the biosorption capacity of Pb ([Bai et al., 2019](#)). While Pb-humic acid complexes are  
23 discussed in the 2013 Pb ISA, the study by [Kostić et al. \(2013\)](#) suggests a mechanism for the binding of  
24 Pb to humic acid may be the "acid-like" nature of Pb(II). Pb(II)-ions strong affinity for humic acid may be  
25 explained by its borderline acid properties and by how humic acids behave as weak acid polyelectrolytes.  
26 Humic acids carry a variety of oxygen-containing functional groups such as carboxylic, hydroxyl,  
27 phenolic and carbonyl groups with oxygen as a donor atom, which helps them form strong bonds with  
28 Pb(II). This is also supported by the study by [Liu et al. \(2022\)](#), which found Pb(II) caused greater  
29 quenching (the decrease of fluorescence by the metal addition) in humic-like DOM compared with  
30 protein-like DOM. The finding was likely due to humic-like components complexing with Pb(II) through  
31 carboxyl and hydroxyl (-COOH and -OH) groups, which generally bonds to Pb(II) preferentially over  
32 protein-like DOM that contains significant amounts of the amino group (-NH<sub>2</sub>).

33 The bioaccumulation capacity for Pb in algae is influenced by the presence of organic acids. [Que](#)  
34 [et al. \(2020\)](#) found that adding organic acids, such as malic acid or citric acid prolonged the adsorption  
35 equilibrium time of the algae-Pb binary system. Citric acid showed a greater bioaccumulation capacity for  
36 Pb in algae than malic acid, due to ternary complex formation. The binding capacity of Pb to OM is also  
37 influenced by solar or UV-B radiation. Pb complexation with representative humic substances (Suwannee

1 River humic acid and Suwannee River fulvic acid) decreased with increasing simulated solar radiation  
2 ([Spierings et al., 2011](#)). This may be due to an increase in the relative abundance of the carboxyl groups  
3 in the photoaltered humic substances and from decreased aromaticity (and thus less electronegativity)  
4 with increasing irradiation doses. The presence of  $Pb^{2+}$  can also increase the photodegradation of  
5 microcystin and thus reduce microcystin accumulation in sediments and in certain fish ([Dai et al., 2017](#)).  
6 Reduced amounts of humic acid were adsorbed to the freshwater microalga *Chlorella kesslerii*, which  
7 then reduced Pb bioavailability to the microalgae because the humic substances increase the  
8 bioavailability of Pb to microalgae by adding supplementary binding sites and because Pb uptake by *C.*  
9 *kesslerii* is controlled by transport across the biological membrane rather than by diffusion in the medium  
10 ([Spierings et al., 2011](#)). However, there was no correlation with an increase in free Pb ions and algal  
11 intracellular Pb content, likely due to the formation of additional binding sites on the photoaltered humic  
12 acids. In additional tests using Elliott humic acid under simulated solar radiation, free Pb ions were  
13 released from the metal-DOM complex as the irradiation dose increased, and there was a 33% increase in  
14 intracellular Pb concentration in *Chlamydomonas reinhardtii* at high irradiance ([Worms et al., 2015](#)).

#### 11.3.2.1.3. pH

15 As described in prior AQCDs and the 2013 Pb ISA, uptake and subsequent toxicity of Pb to  
16 freshwater biota can be affected by pH, either directly or indirectly. Generally, at low pH, there is more  
17  $Pb^{2+}$  available to bind to the biotic ligand. As pH increases, there is increased formation of Pb organic  
18 (DOC) and inorganic ( $OH^-$ ,  $CO_3^{2-}$ ) complexes, which decrease Pb bioavailability. Since the 2013 Pb  
19 ISA, several studies have further characterized Pb complexation and adsorption under changing pH  
20 conditions. There are more binding sites for Pb to humic acids at pH 6 than at pH 4, likely due to the  
21 higher content of dissociated functional groups in humic acids at higher pH, and more favorable  
22 electrostatic attraction when binding surfaces become deprotonated at higher pH ([Bai et al., 2019](#)). [Xu et al. \(2018\)](#)  
23 found that the binding dynamics of DOM groups in response to Pb(II) addition were regulated  
24 by both pH and ionic strength. Specifically, at lower pH and ionic strength (e.g., pH 4.7 and ionic strength  
25 0.01 M), as Pb(II) was added, aryl C-H and carboxyl C = O groups gave the fastest response, followed by  
26 polysaccharide C-OH and chromophoric groups at 265 nm (CDOM<sub>265</sub>). However, when pH was raised  
27 to 6.0, the opposite binding sequence was found, in that the CDOM<sub>265</sub> group was bound first, followed  
28 by the polysaccharide C-OH and carboxyl C = O, and finally the aryl C-H groups. [Hua et al. \(2013\)](#) found  
29 that Pb absorption to biofilms was greatest at pH 9, which was 3.5 times greater than that at the minimum  
30 adsorption (pH = 7).

31 Several studies since the 2013 Pb ISA have tested the effects of changing pH on Pb toxicity to  
32 biota. In the freshwater algal species *Pseudokirchneriella subcapitata*, as pH increased from 6.0 to 7.6,  
33 the 72-hour EC<sub>50</sub> decreased from 72.0 to 20.5 µg filtered Pb/L ([De Schamphelaere et al., 2014](#)). Further,  
34 [Antunes and Kreager \(2014\)](#) observed greater toxicity (more bioavailability) for common duckweed (*L.*

1 *minor*) at higher pH; this was due to less  $H^+$  and competition at the macrophyte binding sites. The  
2 apparent increase in  $Pb^{2+}$  toxicity at  $pH > 7.0$  coinciding with a changing ratio of  $[Pb^{2+}]/[Pb(OH)^+]$  (due to  
3 the marked increase in  $[Pb(OH)^+]$ ) suggests that  $Pb(OH)^+$  also contributed to the toxicological response.

4 In some freshwater invertebrates, recent studies generally support previous understanding that  
5 higher pH is protective; however, these findings vary by the duration of the toxicity bioassays and by  
6 taxa. In a series of chronic reproductive toxicity tests with daphnia *C. dubia* conducted at different pH  
7 values, high pH was protective of Pb toxicity. At the lowest pH tested (pH 6.4), the  $EC_{50} = 99.8 \mu g Pb/L$ ,  
8 while at the highest pH (pH 8.2), the  $EC_{50} = 320 \mu g Pb/L$  (Nys et al., 2014). Similarly, decreasing toxicity  
9 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  
10 24 h- $LC_{50}$  increased from  $784 \mu g Pb/L$  to  $9,473 \mu g Pb/L$ , and the predicted proportion of free  $Pb^{2+}$  ion was  
11 99.75% at pH 5.0 and 2.9% at pH 9.0. High pH was also protective in chronic reproductive toxicity tests  
12 with rotifer *B. calyciflorus*. Both the population growth rate and population size generally decreased with  
13 increasing pH in bioassays conducted at pH values ranging from 6.4 to 8.2 (Nys et al., 2016b). Wang et  
14 al. (2015b) found that for crustaceans, Pb toxicity increased with increasing pH, but for mollusks and  
15 worms, toxicity decreased with increasing pH. For fish, toxicity was least at neutral pH and increased at  
16 lower or higher pH levels. The toxicity of Pb can increase at higher pH when there is less competition  
17 between  $H^+$  and metal binding sites on cell-surface ligands. However, there may be higher toxicity at  
18 lower pH due to increased solubility and altered Pb speciation, which can increase Pb bioavailability for  
19 certain animals. Uptake studies in natural environments have also pointed to the importance of pH in  
20 uptake of Pb. A field study conducted in 36 headwater streams in the Lake District of England reported  
21 statistically significant correlations between total dissolved Pb in stream water and body burdens in the  
22 sampled aquatic insect taxa (*Leuctra* spp., Simuliidae, *Rhithrogena* spp., Perlodidae) (De Jonge et al.,  
23 2014). In the streams,  $H^+$  ion activity was the overriding factor influencing Pb body burden, while DOC  
24 was not a significant factor.

25 In fish, the effects of pH on toxicity were variable in studies cited in the 2013 Pb ISA. For  
26 example, lower pH was shown to result in increased sensitivity to Pb in juvenile fathead minnows  
27 following 30-day exposure to Pb at varying concentrations (Grosell et al., 2006a). Additionally, Birceanu  
28 et al. (2008) determined that fish (specifically rainbow trout) were more susceptible to Pb toxicity in  
29 acidic, soft waters, characteristic of sensitive regions in Canada and Scandinavia. Hence, fish species  
30 endemic to such systems may be more at risk from Pb contamination than fish species in other habitats. In  
31 a study published after the 2013 Pb ISA, Esbaugh et al. (2013) compared three methods used to acidify  
32 laboratory bioassay water on  $LC_{50}$  values in fathead minnow. Pb toxicity varied significantly depending  
33 upon the acidification method used in the experiment. The authors recommended direct acid-base addition  
34 rather than  $CO_2$  or 3-(N-morpholino)propanesulfonic acid buffer. In an approach that linked metal  
35 accumulation with toxicity through a BLM-aided toxicokinetic-toxicodynamic model, Gao et al. (2015)  
36 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<sup>+</sup>] 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 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 the mechanism of action, with some shared binding sites between the two metals. In 5-day postfertilization zebrafish larvae exposed nominally 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 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<sub>50</sub> (= 430 µg Pb/L at the F0 generation) increased to 2,110 µg Pb/L in the F9 generation. The LC<sub>50</sub> 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 Pietroock, 2011](#)). Multiple studies have reported differences in Pb accumulation between parasitized and nonparasitized organisms. European chub (*Squalius cephalus*) showed greater Pb uptake in the gill and bile of nonparasitized host fish compared with parasitized fish, and the highest metal concentrations were detected in acanthocephalan parasites ([Sures et al., 2003](#); [Sures and Siddall, 1999](#)). European perch (*Perca fluviatilis*) infected with two parasites, the acanthocephalan (*Acanthocephalus lucii*) and a tapeworm (*Proteocephalus percae*) showed greater Pb accumulation in the muscle, liver, and hard roe than *P. fluviatilis* parasitized with either *A. lucii* or *P. percae* alone ([Brázová et al., 2015](#)). The number of *P. percae* was negatively correlated with Pb concentration in the muscle of *P. fluviatilis*. 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](#)). In field-collected European chub infected with intestinal parasites (*Pomphorhynchus laevis* or *Acanthocephalus anguillae*), lower metal concentrations were found in infected fish in the postspawning period compared with uninfected individuals, suggesting that the presence of parasites modulate metal accumulation ([Marijic et al., 2014](#)). The concentrations of Pb were lower in trematode-infected *Biomphalaria alexandrina* snails compared with uninfected snails ([Mostafa et al., 2014](#)). These studies suggest that the effects of parasites on host organisms in the presence of Pb are complex.

#### 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 ([Soledad 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. First, [Blankson and Klerks \(2016a\)](#) quantified the transfer of Pb from sediment to overlying water from mesocosms with different numbers of worms. At the end of the 14-day experiment, overlying water Pb concentration increased with increasing density of *L. variegatus*. Next, the researchers added water flea (*Daphnia magna*) to Pb-spiked sediment microcosms with and without *L. variegatus*, restricting some *D. magna* to the water column ([Blankson and Klerks, 2016b](#)). The presence of *L. variegatus* in the microcosms significantly increased the amount of Pb transferred from the sediment to the water column (mean Pb in water column with *L. variegatus* present: 2.51 µg Pb/L versus *L. variegatus* absent: 0.38 µg Pb/L). The turbidity of the water column when *L. variegatus* was present was 85% higher than that in microcosms without *L. variegatus*. Turbidity was unaffected by the containment of *D. magna* and the interaction between these two factors. Microcosms with restricted *D. magna* and those with free-swimming *D. magna* did not affect the concentration of Pb in the water column at the end of the experiment. Transport profiles in microcosms contaminated with Pb-spiked sediments (137.6, 681.9 or 3396.2 mg Pb/kg dry weight) showed the bioturbation activity of *L. variegatus* was affected by increasing Pb concentration; with a decline in bioturbation with worms exposed to 681.9 mg/kg and 3396.2 mg/kg ([Blankson et al., 2017](#)). In 14-day exposure to Pb-spiked mesocosms (104 mg/kg dry weight) with natural sediments collected from different sites, 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. Specifically, [Blankson and Klerks \(2017\)](#) found that organic content and sediment porewater pH had a significant negative effect on the change in worm tissue Pb concentrations, with Pb in worm tissue being 26-fold lower with 5–10% greater OM content. In addition, Pb concentration in worms was about 50% lower in sediment with 10 and 30% silt/clay content compared with unmodified sediment (4.58% silt/clay content) (Pb bioaccumulation was reduced by a factor of 2.6 at an 8-times higher silt/clay content). 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<sub>50</sub> 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

1 feeding ration. Similar results were obtained with the higher feeding ration experiment with multiple  
2 daphnia per experimental unit ( $LC_{50} = 79$  (74–84)  $\mu\text{g Pb/L}$ ) and the single-animal treatment  
3 ( $LC_{50} = 236$   $\mu\text{g Pb/L}$  (no 95% confidence interval could be calculated). Moreover, reproduction (neonate  
4 production) decreased with intraspecific competition at 9 and 14 days in both feeding ration groups  
5 compared with assays with single daphnia where no negative effects on reproduction were observed at  
6 any concentration tested. The authors proposed that individual daphnia modulate their life-history  
7 response in the presence of others of the same species through chemical cues, and this has a modifying  
8 effect on toxicity.

### 11.3.2.1.13. Predator-Stress and Metal Mixture Effects

9 Research published since the 2013 Pb ISA tested the effects of multiple stressors on Pb uptake  
10 and toxicity. Predator stress and the presence of other metals affected the accumulation and sensitivity of  
11 the aquatic sowbug (*A. aquaticus*) to Pb stress ([Van Ginneken et al., 2018](#)). Individual *A. aquaticus*  
12 collected from a stream in Belgium were placed in a control, 0.0232  $\mu\text{mol/L}$ , (4.8  $\mu\text{g Pb/L}$ ), 0.276  $\mu\text{mol/L}$   
13 (57  $\mu\text{g Pb/L}$ ) or 3.08  $\mu\text{mol/L}$  (638  $\mu\text{g Pb/L}$ ) solution with two black alder (*Alnus glutinosa*) leaf discs.  
14 Each Pb treatment and metal mixture (Cu + Pb, Cd + Pb and Cu + Cd + Pb) was crossed with one of two  
15 treatments, either a heterospecific predator cue or conspecific alarm cue. To create the heterospecific  
16 predator cue solution, one damselfly larva (*Calopteryx splendens*) was placed in a container of water for  
17 72 hours. Next, one adult three-spined stickleback (*Gasterosteus aculeatus*) and the ninespine stickleback  
18 (*Pungitius pungitius*) were placed in water for 24 hours. After removing the predators from the water,  
19 equal parts of stimulus water were mixed to create the heterospecific predator cue. To create the  
20 conspecific alarm cue, one *A. aquaticus* was homogenized in solution. Either water (control) or predator  
21 or the conspecific alarm cue solution was added to the control and Pb-contaminated containers with *A.*  
22 *aquaticus* every day for 10 days. Afterward, *A. aquaticus* Pb concentration, growth rate, feeding rate,  
23 percent active time, survival and respiration rate were recorded. Overall, there were no significant effects  
24 of either heterospecific or conspecific predator cues on Pb accumulation in *A. aquaticus*, although  
25 respiration rates did increase when exposed to predator cues. Pb accumulation in the isopods was  
26 positively correlated with Pb free-ion activity. There were no significant effects of predator stress on  
27 isopod body burdens. Metal mixture significantly affected Pb accumulation, as the slope of the  
28 relationship between Pb treatment and Pb body burden decreased when Cu and Cd + Cu were added.  
29 Respiration rates were affected by both Pb exposure and predator stress. Differences in respiration rates  
30 between predator-stress and control treatments were greater when isopods had greater Pb body burdens.  
31 Activity levels decreased as Pb body burden increased, but there was no difference between predator  
32 treatments and the interaction between Pb treatment and predator stress. Growth rate (mg/day) was  
33 negatively correlated with Pb free-ion activity in the water but was not found to vary with predator stress  
34 or body burden. Although Pb body burden did not influence feeding rates (mg/mg/day), the Pb body  
35 concentration of *A. aquaticus* exposed to the Pb + Cd mixture had the greatest effect on feeding rate

1 compared with Pb, Pb + Cu and Pb + Cu + Cd. Finally, activity decreased with increasing Pb body  
2 burden, but was unaffected by predator stress and Pb-metal mixtures.

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### 11.3.2.2. Uptake and Bioaccumulation in Freshwater Plants and Algae

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

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

30 In freshwater floating macrophytes, there is also very little new information on the bioavailability  
31 of Pb. These life forms are important because their roots dangle in the water column instead of being  
32 buried in substrate, and thus, Pb uptake occurs solely through the interface with the water column. One  
33 U.S. study examined the uptake and distribution of metals by a floating macrophyte, water lettuce (*Pistia*

1 *stratiotes* L.), in storm water impoundments in Florida ([Lu et al., 2011](#)). Two stormwater impoundment  
2 ponds were divided into two plots, a control without *P. stratiotes* and one with enough young plants to  
3 initially cover 1/20th of the water surface. While the authors stated that water Pb levels were mostly low  
4 (below the Maximum Daily Limits), they did not provide the concentrations. Even at these low  
5 concentrations, reported BCFs of Pb from the water column into plant roots were higher than 104. Lead  
6 was found inside and adsorbed to plant roots, with approximately 60% of Pb within the root tissue.  
7 Another study by [Chen et al. \(2019\)](#) found that submerged macrophytes in lakes can accumulate Pb,  
8 which is absorbed either from the sediments through roots or from the water by leaves.

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

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### 11.3.2.3. Uptake and Bioaccumulation in Freshwater Invertebrates

19        This section expands on the findings from the 1986 Pb AQCD ([U.S. EPA, 1986](#)), the  
20 2006 Pb AQCD ([U.S. EPA, 2006a](#)) and the 2013 Pb ISA ([U.S. EPA, 2013](#)) on the bioaccumulation and  
21 sequestration of Pb in freshwater invertebrates. Uptake and subsequent bioaccumulation of Pb varies  
22 greatly between species and across taxa, as characterized in previous EPA reviews of this metal. In  
23 invertebrates, Pb can be bioaccumulated from multiple sources, including the water column, sediment and  
24 dietary exposures, and factors such as the proportion of bioavailable Pb (Section 11.1.6) lifestyle, age and  
25 metabolism can affect the accumulation rate. As reviewed by [Wang and Rainbow \(2008\)](#) and supported  
26 by subsequent studies, there are considerable differences between species in the amount of Pb taken up  
27 from the environment and in the levels of Pb retained in the organism.

28        Uptake studies generally show that aquatic invertebrates accumulate Pb from water in a  
29 concentration-dependent manner and may reach an equilibrium depending on the organism's ability to  
30 eliminate or store Pb. In a study reviewed in the 2013 Pb ISA, the tissue concentration of Pb in adult  
31 Eastern Elliptio mussel (*Elliptio complanata*) increased for the first 14 days in an aqueous exposure at an  
32 exposure-dependent rate then did not change significantly for the remainder of the 28-day exposure  
33 ([Mosher et al., 2012b](#)) In another study with the same species conducted after the 2013 Pb ISA, Pb was

1 measured in hemolymph every 7 days during a 28-day exposure, and distinct patterns of response were  
2 observed with Pb concentration. At the lowest concentrations ( $\leq 6 \mu\text{g Pb/L}$ ), Pb gradually increased in the  
3 hemolymph but did not exceed the exposure concentration, at mid-range concentration (up to  
4  $66 \mu\text{g Pb/L}$ ), the mussels appeared to regulate Pb by day 14, whereas at the highest concentration tested  
5 ( $251 \mu\text{g Pb/L}$ ), Pb in hemolymph increased throughout the exposure period ([Mosher et al., 2012a](#)). Pb in  
6 tissue was highly correlated with the exposure concentration at the end of the experiment. The lowest  
7 exposure concentration of  $0.9 \mu\text{g Pb/L}$  resulted in an average tissue concentration of  $1.5 \mu\text{g Pb/g}$  dry  
8 weight.

9         Studies in the 2006 Pb AQCD and 2013 Pb ISA generally showed that the tissue distribution of  
10 Pb in aqueous exposures of freshwater invertebrates is primarily sequestered in the gills, hepatopancreas  
11 and muscle. Recent short-term (3–4-hour) aqueous uptake studies with juvenile snail *L. stagnalis* showed  
12 no significant difference in Pb accumulation among foot, mantle, digestive tract and remaining soft  
13 tissues, suggesting uptake occurred directly across the skin ([Crémazy et al., 2019](#)). *L. stagnalis* was  
14 previously identified as one of the aquatic invertebrates most sensitive to Pb exposure ([Grosell and Brix,](#)  
15 [2009](#); [Grosell et al., 2006b](#)).

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

27         The 2006 Pb AQCD recognized the potential importance of the dietary uptake pathway as a  
28 source of Pb exposure for invertebrates. Additionally, several studies reviewed in the 2013 Pb ISA  
29 quantified water versus dietary uptake of Pb in aquatic invertebrates ([Komjarova and Blust, 2009](#);  
30 [Borgmann et al., 2007](#); [Besser et al., 2005](#)). Since the 2013 Pb ISA, the relative importance of dietary  
31 versus aqueous uptake pathways has been further discerned for some biota. [Camusso et al. \(2012\)](#) applied  
32 a biologically based Biodynamic Model to previously published data and additional unpublished data on  
33 uptake of trace metals in *L. variegatus* from field-collected sediments to assess the main uptake route in  
34 this sediment-dwelling organism. The modeled data suggest that for Pb, both free dissolved concentration  
35 in porewater and dietary uptake contributed to body burden, and the amount of Pb taken up in the gut  
36 appears to be controlled by how tightly Pb is bound to sediment. In *D. magna* fed under two different  
37 dietary regimens (regular diet =  $3 \times 10^5$  *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 *Populus 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](#)).

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#### 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.*



1 *variegatus* pre-exposed for 28-days to the same concentration of Pb as the fish) or simultaneously to  
2 waterborne and dietary Pb ([Alsop et al., 2016](#)). Accumulation of Pb in fish was significantly higher via  
3 the waterborne exposure pathway compared with dietary exposure in all tissues except in the gut, which  
4 accumulated similar amounts of Pb regardless of the exposure route. When fish were exposed to Pb from  
5 both water and their diet, whole-body Pb was reduced up to 61% at 110 µg Pb/L, and Pb accumulation  
6 was significantly reduced at a threshold of ~50 µg Pb/L, with significantly lower concentrations in the  
7 liver and carcass but not the gill or gut. The authors noted that Pb may have altered the nutrient quality of  
8 the prey; carbohydrates and lipid levels in the worms were significantly decreased even at the lowest Pb  
9 concentration.

