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FINAL

FRESHWATER AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA AND ACUTE SALTWATER BENCHMARK FOR

PERFLUOROOCTANE SULFONATE (PFOS)

September 2024

U.S. Environmental Protection Agency Office of Water, Office of Science and Technology, Health and Ecological Criteria Division

Washington, D.C.

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ACRONYMS

6:2 Cl-PFESA	6:2 chlorinated polyfluorinated ether sulfonate
ACR	Acute-to-Chronic Ratio
AFFF	Aqueous film-forming foams
AIC	Akaike information criteria
AMV	Acute Maximum Value
ASW	artificial sea water
AWQC	National Recommended Ambient Water Quality Criteria
BAF	Bioaccumulation factor
C8-PFPA	Perfluorooctyl phosphonic acid
C8/C8-PFPiA	Bis(perfluorooctyl) phosphinic acid
CAS/CASRN	Chemical Abstracts Service Registry Numbers
CC	Chronic Criterion
CCC	Criterion Continuous Concentration
C-F	carbon-fluorine
CMC	Criterion Maximum Concentration
C-R	concentration-response
C-S	carbon-sulfur
CWA	Clean Water Act
DER	Data Evaluation Record
DMSO	dimethyl sulfoxide
dpf	days post fertilization
drc	dose-response curve
dw	dry weight
ECF	Electrochemical fluorination
ECOTOX	ECOTOXicology database
ELS	Early life-stage
EPA	U.S. Environmental Protection Agency
EtFASAAs	*N*-ethyl perfluoroalkane sulfonamidoacetic acids
EtFASAs	*N*-ethyl perfluoroalkane sulfonamides
EtFOSAA	*N*-ethyl perfluorooctane sulfonamidoacetic acid
EtFOSE	*N*-ethyl perfluorooctane sulfonamidoethanol
FACR	Final Acute-to-Chronic Ratio
FASAAs	Perfluoroalkyl sulfonamidoacetic acids
FASAs	Perfluoroalkane sulfonamids
FASEs	perfluoroalkyl sulfonamidoethanols
FAV	Final Acute Value
FCV	Final Chronic Value
FFTG	Canadian Federal Fish Tissue Guideline
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FOSA	Perfluorooctane sulfonamide
FWQG	Federal Water Quality Guideline
GLI	U.S. EPA Great Lakes Initiative
GMAV	Genus Mean Acute Value
GMCV	Genus Mean Chronic Value

HC1	1% Hazardous Concentration
GSD	genus sensitivity distribution
hpf	hours post fertilization
ICE	Interspecies Correlation Estimation
K_{ow}	n-octanol-water partition co-efficient
LOD	limit of detection
LOEC	Lowest Observed Effect Concentration
LOQ	limit of quantification
MATC	Maximum Acceptable Toxicant Concentration
MC	Maximum Criterion
MDL	Method Detection Limit or Minimum Detection Limit
MDRs	minimum data requirements
NAMs	New Approach Methods
NCCA	National Coastal Condition Assessment
NOEC	No Observed Effect Concentration
NPDES	National Pollutant Discharge Elimination System
NRSA	National Rivers and Streams Assessment
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organization for Economic Co-operation and Development
ORD	Office of Research and Development
OSF	Octane sulfonyl fluoride
OW	Office of Water
PFAAs	Perfluoroalkyl acids
PFAS	Per- and polyfluorinated substances
PFCA	Perfluoroalkyl carboxylic acids or Perfluoroalkyl carboxylates
PFDA	Perfluorodecanoate or Perfluorodecanoic acid
PFdiCAs	Perfluoroalkyl dicarboxylic acids
PFdiSAs	Perfluoroalkane disulfonic acids
PFECAs	Perfluoroalkylether carboxylic acids
PFESAs	Perfluoroalkylether sulfonic acids
PFDoA	Perfluorododecanoate or Perfluorododecanoic acid
PFOA	Perfluorooctanoic acid or Perfluorooctanoate
PFOS	Perfluorooctane sulfonate or Perfluorooctane sulfonate acid
PFOSI	Perfluorooctane sulfinic acid
PFOS-K	PFOS potassium salt
PFOS-Li	PFOS lithium salt
PFPAs	Perfluoroalkyl phosphonic acids
PFPiAs	Perfluoroalkyl phosphinic acids
PFSAs	Perfluoroalkane sulfonic acids or Perfluoroalkyl sulfonates
PFSiAs	FASA *N*-glucuronides or Perfluoroalkyl sulfinic acids
pKa	Acid dissociation constant
POSF	Perfluorooctanesulfonyl fluoride
PPAR-α	Nuclear peroxisome proliferator activated receptor-alpha
ppt	parts per thousand
SMACR	Species Mean Acute-to-Chronic Ratio
SMAV	Species Mean Acute Value

SMCV	Species Mean Chronic Value
SNUR	Significant New Use Rules
SOP	Standard Operating Procedure
SSD	Species Sensitivity Distribution
TMDLs	Total Maximum Daily Loads
TSCA	Toxic Substances Control Act
U.S.	United States
UCMR	Unregulated Contaminant Monitoring Rule
web-ICE	Web-based Interspecies Correlation Estimation
WQS	Water Quality Standards
ww	wet weight
WWTPs	Wastewater treatment plants

NOTICES

This document provides information that states and authorized Tribes may consider when establishing water quality standards under the Clean Water Act (CWA) to protect aquatic life from effects of Perfluorooctane sulfonate (PFOS). Under the CWA, states and authorized Tribes establish water quality criteria to protect designated uses. State and Tribal decision makers retain the discretion to adopt approaches that are scientifically defensible that differ from these recommended criteria or benchmarks, including to reflect site-specific conditions. While this document contains the Environmental Protection Agency's (EPA) scientific recommendations regarding ambient concentrations of PFOS that protect aquatic life, the PFOS Criteria Document does not substitute for the Clean Water Act or the EPA's regulations; nor is this document or the values it contains a regulation itself. This document does not establish or affect legal rights or obligations, or impose legally binding requirements on the EPA, states, Tribes, or the regulated community. It cannot be finally determinative of the issues addressed. This document has been approved for publication by the Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use. This document can be downloaded from: https://www.epa.gov/wqc/aquatic-life-criteria-perfluorooctane-sulfonate-pfos.

FOREWORD

The Clean Water Act (CWA) Section 304(a)(l) (P.L. 95-217) directs the Administrator of the EPA to develop and publish water quality criteria recommendations that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including groundwater. This document includes EPA's recommended ambient water quality criteria (AWQC) for the protection of aquatic life based upon consideration of all available information relating to effects of perfluorooctanoic acid on aquatic organisms in freshwaters, as well as an informational acute saltwater benchmark developed under CWA Section 304(a)(2).

Aquatic life benchmarks, developed by the EPA under 304(a)(2) of the CWA, are informational values that EPA generates when there are limited high quality toxicity data available and data gaps exist for several aquatic organism families. EPA develops aquatic life benchmarks to provide information that states and Tribes may consider in their water quality protection programs, including when developing water quality standards. In developing aquatic life benchmarks, data gaps may be filled using new approach methods (NAMs), such as computer-based toxicity estimation tools (e.g., EPA's Web-ICE) or other new approach methods intended to reduce reliance on additional animal testing (https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-vertebrate-animals-chemical), including the use of read-across estimates based on other chemicals with similar structures. Like criteria recommendations developed under Section 304(a)(l), the EPA's aquatic life benchmark values are not regulatory, nor do they automatically become part of a state's water quality standards.

Under CWA Section 303, states or authorized Tribes adopt water quality standards and submit them to EPA for review and approval. If approved by EPA as water quality standards, they become the CWA water quality standards applicable in ambient waters within that state or authorized Tribe. A state or authorized Tribe may, where appropriate, adopt water quality criteria that have the same numerical values as recommended criteria or benchmarks developed by EPA under CWA Section 304. States and authorized Tribes have discretion to adopt criteria that modify EPA's recommended criteria to reflect site-specific conditions, such as the local water chemistry or ecological conditions, or to develop criteria based on other scientifically defensible methods that are protective of designated uses (40 C.F.R. 131.11[b]). Guidelines to assist the states and authorized Tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (see Chapter 3 titled "Water Quality Criteria")(U.S. EPA 2023).

Deborah G. Nagle
Director
Office of Science and Technology

EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) developed the recommended perfluorooctane sulfonate (PFOS) aquatic life ambient water quality criteria and an acute saltwater benchmark in accordance with the provisions of Section 304(a) of the Clean Water Act. This document provides the EPA's basis for and derivation of the national PFOS ambient water quality criteria recommendations to protect aquatic life. The EPA has derived the recommended PFOS aquatic life criteria and benchmark to be consistent with methods described in the EPA's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" (i.e., EPA's 1985 Guidelines; U.S. EPA 1985) and EPA's OCSPP's Ecological Effects Test Guidelines (U.S. EPA 2016b).

PFOS is an organic, human-made perfluorinated compound, consisting of an eight-carbon backbone and a sulfonate functional group. PFOS (and other related chemicals that are perfluoroalkane sulfonic acids) is used in a variety of industrial and commercial products, including surface treatments of soil, surface treatments of textiles, paper, and metals, and in specialized applications such as in firefighting foams. This document provides a critical review of all aquatic toxicity data identified in the EPA's literature search for PFOS, including the anionic form (CAS No. 45298-90-6), the acid form (CAS No. 1763-23-1), potassium salt (CAS No. 2795-39-3), an ammonium salt (CAS No. 56773-42-3), sodium salt (CAS No. 4021-47-0), and a lithium salt (CAS No. 29457-72-5). It also quantifies the toxicity of PFOS to aquatic life and provides criteria to protect aquatic life in freshwater from the acute and chronic toxic effects of PFOS.

The Aquatic Life Ambient Water Quality Criteria for PFOS document includes water column-based acute and a water column-based chronic criteria, as well as chronic tissue-based

criteria for freshwaters. Quantitatively-acceptable estuarine/marine toxicity data only fulfilled five of the eight minimum data requirements (MDRs) for deriving acute estuarine/marine criteria and four of the eight MDRs for deriving chronic estuarine/marine criteria per the 1985 Guidelines. The EPA did, however, include an acute aquatic life benchmark for estuarine/marine environments in Appendix L, using available estuarine/marine species toxicity data and the New Approach Methods (NAMs) application of the EPA Office of Research and Development's (ORD) peer-reviewed web-based Interspecies Correlation Estimate tool (Web-ICE; Version 3.3; https://www.epa.gov/webice/) (Raimondo et al. 2010). The estuarine/marine benchmarks are CWA Section 304(a)(2) information provided for states and authorized Tribes to consider in their state/tribal water quality protection programs. However, the acute estuarine/marine benchmark magnitude is less certain than the freshwater criteria as the benchmark was based on both direct laboratory-based and estimated PFOS acute toxicity data (Appendix L).

The freshwater acute water column-based criterion magnitude is 0.071 mg/L, and the chronic water column-based criterion magnitude is 0.00025 mg/L (250 ng/L). The final chronic freshwater criterion also contains tissue-based criteria with magnitudes of 0.201 mg/kg wet weight (ww) for fish whole-body, 0.087 mg/kg ww for fish muscle tissue, and 0.028 mg/kg ww for invertebrate whole-body tissue. All criteria are intended to be equally protective against adverse PFOS effects and are intended to be independently applicable. The three tissue criteria magnitudes (for fish and invertebrate tissues) are translations of the chronic water column criterion for freshwater using bioaccumulation factors (BAFs) derived from a robust national dataset of BAFs (Burkhard 2021). The assessment of the available data for fish, invertebrates, amphibians, and plants indicates these criteria are expected to protect the freshwater aquatic community.

Table Ex-1. Recommended Perfluorooctane Sulfonate (PFOS) Ambient Water Quality Criteria for the Protection of Aquatic Life in Freshwaters.

Type/Media	Acute Water Column (CMC)[1,4]	Chronic Water Column (CCC)[1,5]	Chronic Invertebrate Whole-Body[1,2]	Chronic Fish Whole-Body[1,2]	Chronic Fish Muscle[1,2]
Magnitude	0.071 mg/L	0.00025 mg/L	0.028 mg/kg ww	0.201 mg/kg ww	0.087 mg/kg ww
Duration	One-hour average	Four-day average	Instantaneous[3]		
Frequency	Not to be exceeded more than once in three years on average	Not to be exceeded more than once in three years on average	Not to be exceeded[6]		

[1] All five of these water column and tissue criteria are intended to be independently applicable and no one criterion takes primacy. All of the above recommended criteria (acute and chronic water column and tissue criteria) are intended to be protective of aquatic life. These criteria are applicable throughout the year.
[2] Tissue criteria are derived from the chronic water-column criterion magnitude (CCC) with the use of bioaccumulation factors and are expressed as wet weight (ww) concentrations.
[3] Tissue data provide instantaneous point measurements that reflect integrative accumulation of PFOS over time and space in aquatic life population(s) at a given site.
[4] Criterion Maximum Concentration; applicable throughout the water column.
[5] Criterion Continuous Concentration; applicable throughout the water column.
[6] PFOS chronic freshwater tissue-based criteria should not be exceeded, based on measured tissue concentrations representing the central tendency of samples collected at a given site and time.

Table Ex-2. Acute Perfluorooctane Sulfonate (PFOS) Benchmark for the Protection of Aquatic Life in Estuarine/Marine Waters.

Type/Media	Acute Water Column Benchmark
Magnitude	0.55 mg/L
Duration	One hour on average
Frequency	Not to be exceeded more than once in three years on average

1 INTRODUCTION AND BACKGROUND

National Recommended Ambient Water Quality Criteria (AWQC) are established by the EPA under the CWA. Section 304(a)(1) states that aquatic life criteria serve as recommendations to states and authorized Tribes by defining ambient water concentrations that are expected to protect against unacceptable adverse ecological effects to aquatic life resulting from exposure to pollutants found in water. States and authorized Tribes may adopt these criteria into their water quality standards (WQS) to protect the designated uses of water bodies. States and authorized Tribes may also modify these criteria before adopting these into standards. After adoption, states/authorized Tribes submit new and revised WQS to EPA for review and approval or disapproval. When approved by EPA, the state's/Tribes WQS become the application WQS for CWA purposes. Such purposes include identification of impaired waters and establishment of Total Maximum Daily Loads (TMDLs) under CWA Section 303(d) and derivation of water quality-based effluent limitations in permits issued under the CWA Section 402 National Pollutant Discharge Eliminations System (NPDES) programs. The EPA recommends the adoption of both the acute and chronic water column criteria as well as the chronic-tissue based criteria to ensure the protection of aquatic life through all exposure pathways, including direct aqueous exposure and bioaccumulation. Aquatic life benchmarks, developed by the EPA under 304(a)(2) of the CWA, are informational values that the EPA generates when there are limited high quality toxicity data available and data gaps exist for several aquatic organism families. The EPA provided an acute estuarine/marine benchmark in Appendix L as additional information on protective values that states and tribes may consider in their water quality programs.

This assessment provides a critical review of all aquatic toxicity data identified in the EPA's literature search for PFOS, including the anionic form (CAS No. 45298-90-6), the acid

form (CAS No. 1763-23-1), a potassium salt (CAS No. 2795-39-3), an ammonium salt (CAS No. 56773-42-3), a sodium salt (CAS No. 4021-47-0), and a lithium salt (CAS No. 29457-72-5). It quantifies the toxicity of PFOS to aquatic life and provides criteria to protect aquatic life in freshwater from the acute and chronic toxic effects of PFOS.

The EPA derived the recommended criteria using the best available data to reflect the latest scientific knowledge on the toxicological effects of PFOS to aquatic life. The EPA developed the criteria following the general approach outlined in the EPA's "*Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses"* (U.S. EPA 1985). The PFOS freshwater criteria, if adopted and implemented, are expected to be protective of most aquatic organisms, including species listed as threated and endangered, in the community and are derived to be protective of aquatic life designated uses established by states and Tribes for freshwaters. The estuarine/marine benchmarks are also intended to be protective of aquatic life designated uses, but as they are based on fewer empirical PFOS data have greater inherent uncertainty. The criteria recommendations presented herein are the EPA's best estimate of the concentrations of PFOS, with associated frequency and duration specifications, that would protect sensitive aquatic life from unacceptable acute and chronic effects.

1.1 Previously Derived PFOS Toxicity Values and Thresholds

Within the U.S., no states or Tribes have CWA Section 303(c) approved water quality standards for the protection of aquatic life from the exposure to PFOS. However, several states have published draft/interim acute and chronic ecological screening level values/benchmarks for the protection of aquatic life. As such, previously published PFOS acute and chronic criteria, benchmarks, and thresholds developed by states and international regulatory authorities were

identified, that included values for both freshwater and marine systems, and are summarized below.

1.1.1 Previously Published Acute Water Protective Values for Direct Aqueous Exposure

Previously published freshwater acute values were available for four states (Florida, Michigan, Minnesota, and Texas) and one geographic region (Europe). These publicly available values for other jurisdictions ranged from 0.021 mg/L in Texas (Giesy et al. 2010; TCEQ 2021) to 0.78 mg/L in Michigan (EGLE 2010). The EPA's freshwater acute PFOS criterion (0.071 mg/L) falls into the middle of the range of state- and European-derived values.

There were two previously derived estuarine/marine acute values, with a benchmark/criterion of 0.0072 mg/L in Europe (RIVM 2010) and 0.21 mg/L in Florida (Stuchal and Roberts 2019) (Table 1-1). These values were derived using safety factors. Consequently, these state values were both lower than the EPA's PFOS acute estuarine/marine benchmark (0.55 mg/L), which used measured and estimated acute toxicity data (Appendix L).

1.1.2 Previously Published Chronic Water Protective Values for Direct Aqueous Exposure

Previously published freshwater chronic values were available for five states (California, Florida, Michigan, Minnesota, and Texas) and three countries or geographic regions (Australia/New Zealand, Canada, and Europe). The publicly available state-derived values ranged from 0.00056 mg/L in California (RWQCB 2020; SERDP 2019; 99% species protection) to 0.14 mg/L in Michigan (EGLE 2010). Overall, the EPA's chronic water column-based PFOS criterion (0.00025 mg/L) is lower than chronic state-derived values because the EPA's chronic PFOS criterion was based on recently published and sensitive insect data.

Internationally, chronic PFOS protective values were 0.000023 mg/L in Europe (RIVM 2010), 0.00013 mg/L in Australia/New Zealand (CRCCare 2017; EPAV 2017) 95% species protection level), and 0.00680 mg/L in Canada (ECCC 2018) (Table 1-1). The EPA's chronic

water-column based PFOS criterion (0.00025 mg/L) is in the middle of the range of chronic PFOS values used in different international jurisdictions.

Previously published estuarine/marine chronic values were available for three states (California, Florida, and Texas) and two geographic regions (Australia/New Zealand and Europe). These publicly available values for other jurisdictions ranged from 0.000294 mg/L for Texas (CRCCare 2017; TCEQ 2021) to 0.013 mg/L in Florida (Stuchal and Roberts 2019) and were 0.0000046 mg/L in Europe (RIVM 2010) and 0.00013 mg/L in Australia/New Zealand (95% species protection; CRCCare 2017; EPAV 2017). The EPA did not derive a chronic PFOS criterion or benchmark for estuarine/marine water because of data limitations.

1.1.3 Previously Published Chronic Fish Tissue Criteria

There was a single previously derived fish tissue value for other jurisdictions. This value was a Canadian Federal Fish Tissue Guideline (FFTG) of 9.4 mg/kg whole-body wet weight (ww) (ECCC 2018). This value was derived by multiplying Canada's Federal Water Quality Guideline of 6.8 µg/L by a BAF of 1,378 L/kg. Canada's fish whole-body based Federal Water Quality Guideline (9.4 mg/kg ww) is significantly larger than the EPA's PFOS fish whole-body tissue criterion (0.201 mg/kg ww) because the EPA's value considered more recently published and relatively sensitive toxicity data that were not available at the time Canada's fish whole-body Water Quality Guideline was derived.

Table 1-1. Previously Derived PFOS Toxicity Values and Thresholds.

State / Country of Applicability	Aquatic Life Protective Value (mg/L unless otherwise indicated)	Criteria or Benchmark and Calculation Approach	Source
Freshwater Acute			
Some European Countries	0.036	Maximum Acceptable Concentration calculated using the lowest acute (LC50) value of 3.6 mg/L for mysid (*Americamysis bahia)* ÷ by assessment factor of 100. Dataset includes freshwater and marine aquatic species, combined.	RIVM (2010)
Texas	0.021	Acute surface water benchmark calculated using U.S. EPA Great Lakes Initiative (GLI; (U.S. EPA 1995)) Tier I Methodology as reported in (Giesy et al. 2010). This is an acute surface water benchmark and does not represent a CWA Section 303(c) approved water quality standard for PFOS.	Giesy et al. (2010); TCEQ (2021)
Minnesota	0.085	Maximum Criterion (MC) calculated as the acute curve-fitted and extrapolated 10-d EC50 for midge (*Chironomus tentans*) of 170 µg/L, which serves as the Final Acute Value or FAV followed by ÷ 2. This draft value does not represent a CWA Section 303(c) approved water quality standard for PFOS.	STS/MPCA (2007)
Florida	0.53	Secondary Acute Value (SAV) calculated using U.S. EPA Great Lakes Initiative (GLI; U.S. EPA 1995) Tier II Methodology. FAV calculated as the lowest GMAV (unspecified) divided by a safety factor of 6.1. This value was released in a White Paper sponsored by Florida Department of Environmental Protection and is considered a draft eco-based surface water screening level. It is not a CWA Section 303(c) approved water quality standard.	Stuchal and Roberts (2019)

State / Country of Applicability	Aquatic Life Protective Value (mg/L unless otherwise indicated)	Criteria or Benchmark and Calculation Approach	Source
Michigan	0.78	FAV of 1,557 µg/L calculated as the lowest Genus Mean Acute Value (GMAV) of 9,500 µg/L for fathead minnow (*Pimephales promelas*) ÷ by a safety factor of 6.1 (following U.S. EPA Great Lakes Initiative [(GLI; U.S. EPA 1995)]. The Acute Maximum Value (AMV) of 0.78 mg/L was then calculated as the FAV ÷ 2. This protective value is a translation of narrative water quality criteria and does not represent a CWA Section 303(c) approved water quality standard for PFOS.	EGLE (2010)
Marine Acute			
Some European Countries	0.0072	Maximum Acceptable Concentration calculated using the lowest acute value (LC_{50}) of 3.6 mg/L for a mysid (*Americamysis bahia*) ÷ by an assessment factor of 500. Dataset includes freshwater and marine aquatic species, combined.	RIVM (2010)
Florida	0.21	Secondary Acute Value (SAV) calculated using U.S. EPA Great Lakes Initiative (GLI; U.S. EPA 1995) Tier II Methodology. FAV calculated as the lowest GMAV (unspecified) divided by a safety factor of 21.9. This value was released in a White Paper sponsored by Florida Department of Environmental Protection and is considered a draft eco-based surface water screening level. It is not a CWA Section 303(c) approved water quality standard.	Stuchal and Roberts (2019)
Freshwater Chronic			
Some European Countries	0.000023	Maximum Permissible Concentration calculated using the lowest value (LOEC) of 0.0023 mg/L for *Chironomus tentans* (MacDonald et al. 2004) ÷ by an assessment factor (100). Dataset includes freshwater and marine aquatic species, combined.	RIVM (2010)

State / Country of Applicability	Aquatic Life Protective Value (mg/L unless otherwise indicated)	Criteria or Benchmark and Calculation Approach	Source
Canada	0.00680	Federal Water Quality Guideline (FWQG) calculated as the fifth percentile value from a Species Sensitivity Distribution (SSD) consisting of 20 species-specific values representing fish (5), amphibians (2), invertebrates (5), and plants and algae (8).	ECCC (2018)
Australia, New Zealand	0.00000023 (99% species protection - high conservation value systems)	Guidelines calculated from Species Sensitivity Distribution (SSD) consisting of 18 species-specific values for fish, amphibians, insects, crustaceans, and algae following the guidance of Warne et al. (2018) and Batley et al. (2014)	CRCCare (2017); EPAV (2017); HEPA (2020)
	0.00013 (95% species protection - slightly to moderately disturbed systems)		
	0.002 (90% species protection - highly disturbed systems)		
	0.031 (80% species protection - highly disturbed systems)		
California	0.00056 (99% species protection)	HC_1 calculated from an acute and chronic NOEC-based SSD as reported in SERDP Project ER18-1614 (SERDP 2019). Acute NOEC values were converted to chronic values using mean acute-to-chronic ratios derived from Giesy et al. (2010). This value represents an "Interim Final Environmental Screening Level" and does not represent a CWA Section 303(c) approved water quality Standard for PFOS.	RWQCB (2020); SERDP (2019)
Texas	0.0051	Acute surface water benchmark calculated using U.S. EPA Great Lakes Initiative (GLI; U.S. EPA 1995) Tier I Methodology as reported in Giesy et al. (2010). This is a chronic surface water benchmark and does not represent a CWA Section 303(c) approved water quality standard for PFOS.	Giesy et al. (2010); TCEQ (2021)

State / Country of Applicability	Aquatic Life Protective Value (mg/L unless otherwise indicated)	Criteria or Benchmark and Calculation Approach	Source
Minnesota	0.019	Chronic Criterion (CC) calculated as the FAV (170 µg/L) ÷ FACR (9.12) per Minnesota Rules Chapter 7050. Two species-specific ACRs and a default ACR were used to calculate the FACR. This draft value does not represent a CWA Section 303(c) approved water quality standard for PFOS.	STS/MPCA (2007)
Florida	0.037	Secondary Chronic Value (SCV) calculated using U.S. EPA Great Lakes Initiative (GLI; U.S. EPA 1995) Tier II Methodology with acute-to-chronic (ACR) of 14.5. SCV = SAV (530 µg/L) ÷ ACR (14.5) = 37 µg/L. This value was released in a White Paper sponsored by Florida Department of Environmental Protection and is considered a draft eco-based surface water screening level. It is not a CWA Section 303(c) approved water quality standard.	Stuchal and Roberts (2019)
Michigan	0.14	Final Chronic Value (FCV) calculated as the FAV (1,557 µg/L) ÷ FACR (11.35) per U.S. EPA Great Lakes Initiative (GLI; U.S. EPA 1995). Two species-specific ACRs and a default ACR were used to calculate the FACR. This protective value is a translation of narrative water quality criteria and does not represent a CWA Section 303(c) approved water quality standard for PFOS.	EGLE (2010)
Marine Chronic			
Australia, New Zealand	0.00000023 (99% species protection - high conservation value systems)	Guidelines calculated from SSD following the guidance of Warne et al. (2018) and Batley et al. (2014) and consisting of nine species-specific values representing fish (2), echinoderms (2), crustaceans (2), mollusc (1), and algae (2). Note: Per HEPA (2020) freshwater values are to be used on an interim basis until final marine guideline values can be set using the nationally agreed process under the Australian and New Zealand Guidelines for Fresh and Marine Water Quality	CRCCare (2017); EPAV (2017); HEPA (2020)
	0.00013 (95% species protection - slightly to moderately disturbed systems)		
	0.002 (90% species protection - highly disturbed systems)		
	0.031 (80% species protection - highly disturbed systems)		

State / Country of Applicability	Aquatic Life Protective Value (mg/L unless otherwise indicated)	Criteria or Benchmark and Calculation Approach	Source
Some European Countries	0.0000046	Maximum Permissible Concentration calculated using the lowest value (LOEC) of 0.0023 mg/L for *Chironomus tentans* divided by an assessment factor (500). Dataset includes freshwater and marine aquatic species, combined.	RIVM (2010)
Texas	0.000294	Default guidelines calculated from SSD following the guidance of Warne et al. (2018) and Batley et al. (2014) and consisting of nine of 16 species-specific values as reported in CRCCare (2017). This is a chronic surface water benchmark and does not represent a CWA Section 303(c) approved water quality standard for PFOS.	CRCCare (2017); TCEQ (2021)
California	0.0026 (99% species protection)	HC_1 calculated from an acute and chronic NOEC-based SSD as reported in SERDP Project ER18-1614 (2019). Acute NOEC values were converted to chronic values using mean acute-to-chronic ratios derived from Giesy et al. (2010). This value represents an "Interim Final Environmental Screening Level" and does not represent a CWA Section 303(c) approved water quality Standard for PFOS.	RWQCB (2020); SERDP (2019)
Florida	0.013	Secondary Chronic Value (SCV) calculated using U.S. EPA Great Lakes Initiative (GLI; U.S. EPA 1995) Tier II Methodology with acute-to-chronic (ACR) of 15.6. SCV = SAV (210 µg/L) ÷ ACR (15.6) = 13 µg/L. This value was released in a White Paper sponsored by Florida Department of Environmental Protection and is considered a draft eco-based surface water screening level. It is not a CWA Section 303(c) approved water quality standard.	Stuchal and Roberts (2019)
Fish Tissue			
Canada	9.4 mg/kg whole body ww fish tissue	Federal Fish Tissue Guideline (FFTG) where FFTG of 9.4 mg/kg ww = (FWQG of 6.8 µg/L) * (BAFgeomean of 1378 L/kg)	ECCC (2018)

1.2 Overview of Per- and Polyfluorinated Substances (PFAS)

PFOS, and its salts, belong to the per- and polyfluorinated substances (PFAS) group of chemicals. PFAS are a large group of structurally diverse anthropogenic chemicals that include PFOA, PFOS, and thousands of other fully or partially fluorinated chemicals. There are many families or subclasses of PFAS, and each contains many individual structural homologues and can exist as either branched-chain or straight-chain isomers (Buck et al. 2011; U.S. EPA 2021a). These PFAS families can be divided into two primary categories: non-polymers and polymers. The non-polymer PFAS include perfluoroalkyl and polyfluoroalkyl substances. Polymer PFAS include fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers (Table 1-2). Several U.S. federal, state, and industry stakeholders as well as European entities have posited various definitions of what constitutes a PFAS. OECD, an international organization comprised of 38 countries, recently published practical guidance regarding the terminology of PFAS (U.S. EPA 2021a). The OECD-led "Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance" workgroup provided an updated definition of PFAS, originally posited in part by Buck et al. (2011), as follows: "PFASs are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e. with a few noted exceptions, any chemical with at least a perfluorinated methyl group (–CF3) or a perfluorinated methylene group (–CF2–) is a PFAS". It is not within the scope of this framework to compare and contrast the various definitions, or the nuances associated with defining or scoping PFAS; rather the reader of this document is referred to OECD (2021) for review. Generally, the structural definition of PFAS includes chemicals that contain at least one of the following three structures:

- R-(CF_2)-CF(R′)R″, where both the CF_2 and CF moieties are saturated carbons, and none of the R groups can be hydrogen (TSCA draft definition);
- R-CF_2OCF_2-R′, where both the CF_2 and CF moieties are saturated carbons, and none of the R groups can be hydrogen; and
- $CF_3C(CF_3)$R′R ″, where both the CF_2 and CF moieties are saturated carbons, and none of the R groups can be hydrogen.