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### 11.3.2.5. Uptake and Bioaccumulation Through Food Web

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

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

23 In a high-elevation lake in the Alps, [Pastorino et al. \(2020b\)](#) examined the accumulation of heavy  
24 metals, including Pb, in sediment, chironomids, and fish. Surface sediment, benthic macroinvertebrates,  
25 and fish were sampled from a glacial-origin lake, Dimon Lake, in Northeast Italy. While there is only a  
26 single fish species in this lake, i.e., the European bullhead (*Cottus gobio*), the benthic macroinvertebrate  
27 community consists of midges (Diptera Chironomidae), worms (Oligochaeta), and leeches (Hirudinea).  
28 The only prey found in the stomachs of *C. gobio* was Diptera Chironomidae, and therefore only these  
29 specimens were used for trace-element analysis. Surface sediment Pb was  $109.6 \pm 1.2$  mg Pb /kg, whole-  
30 body Diptera Chironomidae Pb concentration was  $49 \pm 0.5$  mg Pb/kg, and Pb concentration in *C. gobio*  
31 was  $0.06 \pm 0.03$  mg Pb/kg in the muscle and  $0.03 \pm 0.4$  mg Pb/kg in liver. The BAF and trophic transfer  
32 factor (TTF) in Diptera Chironomidae and *C. gobio* muscle and liver samples were less than 1.0 for Pb,  
33 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  $110 \pm 1.1$  mg Pb/kg (mean  $\pm$  S.D.), while Balma Lake had considerably less Pb ( $41 \pm 1.2$  mg/kg Pb). In addition to lower Pb concentration in the surface sediments, Balma Lake had a lower pH (mean  $\pm$  S.D.; summer:  $6.70 \pm 0.34$ ; autumn:  $7.64 \pm 0.09$ ) than Dimon Lake (summer:  $8.77 \pm 0.12$ ; autumn:  $9.44 \pm 0.05$ ). 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,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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  $\delta^{13}\text{N}$  and  $\delta^{15}\text{N}$  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  $0.03 \pm 0.003$  mg Pb/kg to  $0.045 \pm 0.045$  mg Pb/kg (mean  $\pm$  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. Lead 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 2.94–10.7 mg Pb/kg, which was significantly higher than Pb concentrations in the teneral, 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).

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

1 Few U.S. studies have examined national or regional-scale trends of Pb in freshwater biota. The  
2 1986 AQCD reported the results of [Lowe et al. \(1985\)](#), a nation-wide survey of metal concentrations in  
3 fish from 1979 to 1981. At 112 monitoring stations, they found an average (geometric mean) of  
4 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.  
5 EPA, 1986](#)). In the 2006 AQCD, a representative median and a range of Pb concentrations were reported  
6 in surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L), sediments (median 28 mg Pb/kg dry  
7 weight, range 0.5 to 12,000 mg Pb/kg dry weight) and fish tissues (geometric mean 0.54 mg Pb/kg dry  
8 weight, range 0.08 to 23 mg Pb/kg dry weight [whole body]) in the United States based on a synthesis of  
9 National Ambient Water Quality Assessment data ([U.S. EPA, 2006b](#)). The 2013 Pb ISA reported survey  
10 results from the Western Area Contaminants Assessment Project (2002–2007), which included the  
11 concentration of Pb in fish tissue (0.0033 [fillet] to 0.97 [liver] mg Pb/kg [dry weight]) from a set of  
12 national parks in the western United States ([NPS, 2011](#); [Landers et al., 2008](#)). No recent studies  
13 examining spatial or temporal trends in Pb concentration in freshwater fish or invertebrates from locations  
14 across the United States were identified in this ISA. Many individual studies report Pb concentrations in  
15 aquatic ecosystems and biota from specific sites across the United States; compilation of those data is  
16 outside the scope of this ISA. Pb concentrations in water, sediment and other environmental media are  
17 available in Section 11.1.3 and summarized in Table 11-1.

18 Since the 2013 Pb ISA, a few regional-scale studies, including a study in Canada, have assessed  
19 trends in Pb concentrations in vegetation (peat bogs) or the water column. Peat bogs deposit and preserve  
20 stable layers of accumulated moss and other plant material that can be used to reconstruct a record of  
21 spatial and temporal distribution patterns of air Pb concentrations. Six peat cores collected in 2013 and  
22 2014 in northern Alberta, Canada [Shoty et al. \(2016\)](#) record the rates of atmospheric Pb deposition dated  
23 from 1910 to 2014 using <sup>210</sup>Pb and <sup>14</sup>C dating in models, linking sample depth to age. Peak accumulation  
24 rates were observed between the years 1950 and 2000 in each sample, and overall decreasing rates of Pb  
25 accumulation were observed from 1980. Although this study was not in the United States, decreased Pb  
26 accumulation rates coincided with the introduction of unleaded gasoline in the United States and Canada  
27 in the mid-1970s and nearby potential point sources of Pb air pollution (industrial development including  
28 bitumen mines and upgraders) are not attributed to the increase in Pb accumulation. The uppermost, most  
29 recent, peat layers show near-zero modern atmospheric Pb deposition in the Alberta peat bogs.

30 In a 2015–2017 water quality survey of four Tennessee headwater Appalachian streams [Olson et  
31 al. \(2019\)](#), the maximum observed Pb concentration and sole detectable measurement of this metal was  
32 less than 1 µg Pb/L. Reported mean concentration values at each site were less than the minimum  
33 detection limit of 0.28 µg Pb/L. These observations from remote streams without upstream anthropogenic  
34 Pb sources suggest that long-range atmospheric deposition is not a major source of Pb contamination to

1 this region. Limited evidence from regional studies of temporal trends in freshwater aquatic ecosystems  
2 published since the 2013 Pb ISA suggest that modern atmospheric deposition of Pb is not a major  
3 contributor to Pb concentrations in freshwater aquatic biota and ecosystems in remote locations.

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#### 11.3.4. Effects of Pb in Freshwater Systems

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

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##### 11.3.4.1. Effects on Freshwater Microbes

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

22 In a study from the United States, porewater and sediment Pb concentrations were negatively  
23 correlated with bacterial RNA abundance, but not diversity or richness in Lake DePue, Illinois ([Gough  
24 and Stahl, 2011](#)). Lake DePue is a shallow lake on the Illinois River located near a U.S. EPA Superfund  
25 Site (the DePue/New Jersey Zinc/Mobil Chemical Site). Although the Zn smelting facility and phosphate  
26 fertilizer plant are no longer operational, Lake DePue has received metal-contaminated sediments from  
27 this site for over 80 years. Sediment Pb concentration in the lake was on average 180 mg Pb/kg (range:  
28 68.6 and 541 mg Pb/kg). Porewater and sediment Pb were correlated with a low abundance of archaeal,  
29 bacterial, and eukaryotic terminal restriction fragments (TRF); however, other metals were also correlated  
30 to most of the same TRF profiles (particularly, Zn, As, Cd, Cu, and Fe). Specifically, porewater Pb was

1 significantly correlated with the abundance of putative Mesophilic *Crenarchaeota* (positively correlated  
2 with TRF 191 and negatively correlated with TRF 162 and TRF 510 – 512) but was not correlated with  
3 the diversity of archaeal TRFs. Porewater Pb was also negatively correlated with a putative  
4 *Desulfobacterium* and a eukaryotic TRF 108 with the nearest relative of *Pfiesteria/Scenedesmeaceae*.  
5 Sediment Pb also correlated with putative Mesophilic *Crenarchaeota* (positive correlation with TRF 191  
6 and negative correlation with TRF 162), and unlike porewater, sediment Pb had a significant negative  
7 correlation with archaeal TRF diversity. Sediment Pb did not significantly correlate with any reported  
8 bacterial or eukaryotic TRF abundances. Finally, porewater and sediment Pb were negatively correlated  
9 with relative bacterial RNA abundance. Interestingly, the high positive correlations between TRF 191  
10 (TRF for *Crenarchaeota*) and metals, including Pb, suggest that methanogens, which are an archaeal  
11 population typically expected to be the dominant group in freshwater systems, may not be able to  
12 outcompete other archaeal groups in metal-contaminated sites. Overall, there were some differences in  
13 overall community structure with regard to metal contamination observed using terminal restriction  
14 fragment length polymorphism analysis of 16S rRNA genes, although variation in bacterial diversity,  
15 richness and composition across a metal gradient was not detected. In a follow-up study using the same  
16 samples, [Kang et al. \(2013\)](#) further explored the bacterial communities using a different approach, a  
17 function gene microarray (GeoChip). Overall, the diversity of functional gene variants was similar across  
18 all five sites, suggesting that heavy-metal concentrations in the sediments did not significantly affect  
19 bacterial community structure; however, some individual gene categories were correlated with certain  
20 porewater metal concentration, including Pb. Using a CCA, Pb, Zn, and Cd were all found as important  
21 predictors for sulfate-reducing bacteria communities. Although significant correlations with Pb existed,  
22 functional gene variants had similar relationships with other porewater metal concentrations, including  
23 As, Cd, Cr, and Zn.

24 In another U.S. study, observational evidence suggests that the exchangeable Pb fraction  
25 decreases microbial community diversity, while oxyhydroxide Pb concentration was correlated with an  
26 increase in diversity in the mining district of Lake Coeur d'Alene, Idaho ([Moberly et al., 2016](#)). The  
27 Coeur d'Alene Mining district stretches from Coeur d'Alene, Idaho to Superior, Montana and has had 90  
28 mines in operation. Sediment cores were collected from sites in the Lake Coeur d'Alene delta and from  
29 reference sites in the neighboring St. Joe River delta to characterize metal concentrations (Pb, Fe, Mn, and  
30 Zn) and phase in the sediment and microbial community composition. Pb concentrations in the sediment  
31 were high in the lake, ranging from 1540 to 3422 mg Pb/kg, while the St. Joe River delta site sediment Pb  
32 concentration was 29 mg Pb/kg. More than 70% of the Pb was associated with the  
33 exchangeable/carbonate phase, which is thought to be the most bioavailable phase. Pb in the  
34 exchangeable/carbonate fraction was negatively correlated with the abundance of Aquificae and  
35 Synergistes and positively correlated with candidate phylum LD1PA abundance (a phylum without many  
36 cultured representatives); furthermore, this pattern is similar for Fe and Mn oxyhydroxides, as Pb  
37 exchangeable/carbonate concentrations are highly correlated. Bacteroidetes OTU abundance was  
38 negatively correlated with Pb-exchangeable/carbonate and positively correlated with Pb-(oxy)hydroxide.



1 These results suggest that the phase of Pb is integral in determining the relationship between Pb  
2 concentration in the sediment and microbial communities, as seasonal changes in Pb speciation could  
3 affect microbial diversity.

4 To understand how heterotrophic bacteria in river sediments are affected by Pb, sediments were  
5 collected from sites along three tributaries of the Nagara River in Japan, varying in land use types  
6 (agricultural, industrial, or forested) and contamination ([Du et al., 2018](#)). Sediment samples were brought  
7 into the lab and used in a sequencing batch incubation experiment, in which the sediment and water from  
8 each site were placed in flasks with a C, N, and P source. The flasks were then spiked with a control or  
9 three nominal concentrations of Pb (100 µg/L, 1000 µg/L, or 10000 µg/L). The pH was held constant at  
10 7.0 due to the added P; therefore, the dissolved concentrations for Pb were likely lower than the initial  
11 concentration added but were not measured in this study. Bacterial abundance and activity were sampled  
12 every 3 days for 30 days, and the community structure was sampled on the first and last days of the  
13 experiment (day 0 and day 30). Bacterial abundance (heterotrophic bacterial density) and heterotrophic  
14 activity were not affected by Pb exposure. The dominant species at the end of the experiment differed  
15 from that at the start of the experiment for all treatments; however, there was no difference in the most  
16 abundant bacterial species at the end of the experiment, suggesting little effect of Pb on heterotrophic  
17 bacterial communities. Overall, Pb did not have significant effects on heterotrophic bacteria density,  
18 activity, and community structure after 30 days of an incubation experiment.

19 The Pb enrichment factor, along with other heavy metals, was found to influence bacterial  
20 community structure in the Poyang Lake river system, China ([Zhang et al., 2018](#)). Fifty-nine sediment  
21 samples were collected from five rivers for heavy-metal analysis and characterization of microbial  
22 communities. Mean Pb concentration in the sediments ranged from 29.52 to 40.06 mg Pb/kg. The Pb  
23 enrichment factor, which takes into account Fe as the normalizer element, along with the As and Cd  
24 enrichment factors, pH, OC, and degree of contamination were the main variables affecting bacterial  
25 community structure (redundancy analysis). The Pb enrichment factor, as well as Cd enrichment factor  
26 and the degree of contamination, was strongly associated with higher abundances of Acidobacteria,  
27 suggesting tolerance of the phyla.

28 In another study in freshwater systems in China, Pb concentration in the sediment was found to  
29 negatively correlate with the relative abundance of major bacterial groups, but not with bacterial diversity  
30 ([Li et al., 2020](#)). Pb concentration in the sediment was  $17.3 \pm 7.3$  mg Pb/kg (mean  $\pm$  S.D.) and ranged  
31 from 1.9–25.4 mg Pb/kg across 12 sites in Huangjinxia Reservoir in Shaanxi Province, China. Sediment  
32 Pb concentration was highly correlated with Cr, Zn, and Ni but not significantly correlated with microbial  
33 diversity indices (ACE, Chao1, Shannon, and Simpson's index). However, Pb sediment concentration  
34 was significantly negatively correlated with the relative abundance of Bacteroidota, Nitrospirota, and  
35 Verrucomicrobia and positively correlated with the relative abundance of Chloroflexi. Finally, similar to  
36 Zn and Cr, Pb sediment concentration was negatively correlated with nitrification and aerobic nitrate



1 oxidation, as predicted through Functional Annotation of Prokaryotic Taxa, and with metabolism, as  
2 predicted through phylogenetic investigation of communities by reconstructing unobserved states.

3 Variation in bacterial community composition along an elevation gradient in Yangtze River,  
4 China, was driven by OM, elevation, urbanization, and Pb concentration ([Zhang et al., 2020](#)) Sediments  
5 were collected from 24 sites along the Yangtze River, and environmental parameters (soil pH, total N,  
6 total P, and OM), trace metals (Pb, Cu, Pb, Cd, As) and bacterial diversity (OTU abundance, Shannon  
7 index) were characterized and correlated using Nonmetric multidimensional scaling and Pearson  
8 correlation analysis. Pb concentration in the sediment ranged from  $14.40 \pm 0.80$  mg Pb/kg to  
9  $87.01 \pm 8.00$  mg Pb/kg. Elevation (meters above sea level) was negatively correlated with Pb  
10 concentration in the sediment as were many other physicochemical parameters and metal concentrations.  
11 The population density and urbanization rate were not significantly correlated with Pb. Redundancy  
12 analysis found that the first axis of variation explained 47.9% of the variation in microbial communities  
13 and the second axis explained 18.6% of the variation. OM was the most significant variable, followed by  
14 elevation (10.4%), urbanization rate (9.0%) and Pb (9.5%,). Above 400 meters above sea level (masl),  
15 elevation was the strongest factor correlated with bacterial community structure. Below 400 masl, OM,  
16 urbanization rate and Pb exerted the strongest influence. Bacterial community structure between 50 and  
17 400 masl was most correlated with Pb, and below 50 masl community structure was most correlated with  
18 urbanization rate. Above 400 masl, Pb concentration and OTU abundance were significantly correlated,  
19 while the correlations between Pb and the Shannon index and evenness were not significant. Below 400  
20 masl, the opposite pattern emerged: the relationship between Pb and OTU abundance was negative and  
21 the relationships between Pb and the Shannon index and evenness were nonsignificant. Finally, Pb  
22 concentration was positively correlated with the abundance of certain bacterial genera, negatively  
23 correlated with others, and not correlated with most dominant bacteria taxa.

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

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#### 11.3.4.2. Effects on Freshwater Plants and Algae

29 The toxicity of Pb to freshwater algae and plants has been recognized in earlier EPA reviews of  
30 the metal and the findings are briefly summarized here. In the 1977 Pb AQCD, differences in sensitivity  
31 to Pb among different species of algae were observed, and concentrations of Pb within the algae varied  
32 among genera and within a genus ([U.S. EPA, 1977](#)). The 1986 Pb AQCD ([U.S. EPA, 1986](#)) reported that  
33 some algal species (e.g., *Scenedesmus* sp.) were found to exhibit physiological changes when exposed to

1 high Pb concentrations in situ. Effects of Pb on algae reported in the 2006 Pb AQCD included decreased  
2 growth, deformation, and disintegration of algae cells, and blocking of the pathways that lead to pigment  
3 synthesis, thus affecting photosynthesis. Most studies on effects of Pb in freshwater algal species  
4 reviewed in the 2013 Pb ISA and the AQCDs were conducted with nominal media exposures and effect  
5 concentrations greatly exceeded Pb reported in surface water. In studies in which Pb was quantified,  
6 effect concentrations for growth ( $EC_{50}$ ) for freshwater algae and macrophytes were much higher than  
7 currently reported environmental Pb. Growth endpoints in freshwater algae reviewed in the 2013 Pb ISA  
8 included significant inhibition of chlorophyll a content at 210  $\mu\text{g Pb/L}$  and higher in *Wolffia arrhiza*  
9 ([Piotrowska et al., 2010](#)). An increase in biomass was reported in *L. minor* exposed to 100 or  
10 200  $\mu\text{g Pb/L}$ , with inhibition observed at higher concentrations ([Dirilgen, 2011](#)). There were also  
11 numerous studies conducted at nominal Pb concentration that reported effects on enzyme activities and  
12 protein content in freshwater aquatic plant species. Exposure-response relationships in which increasing  
13 concentrations of Pb lead to increasing effects were consistently observed for freshwater aquatic plants. In  
14 the 2013 Pb ISA, the body of evidence was sufficient to conclude there were likely to be causal  
15 relationships between Pb exposure and freshwater plant physiological stress and between Pb exposure and  
16 reduced freshwater plant growth. The body of evidence was inadequate to conclude there are causal  
17 relationships between Pb exposure and freshwater plant reproduction and between Pb exposure and  
18 freshwater plant survival.

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

28 Additionally, new information on Pb effects on the emergent freshwater macrophyte, the common  
29 reed (*Phragmites australis*), shows an alteration in growth form and propagation strategy under Pb  
30 exposure. In a phytotron experiment, reed plants were exposed to five Pb levels in sediment (measured  
31  $5.9 \pm 0.2$ ,  $304 \pm 4.38$ ,  $508 \pm 7.89$ ,  $1513 \pm 37.28$ ,  $3020 \pm 120.41 \text{ mg Pb/kg}$ ) ([Zhang et al., 2015a](#)). In  
32 addition to decreases in total biomass, photosynthesis and rhizome growth, the addition of Pb caused a  
33 significant alteration in growth form. The numbers of axillary shoot buds and daughter apical rhizome  
34 shoots were increased by Pb addition at the highest concentrations, and the bulk (80%) of daughter shoots  
35 were from daughter axillary shoots. This clonal propagation strategy of increased formation and output of  
36 axillary shoot buds, called the phalanx pattern, is an adaptive response to maintain population stability at  
37 the lowest energetic cost. This same growth pattern alteration was also found in an additional study on the

1 effects of Pb and drought in *P. australis* by the authors ([Zhang et al., 2015b](#)), but clonal modular growth  
2 and reproductive ability were significantly inhibited by the interaction between drought and Pb. These  
3 propagation effects would cause a decline in *P. australis* populations in a dry environment under Pb  
4 pollution.

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

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#### 11.3.4.3. Effects on Freshwater Invertebrates

15 Freshwater aquatic invertebrates are generally more sensitive to Pb exposure than other taxa.  
16 Controlled studies at concentrations near the upper range of representative concentrations of Pb available  
17 from surveys of U.S. surface waters (median: 0.50 µg Pb/L; range 0.04 to 30 µg Pb/L, 95th percentile  
18 1.1 µg Pb/L) ([U.S. EPA, 2006a](#)) (Table 11-1) reviewed in the 1986 AQCD, the 2006 Pb AQCD and the  
19 2013 Pb ISA provide evidence for the effects of Pb on reproduction, growth and survival in sensitive  
20 freshwater invertebrates. Freshwater invertebrate taxa that exhibit sensitivity to Pb include some species  
21 of gastropods, amphipods, cladocerans and rotifers, although the toxicity of Pb is highly dependent upon  
22 water quality variables such as DOC, hardness, and pH. Key studies reported in the 1986 AQCD include  
23 increased mortality as low as 19 µg Pb/L for the snail *Lymnaea palustris* ([Borgmann et al., 1978](#)) and  
24 reproductive impairment at 30 µg Pb/L (nominal values) for *Daphnia* sp. ([Biesinger and Christensen,](#)  
25 [1972](#)). In a 42-day chronic study reviewed in the 2006 Pb AQCD, the LOEC for reproduction was  
26 3.5 µg Pb/L in the amphipod *H. azteca* receiving both waterborne and dietary Pb ([Besser et al., 2005](#)).