It should also be noted that what defines or constitutes a PFAS may change or evolve over time and under different purviews (e.g., federal, state, international).

Table 1-2. Two Primary Categories of PFAS[1].

PFAS Non-polymers	Structural Elements	Example PFAS Families
Perfluoroalkyl acids	Compounds in which all carbon-hydrogen bonds, except those on the functional group, are replaced with carbon-fluorine bonds	Perfluoroalkyl carboxylic and sulfonic acids (e.g., PFOA, PFOS), perfluoroalkyl phosphonic and phosphinic acids, perfluoroalkylether carboxylic and sulfonic acids
Polyfluoroalkyl acids	Compounds in which all carbon-hydrogen bonds on at least one carbon (but not all) are replaced with carbon-fluorine bonds	polyfluoroalkyl carboxylic acids, polyfluoroalkylether carboxylic and sulfonic acids
PFAS Polymers	**Structural Elements**	**Example PFAS Families**
Fluoropolymers	Carbon-only polymer backbone with fluorines directly attached	polytetrafluoroethylene, polyvinylidene fluoride, fluorinated ethylene propylene, perfluoroalkoxyl polymer
Polymeric perfluoropolyethers	Carbon and oxygen polymer backbone with fluorines directly attached to carbon	F-(CmF2mO-)nCF3, where the CmF2mO represents -CF2O, -CF2CF2O, and/or -CF(CF3)CF2O distributed randomly along polymer backbone
Side-chain fluorinated polymers	Non-fluorinated polymer backbone with fluorinated side chains with variable composition	n:1 or n:2 fluorotelomer-based acrylates, urethanes, oxetanes, or silicones; perfluoroalkanoyl fluorides; perfluoroalkane sulfonyl fluorides

1: Amalgamation of information from Figure 9 of OECD (2021) and Buck et al. (2011).

PFOS belongs to the perfluoroalkyl acids (PFAAs) of the non-polymer perfluoroalkyl substances category of PFAS. PFAAs are among the most researched PFAS (Wang et al. 2017).

The PFAA family includes perfluoroalkyl carboxylic, sulfonic, sulfinic, phosphonic, and phosphinic acids (Table 1-3). PFAAs are highly persistent and are frequently found in the environment (Ahrens 2011; Wang et al. 2017). PFAAs may dissociate to their anions in aqueous environmental media, soils, or sediments depending on their acid strength (pK_a value). The protonated and anionic forms may have different physiochemical properties.

Table 1-3. Classification and Chemical Structure of Perfluoroalkyl Acids (PFAAs).[1]

Classification	Functional Group	Examples
Perfluoroalkyl carboxylic acids (PFCAs) Or Perfluoroalkyl carboxylates (PFCAs)	-COOH	Perfluorooctanoic acid (PFOA)
	$-COO^-$	Perfluorooctanoate (PFOA)
Perfluoroalkane sulfonic acids (PFSAs) Or Perfluorokane sulfonates (PFSAs)	$-SO_3H$	Perfluorooctane sulfonic acid (PFOS)
	$-SO_3^-$	Perfluorooctane sulfonate (PFOS)[2]
Perfluoroalkyl sulfinic acids (PFSIAs)	$-SO_2H$	Perfluorooctane sulfinic acid (PFOSI)
Perfluoroalkyl phosphonic acids (PFPAs)	$-P(=O)(OH)_2$	Perfluorooctyl phosphonic acid (C8-PFPA)
Perfluoroalkyl phosphinic acids (PFPiAs)	$-P(=O)(OH)(C_mF_{2m+1})$	Bis(perfluorooctyl) phosphinic acid (C8/C8-PFPiA)
Perfluoroalkylether carboxylic acids (PFECAs)	$CF_3(OCF_2)_nCOOH$	Perfluoro (3,5,7-trioxaoctanoic) acid
Perfluoroalkylether sulfonic acids (PFESAs)	$CF_3(OCF_2)_nSO_3H$	6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA)
Perfluoroalkyl dicarboxylic acids (PFdiCAs)	$HOOC-C_nF_{2n}-COOH$	9:3 Fluorotelomer betaine
Perfluoroalkane disulfonic acids (PFdiSAs)	$HO_3S-C_nF_{2n}-SO_3H$	Perfluoro-1,4-disulfonic acid

[1] Modified from Buck et al. (2011); OECD (2021).

[2] The anionic form is most prevalent in the aquatic environment.

Perfluoroalkane (or -alkyl) sulfonic acids (PFSAs), including PFOS, consist of a general chemical structure (of $C_nF_{2n+1}SO_3H$ for PFOS; see Figure 1-1). This chemical structure makes PFOS extremely strong and stable, and resistant to hydrolysis, photolysis, microbial degradation, and metabolism (see Section 2.3) (Ahrens 2011; Beach et al. 2006; Buck et al. 2011). Furthermore, PFOS has been classified as persistent, bioaccumulative, and toxic (Ahrens 2011; Buck et al. 2011; Lindstrom et al. 2011; OECD 2002).

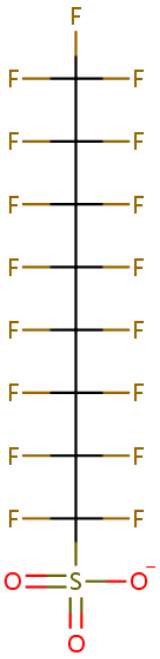

Figure 1-1. Chemical Structure of Linear Perfluorooctane Sulfonate (PFOS).
Source: United States EPA Chemistry Dashboard; https://comptox.epa.gov/dashboard

1.2.1 Physical and Chemical Properties of PFOS

Physical and chemical properties along with other reference information for PFOS are provided in Table 1-4. These physical and chemical properties help to define the environmental fate and transport of PFOS in the aquatic environment.

Table 1-4. Chemical and Physical Properties of PFOS.

Property	PFOS, acidic form[1]	Source
Chemical Abstracts Service Registry Number (CAS No.)	1763-23-1	
Chemical Abstracts Index Name	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid	
Synonyms	Perfluorooctane sulfonic acid; heptadecafluoro-1-octane sulfonic acid; PFOS acid; perfluorooctane sulfonate	
Chemical Formula	$C_8HF_{17}O_3S$	
Molecular Weight (grams per mole [g/mol])	500.13	Lewis (ed. 2004); HSDB (2012); SRC (2016)
Color/Physical State	White powder (potassium salt)	OECD (2002)
Boiling Point	258–260 °C	SRC (2016)
Melting Point	No data	
Vapor Pressure	2.0×10^{-3} millimeters Mercury (mm Hg) at 25°C (estimate)	HSDB (2012)
Henry's Law Constant	Not measurable; not expected to volatilize from aqueous solution ($< 2.0 \times 10^{-6}$)	ATSDR (2015)
K_{OW}	Not measurable	EFSA (2008); ATSDR (2015)
Organic carbon water partitioning coefficient (K_{OC})	2.57	Higgins and Luthy (2006)
Estimated pKa	3.27 (no empirical measurements available)	Brooke et al. (2004)
Solubility in Water	680 mg/L	OECD (2002)
Half-Life in Water	Stable	UNEP (2006)
Half-Life in Air	Stable	UNEP (2006)

[1] PFOS is commonly produced as a potassium salt (CAS No. 2795-39-3). Properties specific to the salt are not included.

PFOS is moderately water soluble, nonvolatile, and stable (Beach et al. 2006; Young and Mabury 2010). PFOS is solid at room temperature with a low vapor pressure. No direct measurement of the acid dissociation constant (pKa) is available. However, PFOS is considered to have a low pKa, which is based on a calculated pKa of 3.27 provided from Finland in a comment to Brooke et al. (2004). Therefore, PFOS is deemed to be a strong acid (Brooke et al. 2004). PFOS introduced as a salt will dissociate into ionic components when in natural water at a

neutral pH and is commonly present as a PFOS anion in solution (Beach et al. 2006; Giesy et al. 2010; Young and Mabury 2010). The PFOS anion forms strong ion pairs with many cations, resulting in less solubility in waters that contain great amounts of dissolved solids. Thus, PFOS solubility in saltwater is approximately 12 mg PFOS/L compared to 589 mg PFOS/L in pure water (Beach et al. 2006). PFOS is reported to have a mean solubility of 56 mg PFOS/L in pure octanol (OECD 2002). These solubility data suggest that any form of PFOS discharged into a water source tends to remain dissolved, unless the PFOS was sorbed to particulate matter or assimilated by organisms (which are both discussed further in Sections 2.2 and 2.5, respectively) (OECD 2002).

Due to the surfactant properties of PFOS, it forms three layers when added to octanol and water in a standard test system used to measure an n-octanol-water partition co-efficient (K_{ow}), thus preventing direct measurement (Giesy et al. 2010; OECD 2002). Although a K_{ow} cannot be directly measured, a K_{ow} for PFOS has been estimated from its individual water and octanol solubilities (Giesy et al. 2010); however, the veracity of such estimates is uncertain (OECD 2002). Lacking a reliable K_{ow} for PFOS precludes application of K_{ow}-based models commonly used to estimate various physiochemical properties for organic compounds, including bioconcentration factors and soil adsorption coefficients. Further, the unusual characteristics of PFOS would bring into question the use of K_{ow} as a predictor of environmental behavior. For example, bioaccumulation of PFOS is thought to be mediated via binding to proteins rather than partitioning into lipids (Giesy et al. 2010; OECD 2002), the latter being the theoretical basis for K_{ow}-based prediction of bioaccumulation.

PFOS is not expected to volatilize from aqueous solution based on its vapor pressure and predicted Henry's law constant $< 2.0 \times 10^{-6}$ (Beach et al. 2006). In 2002, OECD classified PFOS

as a type 2, non-volatile chemical that has a very low or possibly negligible volatility (Beach et al. 2006; Giesy et al. 2010; OECD 2002).

2 PROBLEM FORMULATION

A problem formulation provides a strategic framework for water quality criterion development under the CWA by focusing on the most relevant chemical properties and endpoints. In the problem formulation, the purpose of the assessment is stated, the problem is defined, and a plan for analyzing and characterizing risk is developed. The structure of this problem formulation is consistent with the EPA's Guidelines for Ecological Risk Assessment (U.S. EPA 1998).

2.1 Overview of PFOS Sources

2.1.1 Manufacturing of PFOS

PFOS is used in a variety of products including surface treatments for soil and stain resistance, coating of paper as part of a sizing agent formulation, and in specialized applications such as firefighting foams. PFOS is produced through electrochemical fluorination (ECF) in which an organic raw material, such as octane sulfonyl fluoride (OSF; $C_8H_{17}SO_2F$) in the case of PFOS, undergoes electrolysis in anhydrous hydrogen fluoride solution. This electrolysis leads to the replacement of all the hydrogen atoms by fluorine atoms and results in perfluorooctanesulfonyl fluoride (POSF; $C_8F_{17}SO_2F$), which is the major raw material used to manufacture PFOS (Figure 2-1; Buck et al. 2011). The base-catalyzed hydrolysis of POSF results in PFOS and its salts (Lehmler 2005). ECF results in a mixture of linear and branched chain perfluorinated isomers and homologues, with ratios of linear to branched perfluorinated carbon chains of roughly 70 to 80% linear and 20 to 30% branched for PFOS synthesis depending on how the process is controlled (De Voogt 2010). All compounds produced from POSF and other neutral PFAS with sufficient chain length and a sulfur group have the potential to degrade or transform into PFOS, and therefore have been considered to be "PFOS equivalents" and potential sources of PFOS to the aquatic environment (see Section 2.4)(Ahrens

2011; Lindstrom et al. 2011). PFOS is used in a variety of products including surface treatments for soil and stain resistance, coating of paper as part of a sizing agent formulation, and in specialized applications such as firefighting foams.

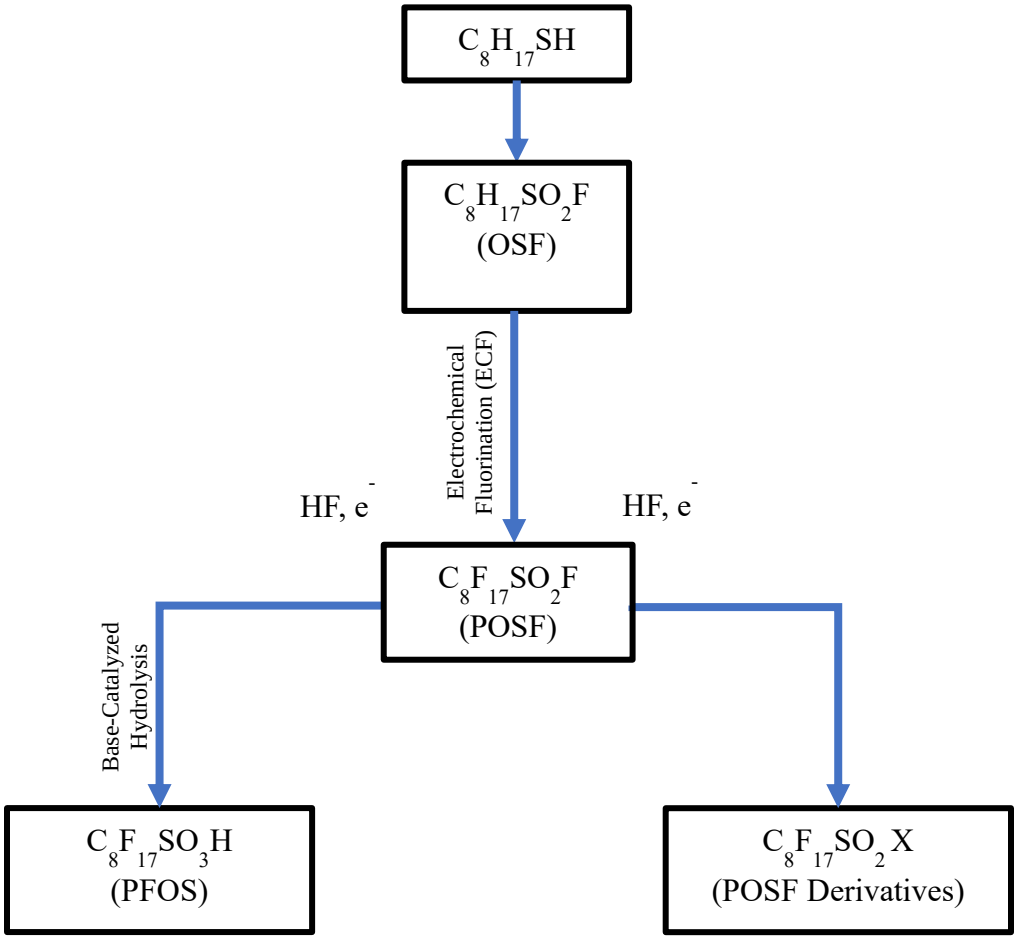

Figure 2-1. Synthesis of PFOS by electrochemical fluorination (ECF).
Modified from Buck et al. (2011).

The manufacture of PFOS started in 1949 with Minnesota Mining and Manufacturing (name changed later to the 3M Company) (3M Company 1999). Prior to 2000, the 3M Company was the major producer of POSF, the raw material used to make PFOS (Figure 2-1), with smaller producers in Europe and Asia (Paul et al. 2009; U.S. EPA 2000a). In 2000, the 3M company manufactured approximately 78% of the estimated global POSF production (approximately

3,665 tons of the 4,650 tons produced globally; (approximately 3,665 tons of the 4,650 tons produced globally; OECD 2002). The estimated total cumulative production of POSF is between 44,000 and 96,000 tons (Paul et al. 2009; Prevedouros et al. 2006; Smithwick et al. 2006). Information on previous and current production of POSF from Asia and other production sources is limited (Paul et al. 2009; Prevedouros et al. 2006; Smithwick et al. 2006).

In May 2000, following negotiations between the EPA and 3M, the 3M Company agreed to voluntary phase out and find substitutes for PFOS chemistry used to produce all but a few small applications (i.e., aqueous film-forming foams (AFFF), and hard chrome plating mist suppression) across their range of products by 2002 (Lindstrom et al. 2011; U.S. EPA 2000a). Starting around the same time, a series of Significant New Use Rules (SNUR) were also put into place by the EPA to restrict the production and use of materials that contain PFOS and its precursors in the U.S. (Lindstrom et al. 2011). In 2009, PFOS and related compounds were listed under Annex B of the Stockholm Convention on Persistent Organic Pollutants; restricting global manufacturing and use of PFOS (Ahrens 2011; OECD 2002). Homologues, neutral precursor compounds, and new classes of PFAS continue to be produced and therefore, are potential sources of PFOS (Ahrens 2011). Assuming there was no step-up production of PFOS and its precursors to offset the phase-out by the 3M Company, the production is estimated to be approximately 1,000 tons from 2002 and onward (Paul et al. 2009). However, while industrialized countries, like the U.S., phased-out the use of PFOS and its precursors, producers in other countries, such as China and Brazil, have scaled up their production to fill remaining demand (Wang et al. 2013). Despite the wide use in an array of industrial and consumer products globally, information on the sources, volumes, and emission of PFOS and its precursors are limited (Paul et al. 2009; Zhang et al. 2016).

2.1.2 Sources of PFOS to Aquatic Environments

Aquatic environments and soil are thought to serve as a reservoir of PFOS, with 42,000 tons emitted to aquatic environments compared to 235 tons released into to air between 1980 and 2002 (Paul et al. 2009). Unlike other contaminants commonly found in aquatic ecosystems, such as metals for example, PFAS are synthetic compounds with no natural source. Thus, the occurrence of any PFAS in the environment is an indication of anthropogenic sources (Ahrens 2011). The occurrence of PFOS in aquatic environments can be attributed to both point and non-point sources, entering aquatic environments from industrial and consumer products during manufacturing, along supply chains, and during product use and/or disposal (Ahrens 2011; Ahrens and Bundschuh 2014; Kannan 2011; Paul et al. 2009). However, quantitative assessments of PFOS production, point and non-point source discharges, and environmental measurements are limited compared to other persistent, bioaccumulative pollutants (Ahrens and Bundschuh 2014; Zhang et al. 2016).

Potential point sources of PFOS to the aquatic environment include both industrial facilities and municipal wastewater treatment plants (WWTPs). Additional point sources may include surface water runoff from industrial use sites such as metal plating facilities, areas that have received AFFF applications, landfills, and contaminated soils. Of these, industrial facilities, specifically those for fluorochemical manufacturing and other use facilities, are a primary source of PFOS to aquatic systems (Ahrens et al. 2011a; Houtz et al. 2016; Sedlak et al. 2017). Estimated total global releases to water arising from discharge of PFOS during manufacturing from 1970 to 2002 ranged between 230 and 1,450 tons (Paul et al. 2009).

Potential non-point PFOS sources to aquatic environments include: dry and wet atmospheric deposition, runoff from contaminated soils, runoff from metal plating facilities, the runoff or discharge of contaminated groundwater, particularly from the use of fire-fighting

foams, and land application of contaminated biosolids (Ahrens 2011; Kannan 2011; OECD 2002; Paul et al. 2009). Identification of non-point PFOS sources and understanding their relative contribution to aquatic ecosystems is difficult due to the lack of sufficient measured environmental data (Ahrens 2011; Paul et al. 2009). Overall, the presence of non-point PFOS sources and their relative contributions are reported to be dependent on the aquatic system, air, groundwater, and soil levels, and nearby land uses. For example, concentrations of PFAS, including PFOS, have been influenced by urban land use (Ahrens 2011; Zhang et al. 2016). Overall, PFOS occurrence in aquatic environments is driven by legacy PFOS sources since PFOS use in the United States was voluntarily phased out by 2002 and SNUR were put into place by the EPA to restrict the production and use of PFOS and its precursors (Lindstrom et al. 2011). However, PFAS concentrations in the environment in general continue to be positively correlated with human population density. For example, PFOS was detected in aquatic systems at elevated concentrations (ranging between 97 and 1,371 ng/L) in densely populated areas of the U.S. and Europe (Zhang et al. (2016) and Loos et al. (2009); respectively), and Paul et al. (2009) estimated the total global PFOS emissions to air and water from 1970 to 2009 resulting from consumer use and disposal to be between 420 and 2,100 tons.

Importantly, PFAS are still produced that can transform or degrade into compounds belonging to the PFSAs family, including PFOS (Ahrens 2011). PFAS precursors such as perfluoroalkyl sulfonamidoacetic acids (FASAAs) and perfluoroalkyl sulfonamidoethanols (FASEs) are known to metabolically transform and degrade to PFOS, respectively (Ahrens and Bundschuh 2014; Benskin et al. 2009; Boulanger et al. 2005b; Buck et al. 2011; Lange 2000; Liu and Mejia Avendano 2013; Plumlee et al. 2008; Rhoads et al. 2008; Wang et al. 2017). However, the understanding of these transformation processes is limited, and additional work is needed to

fully understand these processes and their role as sources of PFOS to aquatic environments (Buck et al. 2011; Lau et al. 2007; Liu and Mejia Avendano 2013; Wang et al. 2017). Degradation of precursors represents a potentially significant source of PFOS to the aquatic environment, particularly since PFOS production within the U.S. has not occurred since 2002 (Buck et al. 2011; Liu and Mejia Avendano 2013). Nevertheless, PFOS-treated articles, such as fabrics, paper, and other treated materials, are still being imported into the U.S. and are ultimately, at least in part, released into the environment (Allred et al. 2015; Lang et al. 2016; Liu et al. 2014d). The importation of PFOS treated articles is considered as production under the Toxic Substances Control Act (TSCA) (U.S. EPA 2020).

2.2 Environmental Fate and Transport of PFOS in the Aquatic Environment

2.2.1 Environmental Fate of PFOS in the Aquatic Environment

PFOS has low volatility in ionized form but can adsorb to particles in air where it can be transported globally, including remote locations (Benskin et al. 2012; Butt et al. 2010). PFOS is water soluble and has been found in surface water, ground water, and drinking water. Because of the relatively low K_{oc} of PFOS, it does not easily adsorb to sediments and tends to stay in the water column (Ahrens 2011; Beach et al. 2006; Giesy et al. 2010; Higgins and Luthy 2006).

PFOS can be re-emitted to aquatic environments from PFOS contaminated soil, groundwater, ice, and sediment (see Section 2.3). Sediment may be an important sink of PFOS in the aquatic environment (Ahrens 2011). The movement of PFOS between groundwater, surface water, and sediment depends on the chemical properties of PFOS and site-specific physiochemical characteristics (including pH, temperature, organic carbon content, and salinity) of the aquatic environment. In general, PFOS may sorb to sediments (with a Kd greater than 1 mL/g; (with a Kd greater than 1 mL/g; Giesy et al. 2010). However, this sorption to sediments is limited and PFOS has a K_{oc} of 2.57 indicating that PFOS is relatively mobile in water and the

physicochemical characteristics of the sediment ultimately influence the sorption of PFOS (Ahrens 2011; Higgins and Luthy 2006). While the release of PFOS from the transformation of other PFAS and historical products still in use (e.g., consumer goods manufactured, imported and/or obtained before the PFOS discontinuation and regulations) are expected to continue into the future, the re-emissions of PFOS from existing sinks are assumed to be decreasing since the restrictions and regulations of PFOS have gone into place (Ahrens 2011; Ahrens and Bundschuh 2014; Paul et al. 2009; Washington and Jenkins 2015; Washington et al. 2015).

In the water column, and other environmental compartments, PFOS is stable and resistant to hydrolysis, photolysis, volatilization, and biodegradation (see Appendix M)(Beach et al. 2006; OECD 2002). The persistence of PFOS has been attributed to the strong carbon-fluorine (C-F) bond. Additionally, there are limited indications that naturally occurring defluorinating enzymes exist that can break a C-F bond. Consequently, no biodegradation or abiotic degradation processes for PFOS are known. The physiochemical properties discussed in Table 1-4 result in PFOS being highly persistent in the aquatic environment (Ahrens 2011). In aquatic environments, the only dissipation mechanisms for PFOS are physical mechanisms, such as environmental dilution, offsite transport, plant uptake, and sorption.

2.2.2 Environmental Transport of PFOS in the Aquatic Environment

The environmental fate of PFOS, outlined in the previous section (Section 2.2.1) plays a role in the environmental transport of PFOS (Ahrens 2011). PFOS is either distributed in biota (via bioaccumulation discussed in Section 2.5) or abiotic matrices (such as water and sediment). Sediment in particular can act as a sink for PFOS. However, the role of sediment as a sink or source by resuspension is not well understood (Ahrens 2011).

The distribution of PFOS is widespread, including to remote regions despite the limited number of manufacturing facilities and/or small population sizes typically found in these areas

(Benskin et al. 2012; Butt et al. 2010). PFOS has been detected in water, sediment, and biota samples from aquatic environments in remote areas (Butt et al. 2010; Giesy and Kannan 2001; Houde et al. 2006b; Yamashita et al. 2008). To date, the dominant transport pathway for PFOS to remote regions has not been conclusively characterized and much of the focus has been on marine systems, with few studies in freshwater environments (Ahrens 2011; Butt et al. 2010; Giesy and Kannan 2002). Additionally, the relative importance of each potential transport pathway is difficult to accurately determine (Butt et al. 2010; Young and Mabury 2010). Many researchers suggest that the dominant mechanism of PFOS transport occurs through water as the anionic form of PFOS, which is the most commonly found form in the aquatic environment, is less volatile (see Section 2.2.1 above) and has a high water solubility. These characteristics make partitioning to and transport through the air less likely (Butt et al. 2010; Giesy and Kannan 2002). However, PFOS transport through water is likely the dominant mechanism on more local scales (e.g., within a waterbody or watershed), and is likely not the prevailing transport pathway of PFOS to remote regions given the considerations of the long distances. Instead, atmospheric transport is likely the main mechanism of PFOS transport to remote regions. Another potential source to remote regions is the indirect formation of PFOS through transformation of other PFAS, particularly volatile precursors (see Section 2.3)(Butt et al. 2010; Wang et al. 2015; Young and Mabury 2010).

Volatile PFOS precursors, which may reach remote locations via atmospheric deposition themselves, may subsequently be metabolized to PFOS in aquatic organisms (Giesy and Kannan 2002). In all likelihood, the continued presence of PFOS in remote areas may be due to multiple exposure pathways, including those caused by direct production and use of PFOS itself as well as degradation and transformation of precursor compounds (Armitage et al. 2009). To better

comprehend both environmental transport and exposure to PFOS, the following needs to be better understood: 1) the potential transformation, metabolism, and bioaccumulation of PFOS and its precursors (particularly partitioning behavior, such as tissue distribution and lipophilicity); 2) explicit biotransformation pathways and pharmacokinetics; and 3) atmospheric fate and transport of PFOS and its precursors (Armitage et al. 2009).