27 In the 2013 Pb ISA, additional studies provided evidence for Pb effects on freshwater  
28 invertebrates at low µg Pb/L concentration. The growth of juvenile freshwater snails (*L. stagnalis*) was  
29 inhibited at an EC<sub>20</sub> of <4 µg Pb/L ([Grosell and Brix, 2009](#); [Grosell et al., 2006b](#)). In fatmucket mussel, *L.*  
30 *siliquoidea* juveniles, a chronic value (geometric mean of no-observed-effect concentration [NOEC] and  
31 LOEC) of 10 µg Pb/L was obtained following 28-day exposures ([Wang et al., 2010](#)). In a 7-day exposure  
32 of the cladoceran *C. dubia* to 50 to 500 µg Pb/L, increased DOC led to an increase in mean EC<sub>50</sub> for  
33 reproduction ranging from approximately 25 µg Pb/L to >500 µg Pb/L ([Mager et al., 2011a](#)). The 48-hour

LC<sub>50</sub> 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<sub>50</sub> was 35 µg Pb/L using neonates hatched from asexual eggs ([Arias-Almeida and Rico-Martínez, 2011](#)). The EC<sub>20</sub> 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<sub>50</sub> of 3,323 µg Pb/L ([Mebane et al., 2008](#)). The EC<sub>10</sub> 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 strengthens the 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 such as SOD, CAT and GPx with aqueous exposure to Pb. Juvenile *D. magna* exposed nominally to 16 µg Pb/L exhibited statistically significant decreased intracellular ROS and increases in total GSH level and SOD activity in 48-hour exposure ([Kim et al., 2018](#)). In the same study, the expression patterns of several molecular biomarker gene transcripts were observed. Daphnid neonates tested with the same concentration as juveniles showed a greater response to Pb exposure, suggesting that the neonate lifestage is more susceptible to Pb. SOD and GPx activities were significantly

1 reduced, and MDA levels were significantly increased in juvenile Oriental river prawn (*Macrobrachium*  
2 *nipponense*) exposed to 25 µg Pb/L for 60 days. CAT activity in the hepatopancreas increased at  
3 12 µg Pb/L and decreased in the 25 µg Pb/L treatment ([Ding et al., 2019](#)). In the same study, reductions in  
4 weight gain and specific growth rate were observed in prawns exposed to 25 µg Pb/L in chronic 60-day  
5 exposure tests. No growth effects were observed in prawns at 12 µg Pb/L (see Section 11.3.5).

6 Physiological stress in freshwater invertebrates was also assessed during sediment exposure to  
7 Pb. Exposure of larval midge *Chironomus riparius* to Pb-spiked sediment (132 mg Pb/kg dry weight and  
8 505.5 mg Pb/kg dry weight) for 16 days resulted in an antioxidant response (increase in metallothionein)  
9 and cellular damage (increase in MDA) ([Arambournou et al., 2013](#)). There was no significant change to  
10 protein concentration, lipid was depleted while glycogen increased with increasing Pb in the sediment. In  
11 the same organisms, Pb exposure via sediment did not result in statistically significant effects on growth,  
12 survival, or number of mentum (mouthpart) deformities. In a separate study in *C. riparius* in Pb-spiked  
13 sediment ranging from 18.1 to 456.9 mg Pb/kg dry weight, no significant differences were observed in the  
14 frequency of mouthpart deformities ([Arambournou et al., 2012](#)). In freshwater snail *Bellamya aeruginosa*  
15 exposed for 28 days to Pb-spiked sediment, CAT activity and metallothionein were significantly induced  
16 at the lowest concentration tested (29.7 mg Pb/kg dry weight) ([Liu et al., 2019b](#)). In the bivalve *Hyridella*  
17 *australis* also exposed 28-days to Pb-spiked sediments (205 ± 9 and 419 ± 16 mg Pb/kg dry mass), the  
18 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,  
19 respectively); however, total antioxidant capacity significantly decreased while ROS and MDA increased  
20 with Pb exposure compared with controls ([Marasinghe Wadige et al., 2014](#)).

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

#### 11.3.4.3.2. Organism-Level Response

32 Organism-level endpoints include effects on behavior linked to Pb neurotoxicity. In the 2013 Pb  
33 ISA, the body of evidence was sufficient to conclude there is a likely to be causal relationship between Pb  
34 exposure and neurobehavioral effects in freshwater invertebrates ([U.S. EPA, 2013](#)) (see Table 11-4 of this

1 appendix). In limited studies available on worms and snails, there is evidence that Pb may affect the  
2 ability to escape or avoid predation. For example, in the tubificid worm *T. tubifex*, the 96-hour EC<sub>50</sub> for  
3 immobilization was 42 µg Pb/L ([Khangarot, 1991](#)). Some organisms exhibit behavioral avoidance while  
4 others do not seem to detect the presence of Pb ([U.S. EPA, 2006b](#)). Additional behavioral endpoints  
5 reported in the Great Lakes Environmental Center draft *Ambient Aquatic Life Water Quality Criteria for*  
6 *Lead* document [GLEC \(2008\)](#) include an EC<sub>50</sub> of 140 µg Pb/L for feeding inhibition in the freshwater  
7 cladoceran *C. dubia*. In a study published since the 2013 Pb ISA, adult amphipods, *G. fossarum* exposed  
8 to Pb for 5 days at a concentration at which survival was unaffected (2.7 µg Pb/L) exhibited sublethal  
9 behavioral and physiological responses. Locomotion was significantly decreased over time (assessed 24,  
10 48 and 120 hours) and respiration rate was significantly lower at 120 hours compared with unexposed  
11 amphipods ([Lebrun et al., 2017](#)). In a separate study with *G. fossarum*, both locomotion and respiration  
12 were significantly decreased following exposure to 2.1 µg Pb/L for 24-hour ([Lebrun and Gismondi,](#)  
13 [2020](#)).

14 As described in the 2013 Pb ISA and previous AQCDs, Pb is neurotoxic to many organisms.  
15 Alterations in neurotransmitter regulation and release may be an underlying mechanism for the behavioral  
16 effects of Pb. Few studies in freshwater invertebrates have reported effects on neurotransmitters at lower  
17 Pb concentrations. In prereproductive freshwater bivalve *Lamellidens jenkinsianus obesa* exposed for  
18 21 days to either 68 or 763 µg Pb/L, AChE activity (assessed on days 1, 7, 15 and 21 of the experiment)  
19 was significantly inhibited at each timepoint compared with control ([Brahma and Gupta, 2020](#)). Several  
20 locomotor behaviors (movement in the form of gliding, foot-siphon extension) were significantly reduced  
21 or ceased completely in the Pb-exposed individuals compared with the control during a separate 5-day  
22 exposure to either 69 or 776 µg Pb/L. In the same study, reproductive-age individuals of another bivalve  
23 species, *Parreysia corrugata*, were exposed to either 26 or 302 µg Pb/L for 21 days. AChE activity was  
24 significantly induced at 26 µg Pb/L and significantly inhibited compared with control at 302 µg Pb/L at  
25 all timepoints. Behavioral response in the form of impaired movement with Pb exposure (25 and  
26 304 µg Pb/L) was also observed in this species. In 28-day chronic exposure of freshwater snail *B.*  
27 *aeruginosa* to Pb-spiked sediment, the activity of the neurotransmitter AChE was significantly induced  
28 starting at day 7 in the lowest concentration (29.7 mg Pb/kg dry weight) ([Liu et al., 2019b](#)).

29 In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship  
30 between Pb exposure and growth in freshwater invertebrates ([U.S. EPA, 2013](#)) (see Table 11-4 of this  
31 appendix). The growth of freshwater snail *L. stagnalis* was identified as one of the most sensitive  
32 organisms and endpoints for Pb toxicity. At the time of the 2013 Pb ISA, the hypersensitivity of this  
33 species to Pb was hypothesized to be from Pb inhibition of Ca<sup>2+</sup> uptake. Subsequent experiments by [Brix](#)  
34 [et al. \(2012\)](#) observed that effects on growth occur prior to effects on net Ca<sup>2+</sup> flux, inhibition of carbonic  
35 anhydrase activity in the snail mantle also showed no effect with Pb; therefore, the mechanism of Pb in  
36 these highly sensitive organisms remains elusive. Additional studies reported in Section 11.3.5, Exposure



1 and Response of Freshwater Species, support Pb effects on the growth of *L. stagnalis* in the low µg Pb/L  
2 range ([Crémazy et al., 2018](#); [Brix et al., 2012](#)).

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

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#### 11.3.4.4. Effects on Freshwater Vertebrates

8 The 1977 Pb AQCD reported Pb effects in both fish and waterfowl. The available Pb studies on  
9 waterfowl investigated exposure to Pb via accidental poisoning or ingestion of Pb shot ([U.S. EPA, 1977](#)).  
10 Studies on aquatic vertebrates reviewed in the 1986 Pb AQCD were limited to hematological,  
11 neurological, and developmental responses in fish ([U.S. EPA, 1986](#)). In the 2006 Pb AQCD, effects on  
12 freshwater vertebrates included consideration of the role of water quality parameters on toxicity to fish, as  
13 well as limited information on the sensitivity of turtles and aquatic stages of frogs to Pb ([U.S. EPA,](#)  
14 [2006a](#)). In the 2013 Pb ISA, the body of evidence was sufficient to conclude there is a causal relationship  
15 between Pb exposure and hematological effects, reproduction and survival in freshwater vertebrates  
16 (based primarily on evidence from fish) ([U.S. EPA, 2013](#)) (see Table 11-4 of this appendix). There were  
17 also likely to be causal relationships concluded between Pb exposure and physiological stress and  
18 neurobehavioral effects. Newly available studies on the effects of Pb in fish and other freshwater  
19 vertebrates are summarized below.

##### 11.3.4.4.1. Fish

##### 11.3.4.4.1.1. Suborganism-level Response

20 A large body of evidence supports sublethal biomarker perturbations with Pb exposure in  
21 freshwater vertebrates; however, few studies were identified for this ISA that reported physiological  
22 response at more environmentally relevant concentrations of Pb ( $\leq 10$  µg Pb/L; Section 11.1.1) or  
23 concurrently assessed response at organism-level endpoints (i.e., from the cellular and subcellular level to  
24 effects on growth, reproduction or survival). Various biomarkers of oxidative stress assessed in carp  
25 (*Carassius auratus gibelio*) after 96 hours and 21 days were significantly altered at analytically verified  
26 concentration of 10 and 30 µg Pb/L ([Khan et al., 2015](#)). For the acute exposure, CAT activities (liver and  
27 kidney) were significantly reduced, and SOD was significantly upregulated in brain, kidney, and muscle  
28 tissue. GP<sub>x</sub> activity in the liver and gill increased significantly, while activity in the muscle and kidney



1 was significantly reduced. Biomarker response in chronic exposure showed significant reduction in CAT  
2 (liver, gill, muscle) at 10 and 30 µg Pb/L, whereas CAT was upregulated in the kidney. There was a  
3 decline in GPx (liver and gill) as well as in SOD (liver, kidney, muscle), while the brain showed an  
4 increase. Acetylcholine, a biomarker of neurotoxic stress, was significantly inhibited following chronic  
5 exposure to 30 µg Pb/L. [Clemow and Wilkie \(2015\)](#) observed no significant effect on respiratory stress,  
6 mean cell hemoglobin concentration, plasma Ca<sup>2+</sup> or Na<sup>2+</sup> ion concentration or plasma protein in juvenile  
7 rainbow trout (*O. mykiss*) over a 5-day exposure to 5.4 µg Pb/L (26.1 nmol/L). In fingerling rainbow  
8 trout, used in the same study for unidirectional Na<sup>+</sup> flux measurement, there was an initial Na<sup>+</sup> loss after  
9 48 hours of exposure that recovered by 72 hours with exposure to 8.3 µg Pb/L (40.2 nmol/L).

10 Hematological effects of Pb on fish reported in the 2013 Pb ISA and AQCDs include a decrease  
11 in red blood cells and inhibition of ALAD with elevated Pb exposure under various test conditions.  
12 Inhibition of ALAD is also reported in environmental assessments of metal-impacted habitats. For  
13 example, as reported in the 2013 Pb ISA, lower ALAD activity has been significantly correlated with  
14 elevated blood Pb concentrations in wild-caught fish from Pb-Zn mining areas, although there are  
15 differences in species sensitivity ([Schmitt et al., 2007](#); [Schmitt et al., 2005](#)). Few studies were identified  
16 since the 2013 Pb ISA that quantify ALAD response in freshwater fish in laboratory exposure at  
17 concentrations considered for this ISA (Section 11.1.1). [Olson et al. \(2018\)](#) reported gene transcription of  
18 ALAD was significantly induced in zebrafish at 100 µg Pb/L and higher nominal exposure. In a field  
19 study of brown trout (*Salmo trutta*) collected from a lake in Norway contaminated with Pb (14 µg Pb/L)  
20 from an abandoned shooting range, ALAD activity in the trout population was approximately 20% of that  
21 of a relatively uncontaminated reference lake (0.76 µg Pb/L) ([Mariussen et al., 2017](#)).

#### 11.3.4.4.1.2. Organism-Level Response

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

31 Since the 2013 Pb ISA, there have been additional studies on neurobehavioral response in  
32 freshwater vertebrates, particularly in zebrafish *D. rerio*. As a widely used model organism in  
33 environmental toxicology, the zebrafish genome shares a high degree of homology with the human  
34 genome ([Dai et al., 2014](#); [Howe et al., 2013](#)). Zebrafish are used as an animal model for human health

1 outcomes associated with Pb exposure such as neurogenerative disease (reviewed in [Lee and Freeman](#)  
2 [\(2014\)](#)) and developmental and neurobehavioral alterations ([Li et al., 2019](#)). Endpoints assessed in these  
3 zebrafish assays, such as decreased locomotor activity and altered social interactions used as surrogates  
4 for autistic behaviors in humans, can affect organism fitness in natural environments. Furthermore, many  
5 of these studies link changes in gene expression, neurotransmitter levels or other molecular and cellular  
6 responses to the observed behavioral outcomes. Experiments conducted in the low  $\mu\text{g/L}$  range are  
7 particularly representative of environmental concentrations (Table 11-1); therefore, zebrafish behavioral  
8 assay studies conducted at low concentrations of Pb are reviewed below.

9 In zebrafish embryos exposed to 5.0, 9.7 or 19.2  $\mu\text{g Pb/L}$  there were no significant effects on  
10 dorsal axon length up to 144 hours postfertilization (hpf); however, there was a significant reduction in  
11 swimming speed at the highest Pb concentration tested ([Zhu et al., 2016](#)). Alterations in the  
12 neurotransmitter gamma-aminobutyric acid (GABA) were observed during development of zebrafish  
13 embryos exposed to Pb (nominally to 10, 50 and 100  $\mu\text{g Pb/L}$  up to 72 hpf, Pb uptake was quantified in  
14 embryos) ([Wirbisky et al., 2014](#)). The levels of this neurotransmitter varied with the dose of Pb and  
15 developmental stage, with all three treatments resulting in a significant decrease in GABA by the end of  
16 embryogenesis (72 hpf). Newly-hatched larval zebrafish exposed to Pb since 2.5 hpf exhibited  
17 neuromuscular responses (increased muscular twitching) at concentrations of 49.6 and 100.7  $\mu\text{g Pb/L}$  at  
18 72 hpf. No twitches were observed at lower concentrations or in the control group ([Kataba et al., 2022](#)). In  
19 another study, locomotor and social behavior responses were assessed in zebrafish larvae exposed to 4.5,  
20 9.6 or 18.6  $\mu\text{g Pb/L}$  at 6 days postfertilization (dpf) during a dark and light photoperiod ([Zhao et al.,](#)  
21 [2020](#)). During the dark period, swimming activity was significantly decreased at 18.6  $\mu\text{g/L}$ , and at both  
22 9.6 and 18.6  $\mu\text{g/L}$ , there was a decrease in clockwise turning; social contact time was significantly higher  
23 in the light period at the highest Pb concentration. Downregulation of genes involved in brain neutrophilic  
24 factor signaling was observed in the Pb-exposed larvae, suggesting an underlying mechanism for the  
25 observed responses. Hyperactivity (increased distance covered and speed) during the light period was  
26 observed in larval zebrafish exposed to Pb (3.2, 93 or 252.6  $\mu\text{g Pb/L}$ ) for 30 minutes in alternating light  
27 and dark intervals of 10 minutes ([Kataba et al., 2020](#)).

28 Several studies in zebrafish have considered the neurobehavioral effects of Pb at multiple  
29 lifestages. [Wang et al. \(2022\)](#) exposed zebrafish embryos from 2 hpf to 120 hpf to nominal concentration  
30 of 20, 50, 100 or 250  $\mu\text{g Pb/L}$  (0, 0.1, 0.25, 0.5  $\mu\text{M}$ ) then examined whether the effects of the early-life  
31 exposures persisted in juveniles and adults. Spinal curvature and hyper swimming activity were observed  
32 in embryos exposed to the lowest concentration. Next, the fish were held in Pb-free conditions and 1-  
33 month old juveniles and 4-month-old adult zebrafish exposed at the two lowest concentrations as embryos  
34 (20 and 50  $\mu\text{g Pb/L}$ ) were evaluated in various behavioral assays. Juvenile fish exposed to Pb in the  
35 embryo stage exhibited significantly elevated hyperactivity and over-response to stimuli compared with  
36 the control fish. Adult fish raised from Pb-exposed embryos were hyperactive and displayed anxiety-like  
37 behaviors consistent with other studies in fish. [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.

Reproductive outcomes in fish may also be affected by Pb-associated neurobehavioral alterations. Courtship behaviors of adult male zebrafish exposed for 2 weeks to Pb (nominal concentration of 1, 10 and 100 µg Pb/L) exhibited a biphasic response to Pb, with hyperactivity observed at low concentrations and inhibitory effects at higher concentrations ([Li et al., 2019](#)). The study used a video system optimized for tracking zebrafish behavior to record the locomotion profiles of male fish interacting with female fish. Movements including total velocity, vertical velocity, turning, and total distance were quantified to evaluate changes in swimming trajectory patterns with Pb exposure. A U-shaped dose-response was reported for total velocity and total distance while turning angle and turning speed were not significantly affected by Pb treatment. Concurrent with the behavioral study, the transcription patterns of key genes involved in testicular steroidogenesis and apoptosis were evaluated in tissue of testes of exposed males. Most genes exhibited upregulation after low-level Pb exposure and downregulation after high-level Pb exposure, consistent with the behavioral assays.

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., 2016](#)). 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. Adult zebrafish exposed nominally to Pb (0.1 µM [20 µg Pb/L], 1.0 µM [200 µg Pb/L] or 10.0 µM [2000 µg Pb/L]) as embryos (to 24 hpf) and then raised in Pb-free medium were tested for avoidance response and conditioning ([Xu et al., 2015](#)). At the lowest concentration, adult zebrafish learned avoidance responses during training and testing, while fish exposed to the higher concentrations of Pb displayed no significant changes in avoidance response. In F3 offspring of the Pb-exposed embryos, these learning deficits persisted at the two higher Pb concentrations.

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

1 exposure (24 months) to 8–49 mg Pb/kg in food pellets, there were no significant differences in fish body  
2 weights or the survival of Prussian carp *C. gibelio* females ([Łuszczek-Trojnar et al., 2013](#)). In another  
3 study with adult female carp *C. carpio* exposed to Pb via diet (68.4 mg Pb/kg dry weight in food pellets),  
4 there were no significant differences in mean body weights at the end of the study (three exposure  
5 seasons), although Pb-exposed fish weighed significantly less than control fish after the first exposure  
6 season ([Łuszczek-Trojnar et al., 2016](#)). This is consistent with dietary studies reviewed in the 2013 Pb  
7 ISA ([Alves and Wood, 2006](#)). In aqueous exposure studies, zebrafish embryos exposed to Pb  
8 (19.3 µg Pb/L) to 6 dpf (144 hpf) showed no significant differences in hatching success, body length or  
9 body weight compared with the control ([Chen et al., 2016b](#)). Similarly, exposure of zebrafish embryos to  
10 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  
11 differences in head length, head width or total body length were observed in 72 hpf embryos exposed  
12 nominally to 10, 50 or 100 µg Pb/L ([Wirbisky et al., 2014](#)).

13 For the effects of Pb on reproduction and development in freshwater vertebrates, the weight of  
14 evidence for the causal relationship in the 2013 Pb ISA was primarily from studies with fish. Pb AQCDs  
15 have reported developmental effects in fish, specifically spinal deformities in brook trout (*Salvelinus*  
16 *fontinalis*) exposed to 119 µg Pb/L for three generations ([U.S. EPA, 1977](#)), as well as in rainbow trout  
17 exposed to concentrations as low as 120 µg Pb/L ([U.S. EPA, 1986](#)). In the 2006 Pb AQCD ([U.S. EPA,](#)  
18 [2006a](#)), decreased spermatocyte development in rainbow trout was reported at 10 µg Pb/L, and testicular  
19 damage occurred in fathead minnow at 500 µg Pb/L. In the 2013 Pb ISA, a 300-day chronic toxicity study  
20 was conducted by [Mager et al. \(2010\)](#) in fathead minnows treated with both 31 and 112 µg Pb/L with  
21 HCO<sub>3</sub> and with 130 µg Pb/L with DOC. The total reproductive output was decreased, and average egg  
22 mass production increased as compared with egg mass size in controls and in low HCO<sub>3</sub> and DOC  
23 treatments with Pb. Other supporting evidence for the causal determination in the 2013 Pb ISA for  
24 reproductive effects in aquatic vertebrates included alteration of steroid profiles and additional  
25 reproductive parameters, although most of the available studies were conducted using nominal  
26 concentrations of Pb. Additionally, a study in frogs in the 2006 AQCD showed Pb delayed  
27 metamorphosis, decreased larval size and caused skeletal malformations at nominal concentration of  
28 100 µg Pb/L; however, tissue concentrations quantified in frogs following exposure fell within the range  
29 of tissue concentrations in wild-caught tadpoles ([Chen et al., 2006](#)).

30 Several new early lifestage fish studies add to the existing evidence for Pb effects on endocrine  
31 and developmental endpoints. In a study that quantified Pb in the exposure water, hatching success rates  
32 in zebrafish embryos were reduced at 4.5, 9.6 and 18.6 µg Pb/L. At 72 hpf, the hatching success rates in  
33 all three concentrations were significantly decreased compared with the control, indicating that Pb caused  
34 a hatching delay, which was also observed at the end of the experiment at 96 hpf ([Zhao et al., 2020](#)).  
35 [Curcio et al. \(2021\)](#) also reported a hatching delay in zebrafish embryos at 102 hpf with nominal exposure  
36 to 5 µg Pb/L. In this study, various embryo developmental effects were noted at 5 µg Pb/L and at the  
37 lower concentration of 2.5 µg Pb/L. All individuals showed spinal and tail deformities after 144 hours of

1 exposure. In contrast, in adult zebrafish exposed nominally to 10 µg Pb/L for 3 months, there were no  
2 significant effects on mortality, malformation, egg production and subsequent growth of larval offspring  
3 ([Chen et al., 2017](#)). In another zebrafish study, endocrine disruption in larvae was assessed by quantifying  
4 changes in thyroid hormone following exposure to Pb (analytically verified concentration of 2, 5, 10, 15,  
5 20, 30 µg Pb/L) in embryos from 2 hpf to 144 hpf ([Zhu et al., 2014](#)). Triiodothyronine (T3) and thyroxine  
6 (T4) levels were significantly reduced at 30 µg Pb/L. Pb did not significantly affect the percentage of  
7 hatched larvae; however, Pb exposure significantly increased malformations and reduced survival at  
8 30 µg Pb/L compared with the control. In comparison to these studies showing reproductive and  
9 endocrine responses in fish early lifestages, no endocrine disruption was observed in adult male common  
10 carp (*C. carpio*) at 7, 14 or 21 days of Pb exposure, even at the lowest analytically verified concentration  
11 (120 µg Pb/L) ([Korkmaz et al., 2022](#)).

12 Reproductive and endocrine effects of exposure to Pb via diet were assessed in dietary exposure  
13 with female Prussian carp *C. gibelio*. At 12 months, there was a significant increase in luteinizing  
14 hormone (LH) secretion after hormonal stimulation at the two highest analytically verified concentrations  
15 (24 and 49 mg Pb/kg), whereas (8 mg Pb/kg) spontaneous LH secretion significantly decreased at the  
16 lowest dose tested ([Łuszczek-Trojnar et al., 2014](#)). At 24 months, differences in LH secretion between  
17 treatment groups were not significant. There were also differences in oocyte size and maturation. At  
18 12 months, oocytes in the 8 mg Pb/kg treatment group were significantly larger than those in the control  
19 and other treatment groups. After 24 months, oocyte maturity and oocyte diameter were not significantly  
20 different between the control and Pb-treated fish.

21 An emerging area of ecotoxicology involves the assessment of pollutant effects on the  
22 microbiome and subsequent fitness of the host organism ([Evariste et al., 2019](#)). Since the 2013 Pb ISA,  
23 gut microbiota as a target for Pb toxicity have been assessed in zebrafish. In adult male zebrafish exposed  
24 for 7 days to a nominal concentration of 10 or 30 µg Pb/L, gut mucus production increased. The relative  
25 abundance of  $\alpha$ -Proteobacteria decreased significantly and the relative abundance of Firmicutes  
26 significantly increased at 30 µg Pb/L relative to the control ([Xia et al., 2018](#)). Approximately 30 kinds of  
27 microorganisms responded to Pb, and concurrent with altered gut microbiota composition, a total of 41  
28 metabolites associated with metabolic pathways and liver function were significantly changed.

#### 11.3.4.4.2. Birds

29 A new study in mallards (*A. platyrhynchos*) expands existing information on Pb effects in birds  
30 frequenting aquatic habitats contaminated with Pb and other metals. Prior AQCDs and the 2013 Pb ISA  
31 include evidence for changes in ALAD activity and other oxidative stress biomarkers. Adding to this  
32 evidence, there was a positive relationship between the lipid peroxidation index and blood Pb in female  
33 mallards sampled in northeastern Spain. Lysozyme levels were negatively correlated with blood Pb  
34 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.

#### 11.3.4.4.3. 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. Two different studies evaluated the effects of Pb-contaminated water on Asiatic toad (*Bufo gargarizans*) tadpole growth and development. [Chai et al. \(2017\)](#) reared Asiatic toad embryos and tadpoles in different nominal concentration of Pb-contaminated water (0, 10, 50, 100, 500, 1000, and 2000 µg Pb/L). At 5 days of exposure, the total length of embryos was significantly lower in 1000 and 2000 µg Pb/L treatments than in controls; however, the total length was significantly higher at 50 µg Pb/L than the length in controls. Similar results were seen in the mean weight of embryos on day 5, with embryos from the two highest exposures being significantly lighter than controls, while embryos from the three lowest treatments were significantly heavier than controls. Malformations (edema in the tail, wavy fin, abdominal edema, stunted growth, hyperplasia, and axial flexures) were observed starting at the 500 µg Pb/L treatment, with the incidence of malformation increasing with Pb concentration. [Yang et al. \(2019\)](#) performed a similar experiment and obtained similar results. Asiatic toads were reared in water with different concentrations of Pb (0, 10, 50, 100, 500, and 1000 µg Pb/L, nominal values; 0, 9.85, 48.73, 97.69, 497.34, and 998.27 µg Pb/L, measured values). On day 10, there was 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 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.

[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 (40, 80, 160, 320, 640, 1280 µg Pb/L nominal values; 38.2, 79.3, 158.4, 318.7, 638.1, 1278.9 µg Pb/L analytically verified concentration) 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,



1 with total malformation rate increasing linearly with Pb concentration. Metamorphosis time was  
2 significantly affected by Pb concentration and exhibited a linear increase with increasing Pb concentration  
3 ( $0 \mu\text{g Pb/L} = 76.4 \pm 0.5$  days,  $160 \mu\text{g Pb/L} = 90.8 \pm 0.5$  days,  $1280 \mu\text{g Pb/L} = 118.4 \pm 0.5$  days). Pb  
4 concentration also significantly affected the survival rate, which decreased with increasing Pb  
5 concentration ( $0 \mu\text{g Pb/L} = 98.3 \pm 1.7\%$ ,  $160 \mu\text{g Pb/L} = 93.3 \pm 1.7\%$ ,  $1280 \mu\text{g Pb/L} = 80.0 \pm 0.3\%$ ).

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

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### 11.3.5. Exposure and Response of Freshwater Species

10 Evidence regarding exposure-response relationships and potential thresholds for Pb effects on  
11 aquatic populations can provide tools for quantitative analyses of risks in freshwater ecosystems (Section  
12 11.1.7.3). Exposure-response data for the reproduction, growth, and survival of freshwater biota  
13 (including microalgae, invertebrate, amphibian, and fish species) were summarized in Table 6-5 of the  
14 2013 Pb ISA ([U.S. EPA, 2013](#)). Additionally, the Annex of the 2006 Pb AQCD ([U.S. EPA, 2006b](#))  
15 summarized data on exposure-response functions for invertebrates (Table AX7 2.4.1) and fish (Table  
16 AX7 2.4.2) available at the time. For Pb exposure-response, there is significant new research reporting  
17 results from bioassays of freshwater algae, invertebrates and fish based on measured rather than nominal  
18 concentration of Pb. In some cases, effects were observed in sensitive species at concentrations  
19 comparable to or lower than those reported in the 2013 Pb ISA (Table 11-5) or earlier EPA reviews of Pb.  
20 Some of the studies report  $\text{LC}_{10}$  and  $\text{LC}_{20}$  toxicity values and/or calculate the free-ion concentration.

21 In the 2006 AQCD and 2013 Pb ISA, available exposure-response data for freshwater plants and  
22 algae did not indicate any effects on growth or survival at environmentally relevant concentrations. In the  
23 2006 AQCD,  $\text{EC}_{50}$  values for growth inhibition in various freshwater algal and aquatic plant species were  
24 between approximately 1000 and  $>100,000 \mu\text{g/L}$  and were mostly based on nominal concentration data  
25 ([U.S. EPA, 2006b](#)). An important advancement since the 2013 Pb ISA is the availability of bioassay data  
26 for algal growth rate in several freshwater species based on measured Pb concentration instead of nominal  
27 concentration, which strengthens confidence in the findings for the concentrations assessed ([De  
28 Schamphelaere et al., 2014](#)). In chronic 72-hour bioassays in standard test media to assess the growth rate  
29 in three commonly tested algal species (*P. subcapitata*, *C. kesslerii*, *C. reinhardtii*), *P. subcapitata* was  
30 the most sensitive, with  $\text{EC}_{50} = 83.9 \mu\text{g Pb/L}$ ,  $\text{EC}_{20} = 45.7 \mu\text{g Pb/L}$  and  $\text{EC}_{10} = 32.0 \mu\text{g Pb/L}$  based on  
31 filtered Pb concentrations ([De Schamphelaere et al., 2014](#)). Furthermore, in subsequent tests with *P.*  
32 *subcapitata* at varying pH, the 72h  $\text{EC}_{50}$  decreased from  $72.0 \mu\text{g filtered Pb/L}$  at pH 6.0 to  $20.5 \mu\text{g filtered}$   
33  $\text{Pb/L}$  at pH 7.6. Inhibitory concentration (IC) values calculated using a specific growth rate at 72 hours



1 with a linear interpolation method for *Raphidocelis subcapitata* (formerly *P. subcapitata*) were  
2  $IC_{10} = 0.15 \mu M$ , (31  $\mu g Pb/L$ ),  $IC_{25} = 0.39 \mu M$  (81  $\mu g Pb/L$ ) and  $IC_{50} = 0.78 \mu M$  (161  $\mu g Pb/L$ ) ([Alho et](#)  
3 [al., 2019](#)).

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

11 For freshwater invertebrates, effects in sensitive species of amphipods, gastropods, cladocerans  
12 and mussels were reported at low  $\mu g Pb/L$  concentrations in exposure-response studies reviewed in the  
13 1986 AQCD, the 2006 AQCD and the 2013 Pb ISA. Additional toxicity data for these taxonomic groups  
14 discussed below support and expand upon what was known in the previous Pb assessment in terms of the  
15 relative sensitivity of these freshwater biota to Pb.

16 Toxicity testing with amphipods reported in the 2006 AQCD and 2013 Pb ISA indicate a  
17 response to Pb at <10  $\mu g Pb/L$  under some water conditions. At higher pH and water hardness, these  
18 organisms are less sensitive to Pb ([U.S. EPA, 2006b](#)). For example, a 7-day  $LC_{50}$  of 1  $\mu g Pb/L$  was  
19 observed in soft water with the amphipod *H. azteca* ([Borgmann et al., 2005](#)). In this same species, the 96-  
20 hour  $LC_{50}$  for Pb at pH 5 was 10  $\mu g Pb/L$  ([Mackie, 1989](#)). In 42-day chronic exposures of *H. azteca*  
21 exposed to Pb via water and diet, the  $LC_{50}$  was 16  $\mu g Pb/L$  ([Besser et al., 2005](#)). In a chronic 42-day  
22 bioassay with *H. azteca*, published after the 2013 Pb ISA, survival was similar to that observed by [Besser](#)  
23 [et al. \(2005\)](#) under two different experimental diets conducted concurrently ( $LC_{20} = 15 \mu g Pb/L$  and  
24  $LC_{20} = 13 \mu g Pb/L$ ) and support the findings of effects in amphipods in the low  $\mu g/L$  range ([Besser et al.,](#)  
25 [2016](#)).

26 Some species of freshwater gastropods have exhibited sensitivity to Pb at <20  $\mu g Pb/L$ . In the  
27 1986 AQCD, [Borgmann et al. \(1978\)](#) found increased mortality at Pb concentration as low as 19  $\mu g Pb/L$   
28 in the freshwater snail *Lymnaea palustris* exposed from hatching to reproductive maturity (approximately  
29 120 days). To follow-up on the set of studies reviewed in the 2013 Pb ISA ([Grosell and Brix, 2009](#);  
30 [Grosell et al., 2006b](#)) that identified the freshwater snail *L. stagnalis* as highly sensitive to Pb  
31 ( $EC_{20} = <4 \mu g Pb/L$  in 30-day exposure experiments) several additional chronic studies have since been  
32 undertaken with this species. In growth bioassays conducted in a variety of natural waters across the  
33 United States with different water chemistries 14-day  $EC_{20}$  and  $EC_{50}$  values ranging from 1.5 to 49.5 and  
34 from 3.6 to 244.6  $\mu g Pb/L$ , respectively, were reported for *L. stagnalis* ([Esbaugh et al., 2012](#)). [Munley et](#)  
35 [al. \(2013\)](#) conducted full life cycle bioassays with a duration of 56 days to assess the effects on survival,  
36 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 life cycle 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 life cycle 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 life cycle 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<sub>20</sub> (biomass) at 8 days of exposure of 3.2 µg l<sup>-1</sup> Pb and 3.5 µg l<sup>-1</sup> Pb after 16 days of exposure. Under similar experimental conditions, Crémazy et al. (2018) reported a 14-day EC<sub>10</sub> of 4 µg Pb/L, an EC<sub>20</sub> of 7.67 µg Pb/L and an EC<sub>50</sub> 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<sub>10</sub> = 0.157 µg Pb/L, EC<sub>20</sub> = 0.320 µg Pb/L and EC<sub>50</sub> = 1.08 µg Pb/L.

New acute data for cladocerans include a 48-hour EC<sub>50</sub> = 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<sub>50</sub> 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<sub>50</sub> 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<sub>50</sub> 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<sub>50</sub> 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<sub>20</sub> for reproduction in *C. dubia* ranged from 12.1 to 223.3 µg Pb/L, and 7-day-EC<sub>50</sub> ranged from 20.1 to 573.4 µg Pb/L (Esbaugh et al., 2012).

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

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

19 In freshwater mussels, sensitivity to Pb has been demonstrated to vary with lifestage. In a study  
20 from the 2013 Pb ISA, newly transformed juvenile freshwater mussels (*Lampsilis siliquoidea*) were more  
21 sensitive than older juveniles in acute exposures. A chronic value (geometric mean of NOEC and the  
22 LOEC) of 10 µg Pb/L was reported in 28-day exposures of 2-month-old juveniles ([Wang et al., 2010](#)). The  
23 lowest median effect concentration for glochidia (larvae) of *L. siliquoidea* at 24 and 48 hours was  
24 >299 µg/L. A more recent study in glochidia of six different freshwater mussel species found in  
25 southeastern Australia (*Hyridella australis*, *Hyridella depressa*, *Velesunio ambiguus*, *Alathyria profuga*,  
26 *Cucumerunio novaehollandiae*, *Hyridella drapeta*) indicated these species were more sensitive in acute  
27 tests than glochidia of *L. siliquoidea* (native to the United States). The 24-hour EC<sub>50</sub> values for valve  
28 closure ranged from 176 to 274 µg Pb/L ([Markich, 2017](#)). Following 72-hour Pb exposure in the same  
29 species, the EC<sub>50</sub> values ranged from 65 to 110 µg Pb/L. Calculated no-effect concentrations (NECs) at  
30 72 hours ranged from 11 to 21 µg Pb/L.

31 Other recent tests with freshwater invertebrates have illustrated the range in the sensitivity of  
32 North American species to Pb. In a battery of acute toxicity tests using resident invertebrates collected  
33 from the South Fork Coeur d'Alene River watershed, Idaho, U.S. and tested in the river water, the lowest  
34 EC<sub>50</sub> concentration for Pb (96-hour EC<sub>50</sub> = 253 µg Pb/L) was obtained with the stonefly *Sweltsa* sp.,  
35 however, in other tests with *Sweltsa* sp., mortalities occurred at Pb concentrations up to three times  
36 greater, indicating a high degree of variability in repeated tests with the same species ([Mebane et al.,](#)  
37 [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<sub>50</sub> values (some invertebrate species were tested multiple times) were: four mayfly species (*Baetis tricaudatus* [96-hour LC<sub>50</sub> = 322 to <1,250 µg Pb/L tested at varying water hardness], *Rhithrogena* sp. [96-hour LC<sub>50</sub> = >166 µg Pb/L], *Drunella* sp. [96-hour LC<sub>50</sub> = >267 µg Pb/L], *Epeorus* sp. [96-hour LC<sub>50</sub> = >346 µg Pb/L] and Leptophlebiidae [96-hour LC<sub>50</sub> = >346 µg Pb/L]), a caddisfly (*Arctopsyche* sp. 96-hour LC<sub>50</sub> = >1,255 µg Pb/L), a Simuliidae black fly (96-hour LC<sub>50</sub> = 415 µg Pb/L), Chironomidae midge (96-hour LC<sub>50</sub> = 1,955 µg Pb/L), a Tipula sp. Crane fly (96-hour LC<sub>50</sub> = >1,035 µg Pb/L), a Dytiscidae beetle (96-hour LC<sub>50</sub> = >1,035 µg Pb/L) and two snail species (*Physa* sp. [96-hour LC<sub>50</sub> = 1,159 µg Pb/L] and *Gyraulus* sp [96-hour LC<sub>50</sub> = 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<sub>50</sub> 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 mg Pb/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<sub>10</sub> = 1,455 mg Pb/kg dry weight) and the biomass of *L. variegatus* (28-day EC<sub>10</sub> = 1,870 mg Pb/kg dry weight) were more sensitive endpoints compared with the growth of *G. pulex* (35-day EC<sub>10</sub> = 2,541 mg Pb/kg dry weight) (Vandeghechuchte 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, 2006b). 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<sub>50</sub> 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<sub>10</sub> = 26 µg Pb/L, EC<sub>20</sub> = 34 µg Pb/L and LC<sub>50</sub> = 55 µg Pb/L (Mebane et al., 2008). Results from a 62-day test, with fish length as the endpoint, were

1 NOEC = 8 µg Pb/L, MATC = 12 µg Pb/L, EC<sub>10</sub> = 7 µg Pb/L, EC<sub>20</sub> = 102 µg Pb/L and LC<sub>50</sub> = 120 µg Pb/L  
2 ([Mebane et al., 2008](#)).

3 New evidence since the 2013 Pb ISA includes additional studies on fish species native to North  
4 America. In 96-h acute toxicity tests with white sturgeon (*A. transmontanus*), which is experiencing  
5 population declines in the U.S. and Canada, two early lifestages (8 and 40 dph) were tested in lab water  
6 and in water from the Columbia River upstream of the Teck Trail smelter facility, British Columbia,  
7 Canada ([Vardy et al., 2014](#)). For 8 dph larvae, 96-hour LC<sub>50</sub> = 177 µg/L (lab water) and 96-hour  
8 LC<sub>50</sub> > 410 µg/L (river water); for 40 dph, 96-hour LC<sub>50</sub> = 528 µg/L (lab water) and 96-hour  
9 LC<sub>50</sub> = 1,556 µg/L (river water) ([Vardy et al., 2014](#)). In 27 dph juvenile white sturgeon exposed to Pb  
10 concentrations in water ranging from 0.03 to 60 µg Pb/L for 28 days, there was an EC<sub>20</sub> > 60 µg Pb/L for  
11 survival, length, and biomass ([Wang et al., 2014a](#)). Considering that the early lifestages of white sturgeon  
12 are in close contact with sediment and porewater [Balistrieri et al. \(2018\)](#) reported an EC<sub>20</sub> = 0.9 nM Pb<sup>2+</sup>  
13 (0.18 µg Pb<sup>2+</sup>/L) developed from predictive response modeling using in situ measurements of Pb in  
14 Columbia River sediment and porewater, free-ion concentrations from equilibrium speciation calculations  
15 and the laboratory toxicity testing results of [Wang et al. \(2014a\)](#) of Pb to the early lifestages of sturgeon.  
16 Similar dose-response curves based on free metal ion concentration were observed for effective mortality  
17 and for reduction in biomass at Pb<sup>2+</sup> concentrations higher than quantified in sediment porewater,  
18 indicating young sturgeon at the sediment-water interface are unlikely to be affected by toxic  
19 concentrations of Pb in the upper reaches of the Columbia River. [Mebane et al. \(2012\)](#) tested westslope  
20 cutthroat trout (*Oncorhynchus clarkii lewisi*) a native subspecies of conservation concern, in a series of  
21 bioassays using water from various locations within the South Fork Coeur d'Alene River watershed,  
22 Idaho. EC<sub>50</sub> values for the effective mortality for this species ranged from 47 to 487 µg Pb/L.

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

36 As discussed in Section 11.1.7.3, the existing U.S. EPA AWQC for Pb for the protection of  
37 aquatic life are CMC of 65 µg Pb/L (for acute exposure) and CCC of 2.5 µg Pb/L (for chronic exposure)

1 at a hardness of 100 mg/L ([U.S. EPA, 1985a](#)). Since these criteria were developed in 1984, there have  
2 been additional acute and chronic toxicity data and improved characterization of modifying factors that  
3 affect Pb bioavailability and toxicity. Taking these advances into consideration [Deforest et al. \(2017\)](#)  
4 proposed updated acute BLM-based aquatic life criteria, ranging from 18.9 to 998 µg Pb/L and chronic  
5 BLM-based Pb freshwater criteria ranging from 0.37 to 41 µg Pb/L (Table 11-5). The lowest criteria were  
6 for water with low DOC (1.2 mg/L), pH (6.7) and hardness (4.3 mg/L as CaCO<sub>3</sub>), and the highest criteria  
7 were for water with high DOC (9.8 mg/L), pH (8.2) and hardness (288 mg/L as CaCO<sub>3</sub>), which  
8 encompasses varying water quality conditions of North American surface waters and the importance of  
9 DOC and pH as modifying factors compared with hardness. The updated data sets in [Deforest et al.](#)  
10 [\(2017\)](#) incorporated toxicity information for *L. stagnalis*, *C. dubia*, *H. azteca* and *P. rapida*, freshwater  
11 invertebrates that are relatively sensitive to Pb exposure. The number of genera with acute toxicity data  
12 for Pb increased from 10 to 32, and for chronic toxicity, from 4 to 13, which enabled the proposed chronic  
13 criteria to be based on bioassay data rather than an acute-to-chronic ratio that was used in 1984 for  
14 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)
<b>Algae/Plants</b>						
Green algae ( <i>Pseudokirchneriella subcapitata</i> ),	<i>P. subcapitata</i> Total Pb: <1, 19, 42, 85, 228.5, 412 Pb µg/L	Standard 3-d toxicity tests conducted in OECD standard test medium with nominal 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 <i>C. kesslerii</i> , <i>C. vulgaris</i> and <i>C. reinhardtii</i> 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 <i>P. subcapitata</i> with varying pH and fulvic acid	Temperature: 24°C  pH = 6	<b>Growth:</b> Interspecies comparison of algal growth rate indicated that <i>P. subcapitata</i> is the most sensitive and <i>C. kesslerii</i> the least sensitive. In <i>P. subcapitata</i> , as pH increased from 6.0 to 7.6, the 72-h EC <sub>50</sub> decreased from 72.0 to 20.5 µg filtered Pb/L	<i>P. subcapitata</i> 2-d EC <sub>50</sub> = 89.9 µg Pb/L 2-d EC <sub>20</sub> = 44.7 µg Pb/L 2-d EC <sub>10</sub> = 29.7 µg Pb/L 3-d EC <sub>50</sub> = 83.9 µg Pb/L 3-d EC <sub>20</sub> = 45.7 µg Pb/L 3-d EC <sub>10</sub> = 32.0 µg Pb/L	<a href="#">De Schamphelae et al. (2014)</a>
Green algae ( <i>Chlorella kesslerii</i> )	Filtered Pb: <1, 16, 37, 77, 201, 418 Pb µg/L					
Green algae ( <i>Chlamydomonas reinhardtii</i> )	<i>C. kesslerii</i> Filtered Pb: <1, 7, 18, 39, 80, 164, 417 µg Pb/L  <i>C. reinhardtii</i> Filtered Pb: <0.8, 9.5, 19.8, 43.3, 89.4, 194, 452, 783, 1613 µg Pb/L				<i>C. kesslerii</i> 2-d EC <sub>50</sub> = 388 µg Pb/L 2-d EC <sub>20</sub> = 185 µg Pb/L 2-d EC <sub>10</sub> = 120 µg Pb/L  <i>C. reinhardtii</i> 2-d EC <sub>50</sub> = 172 µg Pb/L 2-d EC <sub>20</sub> = 108 µg Pb/L 2-d EC <sub>10</sub> = 82.3 µg Pb/L	



Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Green algae ( <i>Raphidocelis subcapitata</i> formerly known as <i>Pseudokirchneriella subcapitata</i> )	(1.20; 2.41; 4.82 and 12.06 µM) Nominal, stock solution analytically verified	72-h toxicity test with triplicates, maintained in a temperature-controlled room. Cell density assessed every 24 h	Temperature: 25 ± 2°C	<b>Growth:</b> 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-h IC10 = 0.15 µM, (31 µg Pb/L) 72-h IC25 = 0.39 µM (81 µg Pb/L) 72-h IC50 = 0.78 µM (161 µg Pb/L)	<a href="#">Alho et al. (2019)</a>
Duckweed ( <i>Lemna minor</i> )	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 <i>L. minor</i> 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 <sub>3</sub> ) <sub>2</sub> . 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 <sub>3</sub> depending on surface water	<b>Growth:</b> 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	<a href="#">Antunes and Kreager (2014)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
<b>Invertebrates</b>						
Amphipod ( <i>Hyalella azteca</i> )	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 yeast, cereal leaves, and trout pellets (YCT) or a diatom + Tetramin (DT) fish food diet. Assays conducted concurrently in test water from the same diluter system	Hardness 100 mg/L as CaCO <sub>3</sub>  pH about 8.2  Alkalinity 95 mg/L	<b>Survival:</b> Survival was similar with aqueous Pb exposure in amphipods fed two different diets  <b>Growth:</b> Biomass significantly reduced in amphipods fed YCT, not significantly reduced in amphipods fed DT up to 63 µg Pb/L  <b>Reproduction:</b> 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: <u>DT diet:</u> 42-d EC <sub>20</sub> = 13 µg Pb/L 42-d NOEC = 5.9 µg Pb/L 42-d LOEC = 13 µg Pb/L <u>YCT diet:</u> 42-d EC <sub>20</sub> = 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 <sub>20</sub> = 8.2 µg Pb/L (total young for the DT test) EC <sub>50</sub> = 6.6 µg Pb/L (biomass for the YCT test)	<a href="#">Besser et al. (2016)</a>
Isopod ( <i>Asellus aquaticus</i> )	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 <sub>2</sub>	Temperature: 20 ± 1°C  Hardness: 117 mg L <sup>-1</sup> CaCO <sub>3</sub>	<b>Survival:</b> Focus of study was on mixture toxicity. Only LC <sub>50</sub> was calculated for Pb-only treatment	10-d LC <sub>50</sub> = 443 µg Pb/L	<a href="#">Van Ginneken et al. (2015)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Isopod ( <i>Asellus aquaticus</i> )	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 <sub>3</sub> ) <sub>2</sub> with adult <i>A. aquaticus</i> . 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	<b>Survival:</b> 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 <sub>10</sub> = 49.7 µg Pb/L LC <sub>10</sub> for FIA = 0.04 µg Pb/L LC <sub>20</sub> = 130 µg Pb/L LC <sub>20</sub> for FIA = 0.31 µg Pb/L LC <sub>50</sub> = 677 µg Pb/L LC <sub>20</sub> for FIA = 9.13 µg Pb/L  7-d survival LC <sub>10</sub> = 97.4 µg Pb/L LC <sub>20</sub> = 602 µg Pb/L LC <sub>50</sub> = 13,562 µg Pb/L  (LC <sub>10</sub> , 20 and 50 values were also calculated for day 1 and day 4).	<a href="#">Van Ginneken et al. (2017)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Cladoceran ( <i>Ceriodaphnia dubia</i> )	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 <sub>2</sub> ) 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	<b>Reproduction</b> 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 <sub>50</sub> 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 <sub>50</sub> 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	<a href="#">Nys et al. (2014)</a>

Cladoceran ( <i>Ceriodaphnia dubia</i> )	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 <sub>3</sub> ) <sub>2</sub> , in a range of representative surface waters across North America <i>C. dubia</i> : (<24-h-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 <i>P. rapida</i> : 4-d chronic Pb toxicity with adults was assessed using a population growth rate endpoint which conformed to classical concentration-dependent responses.  <i>L. stagnalis</i> : 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  <i>C. dubia</i> : pH: 6.51–8.47 DOC: 114–1443 Temperature: 26°C  <i>P. rapida</i> : pH: 7.23–8.44 DOC: 79–1405 Temperature: 26°C  <i>L. stagnalis</i> : pH: 5.79–8.61 DOC: 36–1314 Temperature: 26°C	<b>Reproduction:</b> Highest reproductive toxicity in <i>C. dubia</i> was observed in soft water, most protective water had high DOC. For <i>P. rapida</i> population growth, DOC was not predictive of chronic toxicity  <b>Growth:</b> Effects on growth occurred at low µg/L concentration in <i>L. stagnalis</i> in some of the tested waters. For the snails, the greatest effects on growth occurred with low-DOC waters	<i>C. dubia</i> : 7-d-EC <sub>50</sub> s for reproduction ranged from 20.1 to 573.4 µg/L in representative surface waters of varying chemistries. EC <sub>20</sub> s ranged from 12.1 to 223.3 µg/L.  <i>P. rapida</i> : EC <sub>20</sub> and EC <sub>50</sub> ranged from 3.2–103.3 and 10.6–154.9 µg/L dissolved Pb, respectively  <i>L. stagnalis</i> : EC <sub>20</sub> s and EC <sub>50</sub> 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	<a href="#">Esbaugh et al. (2012)</a>
Rotifer ( <i>Philodina rapida</i> )	For the Ca and pH series: (nominal	Reproductive effects of Pb (PbCl <sub>2</sub> ) assessed in recently	pH:	<b>Reproduction:</b>	For population size in natural waters:	<a href="#">Nys et al. (2016b)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
<i>(Brachionus calyciflorus)</i>	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-h (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	The EC <sub>50</sub> (based on filtered Pb) for population size differed by up 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 <sub>10</sub> ranged from 52 (synthetic reference water) to 231 µg Pb/L EC <sub>20</sub> ranged from 75 (synthetic reference water) to 336 µg Pb/L EC <sub>50</sub> ranged from 138 (synthetic reference water) to 634 µg Pb/L (based on filtered Pb concentration)	
Snail <i>(Lymnaea stagnalis)</i>	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: 23–25°C pH = 7.8	<b>Growth:</b> 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 <sup>2+</sup> flux in the snails, inhibition of carbonic anhydrase activity in the snail mantle also showed no effect with Pb	EC <sub>20</sub> (biomass) at 8 d of exposure was 3.2 µg L <sup>-1</sup> Pb  EC <sub>20</sub> (biomass) was 3.5 µg L <sup>-1</sup> Pb after 16 d of exposure	<a href="#">Brix et al. (2012)</a>



Snail ( <i>Lymnaea stagnalis</i> )	0.18 (control), 1, 2.7 and 8.4 µg Pb/L (measured)	Newly hatched juvenile snails were exposed to Pb (as Pb(NO <sub>3</sub> ) <sub>2</sub> in Milli-Q water) for 56-d in a full life cycle 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	<p><b>Survival:</b> Survival was significantly decreased at the highest concentration (8.4 µg Pb/L) after 21-d exposure to the end of the experiment</p> <p><b>Growth:</b> Growth was significantly decreased, even at the lowest tested concentration (1 µg Pb/L) at 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.</p> <p><b>Reproduction:</b> For the number of egg masses and time until first egg mass, the NOEC&lt;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 life cycle 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)</p>	<p>Survival: 56-d chronic toxicity NOEC = 2.7 µg Pb/L LOEC = 8.4 µg Pb/L</p> <p>Growth: 28-d NOEC&lt;1.0 µg Pb/L LOEC = 1.0 µg Pb/L</p> <p>Reproduction: NOEC&lt;1.0 µg Pb/L LOEC = 1.0 µg Pb/L</p>	<a href="#">Munley et al. (2013)</a>
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Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Snail ( <i>Lymnaea stagnalis</i> )	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 <sub>3</sub> ) <sub>2</sub> ) and binary metal mixtures with juvenile <i>L. stagnalis</i> 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 ± 0.08 mg L <sup>-1</sup>  Alkalinity = 0.80 ± 0.05 mEq·L <sup>-1</sup>	<b>Growth:</b> Inhibition of relative growth rate was observed at low µg Pb/L concentrations, consistent with other bioassays with <i>L. stagnalis</i>	14-d chronic toxicity: EC <sub>10</sub> = 4.0 µg Pb/L EC <sub>20</sub> = 7.67 µg Pb/L EC <sub>50</sub> = 23.4 µg Pb/L  Corresponding chronic effect concentrations based on free-ion activity: EC <sub>10</sub> = 0.157 µg Pb/L EC <sub>20</sub> = 0.320 µg Pb/L EC <sub>50</sub> = 1.08 µg Pb/L	<a href="#">Crémazy et al. (2018)</a>
Mussel ( <i>Hyridella australis</i> )  ( <i>Hyridella depressa</i> )  ( <i>Velesunio ambiguus</i> )  ( <i>Alathyria profuga</i> )  ( <i>Cucumerunio novaehollandiae</i> )  ( <i>Hyridella drapeta</i> )	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 h) with PbCl <sub>2</sub> 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 <sub>3</sub> L <sup>-1</sup>  Alkalinity 22 ± 2 mg CaCO <sub>3</sub> L <sup>-1</sup>	<b>Survival:</b> Pb sensitivity significantly increased with each exposure time and varied by species, with greatest toxicity observed in <i>C. novaehollandiae</i>	24-h EC <sub>50</sub> (for valve closure as a proxy for viability) ranged from 176 to 274 µg Pb  48-h EC <sub>50</sub> ranged from 102–165 µg Pb/L  72-h EC <sub>50</sub> ranged from 65 to 110 µg Pb/L  72-h calculated NEC ranged from 11 to 21 µg Pb/L	<a href="#">Markich (2017)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Prawn ( <i>Macrobrachium nipponense</i> )	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-h 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	<b>Survival:</b> LC <sub>50</sub> 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.  <b>Growth:</b> 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-h LC <sub>50</sub> = 646 µg Pb/L 48-h LC <sub>50</sub> = 250.6 µg Pb/L 72-h LC <sub>50</sub> = 175.6 µg Pb/L 96-h LC <sub>50</sub> = 131.3 µg Pb/L  60-d chronic bioassay: Reduction in weight gain observed at 25 µg Pb/L (approx. 20% of the 96-h LC <sub>50</sub> )	<a href="#">Ding et al. (2019)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
<b>Vertebrates</b>						
Zebrafish ( <i>Danio rerio</i> )	0– 33,300 µg Pb/L (nominal values). 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 with total Pb concentration of 630 µg Pb/L (1,000 µg Pb/L nominal values). Highest concentration tested was 33,300 µg Pb/L (nominal values), which was 3,830 µg Pb/L (measured) in the hard water	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 <sub>3</sub> /L pH: 7.48 Na <sup>+</sup> = 220 M, K <sup>+</sup> = 14 M Ca <sup>2+</sup> = 75 M Mg <sup>2+</sup> = 42 M DOC = 0.9 mg/L.  Hard water: hardness = 141 m g CaCO <sub>3</sub> /L pH = 7.8 Na <sup>+</sup> = 700 M K <sup>+</sup> = 38 M Ca <sup>2+</sup> = 1,350 M Mg <sup>2+</sup> = 336 M, DOC = 3.5 mg/L	<b>Survival:</b> 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-h LC <sub>50</sub> = 52.9 µg Pb/L  Hard water: 96-h LC <sub>50</sub> = >590 µg Pb/L	<a href="#">Alsop and Wood (2011)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Zebrafish ( <i>Danio rerio</i> )	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	<p><b>Reproduction</b></p> <p>No significant effect on percentage of hatched larvae at any of the tested concentrations</p> <p><b>Growth</b></p> <p>Significant increase in prevalence of malformations at 30 µg Pb/L compared with the control</p> <p><b>Survival</b></p> <p>Significant decrease in survival at 30 µg Pb/L compared with the control</p>		<a href="#">Zhu et al. (2014)</a>
Zebrafish ( <i>Danio rerio</i> )	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	<p><b>Reproduction</b></p> <p>No significant difference on hatching success rate at any of the tested concentrations</p> <p><b>Growth</b></p> <p>No significant differences were found for body length or body weight at tested concentrations compared with control</p> <p><b>Survival</b></p> <p>No significant effect on survival</p>		<a href="#">Zhu et al. (2016)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Zebrafish ( <i>Danio rerio</i> )	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	<p><b>Reproduction</b></p> <p>No significant difference on hatching success rate at 19.3 µg Pb/L compared with control.</p> <p><b>Growth</b></p> <p>No significant differences were found for body length or body weight at 19.3 µg Pb/L compared with control</p> <p><b>Survival</b></p> <p>No significant effect on survival at 19.3 µg Pb/L</p>		<a href="#">Chen et al. (2016b)</a>
Zebrafish ( <i>Danio rerio</i> )	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	<p><b>Reproduction:</b></p> <p>Hatching success rate significantly decreased in all concentrations at 72 hpf compared with control; this delay in hatching rate also observed at 96 hpf.</p> <p><b>Survival</b></p> <p>Survival rate of Pb-exposed embryos at all tested concentrations significantly lower than controls at 96 hpf.</p>		<a href="#">Zhao et al. (2020)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
rainbow trout ( <i>Oncorhynchus mykiss</i> )	<p>Waterborne-only study: 4, 10, 20, 80, 240 and 800 µg Pb/L (nominal concentration reported, concentrations analytically verified)</p> <p>Waterborne, diet and combined exposure study: 0, 8.5, 20, 60 and 110 µg Pb/L (measured)</p>	<p>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 <i>Lumbriculus variegatus</i> pre-exposed for 28-d to the same concentration of Pb as the fish) or simultaneously to waterborne and dietary Pb</p>	<p>Temperature: 13°C</p> <p>pH: 7.8–8.0</p> <p>Hardness: 140 mg/L as CaCO<sub>3</sub></p> <p>DOC: 2.5 mg/L</p>	<p><b>Survival:</b> 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%).</p> <p><b>Growth:</b> 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</p>	<p>96-h: LC<sub>10</sub> = 304.3 µg Pb/L LC<sub>20</sub> = 357.7 µg Pb/L LC<sub>50</sub> = 487.3 µg Pb/L</p> <p>7-w: LC<sub>10</sub> = 55.6 µg Pb/L LC<sub>20</sub> = 96.9 µg Pb/L LC<sub>50</sub> = 280.2 µg Pb/L</p>	<p><a href="#">Alsop et al. (2016)</a></p>



Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
rainbow trout ( <i>Oncorhynchus mykiss</i> )	Trout: 0, 10, 20, 40, 80, 160 µg Pb/L (nominal values)	A series of chronic tests with two lifestages (newly hatched larvae and approximately 1-month old juveniles) of trout and sturgeon were conducted in aqueous-only exposure with Pb as Pb-nitrate.	Trout:  Temperature: 12 ± 1°C	<b>Growth/Survival</b>  Note: Effect concentrations reported in this study are based on the most sensitive endpoint (mortality, immobility, fish length or biomass).	Trout:  Acute 4-d EC <sub>50</sub> C1 (larvae): >136 µg Pb/L C2 (juvenile): >143 µg Pb/L CC (larvae) >136 µg Pb/L	<a href="#">Wang et al. (2014a)</a>
white sturgeon ( <i>Acipenser transmontanus</i> )	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	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	Hardness: Approximately 100 mg/L as CaCO <sub>3</sub> , Alkalinity: approximately 90 mg/L as CaCO <sub>3</sub> pH: approximately 8.0  Sturgeon: Temperature: 15 ± 1°C Hardness: Approximately 100 mg/L as CaCO <sub>3</sub> Alkalinity: approximately 90 mg/L as CaCO <sub>3</sub> pH: approximately 8.0	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 <sub>20</sub> s for the survival. However, the EC <sub>20</sub> s based on the length and weight of surviving fish throughout the 53-d exposures were reported	Chronic EC <sub>20</sub> 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 <sub>50</sub> C1 (larvae): >55 µg Pb/L C2 (juvenile): >61 µg Pb/L CC (larvae) >55 µg Pb/L  Chronic EC <sub>20</sub> 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)	

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
white sturgeon ( <i>Acipenser transmontanus</i> )	8 dph	96-h 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	<b>Survival</b>  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	<a href="#">Vardy et al. (2014)</a>
	Lab: 0.1, 0.8, 2.3, 6.4, 19, 65, 210, 414 µg Pb/L		Temperature: 16 ± 0.9°C		96-h LC <sub>50</sub> = 177 µg Pb/L (lab water)	
	Columbia River: 0.2, 0.4, 1.4, 6.1, 17, 60, 191, 410 µg Pb/L		pH 7.5 ± 0.2		96-h LC <sub>50</sub> = >410 µg Pb/L (Columbia River water)	
			Ca <sup>2+</sup> to Mg <sup>2+</sup> Ratio: ~1.3:1		40 dph	
	40 dph		Columbia River Water		96-h LC <sub>50</sub> = 528 µg Pb/L (lab water)	
	Lab: 0.1, 21, 46, 97, 208, 396, 809, 1610 µg Pb/L		Temperature: 16 ± 0.7°C		96-h LC <sub>50</sub> = 1,556 µg Pb/L (Columbia River water)	
	Columbia River: 0.3, 20, 37, 95, 192, 325, 799, 1685 µg Pb/L		pH 7.7 ± 0.1			
			Ca <sup>2+</sup> to Mg <sup>2+</sup> Ratio: ~4:1			

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
Asiatic toad ( <i>Bufo gargarizans</i> )	0, 10, 50, 100, 500 and 1000 µg Pb/L, nominal; 0, 9.85, 48.73, 97.69, 497.34 and 998.27 µg Pb/L, measured	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	<b>Growth</b> 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. <b>Survival</b> No mortality observed in control, 10, 50 or 100 µg Pb/L during 60-d exposure.		<a href="#">Yang et al. (2019)</a>
Dark-spotted frog ( <i>Pelophylax nigromaculata</i> )	40, 80, 160, 320, 640, 1280 µg Pb/L nominal; 38.2, 79.3, 158.4, 318.7, 638.1, 1278.9 µg Pb/L analytically verified concentration	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 19–25°C (room temperature) pH 7.04–7.69, DO 6.8–7.3mg/L Hardness 249–258 mg CaCO <sub>3</sub> /L	<b>Growth</b> Growth was inhibited at higher Pb concentrations; total malformation rate increased linearly with Pb concentration. <b>Survival</b> No significant effect on survival at 40, 80, 160 or 320 µg Pb/L	Lowest threshold concentration = 160 µg Pb /L for effects on metamorphosis time, body mass, snout-vent length, and forelimb length	<a href="#">Huang et al. (2014)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (published since the 2013 Pb ISA)
<b>Multiple</b>						
37 species and 32 genera of invertebrates and fish  (acute toxicity data included in derivation of proposed updated acute freshwater quality criterion for Pb)	Pb was analytically verified in all studies	U.S. EPA guidelines ( <a href="#">U.S. EPA, 1985b</a> ) 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-h EC <sub>50</sub> s. The four lowest genus mean acute values ( <i>Hyaella</i> , <i>Ceriodaphnia</i> , <i>Gammarus</i> and <i>Daphnia</i> ) 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		<b>Acute toxicity endpoints included survival, immobilization, and loss of equilibrium</b>  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.  <b>Chronic toxicity endpoints included survival, growth, and reproduction</b>  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	<a href="#">Deforest et al. (2017)</a>
15 species and 13 genera of invertebrates and fish (chronic toxicity data included in derivation of proposed updated chronic freshwater quality criterion for Pb)		Chronic: Based on EC <sub>20</sub> values from life cycle tests in				

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

Ca<sup>2+</sup> = calcium ion; CaCO<sub>3</sub> = calcium carbonate; d = day; DOC = dissolved organic carbon; dph = days posthatch; DT = diatom + Tetramin; EC<sub>x</sub> = X% effect concentration; hpf = hours postfertilization; K<sup>+</sup> = potassium ion; LC<sub>x</sub> = X% lethal concentration; Mg<sup>2+</sup> = magnesium ion; mo = months; Na<sup>+</sup> = sodium ion; Pb = lead; Pb(NO<sub>3</sub>)<sub>2</sub> = lead nitrate; wk = weeks; YCT = yeast, cereal leaves and trout; yr = year.

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### 11.3.6. Freshwater-Community and Ecosystem Effects

Field studies in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) 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. Macroinvertebrate assemblage from sediments collected from a contaminated region of the lake and a control area were evaluated to assess community tolerance to Pb in 48-hour aqueous exposure to a range of nominal Pb concentrations (0, 20, 200, 2,000, 200,000, or 2,000,000 µg Pb/L) ([Oguma and Klerks, 2017](#)). Mortality for benthos under increasing [Pb<sup>2+</sup>] concentration was lower than for those macroinvertebrates collected from the control site, suggesting community tolerance. The interaction between the collection site (control versus

contaminated site) and  $[Pb^{2+}]$  on survival was nonsignificant. Macroinvertebrate density was similar between the two sediments. When benthic macroinvertebrate species composition was compared between sites, the community at the control site included more metal-sensitive taxa (gastropods and amphipods) compared with sediments from the contaminated site.

In another 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 ( $0.06 \pm 0.01$   $\mu\text{g Pb/L}$ , mean  $\pm$  S.D.) and highest at the mining sites ( $7.85 \pm 1.63$   $\mu\text{g Pb/L}$ ). Sediment Pb concentrations followed the same pattern, with the lowest concentrations at the reference site ( $12.5 \pm 2.1$  mg Pb/kg dry weight), followed by the downstream sites ( $710 \pm 530$  mg Pb/kg dry weight) and the highest concentrations at the mining sites ( $1170 \pm 467$  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



( $12.7 \pm 4.4$  mg Pb/kg dry weight for reference sites and  $720 \pm 276$  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  $\pm$  S.D.,  $66.8 \pm 7.3$  mg Pb/kg dry weight), followed by the downstream sites ( $44.7 \pm 14.4$  mg Pb/kg dry weight) and the reference sites ( $0.55 \pm 0.14$  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  $\times$  m<sup>-2</sup>). 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  $\times$  m<sup>-2</sup>) and crayfish density (number of crayfish  $\times$  m<sup>-2</sup>) 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 ([Obolowski 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<sub>3</sub>, and NH<sub>4</sub>. 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, 2017](#)). Crayfish and fish density was negatively correlated to surface water Pb concentrations and sediment concentrations for crayfish in a river system ([Alert 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](#)).