2.3 Transformation and Degradation of PFOS Precursors in the Aquatic Environment

Transformation and degradation processes of various PFAS are potential sources of PFOS to the aquatic environment (see Section 2.1.2 above). PFAS are still produced that can transform or degrade into compounds belonging to the PFSAs family of PFAS, including PFOS (Ahrens 2011). Thus, transformation and degradation of PFAS should be considered as an ongoing potential source of PFOS to the aquatic environment. Currently, the understanding of these transformation and degradation processes is limited, particularly for PFOS. There is little understanding of which PFAS and how much of each has been or will be released into the aquatic environment (Liu and Mejia Avendano 2013; Wang et al. 2017). Additional work is needed to fully understand the details of these processes and the occurrence of the compounds to better comprehend their role as a source of PFOS to aquatic environments (Lau et al. 2007).

These transformation and degradation pathways are dependent on environmental conditions, degradation kinetics, and the chemical structures and properties of the individual PFAS precursors and volatile PFAS (Buck et al. 2011; Butt et al. 2014; Liu and Mejia Avendano 2013). Of particular importance is the environmental stability of key chemical linkages (such as esters and ethers) as the stability of these chemical linkages determines the stability of the overall PFAS (Liu and Mejia Avendano 2013). The most well studied PFAS precursors are fluorotelomer-based compounds, which are produced through telomerization technology and are

associated with PFOA as the final product (Buck et al. 2011; Liu and Mejia Avendano 2013). In contrast, perfluoroalkane sulfonamido derivatives and other PFAS, such as side-chain-fluorinated polymers, are not as well studied.

It is essential to understand the biodegradation of volatile PFAS, such as perfluroalkane sulfonamido derivatives as their degradation is directly linked with PFOS generation in the environment (Liu and Mejia Avendano 2013). Most published studies on the degradation of perfluoroalkane sulfonamido derivatives focus on those with eight fluorinated carbons since PFOS is a final product (Buck et al. 2011). *N*-ethyl perfluorooctane sulfonamidoethanol (EtFOSE) in particular is the most commonly studied.

2.3.1 Degradation of perfluoroalkane sulfonamido derivatives

Perfluoroalkane sulfonamido derivatives, including perfluoroalkane sulfonamides, sulfonamidoethanols, sulfonamidoethyl acrylates, and sulfonamidoethyl methacrylates, are final products on their own and are important building blocks for further synthesis (Buck et al. 2011). The various derivatives have been found to degrade into PFSAs, such as PFOS when sufficient chain length is present, and are intermediates along the transformation pathway. These derivatives include members of the *N*-ethyl perfluoroalkane sulfonamidoacetic acids (EtFASAAs), *N*-ethyl perfluoroalkane sulfonamides (EtFASAs), perfluoroalkane sulfonamidoacetic acids (FASAAs), perfluoroalkane sulfonamids (FASAs), FASA *N*-glucuronides, and perfluoroalkane sulfinic acids (PFSiAs; Buck et al. 2011). Additionally, in the environment *N*-alkyl perfluoroalkane sulfonamidoethyl acrylates and methacrylates (and polymers based on them) may undergo hydrolysis of the ester linkages to produce *N*-alkyl perfluoroalkane sulfonamidoethanols (FASEs; Buck et al. 2011).

In particular, several studies have demonstrated that EtFOSE, a member of the *N*-alkyl perfluoroalkane sulfonamidoethanols of the perfluoroalkane sulfonamido substances, degrades

into PFOS (Benskin et al. 2009; Boulanger et al. 2005b; Hatfield 2001; Lange 2000; Plumlee et al. 2009; Rhoads et al. 2008). EtFOSE was a product of ECF and was a precursor compound for the synthesis of other products such as phosphate esters that were used to manufacture paper protectors (3M Company 1999). Several studies have investigated the degradation of EtFOSE and all found that it is prone to degradation (Benskin et al. 2009; Boulanger et al. 2005b; Hatfield 2001; Lange 2000; Plumlee et al. 2009; Rhoads et al. 2008).

The overall pathway of EtFOSE degradation was determined to be the major difference between these studies (Figure 2-2). Rhoads et al. (2008) determined that EtFOSA could undergo direct dealkylation to form perfluorooctane sulfonamide (FOSA; as shown by the red arrow in Figure 2-2). Lange (2000) suggested that PFOA could be formed as a minor end product through an abiotic one-electron transfer mechanism from perfluorooctane sulfinic acid (PFOSI; demonstrated by the blue arrow in (Figure 2-2). In contrast, the other studies did not find PFOA to be a degradation product (Benskin et al. 2013; Boulanger et al. 2005b; Rhoads et al. 2008). Further, in the aerobic biodegradation studies, the rate limiting step was determined to be the degradation of *N*-ethyl perfluorooctane sulfonamidoacetic acid (EtFOSAA) and consequently EtFOSAA was the major degradation product rather than PFOS (Liu and Mejia Avendano 2013). Nevertheless, the degradation of EtFOSE resulted in the formation of PFOS as one of the final degradation products. In contrast, in the abiotic degradation studies, PFOS and PFOSI were either present at trace concentrations or were not observed (Hatfield 2001; Plumlee et al. 2009). Instead FOSA was considered to be the stable end product (Plumlee et al. 2009). The differences in the degradation pathways observed in the literature can likely be attributed to environmental conditions (Buck et al. 2011; Liu and Mejia Avendano 2013). Nevertheless, these pathways demonstrated that degradation of EtFOSE resulted in the formation of PFOS and should be

considered a potential source of PFOS to the aquatic environment. However, currently the relative contribution of this potential source to the aquatic environment cannot be quantified (Buck et al. 2011; Liu and Mejia Avendano 2013).

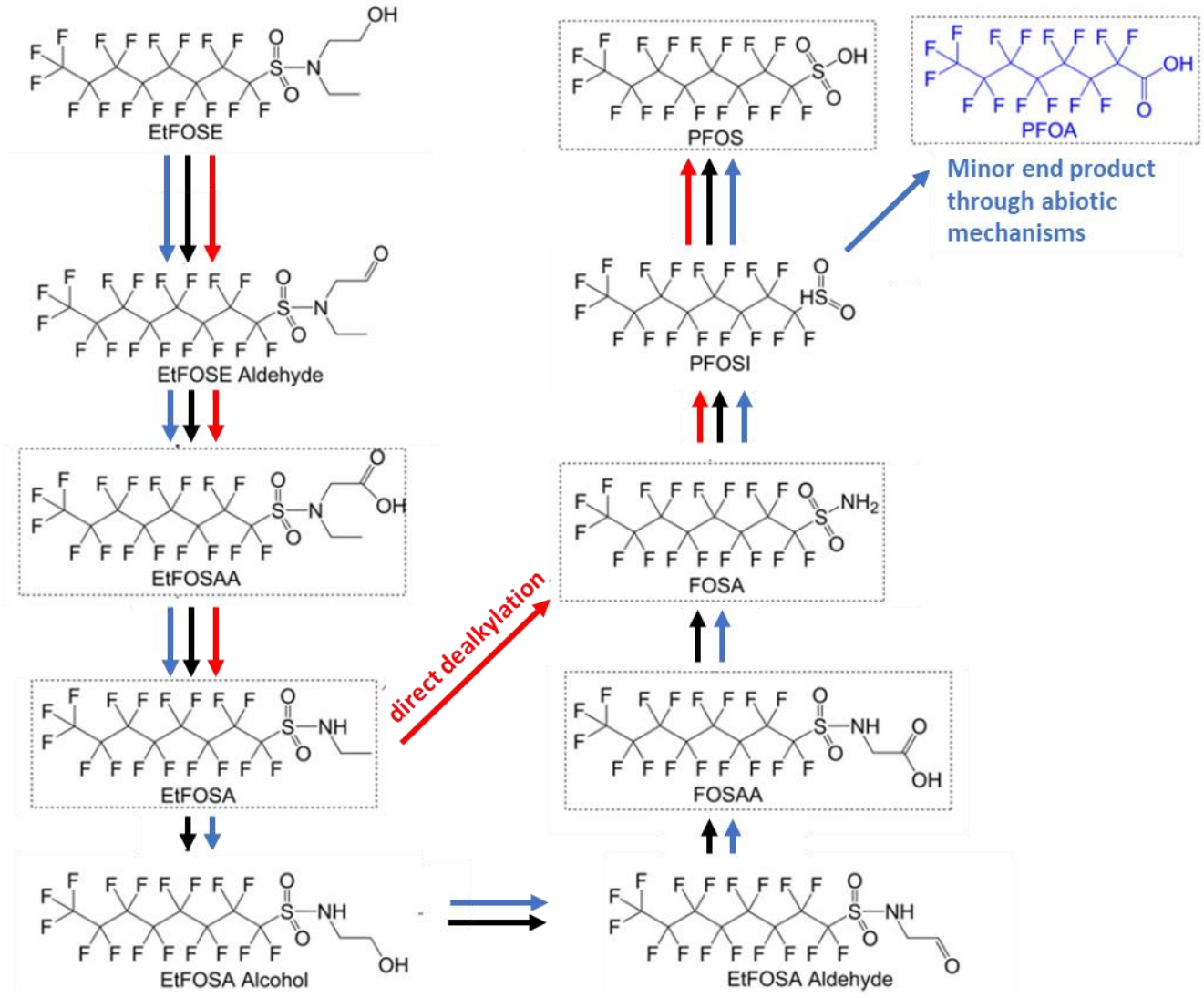

Figure 2-2. Aerobic Biodegradation of EtFOSE in Activated Sludge.
Black arrows show the Aerobic Biodegradation pathway as described by Liu and Mejia Avendano (2013). Blue pathway was observed by Lange (2000). Red pathway was observed by Rhoads et al. (2008). Semi-stable compounds are shown inside boxes.
Modified from: Liu and Mejia Avendano (2013).

2.3.2 Perfluorooctane sulfonamide-based side-chained polymers

In contrast to some other PFAS described in Section 1.2, fluorinated side-chain polymers do not have the per- or polyfluorinated backbone. Instead, fluorinated side-chain polymers consist of a variable composition with per- and polyfluoroalkyl side chains (Buck et al. 2011). The side chains of each of these polymer types may serve to transform into PFAS. Currently, little is known about these transformation processes (Liu and Mejia Avendano 2013). Given the high production volume of perfluorooctane-sulfonamide-based side-chain polymers prior to 2002, these fluorinated side-chain polymers may contribute to the levels of PFAS in the environment. It remains unknown how much these polymers contribute to the PFSAs in the environment (Liu and Mejia Avendano 2013). However, this transformation process is expected to occur over a long period of time (e.g., > 1,000 years) and may be a relatively small contributor of PFAS, including PFOS, in the environment (Buck et al. 2011).

2.3.3 Fluoroalkyl surfactants used in AFFFs

The release of AFFF during firefighting activities has been determined to be a substantial source of PFOS to the aquatic environment (see Section 2.1.2). Since 2002, fluorinated alternatives to PFAAs have been used to manufacture AFFF (Buck et al. 2011; Wang et al. 2013). The ten classes of AFFF chemicals have been identified and show that the new formulations of AFFF include the eight carbon perfluoroalkyl moiety (Place and Field 2012). Some of these fluorinated alternatives may undergo transformation and degradation processes and therefore may contribute to the levels of PFOS occurring in the aquatic environment (Liu and Mejia Avendano 2013). However, additional details about the transformation and degradation processes, including specific transformation pathways, the time to undergo transformation to produce a final product, and the influence of the environmental condition, are lacking at this time (Liu and Mejia Avendano 2013; Wang et al. 2013).

2.4 Environmental Monitoring of PFOS in Abiotic Media

PFOS has been detected in a variety of environmental abiotic matrices in aquatic environments around the globe. These abiotic media include surface water, soils, sediments, groundwater, air, and ice caps (Butt et al. 2010; Lau et al. 2007). Water is expected to be the primary environmental medium in which PFOS is found (Lau et al. 2007). Occurrence and detection of PFOS in surface waters is described below and occurrence in other abiotic media is described in Appendix N.

2.4.1 PFOS Occurrence and Detection in Ambient Surface Waters

2.4.1.1 Summary of PFOS occurrence and concentrations across the U.S.

PFOS is one of the dominant PFAS detected in aquatic ecosystems, along with PFOA (Ahrens 2011; Benskin et al. 2012; Dinglasan-Panlilio et al. 2014; Nakayama et al. 2007; Remucal 2019; Zareitalabad et al. 2013). Despite its wide use and persistence in the aquatic environment, current information on the distribution of PFOS in surface waters of the U.S. is relatively limited (Jarvis et al. 2021). Available data are largely collected from freshwater systems in eastern states, with most of the current, published PFOS occurrence data focused on a handful of study areas with known manufacturing or industrial uses of PFAS and among areas of known AFFF use, such as fire-training areas on military bases (Figure 2-3 and Appendix N).

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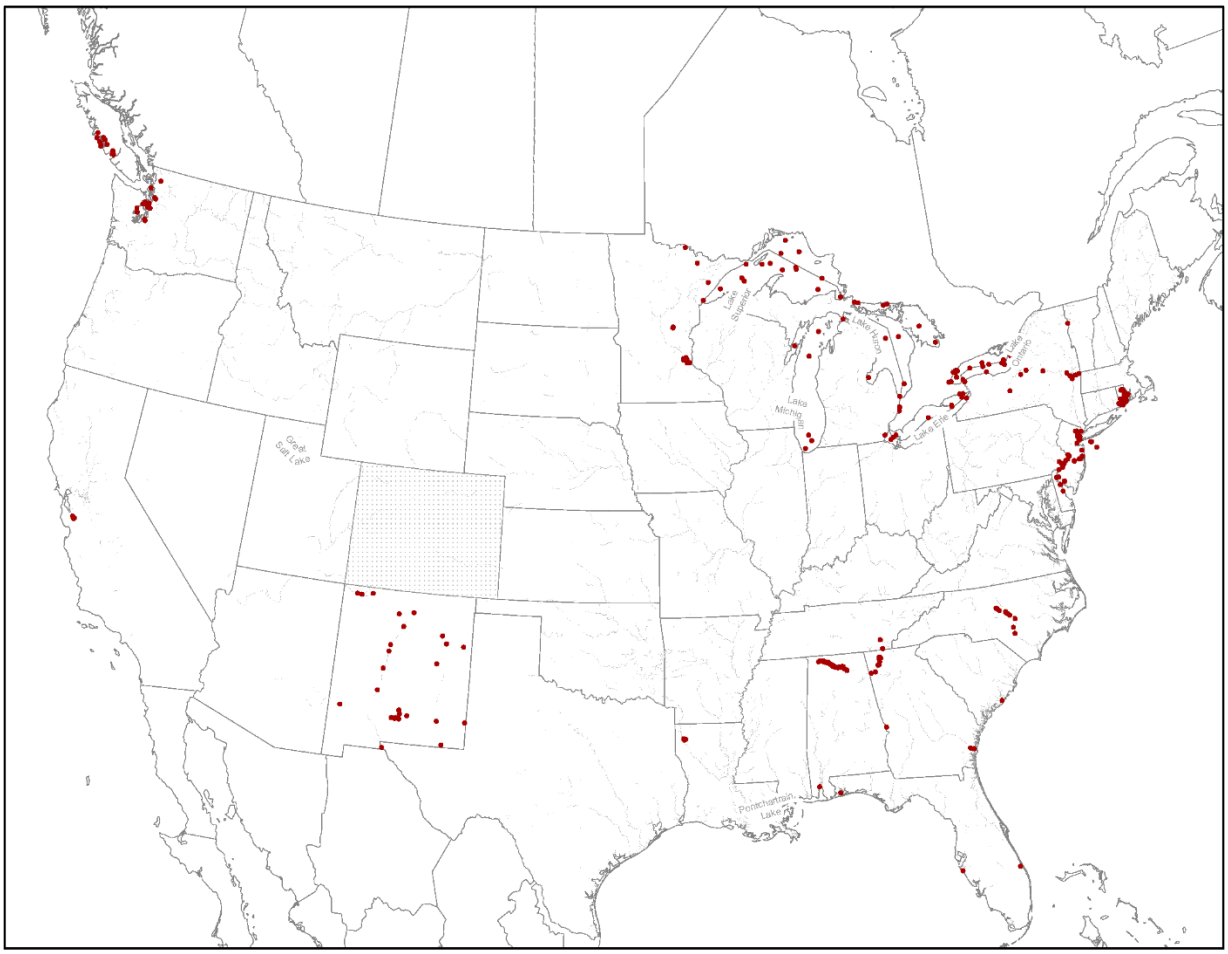

Figure 2-3. Map Indicating Sampling Locations for Perfluorooctane Sulfonate (PFOS) Measured in Surface Waters across the United States (U.S.).

Based on data reported in the current, publicly available literature. Sampling locations for the Colorado data were not available. Therefore, dash marks were used to indicate that measured PFOS surface water concentrations were available for Colorado; however, the exact sampling locations within the state were not publicly available. Detailed information on sampling locations, including references, coordinates (with the exception of Colorado), and sampling site identification numbers and names, are provided in Appendix N.

Modified from: Jarvis et al. (2021).

Concentrations of PFOS in surface waters across the U.S. appear to vary widely, with observed concentrations ranging over eight orders of magnitude. PFOS is generally detected in the picogram and nanogram per liter range with reported concentrations in microgram per liter (or part-per-trillion) ranges (Ahrens 2011; Zareitalabad et al. 2013). For the purposes of this overview, all concentrations reported here are in nanogram per liter (ng/L). Measured surface water concentrations of PFOS in peer-reviewed journal articles and publicly available industry and government reports range between 0.074 and 8,970,000 ng/L with an arithmetic mean concentration of 786.77 ng/L and a median concentration of 3.6 ng/L (Figure 2-4) (Jarvis et al. 2021). However, it should be noted that the mean and median concentrations reported in Jarvis et al. (2021) were calculated from the reported concentrations for individual samples and therefore, are not fully representative of all the measured PFOS concentrations in U.S. surface waters. Additionally, as demonstrated by the median concentration of 3.6 ng/L, a majority (roughly 91%) of measured PFOS concentrations were found to fall below 300 ng/L (Jarvis et al. 2021).

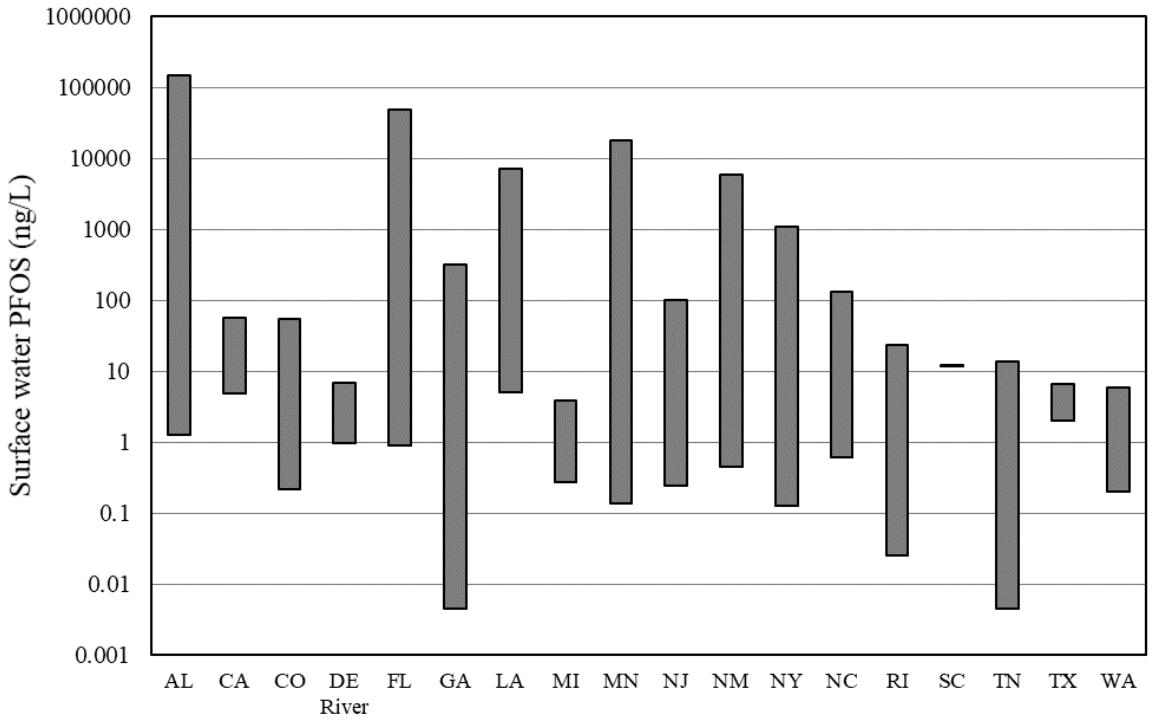

Figure 2-4. Distribution of the Minimum and Maximum Concentrations (ng/L) of Perfluorooctane Sulfonate Measured in Surface Waters for Each State or Waterbody (excluding the Great Lakes) with Reported Data in the Publicly Available Literature.
The distribution is arranged alphabetically by state and waterbody. The measurements in the Delaware River (DE River) could not be contributed to one specific state, and therefore the waterbody is listed.
Modified from: Jarvis et al. (2021).

Numerous available studies report measured PFOS concentrations in surface waters across the U.S. (Appendix N), some of which are summarized in (Jarvis et al. 2021); however, more detailed information on PFOS occurrence in areas not previously sampled and spatial and temporal variability of PFOS remain limited. Prior to the review by Jarvis et al. (2021), there were few analyses of spatial variability of PFOS concentrations in surface water across the U.S (Remucal 2019). Jarvis et al. (2021) indicates that the presence and measured concentrations of PFOS in surface waters are similar between lotic and lentic systems, based on the limited data available (Jarvis et al. 2021)(Appendix N). And as mentioned in the sources of PFOS section above (Section 2.1.2), in contrast with other contaminants commonly found in aquatic ecosystems, PFOS is a synthetic compound with no natural source. Thus, the occurrence of

PFOS in water is a result of the presence of an anthropogenic source, a transport pathway (air, surface water, or ground water), and the persistence and mobility of the PFOS in the environment. Therefore, PFOS concentrations in surface water tend to be dependent on the presence of a nearby source and generally increase with levels of urbanization.

Further, there are insufficient data to quantitatively evaluate temporal trends of PFOS in surface waters across the U.S. (Remucal 2019). However, recent studies have suggested that PFOS concentrations in surface waters with limited sampling sites in northeastern states appear to have decreased since the voluntary phase out of PFOS in 2002 (Pan et al. 2018; Zhang et al. 2016). While these studies observed lower measured PFOS concentrations in surface waters compared to those reported in earlier reports (Hansen et al. 2002; Nakayama et al. 2007), few studies have measured PFOS concentrations from the same sampling sites over time (Jarvis et al. 2021). Eight studies (six focused on the Great Lakes and two in New York on the Hudson River) measured PFOS in the same waterbody over time (Appendix N). Thus, the observed lower concentrations reported in recent literature could be due to trends of PFOS concentrations decreasing since the 2002 PFOS phaseout, differences in sampling site locations, and/or advances in analytical methods for detecting PFOS that reduced detection limits (Jarvis et al. 2021).

Despite the wide use and persistence of PFOS in aquatic ecosystems and unlike the extensive sampling of PFOS in drinking water sources[1], groundwater, and fish tissue monitoring[2], current information on the environmental distribution of PFOS in ambient surface

[1] EPA's database for the Unregulated Contaminant Monitoring Rule (UCMR) that includes data for treated surface waters (https://www.epa.gov/dwucmr).
[2] EPA's National Rivers and Streams Assessment (NRSA; https://www.epa.gov/national-aquatic-resource-surveys/ncca) and the Great Lakes Human Health Fish Tissue Study component of the EPA National Coastal Condition Assessment (NCCA/GL).

waters across the U.S. remains very limited. More recent sampling efforts indicate that PFOS occurrence may be more widespread. PFOS was detected in almost all collected surface water samples, which can likely be attributed to improvements in analytical methods that lowered the PFOS detection limit compared to older analytical methods (Gewurtz et al. 2013).

Thus, from the currently available data, which were largely collected from freshwater systems in eastern states and in the Upper Midwest with known manufacturing or industrial uses of PFAS or use of AFFF, PFOS concentrations measured in U.S. surface waters appear to vary widely, across eight orders of magnitude (Jarvis et al. 2021). PFOS concentrations in remote areas (i.e., areas with little to no PFAS manufacturing and/or industrial uses) range between 0.074 to 23.23 ng/L (Jarvis et al. 2021). This contrasts with PFOS concentrations measured in areas with known PFAS manufacturing, industrial use, and/or application of AFFF, which vary widely and reach up to the maximum observed concentration of 8,970,000 ng/L at a site impacted by AFFF (Appendix N). While current PFAS occurrence data illustrate the prevalence and quantify concentrations of PFOS in surface waters across the U.S., additional data, particularly in central, southwestern, and western freshwaters as well as saltwater systems, are needed to better understand PFOS occurrence in aquatic ecosystems across the U.S. (Jarvis et al. 2021). See Appendix N for further discussion of PFOS occurrence in surface waters and other abiotic media such as aquatic sediments, groundwater, air, and ice.

2.5 Bioaccumulation and Biomagnification of PFOS in Aquatic Ecosystems

PFAS, including PFOS, are found in aquatic ecosystems around the globe (e.g., Ankley et al. 2020; Giesy and Kannan 2001; Houde et al. 2008). Although they were used predominantly in more populated areas, these compounds are resistant to hydrolysis, photolysis, and biodegradation (see Section 2.2), facilitating their long-range transport to aquatic ecosystems in

the remote arctic and mid-oceanic islands (see Section 2.2.2) (Haukas et al. 2007; Houde et al. 2006b). Several physical-chemical properties of PFOS contribute to bioaccumulation within aquatic species once they have entered an ecosystem.

2.5.1 PFOS Bioaccumulation in Aquatic Life

In contrast to many persistent organic pollutants, which tend to partition to fats, PFOS preferentially binds to proteins. Within an organism PFOS tends to bioaccumulate within protein-rich tissues, such as the blood serum proteins, liver, kidney, and gall bladder (De Silva et al. 2009; Jones et al. 2003; Martin et al. 2003a; Martin et al. 2003b). PFOS also binds to ovalbumin, and the transfer of PFOS to such albumin in eggs can be an important mechanism for depuration in female oviparous species, as well as a mechanism for maternal transfer of PFOS to offspring (Jones et al. 2003; Kannan et al. 2005).

The stability of PFOS contributes to its bioaccumulation potential, as it has not been found to undergo biotransformation within the organism (Martin et al. 2003a; Martin et al. 2003b). Within an organism, PFOS undergoes enterohepatic recirculation, in which PFOS is excreted from the liver in bile to the small intestine, then reabsorbed and transported back to the liver (Goecke-Flora and Reo 1996). This process becomes increasingly more efficient the longer the perfluorinated chain length is, resulting in longer biological half-lives for chemicals like PFOS with a relatively long chain length, as they are less readily excreted. PFAS with sulfonate head groups, such as PFOS, are more efficiently resorbed by the small intestine than carboxylate PFAS such as PFOA, resulting in higher bioaccumulation levels (Hassell et al. 2020; Jeon et al. 2010; Martin et al. 2003a).

Sex differences in the elimination rates of PFOS in addition to the transfer of PFOS to albumin in eggs (e.g., Jones et al. 2003; Kannan et al. 2005) have not been well studied. Some research suggests lower PFOS elimination rates in female rats than in male rats (Butenhoff et al.

2012; Chang et al. 2012; Pizzurro et al. 2019), suggesting potentially longer retention of PFOS in females. However, this difference was not observed in mice, rabbits, monkeys, or humans (Pizzurro et al. 2019). In contrast to PFOS, PFOA elimination rates are higher in females than in males for both female fathead minnows (Lee and Schultz 2010) and rats (Pizzurro et al. 2019), suggesting potential longer retention of PFOA in males. These data indicate further research across species and genders for PFAS elimination rates may be useful.

The structure of PFOS also affects its bioaccumulation potential, with linear forms being more bioaccumulative than branched forms (Fang et al. 2014; Hassell et al. 2020). The preferential accumulation of linear PFOS occurs because the elimination rate of branched isomers of PFOS is higher, particularly across gill surfaces (Hassell et al. 2020). This pattern has also been observed in the field, as the proportion of branched isomers was higher in water and sediment compared to fish tissue in Taihu Lake, China (Fang et al. 2014) and Lake Ontario (Houde et al. 2008).

2.5.2 Factors Influencing PFOS Bioaccumulation and Biomagnification in Aquatic Ecosystems

Because of their affinity for binding to proteins, PFAS can enter the base of the food web through sorption to organic matter in sediments or biofilms (Higgins and Luthy 2006; Jeon et al. 2010; Penland et al. 2020), or can bind to blood proteins at gill surfaces of aquatic organisms through respiration (De Silva et al. 2009; Hassell et al. 2020; Martin et al. 2003a; Martin et al. 2003b).