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## 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. Few studies on Pb toxicity have been conducted on saltwater plant and algal 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 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, 2006a](#)). 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<sub>50</sub> = 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 polychaete 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<sub>10</sub> larval development in the mussels *Mytilus trossulus* and *Mytilus galloprovincialis*, was 9 and 10 µg Pb/L respectively, and 72-hour EC<sub>10</sub> 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  $EC_{50} = 44 \mu\text{g Pb/L}$  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  $EC_{50} = 49.5 \mu\text{g Pb/L}$ , 48-hour NEC =  $9.9 \mu\text{g Pb/L}$ ) and *Saccostrea glomerata* (48-hour  $EC_{50} = 52.1 \mu\text{g Pb/L}$ , 48-hour NEC =  $10.1 \mu\text{g Pb/L}$ ) (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, 2006b). 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	
			2013 Pb ISA <sup>a</sup>	2023 Pb ISA <sup>b</sup>
<b>Community and Ecosystem</b>		Community and Ecosystem Effects	Inadequate	<b>Suggestive</b>
<b>Population-level Endpoints</b>	<b>Organism-level Responses</b>	Reproductive and Developmental Effects – Plants	Inadequate	Inadequate
		Reproductive and Developmental Effects – Invertebrates	Suggestive	<b>Likely Causal</b>
		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	<b>Suggestive</b>
	<b>Suborganismal Responses</b>	Neurobehavioral Effects – Invertebrates	Inadequate	Inadequate
		Neurobehavioral Effects – Vertebrates	Inadequate	Inadequate
		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

<sup>a</sup>Conclusions were based on the weight of evidence framework for causal determination in Table II of the 2013 Pb ISA ([U.S. EPA, 2013](#)). 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](#)). <sup>b</sup>Changes from the 2013 Pb ISA are indicated as bolded text.

1

2 The 2013 Pb ISA concluded that the body of evidence was suggestive of a causal relationship

3 between Pb exposure and physiological stress, hematological effects, and reproductive and developmental

4 effects in saltwater invertebrates (Table 11-6). Evidence was inadequate at the time to assess causality for

5 additional effects in saltwater invertebrates and for marine algae and vertebrates. Key uncertainties from

6 the last review for saltwater ecosystems included the uncertainties associated with generalization of

7 effects observed in controlled laboratory studies to conditions in coastal environments where many

8 modifying factors affect Pb bioavailability and toxicity. In general, Pb toxicity to marine or estuarine

9 plants, invertebrates and vertebrates was less well characterized than toxicity to Pb in freshwater systems

1 in the 2013 Pb ISA due to an insufficient quantity of studies on saltwater organisms. Specifically, there  
2 was a lack of chronic toxicity data, and relatively few studies reported analytically verified Pb  
3 concentration in the experimental media. Information regarding the contribution of atmospheric Pb to  
4 total Pb in coastal environments was sparse. This was attributed to multiple sources of Pb, confounding  
5 effects of transport from terrestrial and freshwater systems and the lack of studies connecting the air  
6 concentration of Pb and saltwater ecosystem exposure.

7 Studies published since the 2013 Pb ISA (literature cutoff for inclusion in the 2013 Pb ISA was  
8 September 2011) that characterized bioavailability, uptake, bioaccumulation, and effects of Pb in  
9 saltwater ecosystems or that decreased uncertainties identified in the prior NAAQS review of this criteria  
10 air pollutant are presented throughout the following sections. Saltwater ecosystems considered encompass  
11 a range of salinities from just above that of freshwater (<1 ppt) to that of seawater (generally described as  
12 35 ppt). Coastal ecosystems may receive Pb from multiple sources such as contributions from  
13 atmospheric deposition and via inputs from terrestrial systems including runoff and riverine transport  
14 (Appendix 1: <https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=357282>).

15 Habitats associated with coastal areas include salt marshes, estuaries, shallow open waters, sandy  
16 beaches, mud and sand flats, rocky shores, oyster beds, coral reefs, mangrove forests, river deltas, tidal  
17 pools, and seagrass beds (U.S. EPA, 2016). Estuaries, where freshwater inflows gradually mix with salt  
18 water, are dynamic, heterogeneous environments characterized by gradients of salinity. Salinity is one of  
19 the modifying factors affecting Pb speciation in coastal systems, and changes in salinity affect the ionic  
20 strength of the water (Section 11.4.2). The Pb<sup>2+</sup> ion, which is the most bioavailable form of Pb, is not  
21 common in seawater; rather, Pb primarily exists as a carbonate complex and to a lesser extent as a  
22 chloride complex (Church et al., 2017; Millero et al., 2009).

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

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

32 Factors affecting bioavailability of Pb to saltwater organisms are many of the same factors  
33 affecting bioavailability to freshwater biota (Section 11.3.2), notably OM and pH. Other factors, such as



1 salinity, play a greater role in Pb fate, transport, and bioavailability in saltwater systems, especially in  
2 dynamic estuarine environments characterized by gradients of salinity. Since the 2013 Pb ISA, there is  
3 additional information (summarized below) on these chemical factors which can be quantified and  
4 directly related to toxicity. Studies have further explored the effects of varying DOM composition and  
5 changing pH on Pb uptake and bioaccumulation in saltwater biota. Other factors that affect the uptake and  
6 toxicity of Pb, such as biological adaptations by organisms, are more difficult to link quantitatively to  
7 toxicity. As discussed in previous EPA reviews of Pb, species differences in metabolism, sequestration,  
8 and elimination rates have been shown to control the relative sensitivity and vulnerability of exposed  
9 organisms and influence the potential for effects on survival, reproduction, growth, metabolism, and  
10 development. Diet and lifestage at the time of exposure also contribute significantly to sensitivity and  
11 vulnerability in populations and communities. The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) reviewed the  
12 effects of genetics, age, and body size on Pb toxicity. While genetics appears to be a significant  
13 determinant of Pb sensitivity, the effects of age and body size are complicated by environmental factors  
14 that alter the metabolic rates of saltwater organisms. Literature reviewed in the 2013 Pb ISA corroborated  
15 these findings and discussed seasonal physiological changes and lifestage as important determinants of  
16 differential sensitivity to Pb.

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#### 11.4.2.1. Dissolved Organic Matter

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

33 Several studies published since the 2013 Pb ISA have explored the protective effects of different  
34 types of OM by quantifying enzymatic activity and oxidative response in saltwater invertebrates.

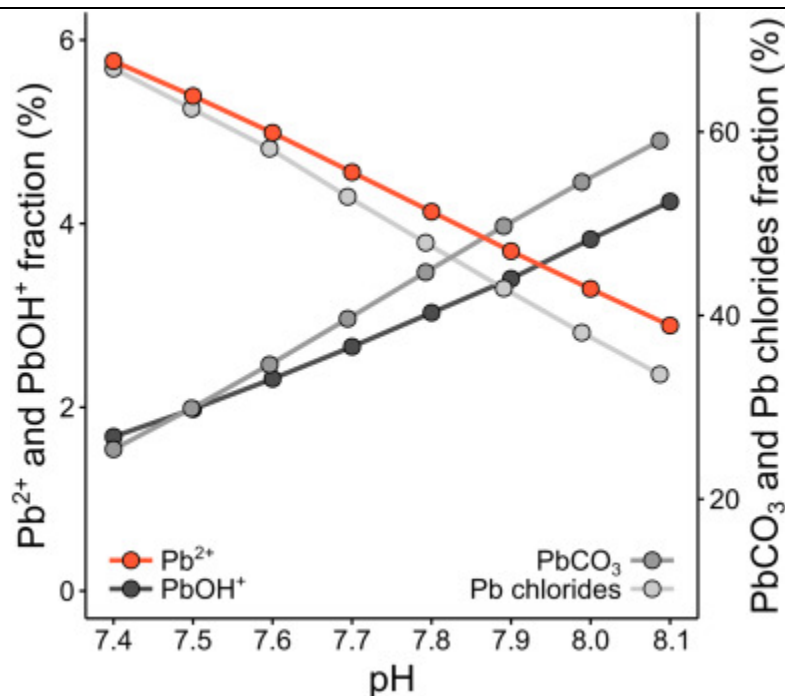


[Nogueira et al. \(2018\)](#) examined the toxicity of Pb alone and in combination with natural OM (NOM) from different sources (allochthonous, autochthonous, and mixed) on larvae of the Canadian native bay mussel (*Mytilus trossulus*). With 48-hour exposure to Pb alone (20 µg Pb/L, nominal value) there 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. trossulus*, 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.

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#### 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, 2006a](#)). 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<sup>2+</sup>) 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 <sup>210</sup>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<sup>2+</sup> in acidified seawater can make Pb<sup>2+</sup> more available to mussels due to decreased competition, and the lower pH means a higher partial pressure of CO<sub>2</sub>, which can result in decreased biomineralization that may facilitate the uptake of Pb.



Source: [Belivermis 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<sup>2+</sup> ([U.S. EPA, 2006a](#); [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<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, 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.

1 In a study reviewed in the 2006 AQCD, [Verslycke et al. \(2003\)](#) exposed the estuarine mysid  
2 *Neomysis integer* to individual metals, including Pb, and metal mixtures under changing salinity. At a  
3 salinity of 5‰, the reported LC<sub>50</sub> for Pb was 1140 µg/L (95% CL = 840, 1440 µg/L). At an increased  
4 salinity of 25‰, the toxicity of Pb was substantially reduced (LC<sub>50</sub> = 4,274 µg/L [95% CL = 3,540,  
5 5710 µg/L]). The reduction in toxicity was attributed to increased complexation of Pb<sup>2+</sup> with Cl<sup>-</sup> ions.  
6 Studies published since the 2013 Pb ISA have further considered salinity as a modifier of Pb uptake and  
7 toxicity in saltwater invertebrates. The relationships between tissue concentration of Pb and inorganic  
8 cations (Na<sup>+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and Ca<sup>2+</sup>) were assessed in the Hong Kong oyster (*Crassostrea hongkongensis*) at  
9 four different salinities at a nominal concentration of 3 µg Pb/L under laboratory conditions ([Yin and](#)  
10 [Wang, 2017](#)). All four cations were negatively correlated with trace metal uptake by oysters; the tissue  
11 concentration of Pb was lower at higher salinities during the 6-week exposure (due to decreasing free-ion  
12 concentration of Pb at higher salinity). For the rotifer *Proales similis*, exposed nominally to Pb (13, 25,  
13 50, 100 µg Pb/L) in 5-day chronic reproductive toxicity tests conducted at four salinity conditions (5, 15,  
14 25 and 35 ppt), population density was highest at the lowest salinity, and toxicity increased with  
15 increasing Pb concentration ([Rebolledo et al., 2021](#)). As salinity increased, population density decreased  
16 in all treatments and the control; however, across all salinities, the population growth rate was lowest at  
17 100 µg Pb/L (the highest tested concentration). In contrast, embryo development assays in larval mussels  
18 (bay and Mediterranean) and pacific purple sea urchins conducted at two salinities (33 ppt and 21 ppt)  
19 reported no effect of salinity on Pb toxicity ([Nadella et al., 2013](#)).

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

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#### 11.4.2.4. Association with Sediments

29 Habitat type is a factor in the bioaccumulation of trace metals, as invertebrates closely associated  
30 with benthic environments have greater contact with porewater and sediments, where metal  
31 concentrations are higher than those in seawater. Several new studies published since the 2013 Pb ISA  
32 reported differences in the bioaccumulation of Pb associated with sediment characteristics. [Belzunce-Segarra et](#)  
33 [al. \(2015\)](#) compared bioaccumulation in the benthic bivalve *Tellina deltoidalis* with two sediment types  
34 (silty, sandy) in the lab and deployed in the field. During the 31-day exposure period, Pb bioaccumulation

1 from sediments generally increased in a linear fashion with increasing sediment Pb concentration and was  
2 greater in sandy sediments. For the silty sediments, there was more bioaccumulation in field-deployed  
3 bivalves compared with bivalves in a parallel laboratory exposure, whereas the opposite was observed  
4 with sandy sediments. Bioaccumulation in bivalves was attributed primarily to dietary exposure via  
5 ingestion of particles due to the poor relationship between dissolved Pb in overlying waters (1 to  
6 2.2 µg Pb/L) and bioaccumulation. The authors noted that under laboratory exposure conditions, the  
7 absence of processes occurring in the natural environment such as sediment resuspension, dilution of  
8 surface sediments by deposition, and avoidance behaviors by organisms, likely lead to overestimation of  
9 bioavailability. [Battuello et al. \(2018\)](#) quantified trace metals in two predaceous marine invertebrates  
10 native to coastal waters of Italy: *Eurydice spinigera* (Isopoda), which burrows in sediments during the day  
11 and rises to feed in the pelagic zone at night, and *Flaccisagitta enflata* (Chaetognatha), a zooplanktonic  
12 species. Although the invertebrates have a similar feeding behavior and occupy the highest invertebrate  
13 trophic level, Pb was an order of magnitude higher in *E. spinigera* (3.1 mg Pb/kg wet weight) compared  
14 with *F. enflata*.

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

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#### 11.4.2.5. Seasonality

25 Seasonal differences in Pb uptake and concentration in bivalves were noted in several European  
26 field monitoring studies included in the 2013 Pb ISA ([Carvalho et al., 2011](#); [Couture et al., 2010](#); [Pearce  
27 and Mann, 2006](#)). These differences could be due to seasonal changes in anthropogenic inputs or to  
28 altered organism physiological condition in warmer versus colder months. Newer studies also reported  
29 seasonal fluctuations in Pb uptake in saltwater invertebrates. Seasonal and spatial variation of trace metal  
30 accumulation was observed in *M. galloprovincialis* mussels collected from sites around Port Phillip Bay,  
31 Australia in the summer and winter ([Shen et al., 2020](#)). In mussels collected from locations identified as  
32 high risk for contamination, Pb body burden was higher in summer than in winter. In mussels collected  
33 from less affected sites, there was no significant difference in Pb burden with season. This suggests that  
34 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. Hernandez-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.

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#### 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 (Hernandez-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  $12.8 \pm 1.7$  mg Pb/kg dry weight (mean  $\pm$  SE) to  $38.6 \pm 4.2$  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  $\delta^{15}\text{N}$  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.

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#### 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](#)).

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#### 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.



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#### 11.4.2.9. Species Sensitivity

1 As is the case for terrestrial and freshwater organisms, there are considerable differences in  
2 response to Pb among saltwater biota. This information serves as the basis for the species sensitivity  
3 distributions (Section 11.4.5) for saltwater invertebrates and fish reported by ([Church et al., 2017](#)). Both  
4 inter and intraspecific differences in Pb uptake and bioaccumulation may occur in macroinvertebrates of  
5 the same functional feeding group ([U.S. EPA, 2013](#)). For example, in the 2013 Pb ISA, data from  
6 20 years of monitoring of contaminant levels in filter-feeding mussels of the *Mytilus* genus and eastern  
7 oysters (*C. virginica*) sampled along the U.S. coast, as part of the NOAA Mussel Watch program, indicate  
8 that Pb is on average three times higher in mussels than in oysters ([Kimbrough et al., 2008](#)). [Wang et al.](#)  
9 [\(2014b\)](#) compared acute toxicity data (hazard toxicity ratios based on LC<sub>50</sub> values; EC<sub>50</sub> values for algal  
10 responses) for temperate and tropical saltwater species sensitivity distributions across five broad  
11 taxonomic groups (algae, crustaceans, fish, mollusks, worms). Based on the hazardous concentration for  
12 10% of the species (HC<sub>10</sub>) ratios, temperate saltwater species are more sensitive to Pb than tropical  
13 saltwater biota. In the meta-analysis, algae were the most sensitive taxa to Pb (HC<sub>10</sub> = 29 µg Pb/L, [95%  
14 CI 9.5, 86], n = 8) followed by fish (HC<sub>10</sub> = 166 µg Pb/L [95% CI 49], n = 10), crustaceans  
15 (HC<sub>10</sub> = 428 µg Pb/L [95% CI, 263, 696], n = 22), mollusks (HC<sub>10</sub> = 1230 µg Pb/L [95% CI 412, 3,660],  
16 n = 7), and worms (HC<sub>10</sub> = 2,430 µg Pb/L [95% CI 1,200, 4,610], n = 9).

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#### 11.4.2.10. Uptake and Bioaccumulation in Saltwater Plants and Algae

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

21 One study examined element concentrations in pelagic *Sargassum* that washed up along the coast  
22 of the Yucatan peninsula in Mexico from the Caribbean ([Rodriguez et al., 2020](#)). Of 63 different samples  
23 collected across eight sites from August 2018 to June 2019, only five samples had Pb levels at 2–  
24 3 mg Pb/kg dry weight (as measured by X-ray fluorescence, which has a detection limit of 2 ppm). Other  
25 metals such as As were detected in much higher amounts. Though Pb is not present in high amounts in  
26 *Sargassum*, the study showed that pelagic seaweed may be an avenue of transport across large distances  
27 and contribute to Pb levels in coastal environments where it washes ashore.

28 An additional area of new research is the uptake of Pb by mangroves and the mechanisms that  
29 may limit or confer tolerance. Mangrove swamps are coastal wetlands found in tropical and subtropical  
30 regions. They are characterized by halophytic woody plants growing in brackish to saline tidal waters.  
31 One greenhouse experiment aimed to investigate the possible function of root lignification/suberization  
32 on Pb uptake and tolerance in two pacific mangrove species with different degrees of root lignification



1 and suberization: holly mangrove (*Acanthus ilicifolius*) and red mangrove (*Rhizophora stylosa*) ([Cheng et](#)  
2 [al., 2015](#)). Plants were grown in pots with three nominal Pb treatments applied to the sediment—low  
3 (250 mg Pb/kg), medium (500 mg Pb/kg) and high (1000 mg Pb/kg)—and one control with no Pb; Pb  
4 exposure was a period of 3 months. In the species with little lignification and suberization, *A. ilicifolius*,  
5 biomass yield decreased significantly as plants were exposed to increasing concentrations of Pb; about 20,  
6 35 and 50% reductions were observed in low, medium, and high Pb treatments when compared with the  
7 respective controls. *R. stylosa*, however, was not affected by low and medium Pb exposure. A significant  
8 decrease in relative Pb was observed within the outer cortex cell layers, indicating that lignified/suberized  
9 exodermis acts as a barrier to the movement of Pb. A further study with six pacific mangrove species  
10 subjected to different levels of a metal mixture (Pb with Zn and Cu) corroborates these findings and  
11 suggests that mangrove species, which possess more extensive lignification and suberization within their  
12 root exodermis, exhibit higher tolerance for heavy metals ([Cheng et al., 2014](#)).

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

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#### 11.4.2.11. Uptake and Bioaccumulation in Saltwater Invertebrates

30 At the time of the 1977 AQCD, it was understood that shellfish concentrate Pb in their tissues and  
31 shells ([U.S. EPA, 1977](#)). Uptake and subsequent bioaccumulation of Pb in marine invertebrates varies  
32 greatly between species and across taxa as previously characterized in the 2006 AQCD ([U.S. EPA,](#)  
33 [2006a](#)) and the 2013 Pb ISA ([U.S. EPA, 2013](#)). In the case of invertebrates, Pb can be bioaccumulated  
34 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<sup>2+</sup>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 1000 mg Pb/kg dry weight) for 30 days, the profile of tissue Pb accumulation was body wall>intestine>respiratory tree ([Wang et al., 2015a](#)). The bioavailability of Pb from food and subsequent

1 trophic transfer is affected by how Pb is stored in the prey. In a feeding study, the common prawn  
2 *Palaemon serratus* was fed for 28 days with either tissues from the mussel (*M. galloprovincialis*) exposed  
3 to 100 µg Pb/L for 48 hours or tissues from the field-collected clam *Dosinia exoleta*, wherein Pb is stored  
4 primarily in nonbioavailable MRG in the kidney ([Sánchez-Marín and Beiras, 2017](#)). Although the Pb  
5 concentration in both food items was similar (15 and 17 mg Pb/kg wet weight, respectively), Pb  
6 accumulation in prawns was 10× higher when fed tissue from the mussels, in which Pb was in a more  
7 soluble subcellular fraction, compared with the prawns consuming *D. exoleta*, in which Pb was in a less  
8 bioavailable form.

9 Pb uptake is influenced by feeding strategy. In the filter-feeding bivalve *Andara trapezia*, uptake,  
10 and bioaccumulation from Pb-spiked sediments (analytically verified concentration of 100 and  
11 300 mg Pb/kg) to the gill and mantle, hemolymph and hepatopancreas were quantified on days 0, 14, 28,  
12 42 and 56 of a 56-day exposure ([Taylor and Maher, 2012](#)). At the end of the experiment, total Pb  
13 concentration in the mollusk was 1 mg Pb/kg at the low concentration and 12 mg Pb/kg at the high  
14 concentration. In the highest Pb treatment, an increase in Pb in hemolymph was observed from day 42 to  
15 day 56, resulting in a doubling of Pb tissue concentration. The authors speculated this could be related to  
16 greater availability of dissolved Pb in porewater over time due to oxidation of the sediments. Generally,  
17 the order of tissue accumulation was hemolymph ≥ gill and mantle > hepatopancreas over the 56-day  
18 exposure. In contrast, the deposit-feeding bivalve *Tellina deltoidealis* exhibited a distinct pattern of Pb  
19 uptake under similar experimental conditions and exposure to spiked sediments (28-day exposure to  
20 analytically verified concentrations of 100 and 300 mg Pb/kg) ([Taylor and Maher, 2014](#)). Individuals in  
21 the 100 mg/kg Pb-spiked sediment rapidly accumulated Pb early in the exposure period (day 3) followed  
22 by continued uptake over the remainder of the experiment, to reach a final tissue concentration  
23 (96 mg Pb/kg) equal to that of the spiked sediment. In the 300 mg Pb/kg microcosm, the bivalves seemed  
24 to exhibit a pattern of uptake and loss over the 28-day period, with the highest Pb concentration at day 21  
25 and a final total Pb concentration of 430 mg Pb/kg.