PFAS binding to the surface of sediment organic matter and biofilms is influenced by both hydrophobic and electrostatic effects, resulting from the hydrophobicity of the perfluorinated chain and the hydrophilicity of the sulfonate or carboxylate head groups (Higgins and Luthy 2006)(see Section 2.2 for further details on the sorption of PFOS). Overall, these results suggest that sorption to sediments should be an important mechanism for PFOS entry into

an aquatic ecosystem, but that subsequent dietary uptake from benthic feeding organisms will be more important for PFOS than PFOA.

The importance of the sediment pathway for PFOS bioaccumulation in aquatic ecosystems has been demonstrated in laboratory studies with *Chironomus riparius* (Bertin et al. 2014), *C. plumosus* (Wen et al. 2016), *Gammarus fossarum* and *G. pulex* (Bertin et al. 2016), and *Lumbriculus variegatus* (Lasier et al. 2011), where PFOS concentrations were positively correlated between sediments and whole-body tissue samples of benthic feeding organisms. The sediment pathway has also been demonstrated in several field studies, where PFOS was measured in sediments and biofilms, and was higher in benthic-feeding invertebrates relative to pelagic-feeding invertebrates (Lescord et al. 2015; Loi et al. 2011; Martin et al. 2004; Penland et al. 2020). In addition, the distribution of PFAS in sediments was more similar to their distribution in the tissues of benthic invertebrates (Lescord et al. 2015) and fish (Thompson et al. 2011) than they were to their distribution in pelagic organisms.

PFAS can also enter aquatic organisms directly from the water column through respiration. Because of its binding affinity to proteins, PFOS can enter the body of gill-breathing organisms by binding to proteins in the blood at gill surfaces (Jones et al. 2003; Martin et al. 2003a; Martin et al. 2003b). The relative distribution of PFOS in tissues is related to the primary route of exposure (dietary or respiratory). In rainbow trout, the rank order of PFOS concentrations following aqueous exposure was blood > kidney > liver (Martin et al. 2003a). In contrast, their rank order following dietary exposure was liver > blood > kidney (Goeritz et al. 2013). Hong et al. (2015) observed the highest concentrations of PFOS in the intestines of green eel goby, soft tissues, shell, and legs of shore crabs; and gills and intestines of oysters, suggesting

bioaccumulation through both dietary and aqueous uptake in invertebrates, and primarily dietary uptake in fish.

In addition to being bioaccumulative, PFOS has been shown to biomagnify with increasing trophic level in a variety of freshwater ecosystems (Kannan et al. 2005; Martin et al. 2004; Penland et al. 2020; Xu et al. 2014) and saltwater ecosystems (de Vos et al. 2008; Houde et al. 2006b; Loi et al. 2011; Powley et al. 2008; Tomy et al. 2004) in North America, Europe, and Asia. PFOS is often the most abundant PFAS in aquatic organisms, and this high relative abundance is at least partially explained by the biotransformation of PFOS precursor chemicals into PFOS (see Section 2.3)(Haukas et al. 2007; Kannan et al. 2005; Kelly et al. 2009; Martin et al. 2004; Tomy et al. 2004). Higher trophic level organisms have a greater capacity to metabolize PFOS precursor chemicals, which have been found in lower concentrations in increasing trophic level (Fang et al. 2014; Kannan et al. 2005; Martin et al. 2004). This suggests that in addition to biomagnification, some of the trophic-level increase in PFOS can be explained by the biotransformation of precursor chemicals.

2.5.3 Environmental Monitoring of PFOS in Biotic Media

PFOS is one of the dominant PFAS detected in aquatic ecosystems, along with PFOA (Ahrens 2011; Benskin et al. 2012; Dinglasan-Panlilio et al. 2014; Remucal 2019; Zareitalabad et al. 2013). PFAS were first detected in human serum samples in the late 1960s, and subsequent studies across several continents demonstrated the global distribution of PFAS in humans (Giesy and Kannan 2001; Houde et al. 2006b). Since then, the global distribution of PFAS in tissues of aquatic species has been demonstrated in studies conducted in freshwater and marine environments across every continent, including remote regions far from direct sources, such as the high arctic, Antarctica, and oceanic islands (Giesy and Kannan 2001; Houde et al. 2006b).

In lentic surface waters of the U.S., one of the most comprehensive studies of PFOS concentrations included fish muscle tissue data from 157 near shore sites across the Great Lakes selected following a probabilistic design as part of the 2010 National Coastal Condition Assessment (Stahl et al. 2014). In this study, PFOS was measured in fish collected at every site, with a median concentration of 15.2 ng/g ww (Stahl et al. 2014). Lake trout (31% of sampled species), smallmouth bass (14%), and walleye (13%) were the most commonly-sampled species from the Great Lakes samples, and the average PFOS concentrations in lake trout muscle were more than twice as high as PFOS concentrations in muscle of smallmouth bass and walleye (Stahl et al. 2014).

Martin et al. (2004) measured PFOS in whole body samples of invertebrates and fish in Lake Ontario, near the town of Niagara-on-the-Lake. PFOS concentrations were much higher in the benthic amphipod *Diporeia hoya* (280 ng/g ww) than in the more pelagic *Mysis relicta* (13 ng/g ww), suggesting sediments are an important source of PFOS in this area (Martin et al. 2004). Among the four fish species sampled, whole body PFOS concentrations were highest in the slimy sculpin (450 ng/g ww), whose preferred food source is *D. hoya* (Martin et al. 2004). Although adult lake trout occupy the highest trophic level at this site, based on nitrogen stable isotope analysis, their PFOS concentrations were less than half (170 ng/g ww) of those measured in sculpin, as their food web is largely pelagic, and not affected by the high sediment PFOS concentrations. Based on stomach content analysis, 90% of the adult lake trout diet consists of alewife, which feed primarily on the more pelagic *M. relicta*, and have the lowest average PFOS concentration (46 ng/g ww) among all fish species (Martin et al. 2004).

Guo et al. (2012) measured PFOS in lake trout muscle tissues in Canadian waters of Lake Superior, Huron, Erie, and Ontario. Average PFOS concentrations correlated with watershed

urbanization, and were 0.85, 8.3, 27, and 46 ng/g ww, respectively (Guo et al. 2012). Delinsky et al. (2010) measured PFOS in bluegill, black crappie, and pumpkinseed muscle tissue in 59 lakes in Minnesota, including four lakes in the Minneapolis-St. Paul metropolitan area. PFOS was detected in muscle tissues of fish collected in 13 of the 59 lakes, and concentrations ranged from 1.08 to 52.4 ng/g ww in lakes where it was detected. In the four lakes in the Minneapolis-St. Paul metropolitan area, PFOS concentrations in fish muscle tissues ranged from 4.39 to 47.3 ng/g ww (Delinsky et al. 2010).

In flowing surface waters of the U.S., one of the most comprehensive fish PFOS monitoring studies included the collection of fish muscle tissue data from 164 urban river sites (5^{th} order or higher) across the conterminous U.S. selected following a probabilistic design. The study was part of the 2008 - 2009 National Rivers and Streams Assessment and the National Coastal Condition Assessment (Stahl et al. 2014). PFOS was detected in 73% of the urban river sites, with a median concentration of 10.7 ng/g ww (Stahl et al. 2014). Largemouth bass (34% of sampled species), smallmouth bass (25%), and channel catfish (11%) were the most commonly sampled species from the urban stream sites, and PFOS concentrations in the muscles of largemouth bass were approximately twice as high as concentrations in the muscles of smallmouth bass (Stahl et al. 2014).

Ye et al. (2008) reported average PFOS concentrations of 83.1, 84.6, and 147 ng/g ww from whole body composite samples of multiple fish species from the Mississippi River, Missouri River, and Ohio River, respectively. Delinsky et al. (2010) sampled PFOS in bluegill, black crappie, and pumpkinseed muscle tissue at several locations along the upper Mississippi River in 2007, and found concentrations ranging from 3.06 ng/g ww at unimpacted sites to 2,000 ng/g ww at Pool 2, a heavily impacted site in the Minneapolis-St. Paul metropolitan area

(Delinsky et al. 2010). Malinsky et al. (2011), as reported in Stahl et al. (2014), measured PFOS concentrations ranging from 41.7 to 180 ng/g ww in fish muscle samples collected along the Mississippi River, with the lowest concentration reported for sauger and the highest reported for bluegill.

Kannan et al. (2005) measured PFOS in invertebrates and vertebrates from two rivers in Southern Michigan (Raisin River, St. Claire River), and one in Northern Indiana (Calumet River). PFOS concentrations were similar across sites for the different taxa and suggested trophic biomagnification for PFOS. Among invertebrate taxa, zebra mussel PFOS soft tissue whole body concentrations ranged from below detection to 3.1 ng/g ww, amphipod whole body concentrations ranged from below detection to 2.9 ng/g ww, and crayfish whole body concentrations ranged from 2.4 to 4.3 ng/g ww. Among fish, PFOS concentrations in round goby whole body samples ranged from 6.6 to 21.5 ng/g ww, and smallmouth bass muscle samples ranged from below detection to 41.3 ng/g ww (Kannan et al. 2005).

In a more recent study, Penland et al. (2020) measured PFAS concentrations in invertebrates and vertebrates along the Yadkin – Pee Dee River, in North and South Carolina in 2015. PFOS was measured in whole body tissues of snails (6.47 ng/g ww) but was not detected in whole body tissues of Asian clam, unionid mussels, or crayfish. The highest concentrations in invertebrates were measured in aquatic insect whole body samples (132.8 ng/g ww) and was hypothesized to result from dietary uptake of aquatic biofilms. PFOS was measured in muscle tissue of all 11 sampled fish species and ranged from 11.42 ng/g ww in channel catfish to 37.36 ng/g in whitefin shiner. The highest concentration that Penland et al. (2020) measured was 482.9 ng/g ww, from the eggs of a single robust redhorse sample, underscoring the preferential binding of PFOS to ovalbumin.

Houde et al. (2006a) measured whole body PFOS in six fish species in Charleston Harbor, South Carolina, and whole body PFOS in zooplankton and five fish species in Sarasota Bay, Florida. Charleston Harbor was the more developed of the two sites and had higher overall PFOS concentrations. Average PFOS concentrations in Charleston Harbor ranged from 19 ng/g ww in pinfish to 92 ng/g in spot. In Sarasota Bay, PFOS concentrations averaged 0.2 ng/g ww in zooplankton and ranged from 3.1 ng/g ww in pigfish to 8.8 ng/g ww in spotted seatrout, suggesting evidence of trophic biomagnification.

Lescord et al. (2015) measured PFOS in chironomids, zooplankton, and juvenile and adult arctic char in six high arctic lakes in Canada. Two of these lakes had been contaminated by PFAS from a nearby airport while the other lakes were free from point source contamination. PFOS in chironomid whole body samples was high at the two contaminated lakes, ranging from 28 to 445 ng/g ww, compared to 5.3 to 14 ng/g ww at the reference lakes (Lescord et al. 2015). Whole body concentrations in pelagic zooplankton were relatively lower, ranging from 49 to 60 ng/g ww, compared to 0.12 to 2.0 ng/g ww at the reference lakes. The higher concentrations of PFOS in the benthic chironomids indicate the importance of sediments as a route of exposure into the base of the food web. PFOS in whole body samples of juvenile char (181 to 224 ng/g ww) and muscle tissue of adult char (24 to 117 ng/g ww) at the two contaminated lakes were lower than whole body PFOS in chironomids, indicating a lack of trophic biomagnification. Additionally, PFOS in whole body samples of juvenile char (0.001 to 15 ng/g ww) and muscle tissues of adult char (below detection to 2 ng/g ww) at the four reference lakes was also lower than whole body PFOS in chironomids at the four reference lakes.

Tomy et al. (2004) measured PFOS in whole body samples of zooplankton (*Calamus hyperboreus*), shrimp (*Pandalus sp.*), clams (*Nya truncata* and *Serripes groenlandica*), and arctic

cod (*Boreogadus saida*); and liver samples of deepwater redfish (*Sebastes mentella*) collected from unimpacted marine locations in the Canadian Arctic. PFOS concentrations were low for all taxa, with the lowest concentrations measured in shrimp (0.3 ng/g ww) and clams (0.04 ng/g ww). PFOS concentrations were similar in zooplankton (1.8 ng/g ww), arctic cod (1.3 ng/g ww), and redfish (1.4 ng/g ww), indicating little, if any biomagnification from invertebrates to fish (Tomy et al. 2004). Haukas et al. (2007) found the average liver PFOS concentration (2.02 ng/g ww) in arctic cod *B. saida* collected in the Barents Sea off the coast of Svalbard in 2004 to be similar to whole body concentrations for this species reported by Tomy et al. (2004). The average whole body PFOS concentration (3.85 ng/g ww) in ice amphipod (*Gammarus wilkitzkii*) samples was higher than the average liver PFOS concentration in in arctic cod, indicating no biomagnification from invertebrates to fish in this ecosystem (Haukas et al. 2007).

Current data indicate that PFOS concentrations measured in aquatic biota vary widely, approximately across four orders of magnitude for both fish (ranging between 0.85 and 2,000 ng/g ww) and aquatic invertebrates (ranging between 0.04 and 445 ng/g ww). Like ambient surface water concentrations, PFOS concentrations in aquatic biota inhabiting remote areas (i.e., areas with little to no PFAS manufacturing and/or industrial uses) appear to be lower than those in areas with known PFAS manufacturing, industrial use, and/or application of AFFF. While current PFAS monitoring data illustrate the prevalence and quantify concentrations of PFOS in aquatic biota across the U.S., additional data are needed to better understand PFOS occurrence and potential bioaccumulation in aquatic ecosystems across the U.S.

2.6 Exposure Pathways of PFOS in Aquatic Environments

There are multiple exposure pathways of PFOS in the aquatic environment, including: 1) direct (dermal and respiratory) aqueous exposure; 2) direct exposure to contaminated sediment

(for benthic organisms); 3) dietary and biomagnification; and 4) maternal-transfer (Ankley et al. 2020). Exposure of PFOS through water and sediment occurs through direct contact with the respective media, such as water passing across the gills, or consumption of suspended and deposited sediments (Prosser et al. 2016). Upon entering an organism, PFAS such as PFOS tend to bind to proteins, and concentrate preferentially within the blood and protein rich tissues, such as liver (Haukas et al. 2007; Xia et al. 2013b). The affinity of PFOS to bind to proteins contributes to the bioaccumulation and biomagnification of PFOS (see Section 2.5 above), resulting in increasing concentrations of PFOS in higher trophic level organisms, such as predatory fish and birds (Custer et al. 2019; Haukas et al. 2007; Xu et al. 2014). However, as noted previously in Section 1.2.1, the lack of a meaningful K_{ow} for PFOS due to its binding primarily to protein, not lipids, precludes application of K_{ow}-based models that are commonly used to estimate bioconcentration factors and predict bioaccumulation for many other important, environmental contaminants (e.g., PCBs). Lastly, elevated PFOS concentrations in eggs and young of aquatic life suggests that PFOS may be maternally transferred to offspring. This exposure pathway may be particularly important among egg-laying species because of the preferential binding of PFOS to egg albumin (Kannan et al. 2005). In summary, PFOS exposure has been found to occur through multiple exposure routes, including via water, sediment, diet, and maternal transfer (Jones et al. 2003; Kannan et al. 2005; Sharpe et al. 2010; Wang et al. 2011).

2.7 Effects of PFOS on Biota

The number of PFOS ecotoxicity studies and data are increasing and study designs are evolving to expand the understanding of the effects of PFOS. Currently, PFOS ecotoxicity studies are primarily focused on fish, aquatic invertebrates, plants, and algae. Fewer studies are

being conducted on aquatic-dependent birds, reptiles, and mammals. Sections 3 and 4 provide study summaries of individual publicly available high quality aquatic life toxicity studies, and Appendix A through Appendix H summarize current PFOS aquatic life ecotoxicity data, both studies used here and unused studies due to quality issues.

2.7.1 Mode of Action and Toxicity of PFOS

The mechanism(s) underpinning the toxicity of PFOS is not well-understood and is an active area of research. Toxicity literature indicate that PFOS causes a wide range of adverse effects in aquatic organisms, including reproductive effects, developmental toxicity, and estrogen, androgen and thyroid hormone disruption (see Sections 3 and 4 and Appendices A.1 through H.1). However, a great deal of research is still needed from a mechanistic perspective to better understand how the different modes of action elicit specific biological responses. Some potential PFOS modes of action in aquatic life appear to include: 1) oxidative stress (Li et al. 2017; Sant et al. 2018; Shi and Zhou 2010); 2) autophagic cell death or apoptosis (Sant et al. 2018; Shi et al. 2008); 3) endocrine modulation of estrogen and thyroid receptors (Benninghoff et al. 2011; Chen et al. 2018; Du et al. 2013; Kim et al. 2011; Shi et al. 2008); 4) interference at the mitochondrial level through the uncoupling of oxidative phosphorylation (ECCC 2018); 5) interference with the homeostasis of DNA metabolism (Hoff et al. 2003); and 6) activation of the nuclear peroxisome proliferator activated receptor-alpha (PPAR-α) pathways (Arukwe and Mortensen 2011; Cheng et al. 2016; Fang et al. 2013; Fang et al. 2012; Yang et al. 2014).

Following exposure to PFOS, molecular level events can perturb estrogen-, androgen- and thyroid-related endocrine systems, as well as neuronal-, lipid-, and carbohydrate-metabolic systems and lead to cellular- and organ-level disturbances and ultimately result in effects on reproduction, growth, and development at the individual organism-level(see Ankley et al. 2020 and Lee et al. 2020 for the latest reviews on the subject) for the latest reviews on the subject).

The mechanisms of PFOS toxicity to fish in particular appear to be related to oxidative stress, apoptosis, thyroid disruption, and alterations of gene expression during development (Lee et al. 2020). Additionally, published research suggested that many of these molecular pathways interact with each other and could be linked. For example, oxidative stress following exposure to PFOS was correlated with effects on egg hatching and larval formation, linking reproductive toxicity, oxidative stress, and developmental toxicity (Lee et al. 2020). The actual mechanism(s) through which PFOS induced oxidative stress operates still requires additional study, but increased ß-oxidation of fatty acids and mitochondrial toxicity are proposed triggers (Ankley et al. 2020; Lee et al. 2020). Thus, the alteration of multiple biological pathways is a plausible explanation for the diversity of observed effects of PFOS reported in the literature (Lee et al. 2020). However, the available data did not allow for a defined adverse outcome pathway-based understanding of the ultimate reductions to survival, growth, and reproduction in the various aquatic taxa in which these effects have been observed or may be expected to occur. Thus, further mechanistic research is warranted.

Notably, PFOS appeared to be related to the disruption of the sex hormone-related endocrine system at the molecular, tissue, and organ levels, resulting in observed adverse reproductive outcomes in freshwater and saltwater fish and invertebrates alike. Further, these effects have been reported after exposure via multiple exposure routes (i.e., waterborne, dietary, maternal; (i.e., waterborne, dietary, maternal; Lee et al. 2020). And these reproductive effects also appeared to be trans-generational, as observed in a multi-generational zebrafish (*Danio rerio*) study by Wang et al. (2011) (see study summary in Appendix Section C.2.5).

PFOS is one of the most studied PFAS in the ecotoxicity literature, with reported adverse effects on survival, growth, and reproduction. However, a great deal of additional research is

needed to better understand the modes of action of PFOS. Specifically, additional research from a mechanistic perspective is needed to better understand how the different modes of action elicit specific biological responses in fish, aquatic invertebrates, and amphibians. Potential effects of PFOS involving multiple biological pathways are a research challenge for PFOS and PFAS in general.

2.7.2 Potential for Interactions with Other PFAS

PFAS occur as mixtures in the environment. Occurrence studies document the presence of complex mixtures of PFAS in surface waters in the U.S. and across the globe (see also Section 2.4.1)(Ahrens 2011; Ahrens and Bundschuh 2014; Giesy and Kannan 2002; Houde et al. 2006b; Keiter et al. 2012; Wang et al. 2017). Although the EPA's PFOS recommended aquatic life water quality criteria are based solely on single chemical exposures to aquatic life, it is recognized that PFAS are often introduced into the aquatic environment as end-use formulations comprised of mixtures of PFAS and/or PFAS-precursors. However, the ecological effects of these potential PFAS mixtures are poorly understood (Ankley et al. 2020). It was useful, therefore, to briefly summarize the types of interactions that might be expected based on the few PFAS mixture studies involving PFOS and one or more PFAS to date. It should be noted that for purposes of this document, the reader is referred to Ankley et al. (2020) and elsewhere for more comprehensive reviews of PFAS mixtures in general, and the challenges they are expected to present in ecological risk assessment. Beyond PFOA and PFOS, systematic reviews of chemical mixture studies across various compound classes indicate that departures from dose additivity are uncommon and rarely exceed minor deviations (~2-fold) from predictions based on additivity (Martin et al. 2021).

Findings of the PFAS-specific studies below are as reported by the study authors without any additional interpretation or analysis of uncertainty. At both the organismal and cellular

levels, studies on zebrafish (*D. rerio*; Ding et al. 2013), a water flea *(D. magna*; Yang et al. 2019), mosquito (*Aedes aegypti*; Olson 2017), a bioluminescent cyanobacteria (*Anabaena sp*.; Rodea-Palomares et al. 2012), or with cultured hepatocytes of the cyprinid (*Gobiocypris rarus*; Wei et al. 2009), demonstrated that the effects observed from *in vivo* and *in vitro* tests on PFAS mixtures vary and can have unpredictable exposure and species-specific effects. PFAS mixture studies on zebrafish reported interactions for combinations of PFOA and PFOS, but departures from additive models were also minor (Ding et al. 2013). Menger et al. (2020) reported zebrafish behavioral effects from a PFAS mixture that were less than individual PFAS, however evaluation of chemical dose response and comparison to mixture models was not conducted. Yang et al. (2019) exposed the water flea, *D. magna*, to single and binary mixtures of PFOS and PFOA. The authors reported synergism in acute and chronic toxic effects. Conversely, Rodea-Palomares et al. (2012) showed a binary PFOS and PFOA mixture as having an antagonistic interaction across the whole range of effect levels tested using the bioluminescent cyanobacterium, *Anabaena.* Olson (2017) exposed larvae of the mosquito, *A. aegypti,* to PFOS and perfluorohexane sulfonate (PFHxS) separately and as a mixture and reported increased toxicity in a manner greater than would be predicted by additivity.

In tests with cultured hepatocytes of the cyprinid, *G. rarus*, co-exposure of PFOS with a mixture of five other PFAS [PFOA, Perfluorononanoate or Perfluorononanoic acid (PFNA), Perfluorodecanoate or Perfluorodecanoic acid (PFDA), Perfluorododecanoate or Perfluorododecanoic acid (PFDoA), and 8:2 FTOH] resulted in highly complex interactions (Wei et al. 2009). A number of genes differentially expressed in the mixture were not differentially expressed in the exposure to the individual chemicals, potentially indicating different modes of action for the mixture compared to the individual chemicals. In this case, the authors reported no

additive responses for the mixture. Consistent with the possible mechanisms of toxicity of PFOS (see Section 2.7.1), the genes identified in the study are involved in multiple biological functions and processes, including fatty acid metabolism and transport, xenobiotic metabolism, immune response, and oxidative stress (Wei et al. 2009). Finally, U.S. EPA (Conley et al. 2022) observed PFOA and PFOS interacting in an additive manner to reduce pup body weight, pup liver weight, and maternal liver weight in the Sprague-Dawley rat.

2.8 Conceptual Model of PFOS in the Aquatic Environment and Effects

A conceptual model depicts the relationship between a chemical stressor and ecological compartments, linking exposure characteristics to ecological endpoints. The conceptual model provided in Figure 2-5 summarizes sources, potential pathways of PFOS exposure for aquatic life and aquatic-dependent wildlife, and possible toxicological effects.

PFOS initially enters the aquatic environment through point sources, including municipal and industrial dischargers and landfill leachate and non-point sources, including land application of contaminated biosolids (see Section 2.1.2). PFOS enters the aquatic environment in dissolved and particle-bound forms and may sorb to surfaces, such as sediment and particulate matter in the water column (see Section 2.2.2), which is depicted in the conceptual model (Figure 2-5). The conceptual model depicted in Figure 2-5 shows exposure pathways for the biological receptors of concern (i.e., aquatic life) and potential effects (e.g., on survival, growth, and reproduction) in those receptors. Both direct (i.e., exposure from the water column which is represented by **) and indirect (i.e., dietary exposure via the food web *) pathways are represented in the conceptual model.

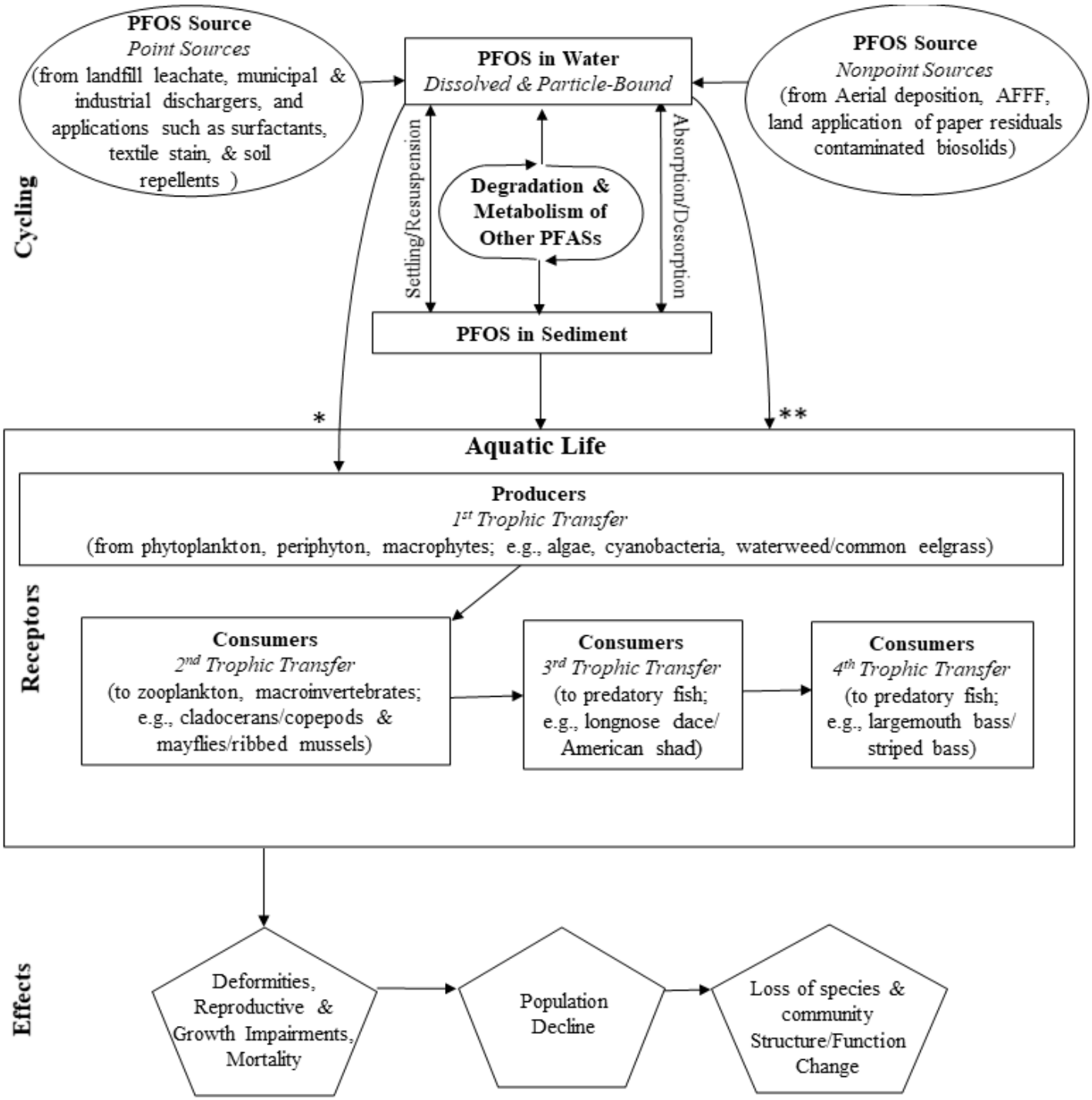

Figure 2-5. Conceptual Model Diagram of Sources, Compartmental Partitioning, and Trophic Transfer Pathways of Perfluorooctane Sulfonate (PFOS) in the Aquatic Environment and its Bioaccumulation and Effects in Aquatic Life.

PFOS sources represented in ovals, compartments within the aquatic ecosystem represented by rectangles, and effects in pentagons. Examples of organisms in each trophic transfer provided as freshwater/marine. Movement of PFOS from water to receptors indicated by two separate pathways: bioconcentration by producers (*) and direct exposure (**) to all trophic levels within box. Relative proportion of PFOS transferred between each trophic level is dependent on life history characteristics of each organism.

2.9 Assessment Endpoints

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected" and are defined by an ecological entity (species, community, or other entity) and its attribute or characteristics (U.S. EPA 1998). Assessment endpoints may be identified at any level of organization (e.g., individual, population, community). In the context of the CWA, aquatic life criteria for toxic pollutants are typically determined based on the results of toxicity tests with aquatic organisms in which unacceptable effects on growth, reproduction, or survival occurred. This information is typically compiled into a sensitivity distribution based on genera and representing the impact on taxa across the aquatic community. Criteria are based on the 5^{th} percentile of genera and are thus intended to be protective of approximately 95 percent of aquatic genera.

The use of laboratory toxicity tests to protect aquatic species was based on the concept that effects occurring to a species in appropriate laboratory tests will generally occur to the same species in comparable field situations. Since aquatic ecosystems are complex and diversified, the 1985 Guidelines recommended acceptable data be available for at least eight genera with a specified taxonomic diversity (the standard eight-family minimum data requirements, or MDRs). The intent of the eight-family MDR was to serve as a surrogate sample community representative of the larger and generally much more diverse natural aquatic community, not necessarily the most sensitive species in a given environment. The 1985 Guidelines note that since aquatic ecosystems can tolerate some stress and occasional adverse effects, protection of all species at all times and places are not deemed necessary (the intent is to protect 95 percent of a group of diverse taxa, and any commercially and recreationally important species; (the intent is to protect 95 percent of a group of diverse taxa, and any commercially and recreationally important species; U.S. EPA 1985).

For more details on aquatic life assessment endpoints for PFOS see Section 3.1 below. This criteria derivation for aquatic life was developed using a genus sensitivity distribution (GSD), which represents the potential for impact to the survival, growth, or reproductive effects on taxa across aquatic communities.

2.10 Measurement Endpoints

2.10.1 Overview of Toxicity Data Requirements

To ensure the protection of various components of an aquatic ecosystem, the EPA compiles acute toxicity test data from a minimum of eight diverse taxonomic groups.

- Acute freshwater criterion require data from the following taxonomic groups:
    a. fish in the family Salmonidae in the class Osteichthyes
    b. a second family of fish in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish)
    c. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian)
    d. a planktonic crustacean (e.g., cladoceran, copepod)
    e. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish)
    f. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge)
    g. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca)
    h. a family in any order of insect or any phylum not already represented

- Acute estuarine/marine criterion require data from the following taxonomic groups:
    a. two families in the phylum Chordata
    b. a family in a phylum other than Arthropoda or Chordata
    c. a family from either Mysidae or Penaeidae
    d. three other families not in the phylum Chordata (may include Mysidae or Penaeidae, whichever was not used above)
    e. any other family

Additionally, to ensure the protection of various animal components of the aquatic ecosystem from long term exposures, chronic toxicity test data are recommended from the same eight diverse taxonomic groups that are recommended for acute criteria. If the eight diverse

taxonomic groups are not available to support the chronic criterion derivation using a genus distribution approach, the chronic criterion may be derived using an acute-to-chronic ratio (ACR) approach. To apply an ACR approach to derive a chronic criterion, a minimum of three taxa are recommended, with at least one chronic test being from an acutely sensitive species. To calculate ACRs, chronic aquatic life criteria require data from the following taxonomic groups:

a. At least one fish
b. At least one invertebrate
c. At least one acutely sensitive freshwater species, for freshwater chronic criterion (the other two may be saltwater species)
d. At least one acutely sensitive saltwater species for estuarine/marine chronic criterion (the other two may be freshwater species)

The 1985 Guidelines also specified at least one quantitative test with a freshwater alga or vascular plant. If plants are among the most sensitive aquatic organisms, toxicity test data from a plant in another phylum should also be available. Aquatic plant toxicity data were examined to determine whether aquatic plants are likely to be adversely affected by the concentration expected to be protective for other aquatic organisms (see Appendix E for freshwater plant toxicity studies).

2.10.2 Measure of PFOS Exposure Concentrations

This PFOS aquatic life AWQC document provides a critical review of all data identified in the EPA's literature search for PFOS, including all forms of PFOS used in toxicity literature (such as the anionic form and salts) and identified in the ECOTOX database:

- the anionic form (CAS No. 45298-90-6)
- the acid form (CAS No. 1763-23-1)
- potassium salt (CAS No. 2795-39-3)
- an ammonium salt (CAS No. 56773-42-3)
- sodium salt (CAS No. 4021-47-0)
- and a lithium salt (CAS No. 29457-72-5)

Typically, studies do not complete an analysis or provide enough information regarding isomer delineation to determine if the PFOS tested was purely linear or branched. However, several PFOS toxicity studies stated that the linear PFOS isomer was used for dosing with fewer studies indicating that the branched isomer was used. Studies reported by researchers that conducted PFOS-only exposures were considered for possible inclusion. For most the EPA aquatic life criteria documents with non-bioaccumulative substances, organisms are exposed to contaminated water but fed a diet grown in uncontaminated media (not spiked with the toxicant prior to introduction into the exposure chambers). Such tests were reviewed, and tests of sufficient quality are included in these PFOS criteria. Toxicity tests conducted with PFOS-spiked diet were also reviewed and considered suitable for deriving a criterion for this bioaccumulative pollutant; however, these toxicity tests were limited in the current PFOS toxicity literature. Consequently, toxicity tests with direct aqueous, dietary, and maternal transfer were included in the EPA's derivation of aquatic life criterion for PFOS (see Section 3). Studies not included in the numeric criteria derivation, including some studies with other PFOS exposures (i.e., *in vitro* studies), were considered qualitatively as supporting information if they were deemed to be of sufficient quality, and are described in the Effects Characterization section below (Section 4.3).

This set of published literature was identified using the ECOTOXicology database (ECOTOX; https://cfpub.epa.gov/ecotox/) as meeting data quality standards. ECOTOX is a source of high-quality toxicity data for aquatic life, terrestrial plants, and wildlife. The database was created and is maintained by the EPA, Office of Research and Development, Center for Computational Toxicology and Exposure. The ECOTOX search generally begins with a comprehensive chemical-specific literature search of the open literature conducted according to ECOTOX Standard Operating Procedures (SOPS; Elonen 2020). The search terms are often

comprised of chemical terms, synonyms, degradates and verified Chemical Abstracts Service (CAS) numbers. After developing the literature search strategy, ECOTOX curators conduct a series of searches, identify potentially applicable studies based on title and abstract, acquire potentially applicable studies, and then apply the applicability criteria for inclusion in ECOTOX. Applicability criteria for inclusion into ECOTOX generally include:

a. The toxic effects are related to single chemical exposure (unless the study is being considered as part of a mixture effects assessment)
b. There is a biological effect on live, whole organisms or *in vitro* preparation including gene chips or omics data on adverse outcome pathways potentially of interest
c. Chemical test concentrations are reported
d. There is an explicit duration of exposure
e. Toxicology information that is relevant to EPA Office of Water (OW) is reported for the chemical of concern
f. The paper is published in the English language
g. The paper is available as a full article (not an abstract)
h. The paper is publicly available
i. The paper is the primary source of the data
j. A calculated endpoint is reported or can be calculated using reported or available information
k. Treatment(s) are compared to an acceptable control
l. The location of the study (*e.g.*, laboratory vs. field) is reported
m. The tested species is reported (with recognized nomenclature)

Following inclusion in the ECOTOX database, toxicity studies were subsequently evaluated by EPA OW. All studies were evaluated for data quality as described by U.S. EPA (1985) and in EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S. EPA 2016b), and EPA OW's internal data quality SOP, which is consistent with OCSPP's data quality review approach (U.S. EPA 2018). Office of Water completed a Data Evaluation Record (DER) for each species by chemical combination from the PFOS studies identified by ECOTOX. This in-depth review ensured the studies used to derive the criteria resulted in robust scientifically-defensible criteria. Example DERs are shown in

Appendix Q with the intent to convey the meticulous level of evaluation, review, and documentation each PFOS study identified by ECOTOX was subject to.

The 1985 Guidelines document indicates that tests used in criteria should be for North American resident species. Due to the EPA's interest in using all available quality data, particularly for data-sparse PFOS (relative to cadmium or ammonia, for example), PFOS toxicity studies were considered for possible inclusion regardless of the test species residential status in North America, as with other published aquatic life criteria. This approach was also based on the relative similarity in sensitivities between resident and non-resident species (see Sections 3 and 4). Moreover, non-North American resident species serve as taxonomically-related surrogate test organisms for the thousands of untested resident species. Supporting analyses to evaluate the influence of including non-resident species on the freshwater criteria magnitudes were conducted by limiting toxicity datasets to North American resident species with established populations in North America (see Section 4.2). These supporting analyses provided an additional line-of-evidence that further suggested it is appropriate to consider non-resident species in PFOS criteria derivation because of their minimal influence of the criteria magnitudes.

Additionally, a substantial number of PFOS toxicity tests reported only nominal, or unmeasured, PFOS concentrations. For PFOS, the EPA has examined the issue of whether nominal (unmeasured) and measured concentrations are in close agreement with each other (Jarvis et al. 2023). While measured PFOS toxicity tests are generally preferred, results of Jarvis et al. (2023) demonstrated that experimental conditions had little influence on observed discrepancies between nominal and measured concentrations for PFOS, with the exception of saltwater tests and freshwater studies that contained substrate. Nominal and measured concentrations in the analysis generally displayed a high degree of linear correlation (>0.95

freshwater, >0.84 saltwater) and relatively low median percent differences (Jarvis et al. 2023). In freshwater tests, when tests with substrate were removed, 89% of the 527 PFOA and PFOS measured concentrations were within 20% of their nominal counterparts Conversely, 65.50% of measured PFOS saltwater concentrations differed from corresponding nominal concentrations by >20% (EPA's OCSPP's Ecological Effects Test Guidelines (U.S. EPA 2016b) consider tests acceptable when measured concentrations are within 20% of nominal, and Rewerts et al. (2021) suggested that PFAS-specific toxicity tests may even be acceptable if measured and nominal concentrations do not differ by up to 30%). Potential dosing errors, differences in experimental design, and/or the presence of substrate were hypothesized to be the primary contributor to these discrepancies (Jarvis et al. 2023). Therefore, when available, measured PFOS concentrations were used; however, for several studies measured PFOS concentrations were not reported, and nominal concentrations were utilized, especially if a concentration-response relationship was observed in another medium where PFOS was measured from the same study (e.g., diet, blood, or eggs).

Typically, per the 1985 Guidelines, acute toxicity data from all measured flow-through tests would be used to calculate species mean acute values (SMAV), unless data from a measured flow-through test were unavailable, in which case the acute criterion would be calculated as the geometric mean of all the available acute values (i.e., results of unmeasured flow-through tests and results of measured and unmeasured static and renewal tests). Chronic unmeasured flow-through tests, as well as measured and unmeasured static and renewal tests are not typically considered to calculate chronic values (an exception being for renewal tests with cladocerans where test concentrations were measured). In the case of PFOS, static, renewal, and flow-through experiments were considered for possible inclusion for both species mean acute

and chronic values regardless of whether PFOS concentrations were measured because PFOS is a highly stable compound (see Section 1.2.1), resistant to hydrolysis, photolysis, volatilization, and biodegradation (see Section 2.3)(Giesy et al. 2010).

Additionally, chronic values were based on endpoints and durations of exposure that were appropriate to the species. Thus, both life- and partial life-cycle tests were utilized for the derivation of the chronic criteria. However, it should be noted that typically, per the 1985 Guidelines, life-cycle chronic tests would be preferred for invertebrates. The chronic studies used in the derivation of the PFOS criteria followed taxa-specific exposure duration requirements from various test guidelines (i.e., EPA's 1985 Guidelines and EPA's OCSPP's Ecological Effects Test Guidelines, (i.e., EPA's 1985 Guidelines and EPA's OCSPP's Ecological Effects Test Guidelines, U.S. EPA 2016b) when available. For example, the EPA's 1985 Guidelines states that daphnid tests should begin with young < 24 hours old and last for not less than 21 days; and this chronic test duration was applied to the consideration of all chronic daphnid tests. When taxa-specific exposure duration requirements were not available for a particular test organism in the PFOS toxicity literature, both life- and partial life-cycle tests were considered in the derivation of the chronic criteria.

PFOS toxicity in aquatic life can be manifested as effects on survival, growth, and/or reproduction. Measurements of fish tissue, such as whole-body, muscle, and eggs, were most closely linked to the chronic adverse effects of PFOS, since PFOS is highly persistent and bioaccumulative. The following subsection of this problem formulation describes the approaches used to establish PFOS effect concentrations in aquatic life in relation to the various criteria derived, including for water and tissue.

2.10.3 Measures of Effect

Each assessment endpoint requires one or more "measures of ecological effect," which are defined as changes in the attributes of an assessment endpoint itself or changes in a surrogate entity or attribute in response to chemical exposure. Ecological effects toxicity test data are used as measures of direct and indirect effects to growth, reproduction, and survival of aquatic organisms.

2.10.3.1 Acute Measures of Effect

The acute measures of effect on aquatic organisms are the lethal concentration (LC_{50}), effect concentration (EC_{50}), or inhibitory concentration (IC_{50}) estimated to produce a specific effect in 50 percent of the test organisms (Table 2-1). LC_{50} is the concentration of a chemical that is estimated to kill 50 percent of the test organisms. EC_{50} is the concentration of a chemical that is estimated to produce a specific effect (e.g., immobilization) in 50 percent of the test organisms. The IC_{50} is the concentration of a chemical that is estimated to inhibit some biological process (e.g., enzyme activity associated with an apical endpoint such as mortality) in 50 percent of the test organisms.

2.10.3.2 Chronic Measures of Effect

The measure of effect for chronic exposures of PFOS was the effect concentration estimated to produce a chronic effect on survival, growth, or reproduction in 10 percent of the test organisms (EC_{10}; Table 2-1). The EPA selected an EC_{10} to estimate a low level of effect that would be both different from controls and not expected to be severe enough to cause severe effects at the population level for a bioaccumulative contaminant, such as PFOS. The use of the EC_{10}, instead of an $EC_{20,}$ is also consistent with the use of this metric for the bioaccumulative pollutant selenium in the recent 2016 Selenium Freshwater Aquatic Life Criteria (U.S. EPA 2016c), and is consistent with the harmonized guidelines from OECD and the generally preferred

effect level for other countries such as Canada, Australia and New Zealand (CCME 2007; OECD 2001; Warne et al. 2018).

Regression analysis was used preferentially to characterize a concentration-response (C-R) relationship and to estimate concentrations at which chronic effects are expected to occur. Author-reported No Observed Effect Concentrations (NOECs) and Lowest Observed Effect Concentrations (LOECs) were only used for the derivation of chronic criterion when a robust EC_{10} could not be calculated for the genus. A NOEC is the highest test concentration at which none of the observed effects are statistically different from the control. A LOEC is the lowest test concentration at which the observed effects are statistically different from the control. When LOECs and NOECs are used, a Maximum Acceptable Toxicant Concentration (MATC, geometric mean of the NOEC and LOEC) is calculated. For the calculation of the chronic criteria, point estimates were selected for use as the measure of effect in favor of MATCs, as MATCs are highly dependent on the concentrations tested. Point estimates also provided additional information that is difficult to determine with an MATC, such as a measure of effect level across a range of tested concentrations.

In conformity with the 2013 Ammonia Freshwater Aquatic Life Criteria (U.S. EPA 2013), a decision rule was also applied to the PFOS toxicity data when an author-reported NOEC or LOEC was used. The decision rule was not to use "greater than" values for concentrations of low magnitude or "less than" values for concentrations of high magnitude because they added little significant information to the analyses. Conversely, if data from studies with only low concentrations indicated a significant effect (suggesting the test material was highly toxic) or studies with high concentrations only found an incomplete response for a chronic endpoint (indicating low toxicity of the test material), those data did significantly enhance the

understanding of PFOS toxicity. Thus, the decision rule was applied as follows: "greater than" (>) high toxicity values and "less than" (<) low toxicity values were included (U.S. EPA 2013). Data that met the quality objectives and test requirements were utilized quantitatively in deriving these criteria for aquatic life and are presented in Table 3-3 and Table 3-7.

Table 2-1. Summary of Assessment Endpoints and Measures of Effect Used in the Criteria Derivation for PFOS.

Assessment Endpoints for the Aquatic Community	Measures of Effect
Aquatic Life: Survival, growth, and reproduction of freshwater and estuarine/marine aquatic life (i.e., fish, amphibians, aquatic invertebrates)	For effects from acute exposure: 1. LC_{50}, EC_{50}, or IC_{50} concentrations in water For effects from chronic exposure: 1. EC_{10} concentrations in water 2. NOEC and LOEC concentrations in water. *Only used when an EC_{10} could not be calculated for a genus.* *Note: only chronic exposures were considered for derivation of the tissue-based criteria since PFOS is a bioaccumulative chemical. These chronic tissue-based criteria are expected to be protective of acute effects, because acute effects were observed at much greater concentrations than chronic effects.*

LC_{50} = 50% Lethal Concentration
EC_{50} = 50% Effect Concentration
IC_{50} = 50% Inhibitory Concentration
NOEC = No-observed-effect-concentration
LOEC = Lowest-observed-effect-concentration
EC_{10} = 10% Effect Concentration

2.10.3.3 Summary of Independent Calculation of Toxicity Values

Where data were available, toxicity values, including LC_{50} and EC_{10} values, were independently calculated using data from the toxicity studies meeting the inclusion criteria described above, via independent statistical analysis conducted by the EPA. Occasionally, individual replicate-level data or treatment-level data were acquired from the study authors to independently calculate toxicity values. All data were analyzed using the statistical software

program R (version 3.6.2) and the associated dose-response curve (drc) package. The R drc package has several models available for modeling a C-R relationship for each toxicity study. The specific model used to calculate toxicity values was selected following the details provided in Appendix K and the models performed well on most or all statistical metrics. The independently-calculated toxicity values used to derive the PFOS aquatic life criteria are included in each quantitative study summary below and were utilized to derive these criteria for aquatic life, where available (for the acute criterion see genus mean values in Table 3-9 and for the chronic criterion, see genus mean values in Table 3-10).

2.11 Analysis Plan

2.11.1 Derivation of Water Column Criteria

During CWA Section 304(a) criteria development, the EPA reviews and considers all relevant toxicity test data. Information available for all relevant species and genera are reviewed to identify: 1) data from acceptable tests that meet data quality standards; and 2) whether the acceptable data meet the minimum data requirements (MDRs) as outlined in the EPA's 1985 Guidelines (U.S. EPA 1985). The MDRs described in Section 2.10.1 were met for acute and chronic freshwater criteria derivation. Acute and chronic MDRs for PFOS estuarine/marine criteria derivation were not met. Consequently, the EPA used the available toxicity data and the EPA's New Approach Methods (NAMs) to generate protective estuarine/marine benchmarks. A minimal number of tests from acceptable studies of aquatic algae and vascular plants were also available. The relative sensitivity of freshwater plants to PFOS exposures indicates plants are less sensitive than aquatic vertebrates and invertebrates so plant criteria were not developed.

2.11.2 Consideration for the Derivation of Tissue-Based Criteria following Chronic PFOS Exposures

Chronic toxicity studies (both laboratory and field studies) were further screened to ensure that they contained the relevant chronic PFOS exposure routes for aquatic organisms (i.e., dietary, maternal, or dietary and waterborne PFOS exposure), measurement of chronic effects, and measurement of PFOS in tissue(s). The EPA considered deriving tissue-based criteria using empirical toxicity tests with studies that exposed test organisms to PFOS via water, diet, and/or maternal transfer and reported exposure concentrations based on measured tissue concentrations. This approach generally corresponded with the 2016 Selenium Aquatic Life Freshwater Criterion, which is the only other EPA 304(a) recommended aquatic life criterion with tissue-based criteria (U.S. EPA 2016c). However, currently, the freshwater chronic PFOS toxicity dataset with measured tissue concentrations is somewhat limited. There were 14 total chronic aquatic life studies considered, six quantitative (three fish, one invertebrate, and two amphibian studies) and eight qualitative studies (see Section 4.5). The quantitative studies provided data for three of the eight MDRs. The qualitative studies provided supporting information for only one additional MDR. Therefore, it was concluded that there are currently insufficient data to derive a chronic tissue criterion using a GSD approach from empirical tissue data from toxicity studies. Thus, the EPA used a Bioaccumulation Factor (BAF) approach for chronic tissue criteria development.

2.11.3 Translation of Chronic Water Column Criterion to Tissue Criteria

To enable use of fish tissue measurements of PFOS in protecting designated uses, chronic tissue criteria for PFOS were derived by translating the chronic freshwater water column criterion (summarized in Section 2.11.1 above) into tissue criteria using bioaccumulation factors (BAFs) and the following equation:

$$Tissue\ Criteria = Chronic\ Water\ Column\ Criterion\ \times BAF \qquad (Eq.\ 1)$$

The resulting tissue criteria correspond to the tissue type serving as the basis of the BAF used in the equation.

2.11.3.1 Aquatic Life Bioaccumulation Factors (BAFs)

A BAF is determined from field measurements and is calculated using the equation:

$$BAF = \frac{C_{biota}}{C_{water}} \qquad (Eq.\ 2)$$

Where:
C_{biota} = PFOS concentration in organismal tissue(s)
C_{water} = PFOS concentration in water where the organism was collected

Given that a BAF is determined from field measurements [as opposed to controlled experiments designed to measure bioconcentration of PFOS using specific test guidelines; (OECD 2001; U.S. EPA 2016c)], a BAF is an expression of all exposure routes, i.e., dietary, water, maternal transfer, and contact with water and sediments via skin and ingestion. Depending upon the tissue residue measurement, BAFs can be based upon residues in the whole organisms, muscle, liver, or any other tissue.

The literature search for reporting on PFOS bioaccumulation was implemented by developing a series of chemical-based search terms. These terms included chemical names and Chemical Abstracts Service registry numbers (CASRN or CAS[3]), synonyms, tradenames, and other relevant chemical forms (i.e., related compounds). Databases searched were Current Contents, ProQuest CSA, Dissertation Abstracts, Science Direct, Agricola, TOXNET, and UNIFY (database internal to U.S. EPA's ECOTOX database). The literature search yielded numerous citations and the citation list was further refined by excluding citations on analytical

[3] Chemical Abstracts Service registry number (CASRN or CAS) for PFOS is 1763-23-1.

methods, human health, terrestrial organisms, bacteria, and where PFOS was not a chemical of study. The citations meeting the search criteria were reviewed for reported BAFs and/or reported concentrations in which BAFs could be calculated. Data from papers with appropriate information were extracted into a PFOS dataset. The studies meeting these inclusion criteria were also screened for data quality.

Four factors were evaluated in the screening of the BAF literature: 1) number of water samples; 2) number of organism samples; 3) water and organism temporal coordination in sample collection; and 4) water and organism spatial coordination in sample collection. Additionally, the general experimental design was evaluated. For further details on BAFs compilation and ranking, see .

Table 2-2 below outlines the screening criteria for study evaluation and ranking. Only BAFs of high and medium quality were used to derive the tissue criteria (Appendix O). For further details on BAFs compilation and ranking, see Burkhard (2021).

Table 2-2. Evaluation Criteria for Screening Bioaccumulation Factors (BAFs) in the Public Literature.

Table modified from Burkhard (2021).

Screening Factor	High Quality	Medium Quality	Low Quality
Number of Water Samples	> 3	2 – 3	1
Number of Organism Samples[1]	> 3	2 – 3	1
Temporal Coordination	Concurrent collection	Within one year	Collection period > 1 year
Spatial Coordination	Co-located collection	Within 1 - 2 km	Significantly different locations (> 2 km)
General Experimental Design			Mixed species tissues samples

[1] Organismal samples from the same species and tissue type.

3 EFFECTS ANALYSIS FOR AQUATIC LIFE

3.1 Toxicity to Aquatic Life

All available, reliable studies relating to the acute and chronic toxicological effects of PFOS on aquatic life were considered in the derivation of the national recommended PFOS criteria. Data for possible inclusion in the PFOS criteria were obtained from published literature reporting acute and chronic exposures of PFOS that were associated with mortality, survival, growth, and reproduction. This set of published literature was identified by the EPA's public ECOTOX database (ECOTOX: https://cfpub.epa.gov/ecotox/) as meeting data quality standards. ECOTOX is a source of high-quality toxicity data for aquatic life, terrestrial plants, and wildlife. The database was created and is maintained by the EPA, Office of Research and Development, Center for Computational Toxicology and Exposure. Studies were then further reviewed by the EPA OW to determine test acceptability for use in the criteria derivation. Additional literature searches were also conducted to ensure all available toxicity data were captured. The latest search was conducted through March 2024.

3.1.1 Summary of PFOS Toxicity Studies Used to Derive the Aquatic Life Criteria

Quantitative data for acute PFOS toxicity were available for 29 freshwater species, representing 20 genera and 17 families in five phyla, and six estuarine/marine species, representing six genera and five families in four phyla. Chronic PFOS toxicity data were available for 19 freshwater species, representing 17 genera and 15 families in four phyla, and five estuarine/marine species, representing five genera and five families in three phyla (Table 3-1).

Table 3-1. Summary Table of Minimum Data Requirements per the 1985 Guidelines Reflecting the Number of Acute and Chronic Genus and Species Level Mean Values in the Freshwater and Saltwater Toxicity Datasets for PFOS.

MDR	Freshwater			
	GMAV	**SMAV**	**GMCV**	**SMCV**
Family Salmonidae in the class Osteichthyes	1	1	1	1
Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species	2	2	2	2
Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)	5	10	3	4
Planktonic Crustacean	2	5	3	4
Benthic Crustacean	3	3	2	2
Insect	1	1	3	3
Family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, or Mollusca)	5	6	2	2
Family in any order of insect or any phylum not already represented	1	1	1	1
Total	**20**	**29**	**17**	**19**
MDR	**Saltwater[a]**			
	GMAV	**SMAV**	**GMCV**	**SMCV**
Family in the phylum Chordata	1	1	1	1
Family in the phylum Chordata	0	0	0	0
Either the Mysidae or Penaeidae family	2	2	1	1
Family in a phylum other than Arthropoda or Chordata	1	1	0	0
Family in a phylum other than Chordata	1	1	1	1
Family in a phylum other than Chordata	1	1	1	1
Family in a phylum other than Chordata	0	0	1	1
Any other family	0	0	0	0
Total	**6**	**6**	**5**	**5**

[a] The 1985 Guidelines require that data from a minimum of eight families are needed to calculate an estuarine/marine criterion. Insufficient data exist to fulfill all eight of the taxonomic MDR groups. Consequently, the EPA cannot derive an estuarine/marine acute criterion, based on the 1985 Guidelines. However, the EPA has developed estuarine/marine benchmarks through use of surrogate data to fill in missing MDRs using the EPA's Web-based Inter-species Correlation Estimation (web-ICE) tool. These benchmarks are provided in Appendix L.

Below are the summarized studies that provided key acute and chronic freshwater toxicity data with effect values that were used quantitatively in deriving the acute and chronic freshwater criteria to protect aquatic life from harmful exposure to PFOS. Study summaries are

also provided for the estuarine/marine toxicity data that could be used quantitatively to derive acute and chronic estuarine/marine criteria if the MDRs were met.

Study summaries for the most sensitive taxa are grouped by acute or chronic exposure and sorted by sensitivity to PFOS. Study data were summarized in tabular form in Appendix A (freshwater acute studies), Appendix B (estuarine/marine acute studies), Appendix C (freshwater chronic studies), and Appendix D (estuarine/marine chronic studies). Key acute and chronic toxicity studies used qualitatively as supporting information are described in the Effects Characterization (Section 4) below and corresponding data are listed in Appendix E, Appendix F, Appendix G and Appendix H while the remaining, unused studies are listed in Appendix J.

Acute and chronic values were presented as reported by the study authors for each individual study. The EPA independently calculated toxicity values if sufficient raw data were available to conduct statistical analyses. All toxicity values, such as LCs, ECs, NOECs, LOECs, and species- and genus-mean values, were given to four significant figures to prevent round-off error in subsequent calculations, not to reflect the precision of the value. The author-reported toxicity values and the EPA's independently-calculated values (where available) were included for each study throughout the document (in the quantitative data study summaries and appendices as applicable), and the specific value utilized to derive the criteria were identified along with a justification. The EPA's independently-calculated toxicity values were used preferentially, where available.