26 Aquatic invertebrate strategies for detoxifying Pb reviewed in the 2006 Pb AQCD and 2013 Pb  
27 ISA included sequestration of Pb in lysosomal-vacuolar systems, excretion of Pb by some organisms and  
28 deposition of Pb to molted exoskeleton. Pb can be stored in two forms: biologically detoxified metal  
29 (which includes MRG) and biologically available metal. Following the biouptake experiments described  
30 above, subcellular partitioning of Pb was determined in the bivalves ([Taylor and Maher, 2014, 2012](#)). Of  
31 the recovered Pb in *A. trapezia* tissues, Pb was associated to the greatest extent with the biologically  
32 detoxified metal fraction (ranging from 66% to 69% in the gill and mantle to 56% in the hepatopancreas),  
33 distributed fairly evenly between the metallothionein-like proteins and MRG fractions ([Taylor and Maher,](#)  
34 [2012](#)). In *T. deltoidealis*, Pb was also primarily found in the biologically detoxified metal fraction  
35 (approximately 70%), with 74% of the total detoxified Pb converted to MRG and the remainder in the  
36 metallothionein-like protein fraction ([Taylor and Maher, 2014](#)).

1 In a 96-hour exposure to analytically verified concentrations of Pb (0–1,800 µg Pb/L),  
2 intracellular partitioning data in adult clams *Venerupis decussata* showed that most Pb accumulated in the  
3 insoluble fraction (>80%), a form not readily bioavailable to consumers at higher trophic levels ([Freitas et](#)  
4 [al., 2014](#)). Total Pb in clams increased with increasing water concentration up to 230 µg Pb/L then  
5 decreased at higher concentrations. The clams bioconcentrated Pb in the soluble fraction more efficiently  
6 at low water concentrations (BCF > 26) compared with higher concentrations (>450 µg Pb/L; BCF<16).  
7 Similar results were observed with the clam *Venerupis corrugata* following 96-hour nominal exposure to  
8 Pb (100 to 800 µg Pb/L). Most of the metal was found in the insoluble fraction and associated with MRG  
9 (42–72%) ([Freitas et al., 2014](#)).

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#### 11.4.2.12. Uptake and Bioaccumulation in Saltwater Vertebrates

10 Studies reviewed in prior AQCDs and ISAs report Pb accumulation in tissues sampled from  
11 seabirds, saltwater fish, and marine mammals ([U.S. EPA, 2013, 2006a, 1977](#)); however, there are fewer  
12 biouptake studies of Pb in saltwater than in freshwater. Because marine fish drink seawater to maintain  
13 osmotic homeostasis, Pb can be taken up from the water column via both the gills and intestine ([Lee et al.,](#)  
14 [2019](#); [Wang and Rainbow, 2008](#)). In the 2013 Pb ISA, storage of Pb in metal granules was reported as a  
15 detoxifying mechanism in mummichogs (*Fundulus heteroclitus*). Fish at more polluted sites stored a  
16 higher amount of Pb in MRG as compared with other detoxifying cellular components such as heat-stable  
17 proteins, heat-denaturable proteins and organelles ([Goto and Wallace, 2010](#)). Since the 2013 Pb ISA,  
18 additional studies have further elucidated the role of subcellular fractions in metal detoxification in  
19 saltwater fish. Metal binding to subcellular fractions in the livers of wild-caught yelloweye rockfish  
20 (*Sebastes ruberrimus*) collected from the southeast coast of Alaska was assessed to gain a better  
21 understanding of the degree to which this long-lived endangered fish species can detoxify nonessential  
22 metals including Pb ([Barst et al., 2018](#)). Combining data from the rockfish, Pb was detected to a greater  
23 extent in the detoxified compartment (46%); however, detoxification was incomplete given that Pb was  
24 also present in metal-sensitive fractions (a total of 35%, divided between heat denatured proteins [12.2%],  
25 mitochondria [11.4%], microsomes and lysosomes [10.8%]). Metals associated with sensitive subcellular  
26 fractions indicate a risk of disruption to cellular processes; however, the concentrations of Pb in rockfish  
27 were low compared with other detected metals. These patterns were consistent with results from  
28 subcellular partitioning in livers of yellow eels native to North America (*Anguilla rostrata*) and Europe  
29 (*Anguilla anguilla*) ([Rosabal et al., 2015](#)). In both eel species, the granule-like detoxification fraction  
30 showed the strongest increase in Pb concentrations among all subcellular fractions, with the metal-  
31 sensitive mitochondrial fraction representing a significant binding compartment for Pb.

32 A novel study exploring the use of fish eyes as an organ for monitoring Pb exposure compared Pb  
33 concentration in mullet (*Liza aurata*) eyes, water column and sediment in a metal-contaminated location  
34 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.

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#### 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, 2006a) 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,  $\delta^{13}\text{C}$  is correlated with the relative proportion of pelagic diet sources, while  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$ , while  $\delta^{13}\text{C}$  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,  $\delta^{15}\text{N}$  was negatively correlated with  $\delta^{13}\text{C}$  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  $0.07 \pm 0.01$  mg Pb/kg (mean  $\pm$  S.D.) in *Glycera dibranchiate* (Polychaeta) to  $1.25 \pm 1.40$  mg Pb/kg in *I. obsoleta* (Gastropoda). There were no significant correlations between trophic level position ( $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$ ) 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 ([Hernandez-Almaraz et al., 2016](#)). Out of the macroalgae and two sea urchins studied, *E. thouarsii* had the highest Pb concentrations (ranging from  $12.8 \pm 1.7$  mg Pb/kg dry weight [mean  $\pm$  SE] to  $38.6 \pm 4.2$  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  $20.86 \pm 5.80$  mg Pb/kg (mean  $\pm$  S.D.),



1 and the suspended load Pb concentration was  $16.59 \pm 2.79$  mg Pb/kg in the San Pedrito Lagoon, which is  
2 impacted by wastewater discharge and petroleum extraction. Pb concentration was only above the limit of  
3 detection in two plant species, spider lily (*Hymenocallis littoralis*) and mangrove fern (*Acrostichum*  
4 *aureum*), and fish samples (2.9 mg Pb/kg) from the lagoon. In general, BCFs of Pb were low, and BCFs  
5 were higher in plants than in fish, suggesting trophic dilution.

6 Bioaccumulation, but not biomagnification, was found in a semiarid coastal lagoon in Sonora,  
7 Mexico along the Gulf of California ([Jara-Marini et al., 2020](#)). The community consists of primary  
8 producers (phytoplankton, algae, mangrove), primary consumers (zooplankton, barnacles, oysters, clams,  
9 snails, shrimp, crab, snapper, and juveniles of flathead mullet), secondary consumers (adults of flathead  
10 mullets, crab, snapper, mojarra, and grunt), and tertiary consumers (night herons, great blue herons,  
11 magnificent frigate, and cormorants). BMF was corrected for the trophic position, and the trophic BMF  
12 (TBMF) was estimated from the antilogarithm of the slopes of the linear correlation between the trophic  
13 level and the metal concentration. BMF and TBMF values above 1.0 indicate that a metal is being  
14 transferred upward through the trophic levels, while values below 1.0 indicate biodilution along the food  
15 web. Pb values in suspended particulate matter and sediment varied between seasons, ranging from  
16 0.70 mg Pb/kg in autumn to 1.03 mg Pb/kg in winter. Pb concentrations among primary producers (range:  
17 0.63 to 1.03 mg Pb/kg), secondary consumers (range: 0.80 to 1.53 mg Pb/kg), and most tertiary  
18 consumers did not vary seasonally. Only two tertiary consumers, neotropic cormorant (*Phalacrocorax*  
19 *brasilanus*) and magnificent frigatebird (*Fregata magnificens*), showed the highest Pb concentrations in  
20 the summer. Pb only showed a positive relationship between log-transformed Pb concentrations and  
21 trophic level in the summer. Pb concentrations generally decreased through the food web, depending on  
22 the season. The BMF ranged from 0.50 to 3.57 for Pb across organisms, and TBMF ranged from 1.02 to  
23 1.15. Although TBMF values were above 1.0, biomagnification was unlikely because the relationship  
24 between trophic level and Pb concentration was only significantly positively correlated in the summer.

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

34 Although most observational studies suggest biodilution of Pb occurs through marine food webs,  
35 Pb was found to bioaccumulate in mummichog (*Fundulus heteroclitus*) in the Goose Pond estuary in  
36 Brooksville, Maine ([Broadley et al., 2013](#)). The Goose Pond estuary was impacted by the former Callahan  
37 Mine, which is one of the few documented open-pit hard-rock mining sites in an intertidal zone. The



1 sediment concentration of Pb was above the probable effects level in some of the sample sites. BAFs  
2 ranged from 3.3–3.65 across Goose Pond sites and 2.62–3.77 at the reference sites. The reference site  
3 values bioaccumulation factors were conservative as they included water and tissue Pb concentrations  
4 below the instrument detection limit. The sediment-to-*F. heteroclitus* and water-to-*F. heteroclitus* ratios  
5 were high for Pb. The ratio of metal enrichment to background levels (concentrations at the Goose Pond  
6 site adjacent to the tailings pile / the mean concentrations at a reference site) were 34.2 for sediment, 32.3  
7 for water, and 45.6 for *F. heteroclitus*.

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

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

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

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

31 Quantification of chemical variation in relative presence of Pb and of other elements taken up and  
32 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 1949–2002 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) ( $\bar{x} = 0.289 \pm 0.068$  mg Pb/kg) and to the data from 1999 to 2000 ( $\bar{x} = 0.0353 \pm 0.00496$  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 2006–2015, [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.

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

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#### 11.4.4. Effects of Pb in Saltwater Systems

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

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##### 11.4.4.1. Effects on Saltwater Microbes

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

18 Pb negatively affected microbial diversity and structure in rhizosediments of sea rush (*Juncus*  
19 *maritimus*) and the common reed (*P. australis*) collected from the Lima estuary, Portugal ([Mucha et al.,](#)  
20 [2013](#)). Rhizosediments colonized with *Juncus maritimus* (a phytostabilizer that retains metal in the roots  
21 and rhizomes) and *P. australis* (a phytoextractor, which accumulates metals in the aboveground tissue)  
22 were analyzed for heavy-metal concentrations, physicochemical properties, and microbial abundance.  
23 Sediments were then exposed to a nominal gradient of Pb (0 mg Pb/kg, 218 mg Pb/kg, 2180 mg Pb/kg or  
24 10900 mg Pb/kg and incubated for 7 days using artificial seawater). Rhizosediments collected initially  
25 contained 63 ± 2 mg Pb/ kg (mean ± S.D.) and 70 ± 11 mg Pb/ kg for *P. australis* and *J. maritimus*,  
26 respectively. Only 0.02–1.3% of the initially added Pb remained in solution at the end of the experiment.  
27 At the end of the experiment, bacterial total cell count (cells/g wet sediment) was higher in the  
28 218 mg Pb/kg treatment than in the control for *J. maritimus*, while total cell count was unaffected by Pb  
29 exposure for *P. australis*. *Juncus maritimus* rhizosediment exhibited lower OTU number, diversity,  
30 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. Although only a subset of variables was used in the analysis of bacterial community structure, all sediment and water column predictors were used for the bacterial community diversity analysis. Pb and Cu sediment concentration were the most important predictors for bacterial diversity. Out of all 28 predictors used in boosted regression tree (BRT) models, 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](#)). Sediments were collected and analyzed for heavy metals, bacterial communities were analyzed using 16S rDNA sequencing, and potential functional genes associated with heavy-metal transport and elimination were examined using GeoChip analysis. Sediment Pb concentrations ranged from  $0.142 \pm 0.094$  mg Pb/kg to  $3.257 \pm 0.094$  mg Pb/kg (mean  $\pm$  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, and few Pb-transporting genes were found.

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. Sediment heavy-metal concentration (V, Ni, U, Mo, Zn, Se, antimony [Sb], Co, Cr, Cd, Pb, As, Cu, and Hg) and the bacterial abundance and community

1 structure (polymerase chain reaction denaturing gradient gel electrophoresis fingerprinting) were  
2 quantified. The concentration of Pb varied among the three sites (mean  $\pm$  S.D. site Shilaoren Beach:  
3  $19.09 \pm 1.86$  mg Pb/kg, site Haibohe estuaries:  $38.65 \pm 9.26$  mg Pb/kg and site Licunhe estuaries:  
4  $72.87 \pm 17.56$  mg Pb/kg). The concentrations of Co, Zn, Hg, As, and Se explained more of the variation  
5 in bacterial community composition in this system, and Pb concentration was not one of the top three  
6 strongest predictors of bacterial diversity at any site.

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

20 Finally, Pb concentration in the water column did not affect bacterioplankton community  
21 composition in the Toulon Bay, France ([Coclet et al., 2019](#)). Mn, DOC, salinity, Cu, and Cd explained the  
22 most variation in the bacterioplankton community composition (range in variance: 1.01%–1.22%), while  
23 Pb concentration only explained 0.51% of the variance in bacterioplankton community composition.  
24 *Rhodobacteraceae*, SAR11 (Alphaproteobacteria), *Balneola* (Bacteroidetes), and *Synechococcus*  
25 (Cyanobacteria) were negatively correlated with either Cd, Cu, Pb or Zn, while *Candidatus aquilina*  
26 (Actinobacteria) was positively correlated with Pb.

27 In summary, several experimental and observational studies since the 2013 Pb ISA ([U.S. EPA,  
28 2013](#)) reported negative relationships between sediment or saltwater Pb concentration and microbial  
29 abundance and diversity ([Meng et al., 2021](#); [Mucha et al., 2013](#)), while other studies found no relationship  
30 ([Coclet et al., 2019](#); [Zhuang et al., 2019](#); [Yao et al., 2017](#)).

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#### 11.4.4.2. Effects on Saltwater Plants and Algae

31 In the 2013 Pb ISA, evidence was inadequate to infer a causal relationship between Pb exposure  
32 and endpoints relevant to saltwater plants and algae (growth, survival, physiological stress) (Table 11-6).  
33 Key studies in the 2013 Pb ISA included a 72-hour EC<sub>50</sub> 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<sub>10</sub> 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. frutescens*, 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.

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#### 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, 2006a). 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



1 physiological perturbations associated with Pb exposure, including a few observations in previously  
2 untested taxa. Only a few of the many studies identified in the literature search on suborganism-level  
3 responses to Pb exposure in saltwater invertebrates were conducted in the low  $\mu\text{g Pb/L}$  range and hence  
4 met the criteria for inclusion in the ISA (Section 11.1.1).

#### 11.4.4.3.1. Suborganism-Level Response

5 The majority of studies in saltwater invertebrates do not link the effects reported at the molecular  
6 and cellular levels to effects at the organism level of biological organization (e.g., survival, growth,  
7 reproduction). One study in Tiger prawn (*Penaeus monodon*) exposed to a range of Pb concentrations (14  
8 to 232  $\mu\text{g Pb/L}$ ) in seawater for 30 days reported an increase in lipid peroxidation starting at the  
9 56  $\mu\text{g Pb/L}$  exposure concentration. Chronic exposure yielded NOEC = 14  $\mu\text{g Pb/L}$  and  
10 LOEC = 29  $\mu\text{g Pb/L}$  for survival in this species ([Hariharan et al., 2012](#)).

11 For the endpoint of physiological stress, many studies from the 2013 Pb ISA, especially those that  
12 considered enzymatic responses to Pb exposure, were conducted with nominal Pb concentrations in  
13 mollusks. In several studies published since the 2013 Pb ISA, perturbations in biomarkers of oxidative  
14 stress were observed at nominal concentration of 10  $\mu\text{g Pb/L}$ . In an 8-day exposure study, there were  
15 changes in the activity of CAT, SOD and GST in the gill and digestive gland of oysters (*Crassostrea*  
16 *madrasensis*) ([Shenai-Tirodkar et al., 2017](#)). In Manila clam *Ruditapes philippinarum* ([Aouini et al.,](#)  
17 [2018](#)) (exposed 7 days nominally to 10  $\mu\text{g Pb/L}$ ), biomarkers assessed in this species showed a response  
18 at 10  $\mu\text{g Pb/L}$  including inhibition of GST and a significant increase of lipid peroxidation.

19 New information on molecular biomarker responses to Pb in an additional taxonomic group  
20 (marine ciliate protozoans) has become available since the 2013 Pb ISA. Intracellular reactive oxidative  
21 species and GSH content was quantified in *Euplotes crassus*, a single-celled eukaryote ([Kim et al., 2014](#))  
22 following 8-hour nominal exposure to Pb ranging from 25  $\mu\text{g Pb/L}$  to 250  $\mu\text{g Pb/L}$ . ROS levels were  
23 significantly increased at the lowest concentration tested and decreased to the same levels as the control at  
24 100  $\mu\text{g Pb/L}$ . Total GSH was significantly induced at 25  $\mu\text{g Pb/L}$  and 100  $\mu\text{g Pb/L}$ , although the increase  
25 was greatest at the lowest concentration. Concurrent gene expression of the glutathione-related genes  
26 (glucocorticoid receptor and GPx) was observed with Pb exposure in ciliates.

27 Since the 2013 Pb ISA, additional studies have explored the mechanisms of Pb-induced  
28 physiological stress in saltwater invertebrates by linking observed responses to changes in gene  
29 expression. Over the course of a 4-week exposure of the mussel *M. edulis* to 111.68  $\mu\text{g Pb/L}$  (0.54  $\mu\text{M}$ ),  
30 transcripts involved in the unfolding protein response were differentially expressed with Pb, which  
31 correlated with the bioaccumulation of Pb in gill tissue ([Poynton et al., 2014](#)). In addition, a sequence of  
32 unknown function showed a statistically significant relationship with Pb concentration in gill tissue, and  
33 the authors proposed the sequence may be identified in the future as a dose-dependent Pb-specific



1 biomarker in this species. In the marine polychaete *Perinereis nuntia*, the expression of three different  
2 SOD genes was significantly upregulated following 48-hour nominal exposure to Pb (50 µg Pb/L), and  
3 the expression patterns differed from those observed with exposure to Ni, As and combinations of these  
4 metals, suggesting the suitability of SOD genes as a molecular biomarker for in situ monitoring of sites  
5 contaminated with multiple metals ([Won et al., 2014](#)). [Meng et al. \(2018\)](#) conducted a detailed analysis of  
6 *C. gigas* (pacific oyster) gene expression and physiological response in gill and digestive gland tissue  
7 following 9-day nominal exposure to 5 µg Pb/L. In both tissues, tumor necrosis factor alpha, a marker of  
8 immune response, was significantly inhibited under Pb exposure. The mechanism of Pb toxicity in the gill  
9 was altered Ca<sup>2+</sup> homeostasis in the endoplasmic reticulum, which led to induced expression of stress  
10 chaperones. In digestive glands, a significant increase in ROS, lipid peroxidation products and MDA  
11 content compared with control suggested the primary mechanism of Pb toxicity was oxidative stress.

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

28         Evidence in the 2013 Pb ISA was suggestive of a causal relationship between Pb exposure and  
29 hematological effects, primarily based on field studies that correlated ALAD activity to measured Pb  
30 levels in bivalve tissue ([Company et al., 2011](#); [Kalman et al., 2008](#)). Generally, these studies have noted  
31 that Pb content varies significantly among species and is related to habitat and feeding behavior. A few  
32 additional studies have reported inhibition of ALAD activity in Pb-exposed saltwater invertebrates;  
33 however, the concentration at which enzyme activity is affected appears to be higher than the  
34 concentration of Pb typically encountered in seawater (Table 11-1). In *R. philippinarum* gill tissue,  
35 ALAD activity decreased significantly after 7-day nominal exposure to 10 µg Pb/L, and activity did not  
36 recover in a 7-day depuration period following the initial exposure ([Aouini et al., 2018](#)). ALAD activity  
37 was higher in the gill compared with the digestive glands. [Duarte et al. \(2020\)](#) demonstrated various

sublethal biomarkers were activated during 28-day exposure at a low concentration of Pb (10 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 to Pb (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). A few additional studies that provide information on these endpoints are discussed here. Dietary exposure of sea cucumber (*A. japonicus*) to a Pb-amended diet (100, 500 or 1000 mg Pb/kg dry weight) for 30 days resulted in no significant effects on growth or survival; however, antioxidant enzyme activity was significantly lower in the treatment groups compared with the control (Wang et al., 2015a). In sea hare (*A. californica*) exposed to Pb solely through diet over 2 or 3 weeks (green seaweed *U. lactuca* previously exposed to 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).

Brine shrimp (*Artemia franciscana*) exposed nominally to Pb (8, 16, 32, 64 µg Pb/L) for 20 days (from recently hatched nauplii to adult lifestage) exhibited a significant decrease in mating behavior (sexual couples assessed during the last 3 days of the experiment) at all concentrations, compared with nonexposed shrimp (Frías-Espéricueta et al., 2022). In the marine ascidian *Ciona intestinalis*, various reproductive and developmental parameters were assessed at nominal concentration of 10, 20 and 100 µg Pb/L (Gallo et al., 2011). Hatching rate and embryo development were unaffected in all treatment groups. Significant inhibition of oocyte voltage gated Na<sup>+</sup> currents and postfertilization contraction were observed at the two highest Pb concentrations, suggesting an effect on the mechanisms of fertilization.

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#### 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 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.