3.1.1.1 Summary of Acute PFOS Toxicity Studies Used to Derive the Freshwater Aquatic Life Criterion

Acute toxicity data were available for all of the freshwater MDRs. Acceptable data on the acute effects of PFOS in freshwater were available for a total of 29 species representing 20 genera and 17 families in five phyla (Appendix A: Acceptable Freshwater Acute PFOS Toxicity

Studies). More specifically, quantitative data for acute PFOS toxicity were available for three freshwater fish species (two of the eight MDRs), 16 freshwater invertebrate species (five of the eight MDRs), and 10 freshwater amphibian species (one of the eight MDRs). Ranked genus mean acute values (GMAVs) for PFOS in freshwater based on acute toxicity were identified in Table 3-2 (4 most sensitive genera) and Table 3-3 (all genera) and plotted in Figure 3-1.

Table 3-2. The Four Most Sensitive Genera Used in Calculating the Acute Freshwater Criterion (Sensitivity Rank 1-4).
Ranked below from most to least sensitive.

Rank	Genus	Species	GMAV (mg/L)
1	*Neocloeon*	Mayfly, *N. triangulifer*	0.07617
2	*Moina*	Cladoceran, *M. microcopa* and *M. micrura*	3.075
3	*Pimephales*	Fathead minnow, *P. promelas*	6.950
4	*Oncorhynchus*	Rainbow trout, *O. mykiss*	7.515

3.1.1.1.1 Most Sensitive Freshwater Genus for Acute Toxicity: Neocloeon (mayfly)

Soucek et al. (2023) conducted a 96-hour acute toxicity test to determine the effects of PFOS-K (PFOS potassium salt, CAS # 2795-39-3, 98% purity) in water on the parthenogenetic mayfly, *Neocloeon triangulifer*. The test was performed under static, nonrenewal conditions beginning with < 24 hour old nymphs. Mayflies were fed live diatom biofilm scraping beginning on day 0. Feeding only occurred on day 0. The authors indicated test organisms required food to survive the entire 96-hour exposure, with previous studies demonstrating greater than 80% mortality at 48 hours with no food (Soucek and Dickinson 2015). Percent survival in the control treatment after 96 hours was 100%. The EPA was able to independently calculate a 96-hour LC_{50} of 0.07617 mg/L (0.06546 - 0.08688 mg/L; 95% CI) for this study. The EPA's independently-

calculated LC_{50} is in line with the author-reported LC_{50} of 0.08 mg/L. Therefore, the independently-calculated LC_{50} of 0.07617 mg/L was used quantitatively to derive the freshwater acute water column criterion for PFOS.

3.1.1.1.2 Second Most Sensitive Freshwater Genus for Acute Toxicity: Moina (cladoceran)

Ji et al. (2008) performed a 48-hour static, unmeasured acute test of PFOS (purity unreported) with *Moina macrocopa*. The test followed the EPA's Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms [U.S. EPA/600/4-90/027F; (U.S. EPA 2002)]. The test involved four replicates of five neonates each in five nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 6.25, 12.5, 25, 50 and 100 mg/L. Survival of organisms in the negative control was not reported in the paper. However, raw data were obtained by the EPA from the study authors and control survival was 100% in the acute test. The study authors reported a 48-hour EC_{50} value of 17.95 mg/L for *M. macrocopa*. The 48-hour EC_{50} value was independently-calculated by the EPA as 17.20 mg/L. The independently-calculated acute toxicity value was quantitatively used in the derivation of the freshwater acute water column criterion.

Razak et al. (2023) tested the acute toxicity of perfluorooctanesulfonate (PFOS, ≥98% purity) on *Moina micrura* for 48 hours in a measured, static experiment. Testing methods followed OECD 202 (OECD 2004) with nominal testing concentrations of 10, 25, 50, 75, 100, 250, 500, 750, 1,000, 2,500, 5,000, 7,500, and 10,000 µg/L, plus a control, with four replicates per treatment. Test water was filtered lake water. Each replicate consisted of 10 neonates (<48 hours old) in 50 mL of solution in a 100 mL beaker, and organisms were not fed during the study. The lethal effect concentrations (LC) were calculated using Probit analysis, and the 48-

hour LC_{50} value of 549.6 µg/L, or 0.5496 mg/L was determined to be acceptable for quantitative use.

The geometric mean of the two SMAVs for *Moina macrocopa* (17.20 mg/L) and *Moina micrura* (0.5496 mg/L) were used to calculate the GMAV of 3.075 mg/L for the genus *Moina*. If the EPA excluded the *M. micrura* SMAV on the basis of it being an overly sensitive outlier (relative to *M. macrocopa* and the overall acute data except for *N. triangulifer*) that would result in the final PFOS acute criterion potentially being underprotective of untested sensitive invertebrate or other taxa, considering that the available data serve as surrogate information for the thousands of untested freshwater species. Conversely, excluding the *M. macrocopa* SMAV on the basis of it being a tolerant outlier (relative to *M. micrura*) would result in the Moina GMAV being highly influenced by a single test/species with an LC_{50} that was relatively sensitive (i.e., *M. micrura* SMAV = 0.5496 mg/L) compared to the overall acute data with the exception of *N. triangulifer*. Averaging the *M. micrura* and *M. moina* SMAVs resulted in a GMAV (3.075 mg/L) that was the second most sensitive GMAV, still in the general range of the data overall.

3.1.1.1.3 Third Most Sensitive Freshwater Genus for Acute Toxicity: Pimephales (fathead minnow)

Drottar and Krueger (2000c) evaluated the acute effects of PFOS-K (PFOS potassium salt, CAS# 2795-39-3, Lot # 217 (T-6295) obtained from the 3M Company, 90.49% purity) on juvenile fathead minnows (*Pimephales promelas*) during a 96-hour measured, static study. Researchers followed protocols from U.S. EPA Series 850, OPPTS 850.1075 and OECD Guideline 203. All fish used in the test were from the same source and year class, and the total length of the longest fish was no more than twice the length of the shortest. The authors reported an LC_{50} of 9.5 mg/L PFOS. The EPA's independently-calculated 96-hour LC_{50} was 9.020 mg/L and was used quantitatively to derive the freshwater acute water column criterion for PFOS. 3M

Company (2000) provides the results of a 96-hour static, unmeasured acute toxicity test with the fathead minnow and PFOS-Li (PFOS lithium salt, CAS # 29457-72-5). Fish were 79 days old at test initiation with an average length of 2.1 cm and weight of 0.069 g. No mortality occurred in the control treatment and 100% mortality was observed in the highest treatment (56 mg/L). The study authors reported that the test sample containing 24.5% PFOS-Li exhibited a 96-hour LC_{50} of 19 mg/L, which equates to 4.655 mg/L as PFOS. The independently-calculated 96-hr LC_{50} value was 21.86 mg/L, which equates to 5.356 mg/L as PFOS, and was used quantitatively to derive the freshwater acute water column criterion for PFOS.

The geometric mean of the two acute toxicity values described above for *P. promelas* (9.020 and 5.356 mg/L) were used to calculate an SMAV and GMAV of 6.950 mg/L, which represents the second most sensitive GMAV in the EPA's freshwater acute dataset for PFOS.

3.1.1.1.4 Fourth Most Sensitive Freshwater Genus for Acute Toxicity: Oncorhynchus (trout)

Sharpe et al. (2010) evaluated the acute effects of PFOS-K (potassium salt, CAS # 2795-39-3, 98% purity) to *Oncorhynchus mykiss*, rainbow trout, via a 96-hour renewal exposure with measured concentrations (renewal was not stated in paper, but assumed based on other information provided, including the test Guideline protocol that the authors cited as the protocol that was used). There were limited details in the publication about the test protocol; however, it was noted that the Organization for Economic Co-operation and Development (OECD) Guideline 203 was followed, and the study authors did not identify any deviations from these test guidelines. The EPA obtained clarification from the study authors on the experimental design regarding the biomass loading rate, which was 1 to 1.5 g/L (based on four fish weighing a total of 2 to 3 g per 2 L tank; personal communication with Greg Goss and Rainie Sharpe, March 2021). This biomass loading rate was slightly higher than that stated in OECD Guidelines of 0.8

g/L (OECD 1992). The author-reported 96-hour LC_{50} for the study was 2.5 mg/L. The authors do not specify if this concentration was nominal or measured. Given the clarifications regarding the biomass loading, the LC_{50} from this study was used quantitatively to derive the freshwater acute water column criterion for PFOS.

Palmer et al. (2002a) evaluated the acute effects of PFOS-K (potassium salt, identified as FC-95 obtained from 3M Company) to rainbow trout via a 96-hour static exposure with measured concentrations. The study author-reported 96-hour LC_{50} for the study was 22 mg/L. The independently-calculated 96-hour LC_{50} value was 22.59 mg/L. The independently-calculated LC_{50} was used quantitatively to derive the freshwater acute water column criterion for PFOS.

The geometric mean of the two toxicity values described above (2.5 and 22.59 mg/L), was used to calculate the SMAV and GMAV of 7.515 mg/L for rainbow trout, *O. mykiss*. The GMAV of 7.515 mg/L is consistent with the acute rainbow trout studies cited in OECD's 2002 PFOS Hazard Assessment, from which the LC_{50} values for rainbow trout range from 7.8 to 22 mg/L (OECD 2002).

Table 3-3. Ranked Freshwater Genus Mean Acute Values.

Rank[a]	GMAV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOS)
1	0.07617	F	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	0.07617
2	3.075	D	*Moina*	Cladoceran, *Moina macrocopa*	17.20
				Cladoceran, *Moina micrura*	0.5496
3	6.950	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	6.950
4	7.515	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	7.515
5	13.50	G	*Ligumia*	Black sandshell, *Ligumia recta*	13.50

Rank[a]	GMAV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOS)
6	15.61	E	*Neocaridina*	Japanese swamp shrimp, *Neocaridina denticulata*	15.61
7	15.99	C	*Xenopus*	African clawed frog, *Xenopus laevis*	15.99
8	16.50	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	16.50
9	19.88	C	*Hyla*	Gray treefrog, *Hyla versicolor*	19.88
10	22.48	G	*Dugesia*	Planaria, *Dugesia japonica*	22.48
11	27.86	B	*Danio*	Zebrafish, *Danio rerio*	27.86
12	43.15	D	*Daphnia*	Cladoceran, *Daphnia carinata*	11.56
				Cladoceran, *Daphnia magna*	51.86
				Cladoceran, *Daphnia pulicaria*	134.0
13	47.40	C	*Ambystoma*	Jefferson salamander, *Ambystoma jeffersonianum*	51.71
				Small-mouthed salamander, *Ambystoma texanum*	30.00
				Eastern tiger salamander, *Ambystoma tigrinum*	68.63
14	48.81	E	*Pontastacus*	Crayfish, *Pontastacus leptodactylus*	48.81
15	56.49	C	*Anaxyrus*	American toad, *Anaxyrus americanus*	56.49
16	59.87	E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	59.87
17	61.80	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	61.80
18	64.35	G	*Elliptio*	Eastern elliptio, *Elliptio complanata*	64.35
19	109.2	C	*Lithobates*	American bullfrog, *Lithobates catesbeiana*	133.3
				Green frog, *Lithobates clamitans*	113.0
				Northern leopard frog, *Lithobates pipiens*	72.72
				Wood frog, *Lithobates sylvatica*	130.0

Rank[a]	GMAV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOS)
20	172.1	G	*Physella*	Bladder snail, *Physella acuta*	183.0
				Snail, *Physella heterostropha pomilia*	161.8

[a] Ranked from the most sensitive to the most tolerant based on Genus Mean Acute Value.
[b] From Appendix A: Acceptable Freshwater Acute PFOS Toxicity Studies.
[c] MDR Groups identified by list provided in Section 2.10.1 above.

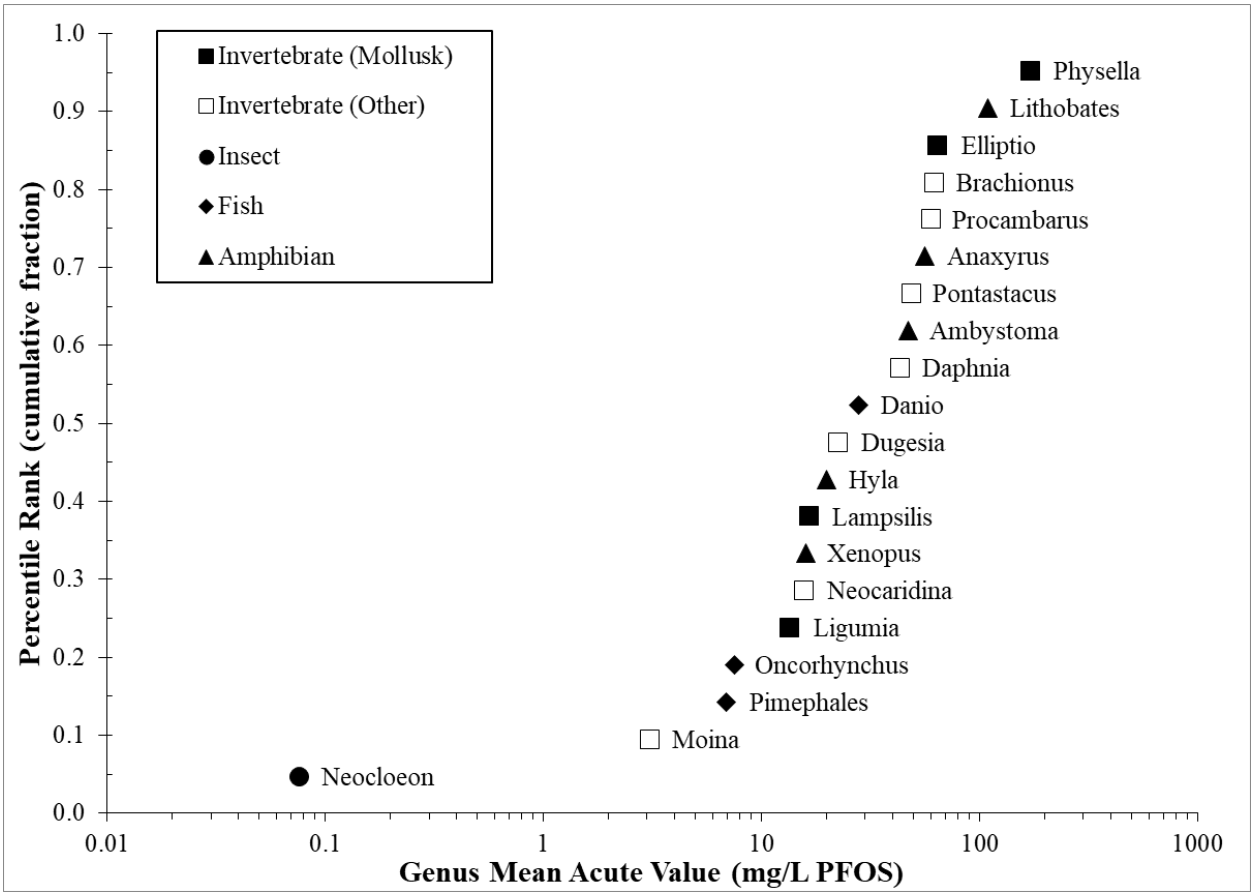

Figure 3-1. Freshwater Acute PFOS GMAVs Fulfilling the Acute MDRs.

3.1.1.2 Summary of Acute PFOS Toxicity Studies Used to Derive the Estuarine/Marine Aquatic Life Criterion

Quantitative empirical data for acute PFOS toxicity were available for six saltwater species, representing six genera and five families. The data available for saltwater species fulfilled only five of the eight MDRs. In the interest of providing information to states/authorized Tribes on protective values, the EPA developed an estuarine/marine acute benchmark using the

available empirical data supplemented with toxicity values generated through the use of NAMs, specifically through the use of the EPA Office of Research and Development's peer-reviewed publicly-available Web-based Interspecies Correlation Estimation (WebICE) tool (Raimondo et al. 2010). These benchmarks are provided in Appendix L. Table 3-4 below shows the four most sensitive acute estuarine/marine genera that could be used quantitatively to derive acute criteria if the MDRs were met. Ranked GMAVs for saltwater organisms based on acceptable acute toxicity values were identified in Table 3-5 and plotted in Figure 3-2.

Table 3-4. The Four Most Sensitive Acute Estuarine/Marine Genera.
Ranked Below from Most to Least Sensitive.

Rank	Genus	Species	GMAV (mg/L PFOS)
1	*Mytilus*	Mediterranean mussel, *M. galloprovincialis*[1]	1.1
2	*Strongylocentrotus*	Purple sea urchin, *S. purpuratus*	1.7
3	*Paracentrotus*	Sea urchin, *P. lividus*[2]	1.795
4	*Americamysis*	Mysid, *A. bahia*	4.914

[1] Not a resident species in North America, but other species in this genus are resident and commercially or ecologically important species.
[2] Not a resident species in North America, but other species in this family (Echinidae) are common ecotoxicity test species that serves as a surrogate for untested urchin species residing in North America.

3.1.1.2.1 Most Sensitive Estuarine/Marine Genus for Acute Toxicity: Mytilus (mussel)

The acute toxicity of perfluorooctane sulfonate (PFOS, purity not provided) on the Mediterranean mussel, *Mytilus galloprovincialis* was evaluated by **Fabbri et al. (2014)**. This species is not resident to North America, but is a surrogate for North American mussel species, including the widespread, commercially and ecologically important blue mussel, *Mytilus edulis.* The test endpoint was the percentage of normal D-larvae in each well, including malformed larvae and pre-D stages, at test termination (48 hours). The acceptability of test results was based

on controls for a percentage of normal D-shell stage larvae, > 75% (ASTM 2004b). The percentage of normal D-larva decreased with increasing test concentrations. The NOEC and LOEC reported for the study were 0.00001 and 0.0001 mg/L, respectively. However, the test concentrations failed to elicit a 50% reduction in malformations in the highest test concentration (1 mg/L), and an EC_{50} was not determined. Therefore, the EC_{50} for the study was greater than the highest test concentration (1 mg/L). The 48-hour EC_{50} based on malformation of > 1 mg/L was acceptable for quantitative use.

Hayman et al. (2021) report the results of a 48-hour static, measured test on the effects of PFOS-K (PFOS potassium salt, CAS # 2795-39-3, 98% purity) on *M. galloprovincialis*. Authors noted that tests followed U.S. EPA (1995) and ASTM (2004a) protocols. Larvae were enumerated for total number of larvae that were alive at the end of the test as well as number of normally-developed D-shaped larvae. There were no significant differences between solvent control and filtered seawater, suggesting no adverse effects of methanol. The author-reported 48-hr EC_{50}, based on normal development, was 1.1 mg/L. The EPA was not able to independently calculate a 48-hour EC_{50} value as the curve fitted model did not result in a good fit. Therefore, the author-reported EC_{50} of 1.1 mg/L was considered for quantitative use.

The two EC_{50} values from the two studies both indicated sensitivity of the Mediterranean mussel to acute exposure of PFOS is above 1 mg/L. However, the EC_{50} for *M. galloprovincialis* from **Fabbri et al. (2014)** was unbounded while the EC_{50} from **Hayman et al. (2021)** was definitive, and therefore the latter EC_{50} (1.1 mg/L) serves as the basis for the SMAV and GMAV used to derive the acute estuarine/marine benchmark for PFOS.

3.1.1.2.2 Second Most Sensitive Estuarine/Marine Genus for Acute Toxicity: Strongylocentrotus (sea urchin)

The **Hayman et al. (2021)** study also included the results of a 96-hour static, measured test on the effects of PFOS-K (PFOS potassium salt, CAS # 2795-39-3, 98% purity) on the purple sea urchin, *Strongylocentrotus purpuratus*. Authors noted that tests followed U.S. EPA (1995) and ASTM (2004a) protocols. At test termination (96 hours), the first 100 larvae were enumerated and observed for normal development (4-arm pluteus stage). As with the other tests in the study with different species, there were no significant differences between solvent control and filtered seawater, suggesting no adverse effects of methanol. The author-reported 96-hour EC_{50}, based on normal development, was 1.7 mg/L. The EPA was not able to independently calculate a 96-hour EC_{50} value as the curve fitted model did not result in a good fit. Therefore, the author-reported EC_{50} of 1.7 mg/L mg/L was thus applied for quantitative use and was utilized as the SMAV and GMAV to derive the acute estuarine/marine benchmark for PFOS.

3.1.1.2.3 Third Most Sensitive Estuarine/Marine Genus for Acute Toxicity: Paracentrotus (sea urchin)

A 72-hour static, unmeasured PFOS (purity not provided) toxicity test with the sea urchin, *Paracentrotus lividus* (a non-North American species) was conducted by **Gunduz et al. (2013)**. The 72-hour EC_{50} based on normal development to the pluteus stage was 1.795 mg/L PFOS and was acceptable for quantitative use; however, additional consideration needs to be given to the use of this value in benchmark derivation due to the short test duration.

3.1.1.2.4 Fourth Most Sensitive Estuarine/Marine Genus for Acute Toxicity: Americamysis (mysid)

Along with the Mediterranean mussel and purple sea urchin, **Hayman et al. (2021)** conducted a 96-hour static, measured test to determine the effects of PFOS-K on the mysid, *Americamysis bahia*. Authors noted that tests followed U.S. EPA (1995) and ASTM (2004a)

protocols. Only two of the sixty organisms (3.3%) were found dead in the controls at test termination. The author-reported 96-hour LC_{50} is 5.1 mg/L PFOS-K. The independently-calculated 96-hr LC_{50} value was 4.914 mg/L and is acceptable for quantitative use in the derivation of the acute estuarine/marine benchmark for PFOS.

Table 3-5. Ranked Estuarine/Marine Water Genus Mean Acute Values.

Rank[1]	GMAV (mg/L PFOS)	MDR Group[3]	Genus	Species	SMAV[2] (mg/L PFOS)
1	1.1	D	*Mytilus*	Mussel, *Mytilus galloprovincialis*	1.1
2	1.7	F	*Strongylocentrotus*	Purple sea urchin, *Strongylocentrotus purpuratus*	1.7
3	1.795	E	*Paracentrotus*	Sea urchin, *Paracentrotus lividus*	1.795
4	4.914	C	*Americamysis*	Mysid, *Americamysis bahia*	4.914
5	6.9	C	*Siriella*	Mysid, *Siriella armata*	6.9
6	>15	A	*Cyprinodon*	Sheepshead minnow, *Cyprinodon variegatus*	>15

[1] Ranked from the most sensitive to the most tolerant based on Genus Mean Acute Value.
[2] From Appendix B: Acceptable Estuarine/Marine Acute PFOS Toxicity Studies.
[3] MDR Groups identified by list provided in Section 2.10.1 above.

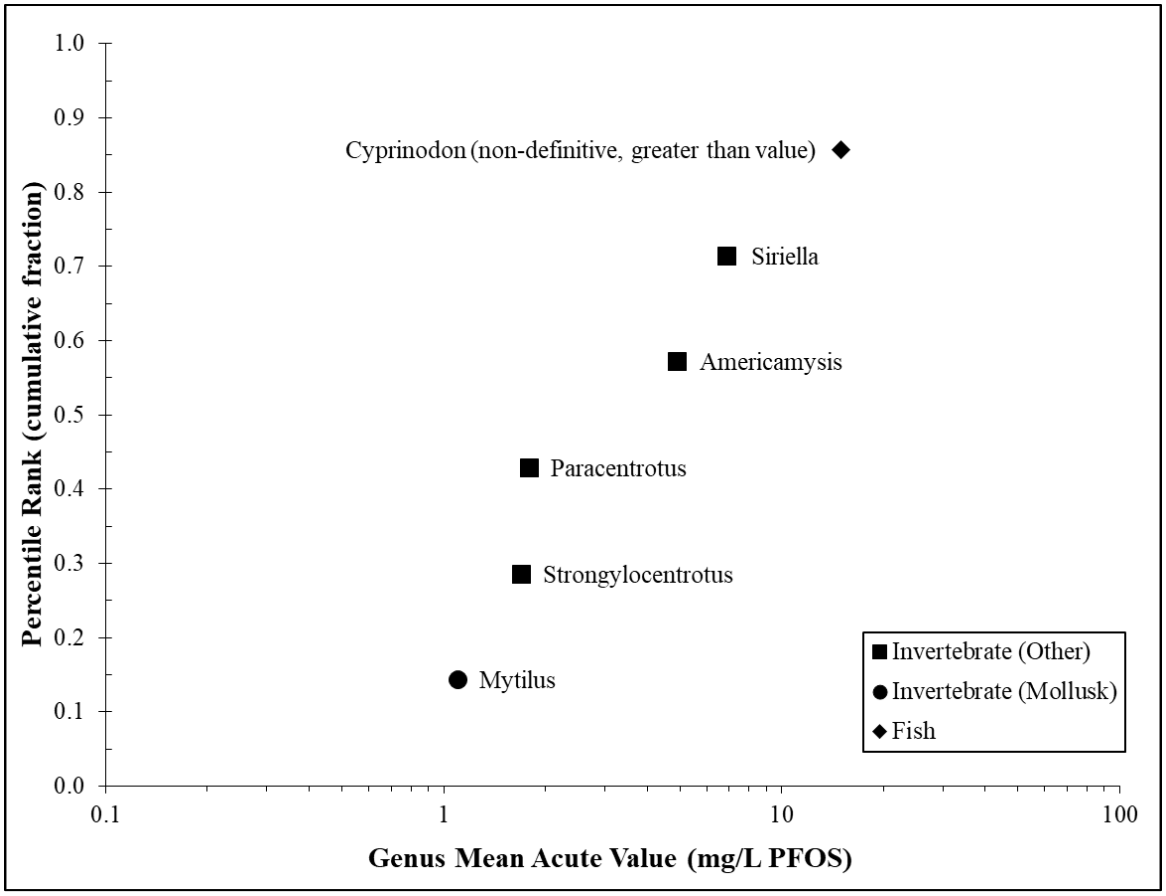

Figure 3-2. Acceptable Estuarine/Marine GMAVs.

3.1.1.3 Summary of Chronic PFOS Toxicity Studies Used to Derive the Freshwater Aquatic Life Criterion

Chronic toxicity data were available for all of the freshwater MDRs. Chronic PFOS toxicity data were available for 19 freshwater species, representing 17 genera and 15 families in four phyla. More specifically, quantitative data for acute PFOS toxicity were available for four freshwater fish species, representing four genera and three families (two of the eight MDRs), 11 freshwater invertebrate species, representing 11 genera and ten families (five of the eight MDRs), and three amphibian species, representing two genera in two families (one of the eight MDRs). Ranked GMCVs for PFOS in freshwater based on chronic toxicity are listed in Table 3-6 (four most sensitive genera) and Table 3-7 (all genera) and plotted in Figure 3-3.

Table 3-6. The Four Most Sensitive Genera Used in Calculating the Chronic Freshwater Criterion.

Ranked Below from Most to Least Sensitive.

Rank	Genus	Species	GMCV (mg/L PFOS)[1]
1	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	0.000226
2	*Chironomus*	Midge, *Chironomus dilutus*	0.005198
3	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	0.01768
4	*Enallagma*	Blue damselfly, *Enallagma cyathigerum*	0.03162

[1]Other values were used in additional analyses supporting the criterion calculation to examine the effects of less certain toxicity studies and non-resident species on the chronic freshwater criterion. See Section 4.1 below for more details.

3.1.1.3.1 Most Sensitive Freshwater Genus for Chronic Toxicity: Neocloeon (mayfly)

Soucek et al. (2023) conducted a chronic life-cycle test to determine the effects of PFOS-K (PFOS potassium salt, CAS # 2795-39-3, 98% purity) on the parthenogenetic mayfly, *Neocloeon triangulifer*. The test was performed under renewal conditions over 27 days beginning with < 24 hour old nymphs. There were sixteen (with one mayfly per replicate) replicates per test concentration and control. Replicates one through eight were destructively sampled on day 14 and replicates nine through sixteen continued until the end of the test (when all mayflies either molted into imago stage or died). The endpoints that were evaluated included survival for all replicates, 14-d length and calculated dry weight (using a previously published body dry weight equation; Besser et al. 2021) for replicates 1 through 8, and percent survival to pre-emergent nymph (PEN) stage, number of days until PEN stage, percent emergence (to imago stage), and pre-egg laying live weight of imago for replicates 9 through 16. Percent survival in the control after 14 days was 100%. Percent survival of mayflies after 14 days in the remaining seven test concentrations ranged from 79 to 100%. The most sensitive endpoint was 14-day dry weight. The study authors reported three different 14-day dry weight EC_{10} values that were

calculated using various point-estimation approaches. The author-reported 14-day dry weight EC_{10} values produced by the various approaches were relatively similar to one another, ranging from 0.000226 (using TRAP [2 parameter, threshold sigmoidal curve]) to 0.000272 mg/L (using log-linear regression, controls excluded). The EPA was not able to fit a reliable model with significant model parameters to the 14-day dry weight C-R dataset and, therefore, relied on the author-reported EC_{10} of 0.000226 mg/L (based on TRAP) as the primary effect concentration. The EPA selected the TRAP-based EC_{10} preferentially over the EC_{10} values based on the two other point estimation approaches (i.e., log-linear regression with and without controls) because the TRAP-based model (1) considered control responses; (2) was more fundamentally consistent with the maximum likelihood regression approaches used by the EPA to assess C-R datasets throughout this PFOS aquatic life AWQC document, and; (3) relied on replicate-level data, which the EPA used preferentially over treatment-mean data in assessing C-R datasets throughout the PFOS aquatic life criteria derivation process. The author-reported EC_{10} of 0.000226 mg/L (TRAP-based) was used quantitatively to derive the freshwater chronic water column criterion for PFOS.

3.1.1.3.2 Second Most Sensitive Freshwater Genus for Chronic Toxicity: Chironomus (midge)

MacDonald et al. (2004) conducted sub-chronic, partial-life cycle tests on larva and chronic life-cycle tests to determine the effects of PFOS-K (PFOS potassium salt, 95% purity) on the midge, *Chironomus dilutus* (formally known as *Chironomus tentans*). The test was performed under renewal conditions over 10 days for the larval test and greater than 50 days for the life-cycle test. The tests followed the general guidance given by EPA-600-R99-064 (U.S. EPA 2000b) and ASTM E 1706-00 (ASTM 2002).

The author-reported 10-day growth and survival EC_{10}s for the study were 0.0492 and 0.1079 mg/L, respectively. The study authors also reported NOECs of 0.0491 mg/L, LOECs of 0.0962 mg/L, and MATCs of 0.0687 mg/L for both endpoints. The author-reported 20-day EC_{10}s for growth, survival, and total emergence were 0.0882, 0.0864, and 0.0893 mg/L, respectively. The study authors also reported NOECs of 0.0217 mg/L for growth and survival and < 0.0023 mg/L for emergence, LOECs of 0.0949 mg/L for growth and survival and 0.0271 mg/L for emergence, and MATCs of 0.0454 mg/L for growth and survival and 0.0071 mg/L for emergence. It is noted here that the paper reported contrasting NOECs for 20-day survival. The text in the paper stated that the NOEC was 0.0271 mg/L and Table 2 of the paper provided a value of 0.0949 mg/L. The EPA assumed the NOEC in Table 2 of the paper was not correct and that 0.0217 mg/L was the correct NOEC based on the data presented in Figure 3A of the paper, since the EPA was unable to gain confirmation from the study authors. This assumption was applied to the summary of the study results presented in this PFOS aquatic life AWQC document. The EPA was able to independently calculate an EC_{10} for 10-day growth of 0.05896 mg/L for the study. The independently-calculated 10-day EC_{10} value for growth of the midge was used quantitatively to derive the freshwater chronic water column criterion for PFOS.

McCarthy et al. (2021) conducted two chronic toxicity tests with PFOS (98% purity) on the midge, *C. dilutus*, a 10-day and a 20-day exposure, following standard protocols from U.S. EPA (2000b) and ASTM (2002) with slight modifications. The 10-day exposure was considered a range finding test, with concentrations spaced by ~100x and only mortality measured, whereas the 20-day exposure measured both survival and growth and was termed an "abbreviated full life cycle test" by the study authors. The 20-day exposure is less than the recommended 50 – 65 day full-life cycle method outlined in U.S. EPA (2000b) and used in MacDonald et al. (2004), and

since exposures of midges started on day two or four, the actual exposure duration is only 16 or 19 days long. The most sensitive endpoint was survival from the "abbreviated full life cycle test" with an author-reported 16-day EC_{10} of 0.00136 mg/L PFOS. Additionally, the study authors reported EC_{10}s of 0.00162 and 0.00323 mg/L PFOS for growth as mean biomass and mean weight, respectively. The EPA was unable to independently calculate EC_{10}s for survival and mean weight. However, the EPA was able to independently calculate an EC_{10} value for mean biomass of 0.001588 mg/L PFOS. The independently-calculated 16-day EC_{10} for mean biomass was used quantitatively to derive the freshwater chronic water column criterion for PFOS.

Krupa et al. (2022) conducted a partial-life cycle chronic toxicity test with the midge, *C. dilutus*, and PFOS-K (perfluorooctanesulfonate potassium salt, > 98% purity, CAS No. 2795-39-3. The larvae were exposed to PFOS for 16 days. The measured exposure concentrations were < the limit of detection (LOD), 0.001, 0.0025, 0.004, 0.0075, 0.016 and 0.03 mg/L. At test termination, larval survival was assessed, and ash-free dry weight (AFDW) was determined following ASTM (2019). The AFDW of five groups of 12 larvae was measured at test initiation to establish a baseline for growth. The author-reported 16-day growth EC_{10} was 0.0015 mg/L PFOS-K. The EPA was unable to fit a reliable model for any of the chronic endpoints from this test. Therefore, the author-reported EC_{10} value of 0.0015 mg/L for growth presented in the paper was used quantitatively to derive the freshwater chronic water column criterion for PFOS.

The most sensitive endpoints from the two toxicity studies with *C. dilutus* that could be independently-calculated (see details in Appendix C.2.2) were for 10-day growth with an EC_{10} of 0.05896 mg/L (MacDonald et al. 2004) and 16-day mean biomass with an EC_{10} of 0.001588 mg/L (McCarthy et al. 2021). The EPA could not independently calculate the 16-day growth EC_{10} of 0.0015 mg/L (Krupa et al. 2022). Although over an order of magnitude difference exists

between MacDonald et al. (2004) and the other two studies, all three EC_{10}s were used quantitatively to derive the chronic aquatic life criterion with a SMCV and GMCV equal to the geometric mean of the three values or 0.005198 mg/L.

As mentioned in the Bots et al. (2010) summary (Section 3.1.1.3.4), the observed effects of PFOS on aquatic insects appeared to be consistent across the available data for chironomids and odonates. However, Bots et al. (2010) did not measure the effects of PFOS on nymph growth and therefore, the observed effects in that study cannot be compared with the results of MacDonald et al. (2004), McCarthy et al. (2021), and Krupa et al. (2022). The remainder of the toxicity values available for aquatic insects were used as supporting information to corroborate the toxicity value used to derive the freshwater chronic criterion and to better understand the effects of PFOS on aquatic insects in general. No other quantitative toxicity values were available for this species or genus.

3.1.1.3.3 Third Most Sensitive Freshwater Genus for Chronic Toxicity: Lampsilis (mussel)

Hazelton (2013); Hazelton et al. (2012) conducted a test of the long-term effects of PFOS (acid form, > 98% purity) on glochidia and juvenile life stages from the mussel *Lampsilis siliquoidea* using a unique experimental design for which standard methods have not been established. The test exposed brooding glochidia (in marsupia) for 36 days followed by a 24-hour exposure of free glochidia in a factorial design. As such, the free glochidia from the control group of the marsupia exposure were divided between a control and the two PFOS treatments and the PFOS treatments were split into control and the same PFOS treatment group as the marsupia exposure. This factorial design allowed for the comparison of PFOS effects in two different life-stages. See Appendix C.2.3 for additional details on the experimental design and considerations for the utilization of this study in the criterion derivation.

The data presented in the paper for metamorphosis success were considered for quantitative use in the derivation of the chronic criterion for PFOS (see Appendix C.2.3). The author-reported NOEC was 0.0045 mg/L and LOEC was 0.0695 mg/L. The reduction in metamorphosis success at the LOEC was estimated to be 35.4%. However, this was not a definitive test in that both the study design (which only included two treatment groups) and level of data presented (which are only presented graphically in Figure 2 of the paper) in the publication lack the details needed to fully understand the effects of chronic PFOS exposures to the glochidia and juvenile life stages of *L. siliquoidea.* Additionally, as there were only two PFOS treatment groups and the gap in these exposure concentrations is large (about 15-fold), the EPA was not able to fit a curve to estimate an EC_{10} in a manner similar to the other toxicity studies used to derive this criterion. Instead, both the use of an MATC and an estimated EC_{10} were considered for the chronic value. An EC_{10} was estimated by assuming the 0.0695 mg/L treatment represents an $EC_{35.4}$ and estimating the EC_{10} using the exposure response slope from another PFOS toxicity study focused on another mussel species (*Perna viridis*). Specifically, the chronic exposure of *Perna viridis* reported by Liu et al. (2013), which is summarized in Section 3.1.1.4.1, was used to derive a $EC_{10}/EC_{35.4}$ ratio from that study, which was 0.0033/0.0186, or 0.1770. Applying this ratio to Hazelton et al. (2012) yields an estimated EC_{10} of 0.0123 mg/L. Given the similarity between this EC_{10} and the author-reported MATC for Hazelton et al. (2012), the MATC of 0.01768 mg/L was used to derive the chronic criterion for PFOS. The EPA hopes to further refine the estimated EC_{10} by obtaining the treatment level data from the study authors and exploring additional exposure response slopes from the PFOS dataset. No other quantitative chronic toxicity values were available for this species or genus; therefore, the

MATC of 0.01768 mg/L was used quantitatively to derive the freshwater chronic water column criterion for PFOS.

3.1.1.3.4 Fourth Most Sensitive Freshwater Genus for Chronic Toxicity: Enallagma (damselfly)

Bots et al. (2010) conducted a 320-day partial life-cycle study under renewal test conditions to examine the effects of PFOS (tetraethylammonium salt, 98% purity) on the damselfly *Enallagma cyathigerum.* Approximately 40% of the nymphs in the control treatment died during the first 60 days and similar mortality levels were observed in the other treatments. However, it appeared that control survival plateaued between 60 and 200 days, with 82.57% of the remaining nymphs in the control treatment surviving during this time, indicating that survival settled out during this phase of the experiment. The initial drop in nymph survival could likely be attributed to the handling of the test organisms between the various phases of the experiment. This would explain the observed plateau between 60 and 200 days, as the nymphs were not handled during this time. The observed control survival in this test was consistent with other odonate tests and excessive mortality of nymphs is typically expected within the first 200 days given the difficulty in maintaining odonates in a lab setting (Abbott and Svensson 2007; Rice 2008). Therefore, the observed control survival for this study was considered within the acceptable range for this species up to the 200-day exposure duration. Further, the control survival observed in this study was largely consistent with the toxicity testing guidelines for chironomids (requiring 70% control survival: ASTM 2002; U.S. EPA 2000b), which are currently the only test guidelines for an emergent aquatic insect as there currently is no test guideline for odonates. Therefore, considerations regarding the use of these data for chronic criterion derivation were based on best scientific judgement and were restricted to the first 200 days of the experiment.

The observed effects of PFOS on *E. cyathigerum* reported in the paper by the study authors include decreased survival over the exposure duration and decreased metamorphosis success. The MATC based on metamorphic success was less sensitive than for survival. As such, the MATC author-reported value of 0.03162 mg/L for nymph survival was considered quantitatively in the derivation of the aquatic life criteria. The remainder of the toxicity values were used as supporting information to corroborate the toxicity value used to derive the freshwater chronic criterion and to better understand the effects of PFOS on aquatic insects. As no other quantitative toxicity values were available for this species or genus, the author-reported MATC of 0.03162 mg/L served directly as the SMCV/GMCV. Additionally, the EPA ran additional analyses with some of the other toxicity values for *E. cyathigerum* to understand the influence of this study on the overall chronic criterion (see Section 4.1 below).

Table 3-7. Ranked Freshwater Genus Mean Chronic Values.

Rank[a]	GMCV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMCV[b] (mg/L PFOS)
1	0.000226	F	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	0.000226
2	0.005198	F	*Chironomus*	Midge, *Chironomus dilutus*	0.005198
3	0.01768	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	0.01768
4	0.03162	F	*Enallagma*	Blue damselfly, *Enallagma cyathigerum*	0.03162
5	0.03217	B	*Danio*	Zebrafish, *Danio rerio*	0.03217
6	0.06519	D	*Daphnia*	Cladoceran, *Daphnia carinata*	0.003162
				Cladoceran, *Daphnia magna*	1.344
7	> 0.1	A	*Salmo*	Atlantic salmon, *Salmo salar*	> 0.1
8	0.1098	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	0.1098

Rank[a]	GMCV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMCV[b] (mg/L PFOS)
9	0.167	E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	0.167
10	0.1789	D	*Moina*	Cladoceran, *Moina macrocopa*	0.1789
11	0.25	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	0.25
12	0.5997	C	*Xiphophorus*	Swordtail fish, *Xiphophorus helleri*	0.5997
13	0.7507	C	*Xenopus*	African clawed frog, *Xenopus laevis*	> 0.7160
				Clawed frog, *Xenopus tropicalis*	0.7871
14	1.316	C	*Lithobates*	Northern leopard frog, *Lithobates pipiens*	1.316
15	2.899	E	*Hyalella*	Amphipod, *Hyalella azteca*	2.899
16	8.527	G	*Physella*	Snail, *Physella heterostropha pomilia*	8.527
17	8.640	D	*Ceriodaphnia*	Cladoceran, *Ceriodaphnia dubia*	8.640

[a] Ranked from the most sensitive to the most tolerant based on Genus Mean Chronic Value.
[b] From Appendix C: Acceptable Freshwater Chronic PFOS Toxicity Studies
[c] MDR Groups identified by list provided in Section 2.10.1 above.

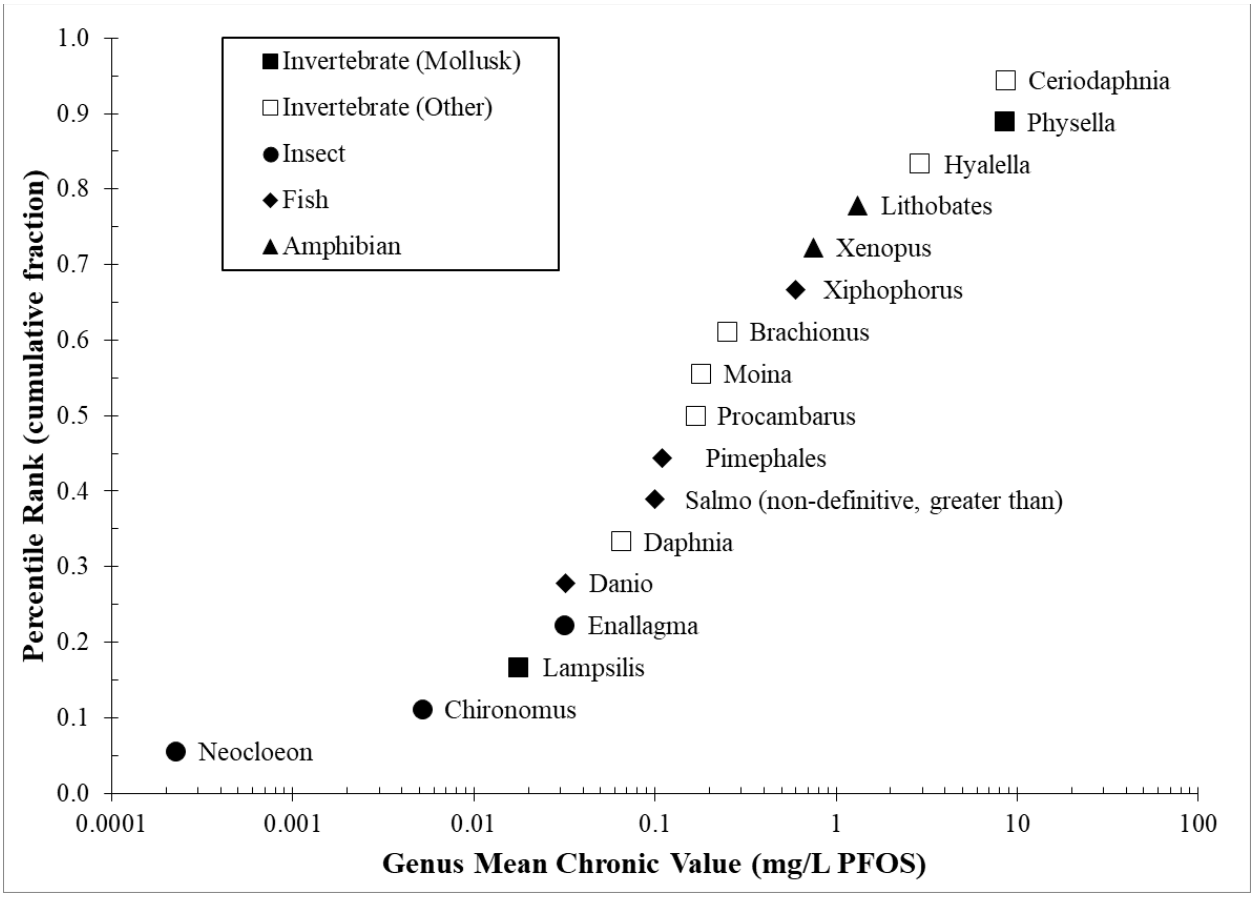

Figure 3-3. Ranked Freshwater Chronic PFOS Used Quantitatively to Derive the Criterion.

3.1.1.4 Summary of Chronic PFOS Toxicity Studies Used to Derive the Saltwater Aquatic Life Criterion

Data for chronic PFOS toxicity were available for five saltwater invertebrate species, representing five genera and five families. The data available for saltwater fish fulfilled only five of the eight MDRs.

Table 3-8. The Four Ranked Estuarine/Marine Genus Mean Chronic Values.
Ranked Below from Most to Least Sensitive.

Rank	Genus	Species	GMCV (mg/L PFOS)	Comments
1	*Perna*	Asian green mussel, *Perna viridis*	0.0033	Not a resident species in North America
2	*Austrochiltonia*	Amphipod, *Austrochiltonia subtenuis*	0.01118	Not a resident species in North America, but other species in this Order (Amphipoda) are common ecotoxicity test species that serves as a surrogate for untested amphipod species residing in North America.
3	*Americamysis*	Mysid, *Americamysis bahia*	0.3708	North American resident species
4	*Tigriopus*	Copepod, *Tigriopus japonicus*	0.7071	Not a resident species in North America, but other species in this genus (*Tigriopus*) are common ecotoxicity test species that serves as a surrogate for untested copepod species residing in North America.

3.1.1.4.1 Most Sensitive Estuarine/Marine Genus: Perna (mussel)

Liu et al. (2013) evaluated the chronic effects of PFOS-K (PFOS potassium salt, CAS# 2795-39-3, 98% purity) on green mussels, *Perna viridis*, via a 7-day measured, static-renewal study. Mussels were exposed at a salinity of 25 ppt (artificial seawater) and a temperature of 25°C. PFOS concentrations were verified through water and muscle tissue samples via liquid chromatography-tandem mass spectrometry. Weights and lengths were determined on days zero and seven. An author-reported NOEC of 0.0096 mg/L and a LOEC of 0.106 mg/L was determined for the growth condition index. The EPA's independently-calculated EC_{10} for growth condition index is 0.0033 mg/L. This EC_{10} is used quantitatively to represent the chronic sensitivity of this species to PFOS exposure in the marine/estuarine aquatic life dataset.

3.1.1.4.2 Second Most Sensitive Estuarine/Marine Genus: Austrochiltonia (amphipod)

Sinclair et al. (2022) tested perfluorooctane sulfonic acid (PFOS, purity not reported) on amphipods (*Austrochiltonia subtenuis*) in a 7-day unmeasured, static experiment. The 7-day experiment consisted of five controls, one solvent control (methanol 0.25 mL/L), and five nominal PFOS concentrations (0.04, 0.2, 1.0, 5.0, 25 µg/L). Test vessels were 600 mL beakers with 400 mL of test material and a 2x2 cm gauze substrate. Each test vessel included 20 amphipods, and all test material was dissolved in modified standard artificial media. The NOEC and LOEC for 7-day survival were 0.005 mg/L and 0.025 mg/L, respectively, and the resulting MATC of 0.01118 mg/L was determined to be acceptable for quantitative use.

3.1.1.4.3 Third Most Sensitive Estuarine/Marine Genus: Americamysis (mysid)

Drottar and Krueger (2000h) reported the results of a 35-day flow-through, measured life-cycle test of PFOS-K (potassium salt, 90.49% purity) with *Americamysis bahia* (formerly *Mysidopsis bahia*). The 35-day NOEC (reproduction and growth) was 0.25 mg/L, and the corresponding 35-day LOEC was 0.55 mg/L. An independently-calculated EC_{10} could not be defined at this time given the level of data that was presented in the paper (Appendix D). The calculated MATC for the test was 0.3708 mg/L. This chronic value was considered acceptable for quantitative use despite the control survival of 78% because it was only slightly below the 80% survival threshold, and because there were no other deficiencies in the study design.

3.1.1.4.4 Fourth Most Sensitive Estuarine/Marine Genus: Tigriopus (copepod)

A 20-day renewal, unmeasured full life-cycle test with PFOS (analytical grade) was conducted on the copepod, *Tigriopus japonicus* (non-North American species) by **Han et al. (2015)**. The development of the copepod's growth from nauplii to copepodite and from nauplii to adults was determined daily based on morphological characteristics. Results were presented as

the number of days needed to reach the normal development stages. The highest test concentration (1 mg/L PFOS) significantly increased the amount of time it took the copepods to reach the development stage. Additionally, the authors assessed the reproduction of the copepods by counting the nauplii produced by eight ovigerous females for 10 days in each well exposed to PFOS. However, it was unclear if this was a subsampling of the organisms used in the 20-day developmental test or if an independent assay with adult females was run. Results are presented graphically as daily nauplii production/individual. There was a statistically significant decrease in production (daily nauplii production/individual) in the 0.25, 0.5 and 1.0 mg/L PFOS concentrations compared to the control. Production was decreased by approximately 50% in the highest concentration (1 mg/L). The 20-day MATC based on time to reach development stage was 0.7071 mg/L and was acceptable for quantitative use in the marine/estuarine chronic aquatic life dataset.

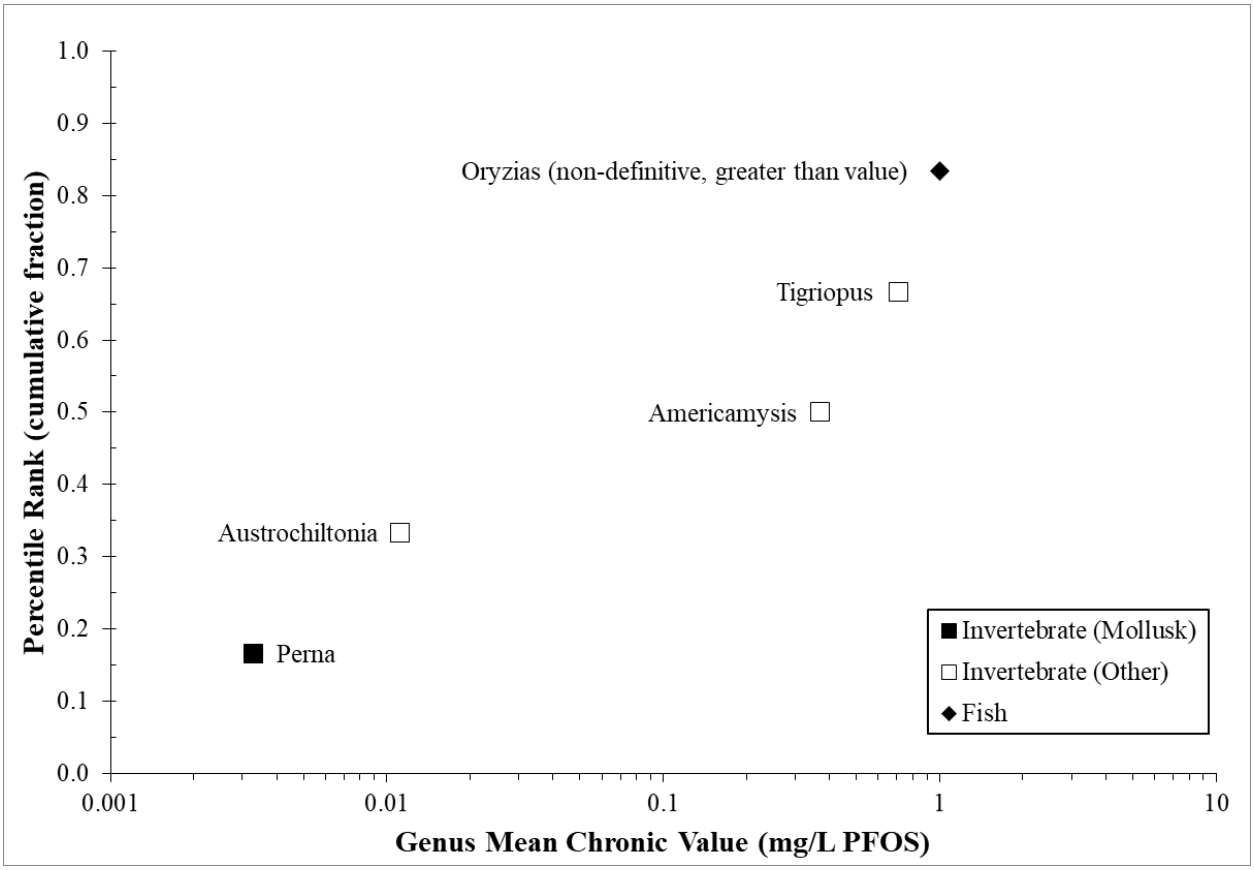

Figure 3-4. Acceptable Estuarine/Marine GMCVs.

3.2 Derivation of the PFOS Aquatic Life Criteria

3.2.1 Derivation of Water Column Criteria for Direct Aqueous Exposure

3.2.1.1 Derivation of Acute Water Column Criterion for Freshwater

The PFOS acute dataset for freshwater based on direct aqueous exposures contained 20 genera representing all eight MDRs. GMAVs for the 20 freshwater genera are provided in Table 3-3, and the four most sensitive genera were within a factor of ~100 of each other. The lowest acute value for the mayfly, *Neocloeon triangulifer*, is 40 times lower than the second most sensitive genus (Figure 3-5). The freshwater FAV, the 5th percentile of the genus sensitivity distribution, for PFOS was 0.1413 mg/L, and was calculated using the procedures described in the 1985 Guidelines (U.S. EPA 1985). The FAV was lower than all of the GMAVs of the tested species, except the mayfly, *Neocloeon triangulifer*. The FAV was then divided by two to obtain a

concentration yielding minimal effects (see Section 2.9). The FAV/2, which is the acute freshwater criterion (or criterion maximum concentration, CMC), was 0.071 mg/L PFOS (rounded to two significant figures), and is expected to be protective of approximately 95% of freshwater genera potentially exposed to PFOS via direct aqueous exposure, under short-term duration conditions of one-hour, when the criterion magnitude is not exceeded more than once in three years on average (Table 3-9).

Table 3-9. Freshwater Final Acute Value and Criterion Maximum Concentration.