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#### 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<sub>50</sub> of 105 µg Pb/L reported for *Chaetorceros* spp. Other tested species were considerably less sensitive (72-hour EC<sub>50</sub> = 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<sub>50</sub> = 1,810 µg Pb/L for growth

inhibition ([Zamani-Ahmadm Mahmoodi 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  $EC_{10} = 152 \mu\text{g Pb/L}$  for *Cryothecomonas armigera* ([Koppel et al., 2017](#)) and a 10-day  $IC_{10} = 260 \mu\text{g Pb/L}$  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  $EC_{50} = 197 \mu\text{g Pb/L}$  for the growth of larvae and  $EC_{50} = 297 \mu\text{g Pb/L}$  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 polychaete *Hydroides elegans*; in eggs pretreated with 48  $\mu\text{g Pb/L}$ , 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  $\mu\text{g Pb/L}$ . 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  $EC_{50} = 221 \mu\text{g Pb/L}$  and LOEC = 50  $\mu\text{g Pb/L}$  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  $\mu\text{g Pb/L}$  ([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  $\geq$  scallops  $\geq$  cockles  $\geq$  clams ([Markich, 2021](#)). The oysters *M. gigas* (48-hour  $EC_{50} = 49.5 \mu\text{g Pb/L}$ , 48-hour NEC = 9.9  $\mu\text{g Pb/L}$ ) and *S. glomerata* (48-hour  $EC_{50} = 52.1 \mu\text{g Pb/L}$ , 48-hour NEC = 10.1  $\mu\text{g Pb/L}$ ) were most sensitive while the clam *Irus crenatus* (48-hour  $EC_{50} = 196 \mu\text{g Pb/L}$ , 48-hour NEC = 39.8  $\mu\text{g Pb/L}$ ) 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. trossulus*, 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*- $EC_{50} = 63 \mu\text{g Pb/L}$ ,  $EC_{20} = 19 \mu\text{g Pb/L}$ ;  $EC_{10} = 10 \mu\text{g Pb/L}$ , NOEC = 3.2  $\mu\text{g Pb/L}$  and *M. trossulus*,  $EC_{50} = 45 \mu\text{g Pb/L}$ ;  $EC_{20} = 16 \mu\text{g Pb/L}$ ;  $EC_{10} = 9 \mu\text{g Pb/L}$ ; NOEC = 3.4  $\mu\text{g Pb/L}$ ). In the 72-hour embryo-larval toxicity test in the sea urchin *S. purpuratus*, the  $EC_{50} = 74 \mu\text{g Pb/L}$ ,  $EC_{20} = 31 \mu\text{g Pb/L}$ ,  $EC_{10} = 19 \mu\text{g Pb/L}$  and NOEC = 2.7  $\mu\text{g Pb/L}$ . In a similar 72-hour larval development toxicity test with the sea urchin *Evechinus chloroticus*, the  $EC_{50} = 52.2 \mu\text{g Pb/L}$ , with skeletal abnormalities observed in the lower range of concentrations (10  $\mu\text{g Pb/L}$  and 20  $\mu\text{g Pb/L}$ ) ([Rouchon and](#)



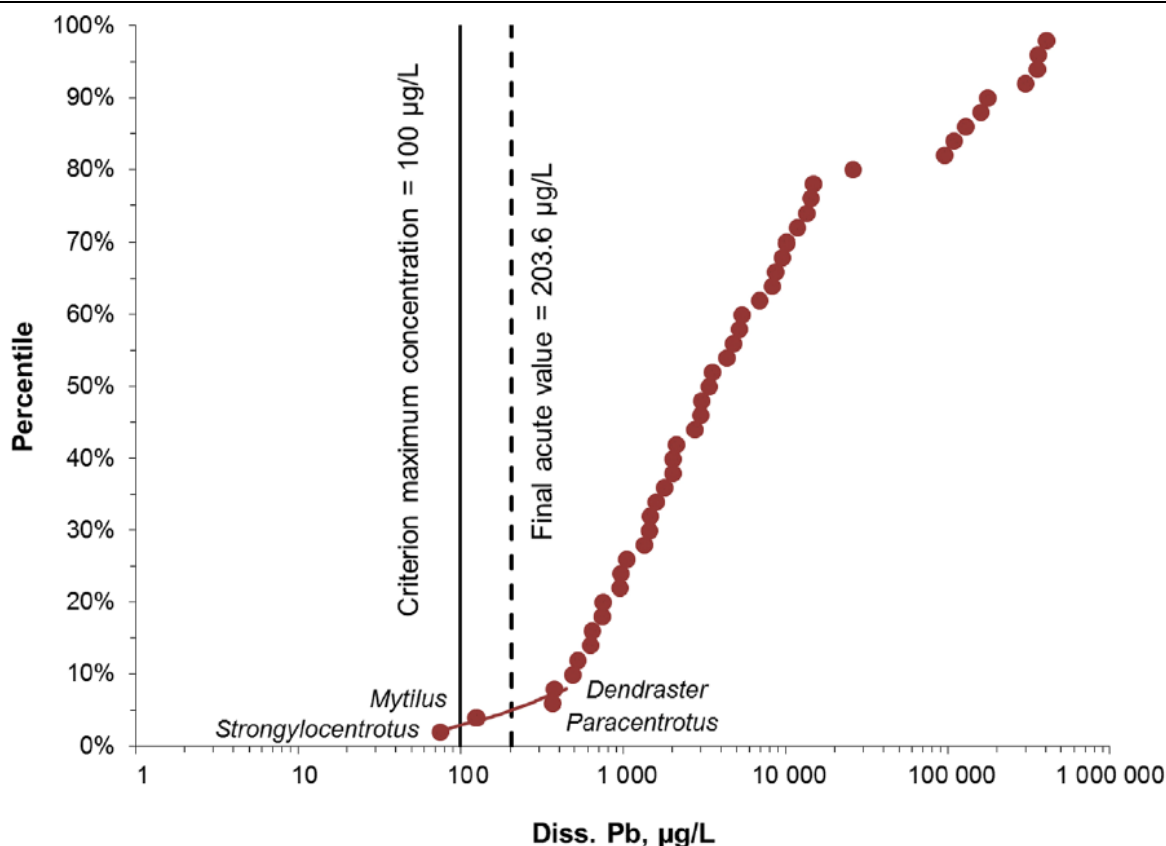
1 [Phillips, 2017](#)). However, Pb in the exposure water was not analytically verified in the study.  
2 Developmental endpoints in oyster *C. gigas* were less sensitive to Pb, with  $EC_{50} = 660.3 \mu\text{g Pb/L}$  for  
3 embryo toxicity, 96-hour  $LC_{50} = 699.5 \mu\text{g Pb/L}$  for larval mortality and  $LOEC = 96.7 \mu\text{g Pb/L}$  for  
4 significant increase of abnormal D-shaped larvae ([Xie et al., 2017](#)). In a series of fertilization bioassays  
5 with the marine polychaete broadcast spawner *Galeolaria caespitosa*, the  $EC_{10}$  for reproduction varied  
6 with the density of sperm used in the bioassays and ranged from 65 to 910  $\mu\text{g Pb/L}$ . The toxicity of Pb  
7 was significantly decreased at higher sperm density ([Lockyer et al., 2019](#)). The  $EC_{10}$  was calculated to be  
8 30  $\mu\text{g Pb/L}$  at a sperm density required to achieve 50% of the maximum fertilization.

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

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

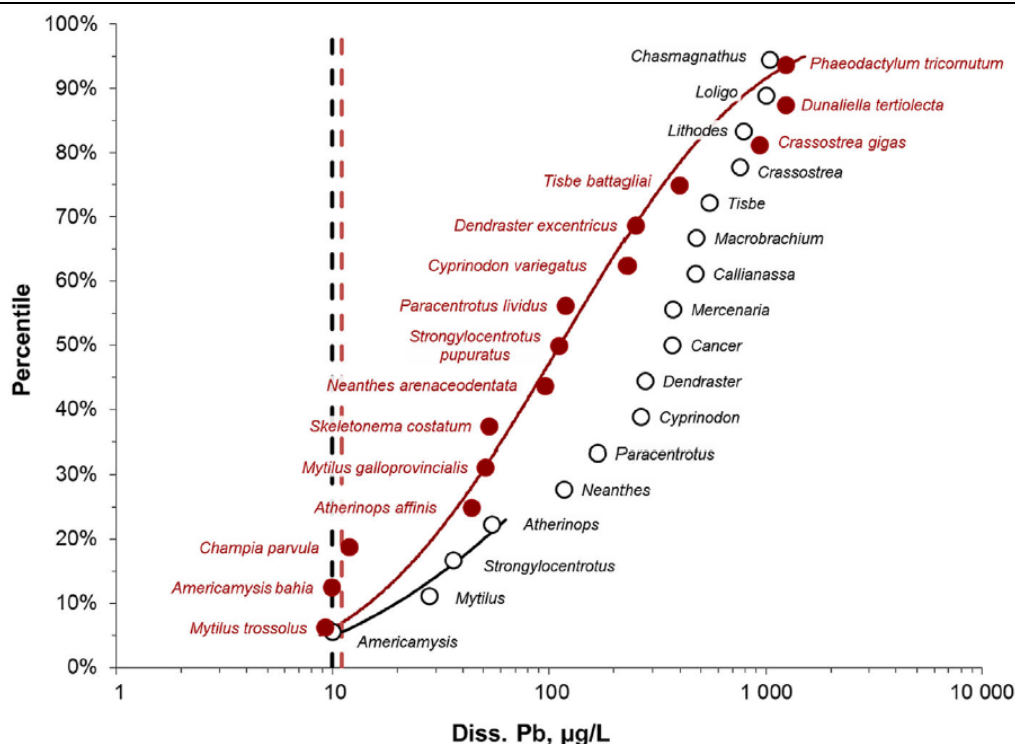


Dissolved Pb and triangular distribution fit to the four lowest genus mean acute values following U.S. EPA guidelines. Genus mean acute values (red circles); solid curved line = triangular distribution; dashed vertical line = final acute value; solid vertical line = criterion maximum concentration (proposed 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<sub>20</sub> or, in some cases, EC<sub>50</sub> data divided by a factor of two when EC<sub>20</sub> 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 (*Mytilus* 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 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<sub>20</sub> = 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)
<b>Invertebrates</b>						
Mussel ( <i>Mytilus galloprovincialis</i> )	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-h postfertilization) and sea urchin (to 72-h postfertilization) conducted using ASTM protocols in 100% sea water	DOC: 1.79 ± 0.02 mg/L Additional toxicity tests were conducted with added DOC	<b>Reproduction:</b> Development of larvae: The percentage of embryos exhibiting normal development was assessed after 48-h (mussels) or 72-h (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	<b>M. galloprovincialis</b> 48-h EC <sub>50</sub> = 63 µg Pb/L 48-h EC <sub>20</sub> = 19 µg Pb/L 48-h EC <sub>10</sub> = 10 µg Pb/L 48-h NOEC = 3.2 µg Pb/L	<a href="#">Nadella et al. (2013)</a>
Mussel ( <i>Mytilus trossulus</i> )			Salinity: 33 ppt Developmental assays conducted over a range of salinities from 15–33 ppt			
Sea urchin ( <i>Strongylocentrotus purpuratus</i> )			Temperature: 20°C ± 1°C for mussels 15°C ± 1°C for sea urchin		<b>M. trossulus</b> 48-h EC <sub>50</sub> = 45 µg Pb/L 48-h EC <sub>20</sub> = 16 µg Pb/L 48-h EC <sub>10</sub> = 9 µg Pb/L 48-h NOEC = 3.4 µg Pb/L  <b>S. purpuratus</b> 72-h EC <sub>50</sub> = 74 µg Pb/L 72-h EC <sub>20</sub> = 31 µg Pb/L 72-h EC <sub>10</sub> = 19 µg Pb/L 72-h NOEC = 2.7 µg Pb/L	
Scallop ( <i>Argopecten purpuratus</i> )	7 (control), 25, 50, 100, 140, 570, 730, 1000,	48-h embryo-larval development assay with Pb-nitrate conducted in 100%	Salinity: 35 ppt	<b>Reproduction:</b> Embryos exhibited abnormal development (impaired D-larvae	Embryo: 48-h EC <sub>50</sub> = 44 µg Pb/L	<a href="#">Romero-Murillo et al. (2018)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
	1590 µg Pb/L (measured)	sea water. In addition, a 96-h acute toxicity test was conducted with juveniles (21 mm in shell length)	pH: 8.0  Temperature (embryo exposure) 19°C ± 1°C	development) following Pb exposure. <b>Survival:</b> Assessed in juvenile lifestage only	Juvenile: 96-h LC <sub>50</sub> = 1,420 µg Pb/L	
Oyster ( <i>Magallana gigas</i> )	Each test with 1.5 to 2-h-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-h 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%	<b>Reproduction:</b> 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	<b><i>M. gigas</i></b> 48-h EC <sub>50</sub> = 49.5 µg Pb/L 48-h NEC = 9.9 µg Pb/L	<a href="#">Markich (2021)</a>
Oyster ( <i>Saccostrea glomerata</i> )			pH: 7.85 ± 0.05		<b><i>S. glomerata</i></b> 48-h EC <sub>50</sub> = 52.1 µg Pb/L 48-h NEC = 10.1 µg Pb/L	
Mussel ( <i>Xenostrobus securis</i> )			Temperature: 21°C ± 1°C		<b><i>X. securis</i></b> 48-h EC <sub>50</sub> = 59.9 µg Pb/L 48-h NEC = 12 µg Pb/L	
Scallop ( <i>Scaechlamys livida</i> )			DO: 80 to 95% saturation		<b><i>S. livida</i></b> 48-h EC <sub>50</sub> = 67.2 µg Pb/L 48-h NEC = 13.7 µg Pb/L	
Cockle ( <i>Anadara trapezia</i> )					<b><i>A. trapezia</i></b> 48-h EC <sub>50</sub> = 84.9 µg Pb/L 48-h NEC = 16.8 µg Pb/L	
Cockle ( <i>Fulvia tenuicostata</i> )					<b><i>F. tenuicostata</i></b> 48-h EC <sub>50</sub> = 108 µg Pb/L 48-h NEC = 22.3 µg Pb/L	
Clam ( <i>Hiatula alba</i> )						

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
Clam ( <i>Barnea australasiae</i> )					<b><i>H. alba</i></b> 48-h EC <sub>50</sub> = 129 µg Pb/L 48-h NEC = 24.8 µg Pb/L	
Clam ( <i>Spisula trigonella</i> )					<b><i>B. australasiae</i></b> 48-h EC <sub>50</sub> = 140 µg Pb/L 48-h NEC = 28 µg Pb/L	
Clam ( <i>Irus crenatus</i> )					<b><i>S. trigonella</i></b> 48-h EC <sub>50</sub> = 177 µg Pb/L 48-h NEC = 36.7 µg Pb/L  <b><i>I. crenatus</i></b> 48-h EC <sub>50</sub> = 196 µg Pb/L 48-h NEC = 39.8 µg Pb/L	
Prawn ( <i>Penaeus monodon</i> )	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 ± 0.6mg/L  pH: 7.1 ± 0.5	<b>Survival:</b> Survival of <i>P. monodon</i> was significantly decreased at the higher exposure concentrations	30-d NOEC = 14 µg Pb/L 30-d LOEC = 29 µg Pb/L	<a href="#">Hariharan et al. (2012)</a>
<b>Vertebrates</b>						
Topsmelt ( <i>Atherinops affinis</i> )	<b>Measured: Mean ± SD</b>	Larval fish (≤ 3 day old) were tested in two different salinities	<b>Low Salinity larval fish:</b>	<b>Survival:</b> Pb was consistently more toxic to larva fish at the lower salinity	28-d survival of larval fish at 14 ppt salinity	<a href="#">Reynolds et al. (2018)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
	Low salinity, larval fish: Total Pb BDL, 17 ± 1 µg Pb/L, 34 ± 1 µg Pb/L, 69 ± 4 µg Pb/L, 8 5 ± 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 , 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 ,	(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)	Salinity 14.1 ± 0.1 ppt Temperature: 18.2 ± 0.3°C Alkalinity: 58 ± 5 mg/L as CaCO <sub>3</sub> pH: 7.96 ± 0.17 DO: 7.58 ± 0.39  <b>High Salinity larval fish:</b> Salinity 28.1 ± 0.6 ppt Temperature: 18.1 ± 0.2°C Alkalinity: 105 ± 8 mg/L as CaCO <sub>3</sub> pH: 7.92 ± 0.07 DO: 6.88 ± 0.60	(14 ppt) compared with the higher salinity and larvae were more sensitive than juvenile fish at 28 ppt. Free Pb <sup>2+</sup> ion concentrations, the most bioavailable form of Pb, were higher in the lower salinity water based on Pb speciation calculations. <b>Growth:</b> Growth effects in larval fish (assessed as standard length) were more pronounced at the lower salinity (EC <sub>10</sub> = 16.4 µg Pb/L) compared with the higher salinity (EC <sub>10</sub> = 82.4 µg Pb/L)	LC5 = 7.7 µg Pb/L LC <sub>10</sub> = 8.3 µg Pb/L LC15 = 9.9 µg Pb/L LC <sub>20</sub> = 10.7 µg Pb/L LC25 = 11.5 µg Pb/L LC40 = 13.6 µg Pb/L LC <sub>50</sub> = 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 <sub>10</sub> = 43.4 µg Pb/L LC15 = 48.8 µg Pb/L LC <sub>20</sub> = 53.5 µg Pb/L LC25 = 58.0 µg Pb/L  LC40 = 70.8 µg Pb/L LC <sub>50</sub> = 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 <sub>10</sub> = 110.9 µg Pb/L LC15 = 116.8 µg Pb/L LC <sub>20</sub> = 123 µg Pb/L LC25 = 129.5 µg Pb/L	

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
	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				LC40 = 151.2 µg Pb/L LC50 = 167.6 µg Pb/L  28-d EC <sub>10</sub> 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 ( <i>Mugil cephalus</i> )	7, 16, 34, 65, 136 µg Pb/L ( <i>M. cephalus</i> )	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 DO: 6.5 ± 0.6	<b>Survival:</b> Survival of <i>M. cephalus</i> and <i>T. jarbua</i> decreased with the increase in exposure concentrations	Grey mullet: 30-d NOEC = 14 µg Pb/L 30-d LOEC = 29 µg Pb/L	<a href="#">Hariharan et al. (2016)</a>
Tiger perch ( <i>Terapon jarbua</i> )	7, 15, 29, 60, 118 µg Pb/L ( <i>T. jarbua</i> )				Tiger perch: 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	Pb was analytically verified in all studies	U.S. EPA guidelines ( <a href="#">U.S. EPA, 1985b</a> ) were used to identify acceptable studies. Acute: All included assays were embryo-larval toxicity studies reporting 48 to 96-h EC <sub>50</sub> s. The four		<b>Acute toxicity endpoints included survival, immobilization, and embryo-larval development</b> The proposed updated acute criterion is lower than the current EPA acute saltwater criterion of 210 µg Pb/L due to embryo-larval	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.	<a href="#">Church et al. (2017)</a>

Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
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)		lowest genus mean acute values ( <i>Strongylocentrotus purpuratus</i> = 75 µg Pb/L; <i>Mytilus</i> spp = 123 µg Pb/L; <i>Paracentrotus lividus</i> = 363 µg Pb/L; and <i>Dendraster excentricus</i> = 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 <sub>20</sub> from life cycle tests or EC <sub>50</sub> data divided by a factor of 2 when EC <sub>20</sub> data could not be calculated and augmented with 48-h toxicity data in some cases. The four lowest genus mean chronic values ( <i>Americamysis bahia</i> = 10 µg Pb/L; <i>Mytilus</i> spp. = 28 µg Pb/L;		toxicity tests with sensitive echinoderm and mussel species.  <b>Chronic toxicity endpoints included survival, growth, development, and reproduction</b>  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 chronic criterion has decreased due to increased availability of studies; an acute-to-chronic ratio was not used.	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	



Species	Concentration	Exposure Method	Modifying Factors	Effects on Endpoint	Effect Concentration	Reference (Published since the 2013 Pb ISA)
		<i>Strongylocentrotus purpuratus</i> = 36 µg Pb/L; <i>Atherinops affinis</i> = 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 EPA guidelines				

ASTM = American Society for Testing and Materials; BDL = below the method detection limit; CaCO<sub>3</sub> = calcium carbonate; DO = dissolved oxygen; DOC = dissolved organic carbon; EC = effect concentration; LC = lethal concentration; LOEC = lowest observed effect concentration; mo = months; NEC = no-effect concentration; NOEC = no observed effect concentration; Pb = lead.

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#### 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, 2006a](#)) 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 mesocosm and observational 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.

Reductions in benthic foraminiferal and meiofaunal community richness, diversity, and the abundance of certain taxa under Pb exposure were supported by a mesocosm study conducted in Italy ([Frontalini et al., 2018](#)). Sediment was collected from a relatively undisturbed coastal area and placed in mesocosms with artificial seawater exposed to nominal concentrations of 0, 10, 100, 200, 500, 1000, 5000, or 10000 µg Pb/L. Geochemical parameters, meiofaunal communities, and foraminiferal communities were sampled after 1, 2, 3, 4, 6, and 8 weeks. Meiofaunal density, richness, and Nematoda abundance decreased over time at higher concentrations of Pb. Although most meiofaunal taxa abundance and richness decreased in a dose-dependent manner with increasing Pb concentrations in the sediment, Ostracoda exhibited an increase in abundance with increasing Pb sediment concentration. Pb water concentration was negatively correlated with Bivalvia abundance, while positive correlations were found between Pb water content and Gastropoda, Copepoda, and Polychaeta. Finally, the abundance of most benthic foraminiferal species, the Shannon-Weiner diversity index, and Pielou's evenness, were negatively correlated with Pb concentrations in the sediment, whereas positive correlations were observed with *Ammonia tepida* and *Bolivina spathulata*.

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 ([Martinez-Colon 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  $36.98 \pm 11.18$  mg Pb/kg (range: 13.5–62.9 mg Pb/kg). Trace metal concentrations in the sediment of Pb,

1 Cu, Co, Cr, Ni, V and Zn were negatively correlated with the Shannon-Weaver index, Fisher  $\alpha$  index,  
2 species richness, and abundance of certain foraminiferal species. The CCA demonstrated that Pb  
3 explained 7.5% of variation in the foraminiferal community.

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

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

26 Pb enrichment factors were slightly positively correlated with *Ammonia* spp. (*Ammonia beccarii*,  
27 *A. gaimardii*, *A. tepida*, and *A. parkinsoniana*) and low-oxygen foraminiferal assemblages in the Gulf of  
28 Milazzo, Italy, but not to total deformed foraminifera, foraminiferal density, or the abundance of other  
29 foraminiferal taxa ([Cosentino et al., 2013](#)). Pb concentrations in the sediment ranged from 4.75 to  
30 49.19 mg Pb/kg. Finally, Pb and Al were negatively correlated with foraminiferal abundance across a  
31 gradient of sites in the Strait of Malacca, Malaysia ([Minhat et al., 2020](#)), with Pb showing the greatest  
32 enrichment among all metals, with values ranging from 8.8–29.2 mg Pb/kg. Overall, dissolved oxygen,  
33 depth, Al, and Pb concentrations explained the most variation in foraminiferal species distributions. The  
34 abundance of *Ammonia tepida*, which was the highest, was not significantly correlated with Pb sediment  
35 concentration, while those of *Bulimina marginata*, *Pararotalia ozawai*, and *Nonion subturngidum* were  
36 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  $68.27 \pm 22.14$  mg Pb/kg (mean  $\pm$  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](#); [Frontalini et al., 2018](#); [Martinez-Colon et al., 2018](#)). Other studies found positive or null correlations ([Barik et al., 2022](#); [Martinez-Colon et al., 2018](#); [Cosentino et al., 2013](#); [Martins et al., 2011](#)).

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