Calculated Freshwater FAV based on 4 lowest values: Total Number of GMAVs in Dataset = 20						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Neocloeon*	0.07617	-2.57	6.63	0.048	0.218
2	*Moina*	3.075	1.12	1.26	0.095	0.309
3	*Pimephales*	6.950	1.94	3.76	0.143	0.378
4	*Oncorhynchus*	7.515	2.02	4.07	0.190	0.436
		Σ (Sum):	**2.50**	**15.72**	**0.48**	**1.34**

S^2 = 534.58 **S = slope**
L = -7.127 **L = X-axis intercept**
A = -1.957 **A = lnFAV**
FAV = 0.1413 **P = cumulative probability**
CMC = 0.071 mg/L PFOS (rounded to two significant figures)

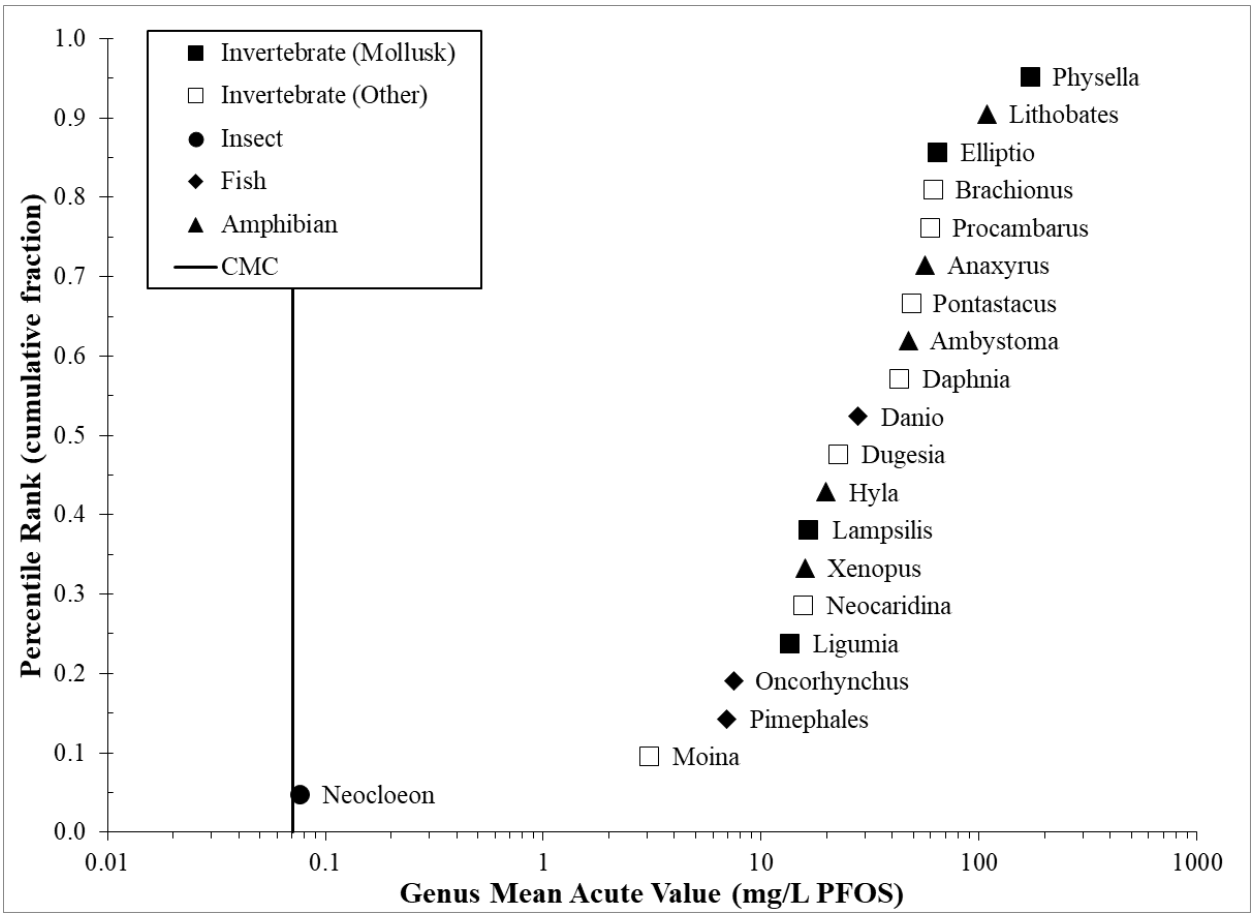

Figure 3-5. Ranked Freshwater Acute PFOS GMAVs Used Quantitatively to Derive the Criterion.

3.2.1.2 Derivation of Acute Water Column Criterion for Estuarine/Marine Water

The estuarine/marine acute dataset for PFOS contained six genera (Table 3-5 and Appendix B) representing only five of the eight taxonomic MDR groups. The missing MDR groups included one family in the phylum Chordata, a family in a phylum other than Chordata, and another family not already represented. The GMAVs of the four most sensitive definitive estuarine/marine genera were within a factor of 4.5 of each other (Table 3-5).

Because data were available for only five of eight MDRs, the EPA developed an estuarine/marine acute benchmark using the available empirical data supplemented with toxicity values generated through the use of NAMs, specifically through the use of the EPA Office of

Research and Development's peer-reviewed publicly-available web-ICE tool (Raimondo et al. 2010). This benchmark is provided in Appendix L.

3.2.1.3 Derivation of Chronic Water Column Criterion for Freshwater

The PFOS chronic dataset based on direct aqueous exposures contained data for all eight MDRs, thus the Final Chronic Value (FCV) can be calculated directly without the use of an ACR. There were GMCVs for 17 freshwater genera (Table 3-7). The four most sensitive genera were within a factor of 140 of each other. The lowest chronic value for the mayfly, *Neocloeon triangulifer*, is over an order of magnitude lower than the second most sensitive genus (Figure 3-6). The freshwater FCV for PFOS of 0.0002491 mg/L was calculated using the procedures described in the 1985 Guidelines (U.S. EPA 1985). The FCV is the 5th percentile of the genus sensitivity distribution and is intended to be protective of 95 percent of the genera. The FCV was lower than all of the GMCVs of the tested species, except the mayfly, *Neocloeon triangulifer*. Unlike the FAV, the FCV was not divided by two, as it already represents a low effect level, and was equal to the water column chronic criterion (or criterion continuous concentration, CCC; Table 3-10). The freshwater CCC had a magnitude 0.00025 mg/L PFOS (rounded to two significant figures), or 0.25 µg/L, and is expected to be protective of 95% of freshwater genera potentially exposed to PFOS through direct aqueous exposure under long term conditions of four days, if not exceeded more than once every three years on average (Table 3-10).

Table 3-10. Freshwater Final Chronic Value and Criterion Continuous Concentration.

Calculated Freshwater FCV based on 4 lowest values: Total Number of GMCVs in Dataset = 17						
Rank	**Genus**	**GMCV (mg/L)**	**ln(GMCV)**	**ln(GMCV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Neocloeon*	0.000226	-8.39	70.48	0.056	0.236
2	*Chironomus*	0.005198	-5.26	27.66	0.111	0.333
3	*Lampsilis*	0.01768	-4.04	16.28	0.167	0.408
4	*Enallagma*	0.03162	-3.45	11.93	0.222	0.471
		Σ (Sum):	**-21.14**	**126.35**	**0.56**	**1.45**

S^2 = 472.36 **S = slope**
L = -13.157 **L = X-axis intercept**
A = -8.297 **A = lnFCV**
FCV = 0.0002491 **P = cumulative probability**
CCC = 0.00025 mg/L PFOS (rounded to two significant figures)

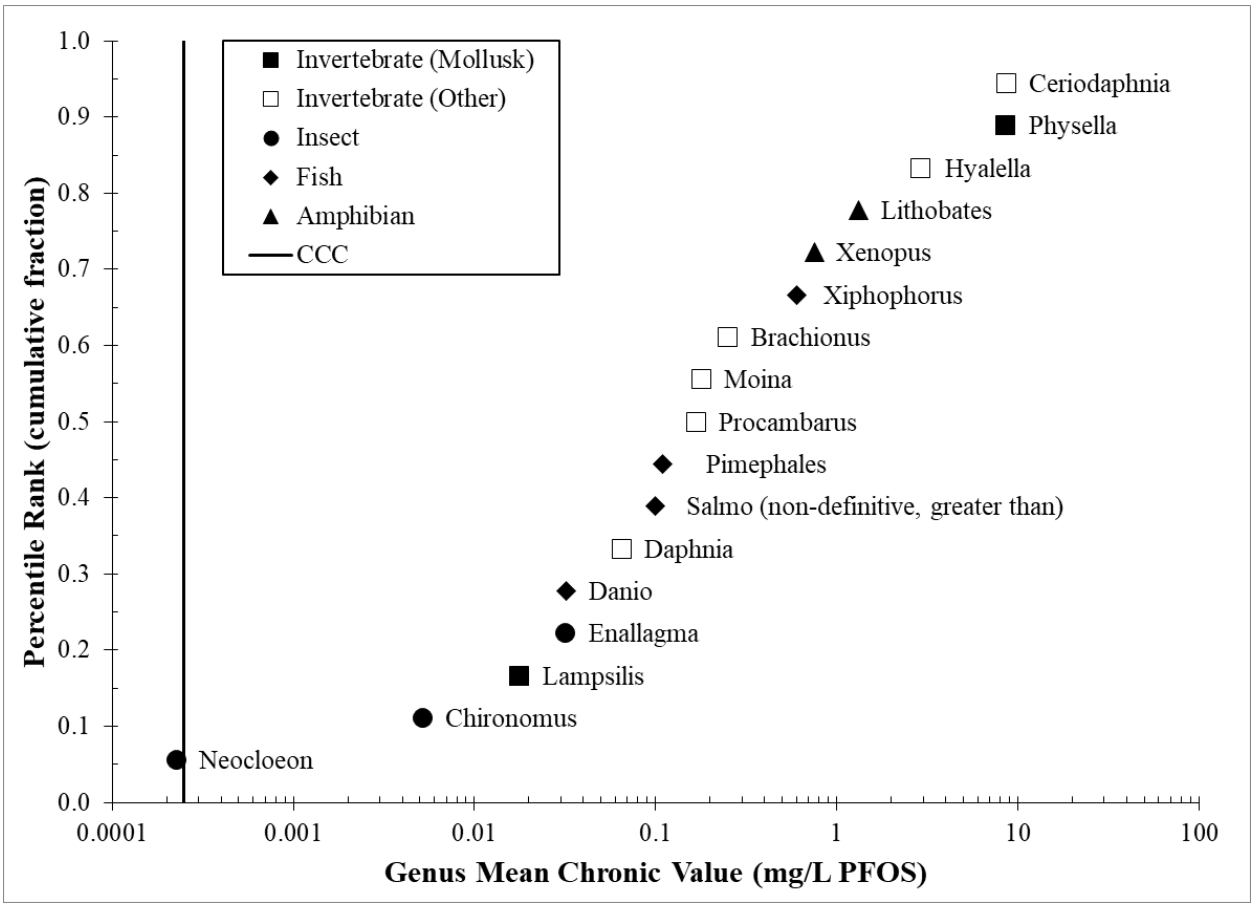

Figure 3-6. Ranked Freshwater Chronic PFOS GMCVs Used Quantitatively to Derive the Criterion.

3.2.1.4 Deriving A Protective Duration Component of the Chronic Water Column-Based Criterion

Effects to sensitive life stages was a primary reason why the 1985 Guidelines (U.S. EPA 1985) recommended a 4-day duration for most water column-based criteria. U.S. EPA (1985) states, "*An averaging period of four days seems appropriate for use with the CCC for two reasons. With one of the two reasons specify being, "for some species it appears that the results of chronic tests are due to the existence of a sensitive life stage at some time during the test*."

The SMCV for *C. dilutus*, representing the *Chironomus* GMCV (second most sensitive genus) is based on the EPA's independently-calculated EC_{10} of 0.05896 mg/L for a 10-day larval growth endpoint by MacDonald et al. (2004), an EC_{10} of 0.001588 mg/L for a 16-day larval mean biomass endpoint by McCarthy et al. (2021), and an EC_{10} of 0.0015 mg/L for a 14-day larval growth endpoint by Krupa et al. (2022). The EC_{10} for a 10-day larval growth by MacDonald et al. (2004) is slightly higher than the author-reported EC_{10} for this effect in the study. The author-reported EC_{10}s for the 20-day test by MacDonald et al. (2004) were higher than those for the 10-day test, which is an atypical outcome, and were not used for criteria derivation. Consequently, there was no clear influence of exposure time on the effects of PFOS on this species.

The SMCV for *L. siliquoidea,* representing the *Lampsilis* GMCV (third most sensitive genus) is based on a 36-day study by Hazelton (2013); Hazelton et al. (2012) using glochidia and juvenile life stages. The test exposed brooding glochidia (in marsupia) for 36 days followed by a 24-hour exposure of free glochidia. The 24-hour free glochidia exposure consisted of a factorial design, such that free glochidia from the control group of the marsupia exposure were divided between a control and the two PFOS treatments and the PFOS treatments were split into control

and the same PFOS treatment group as the marsupia exposure. This factorial design allowed for the comparison of PFOS effects in two different life-stages.

Given the limitations of time points that could be discerned by the test, it appeared that for reduced viability and or metamorphosis success of free glochidia to occur at concentrations near the chronic value for the test (0.01768 mg/L), the test's 36-day exposure period would also be needed. For example, the study authors determined that the *in-marsupia* (36-day) exposure held the greatest weight of evidence and explained 78% of the variability in the glochidia viability (AIC = 22843, wi = 0.78) and 83% of the metamorphosis success (AIC = 21955, wi = 0.83). As a result, this species appears to be protected by the chronic 4-day duration component of the water column criterion. It should also be noted, brief PFOS exposures at elevated concentrations consistent with the magnitude and four-day duration of the chronic criterion are not expected to cause effects to free swimming glochidia based on the 24-hour acute toxicity data for glochidia.

The SMCV for *Enallagma cyathigerum*, representing the *Enallagma* GMCV (fourth most sensitive genus) are based on a 320-day partial life-cycle test by Bots et al. (2010). Only a single treatment, 0.1 mg/L, showed partial effects. The treatment 10X higher (i.e., 1 mg/L) yielded 100% mortality within 20 days. The treatment 10X lower (0.01 mg/L) showed no effects over the entire test. The authors provided the time course of mortality throughout the entire test. At 0.1 mg/L a marked reduction in survival began at 130 days, and reached zero survival at 250 days, suggesting a relatively long time-to-effect. Because 0.1 mg/L is more than 3-fold higher than the estimated chronic value for the test, 0.03162 mg/L, it is postulated that the time course of mortality observed at 0.1 mg/L would be substantially faster than what would be expected to occur at 0.03162 mg/L. Given the relatively slow manifestation of chronic effects observed in

this study, this species appears to be protected by the chronic 4-day duration component of the water column criterion.

No chronic PFOS toxicity tests specifically evaluated time-to-effect, reported effect data at time intervals at a high enough resolution to model the speed of toxic action, assessed time variable PFOS exposures, or provided insight into the potential for latent toxicity. However, chronic tests, including life-cycle tests with relatively sensitive species, suggested chronic effects may occur at durations shorter than those of standard chronic toxicity tests (e.g., 28-day ELS tests) and a chronic 4-day duration component of the water column criterion was considered protective for these species/genera. Therefore, the EPA has set the duration component of the PFOS chronic water column criterion at four days to reflect the chronic criterion duration recommended in the 1985 Guidelines. This 4-day duration component of the chronic water column is also consistent with U.S. EPA (1991), which considered the default 4-day chronic averaging period as "*the shortest duration in which chronic effects are sometimes observed for certain species and toxicants*", and concludes that 4-day averaging "*should be fully protective even for the fastest acting toxicants*."

3.2.1.5 Derivation of Chronic Water Column Criterion for Estuarine/Marine Water

The estuarine/marine chronic dataset for PFOS contained GMCVs for five genera. GMCVs for five estuarine/marine genera are summarized in Section 3.1.1.4 and shown in Figure 3-4. The eight-family taxonomic (MDR) requirement was not met by the chronic dataset, as acceptable chronic studies for species representing three MDR groups are not available (one family in the phylum Chordata, a family in a phylum other than Arthropoda or Chordata, and another family not already represented). The 1985 Guidelines allow the use of a Final Acute-Chronic Ratio (FACR) to convert a FAV to an FCV (i.e., FAV/FACR = FCV), which is equivalent to a CCC. However, since an FAV could not be calculated with the available data, an

FCV also could not be calculated. Consequently, the EPA could not derive estuarine/marine chronic criteria for PFOS (see Appendix L for derivation of acute estuarine/marine benchmarks).

3.2.2 Derivation of Freshwater Chronic Tissue criteria for PFOS

Currently, the freshwater chronic PFOS toxicity data with measured tissue concentrations were somewhat limited. There are 14 total freshwater aquatic life studies considered for either quantitative (six studies – three fish, one invertebrate, and two amphibian studies) or qualitative (eight studies) use in this aquatic life criterion. The quantitative studies only comprised data for three of the eight MDRs. The qualitative studies provided supporting information for only one additional MDR. Therefore, it was concluded that there is currently insufficient data to derive a chronic tissue criterion using a GSD approach from empirical tissue data from toxicity studies. However, these studies provided context to the translation of tissue criteria as described in Section 3.2.3 below. This comparison is provided in the Effects Characterization (Section 4.5).

3.2.3 Translation of Chronic Water Column Criterion to Tissue Criteria

As described in Section 3.2.2 above, there are currently insufficient freshwater chronic toxicity data with measured tissue concentrations to derive a chronic PFOS tissue criterion using a GSD approach. Therefore, the chronic tissue criteria for PFOS were derived by translating the chronic freshwater water column criterion (see Section 3.2.1.3) into tissue criteria using bioaccumulation factors (summarized in Section 3.2.3.1 below) and the following equation:

$$Tissue\ Criteria = Chronic\ Water\ Column\ Criterion\ \times BAF \qquad (Eq.\ 1)$$

The resulting tissue criteria corresponded to the tissue type associated with the BAF used in the equation.

3.2.3.1 PFOS Bioaccumulation Factors (BAFs)

Section 2.11.3.1 above summarizes the literature search, calculation, and evaluation of the PFOS BAFs for aquatic life. These BAFs were compiled by and can be found in Burkhard

(2021). BAFs used in the derivation of the PFOS tissue criteria consisted of two or more water and organism samples each and were collected within one year and 2 km distance of one another. In order to derive more protective tissue criteria and to limit the effects of site-specific differences in BAFs, the distributions of BAFs used to derive tissue criteria were based on the lowest species-level BAF reported at a site. When more than one BAF was available for the same species within the same waterbody, the species-level BAF was calculated as the geometric mean of all BAFs for that species at that site. Summary statistics for the PFOS BAFs used in the criteria derivation are presented in Table 3-11 and individual BAFs are provided in Appendix O.

Table 3-11. Summary Statistics for PFOS BAFs in Fish and Invertebrates[1].

Category	n	Geometric Mean BAF (L/kg-ww)	Median BAF (L/kg-ww)	20th Centile BAF (L/kg-ww)	Minimum (L/kg-ww)	Maximum (L/kg-ww)
Invertebrates	28	771.6	924	111.5	2.69	100,000
Fish (Whole-Body)	28	3,739	5,905	803.9	4.79	46,098
Fish (Muscle)	21	1,069	1,048	346.4	8.72	50,234

[1] Based on the lowest species-level BAF measured at a site (i.e., when two or more BAFs were available for the same species at the same site, the species-level geometric mean BAF was calculated, and the lowest species-level BAF was used).

The fish tissue criteria were developed for muscle and whole-body to accommodate the most commonly sampled tissue types in monitoring programs. Additional tissue values for various other tissue types (e.g., liver and blood) were also calculated and can be found in Appendix P.

3.2.3.2 Deriving Protective Tissue Concentrations from the Chronic Water-Column Criterion

Invertebrate whole-body and fish muscle and whole-body tissue criteria were derived separately by multiplying the freshwater chronic water-column criterion (see Section 3.2.1.3) by the respective 20th centile of the distribution of BAFs using Equation (Eq. 1) from Section 3.2.3. The 20th centile BAF was used to derive tissue-based criteria as a relatively conservative BAF

estimate in order to protect species across taxa and across water bodies with variable bioaccumulation conditions. That is, use of the 20th centile BAF protects species and conditions where the bioaccumulation of PFOS and resultant tissue-based exposures is relatively low as well as those conditions with the bioaccumulation potential of PFOS is relatively high.

The invertebrate whole-body tissue criterion was calculated by multiplying the 20th centile BAF of 111.5 L/kg ww by the PFOS freshwater chronic water criterion of 0.00025 mg/L, resulting in an invertebrate whole-body tissue criterion of 0.028 mg/kg ww. The fish whole-body tissue criterion was calculated by multiplying the 20th centile BAF of 803.9 L/kg ww by the PFOS freshwater chronic water criterion of 0.00025 mg/L, resulting in a fish whole-body tissue criterion of 0.201 mg/kg ww. The fish muscle tissue criterion was calculated by multiplying the 20th centile BAF of 346.4 L/kg ww by the PFOS freshwater chronic water criterion of 0.00025 mg/L, resulting in a fish muscle tissue criterion of 0.087 mg/kg ww. The chronic tissue-based criteria are expected to be protective of 95% of freshwater genera potentially exposed to PFOS under long-term exposures if the tissue-based criteria are not exceeded. The duration component of the tissue-based criteria is expressed as an instantaneous duration because the tissue-based criteria are protective of long-term conditions and represent an integrated measure of bioaccumulated PFOS concentrations over time.

The EPA acknowledges that there is uncertainty in deriving protective tissue criteria magnitudes by transforming the chronic water column criterion (which was based on tests that only added PFOS to the water column) into tissue concentrations through field-measured bioaccumulation data of paired water and tissue concentrations in waterbodies. Nevertheless, the chronic water column criterion is based on chronic toxicity tests where test organisms were fed. In these tests, PFOS can directly affect species based on direct water column exposure and/or

sorb to added food that is consumed by test organisms before eliciting chronic effects from dietary exposure. Therefore, the chronic water column criterion magnitude accounts for water column-based and, to a possible lesser extent, dietary-based effects, while the field-based BAFs account for water column- and dietary-based PFOS exposure and subsequent accumulation in tissues. The chronic tissue criteria will allow states, Tribes, and stakeholders monitoring PFOS in freshwater lentic and lotic systems to evaluate potential effects to aquatic organisms based on the aquatic tissue monitoring data collected. Quantitatively acceptable data on the effects of dietary exposures to aquatic species were relatively limited, thus the EPA chose to develop protective values for freshwater aquatic organisms based on the observed relationship between water column concentrations and tissue concentrations and observed PFOS toxicity in chronic tests where PFOS was only added directly to the water column.

3.2.3.3 Deriving A Protective Duration and Exceedance Frequency for the Tissue-based Chronic Criteria

3.2.3.3.1 Duration: Chronic Tissue-Based Criteria

PFOS concentrations in tissues are generally expected to change only gradually over time in response to environmental fluctuations. The chronic tissue-based criteria averaging period, or duration, was therefore specified as instantaneous, because tissue data provide point, or instantaneous, measurements that reflect integrative accumulation of PFOS over time and space in population(s) at a given site.

3.2.3.3.2 Frequency: Chronic Tissue-Based Criteria

The PFOS tissue-based criteria frequencies are set as “not to be exceeded” to ensure protection of freshwater aquatic organisms. The “not to exceed” condition for frequency is meant to account for the many variables influencing ecological recovery and uncertainty due to the complete lack of PFAS-specific ecological recovery case studies available to inform recovery

rates following elevated PFOS concentrations in aquatic biota. Ecological recovery times following chemical disturbances in general are situational-specific, being largely dependent on: (1) biological variables such as the presence of nearby source populations or generational time of taxa affected; (2) physical variables such as lentic and lotic habitat considerations where recovery rates in lentic systems may be slower than lotic systems where the pollutant may be quickly flushed downstream, and; (3) chemical variables such as the persistence of the chemical and potential for residual effects.

PFOS-specific case studies are unavailable to directly inform rates of ecological recovery following elevated concentrations in fish and aquatic invertebrates. Metals and other chemical pollutants may be retained in the sediment and biota, where they can result in residual effects over time that further delay recovery. Few studies are available concerning PFOS elimination or depuration half-life in aquatic animals, however the data that exist indicate a short half-life. For example, the elimination half-life for PFOS in adult rainbow trout exposed to PFOS for 28 days via the diet followed by 28 days depuration was estimated to be 8.4 days in muscle tissue (Falk et al. 2015), while the terminal half-life in rainbow trout receiving a one-time intra-arterial injection of PFOS was 86.8 days (Consoer 2017). Additionally, the depuration half-life in northern leopard frog tadpoles via a 40-day aqueous exposure to 0.01 mg/L PFOS was estimated to be 2.2 days (Hoover et al. 2017). It's unclear whether PFOS half-life in aquatic organism tissues is the mechanistic result of rapid depuration or an artifact of these measurements taken during relatively short testing times (e.g., 28 days) where steady state between PFOS and water and tissues has not occurred. Long-term uptake and subsequent excretion rates of PFOS has been extensively studied in humans relative to aquatic life. Li et al. (2018) reported a median PFOS half-life of 3.4 years in human serum following exposure to PFOS in drinking water, which the

authors stated was in the range of previously published estimates. Due to chemical retention in tissues, ecosystems impacted by discharges of bioaccumulative pollutants (such as selenium) generally recover from chemical disturbances at relatively slow rates. For example, Lemly (1997) concluded that although water quality in Belews Lake in North Carolina (a freshwater reservoir) had recovered significantly in the decade since selenium discharges were halted in 1985, the threat to fish had not been eliminated. The selenium discharges that led to severe reproductive failure and deformities in fish were still measurable (as fish deformities) in 1992 (seven years later) and in 1996 (ten years later). Lemly (1997) estimated based on these data that "*the timeframe necessary for complete recovery from selenium contamination from freshwater reservoirs can be on the order of decades.*"

Beyond bioaccumulation, chemical-specific considerations such as degradation versus persistence may also provide a mechanism influencing ecological recovery rates. The persistence of PFOS in the environment has been attributed to the strong C-F bond, with no known biodegradation or abiotic degradation processes for PFOS (refer to Section 2.3). Somewhat similarly to PFOS, metals do not degrade and may persist in aquatic systems following elevated discharge. The persistence of metals may explain why metals had the second longest median recovery time of any disturbance described in a systematic review of aquatic ecosystem recovery (Gergs et al. 2016). Gergs et al. (2016) showed recovery times following metal disturbances ranged from roughly six months to eight years (median recovery time = 1 year; 75^{th} centile ~ 3 years; n = 20). Unlike metals, however, PFOS is not naturally occurring, and aquatic organisms possess no evolved detoxification mechanisms to aid in recovery at the individual level. Furthermore, the degradation of other PFAS into PFOS may represent an additional source of PFOS in aquatic systems that further delays recovery.

The persistence and bioaccumulative/human-made nature of PFOS in aquatic systems, in combination with the documented recovery times of pollutants with similar chemical attributes (Gergs et al. 2016; Lemly 1997; Mebane 2022), suggests aquatic systems may recover from PFOS tissue criteria exceedances on the order of 5 to 10 years, if sources were eliminated. However, recovery times could be longer, if the sources of PFOS and other PFAS that degrade into PFOS have not been removed. Specifying a time interval associated with ecological recovery from exceedances of PFOS tissue criteria, then, is highly uncertain given the lack of PFOS-specific examples of ecological recovery and the many situational-specific factors influencing recovery (Mebane 2022). For example, the lack of PFOS degradation in the environment, and the fact that other PFAS in the environment can degrade into PFOS could act as ongoing PFOS sources that further delay recovery. Given these uncertainties, the PFOS tissue-based criteria frequencies are set as "not to be exceeded" to ensure protection of aquatic life populations. Moreover, if tissue-based criteria were exceeded, then PFOS has likely built up through the food web and PFOS source reservoirs are likely to exist, representing a broad level of PFOS contamination throughout the aquatic ecosystem.

The "not to exceed" frequency components of the tissue-based criteria do not suggest aquatic ecosystems could never recover from an exceedance of the PFOS tissue-based criteria under the right conditions. Ecological recovery from such an exceedance could begin once PFOS source reservoirs existing within the ecosystem are eliminated or decreased, including other PFAS that degrade into PFOS; became permanently isolated from possible uptake by the ecosystem (e.g., long-term burial with no benthic disturbances under certain environmental conditions); and, unaffected organisms were able to repopulate the system through immigration and/or reproductive events that yield generations that are no longer exposed to PFOS.

Evaluation of PFOS concentrations in tissues would likely include evaluating the central tendency of samples for a given species, collected at a specific site and time. Considering a measure of central tendency to assess tissue-based exposures in the field relative to the criteria is appropriate because the criteria are intended to protect aquatic life populations.

3.3 Summary of the PFOS Freshwater Aquatic Life Criteria and Acute Estuarine/Marine Benchmark

The PFOS aquatic life criteria were developed to protect freshwater aquatic life against adverse effects, such as mortality, altered growth, and reproductive impairments, associated with acute and chronic exposure to PFOS. The nationally recommended criteria include water column based acute and chronic criteria for fresh waters. The freshwater acute water column-based criterion magnitude is 0.071 mg/L, and the chronic water column-based criterion magnitude is 0.00025 mg/L (Table 3-12). The chronic freshwater tissue-based criteria magnitudes are 0.201 mg/kg wet weight (ww) for fish whole-body, 0.087 mg/kg ww for fish muscle tissue and 0.028 mg/kg ww for invertebrate whole-body tissue. These PFOS aquatic life criteria are expected to be protective of aquatic life on a national basis (Table 3-12). All of these water column and tissue criteria are intended to be independently applicable and no one criterion takes primacy. All of the recommended criteria (acute and chronic water column and tissue criteria) are intended to be protective of aquatic life. Acute and chronic water column criteria for estuarine/marine waters could not be derived at this time due to data limitations; however, an estuarine/marine acute benchmark protective of aquatic life is provided in Appendix L.

The freshwater chronic water-column criterion is more strongly supported than the chronic tissue-based criteria because the water column-based chronic criterion was derived directly from the results of empirical toxicity tests. The chronic tissue-based criteria are relatively less certain because they were derived by transforming the chronic water-column

criterion into tissue concentrations through BAFs, with any uncertainty and variability in the underlying BAFs then propagating into the resultant tissue-based criteria magnitudes.

Table 3-12. Recommended Perfluorooctane Sulfonate (PFOS) Ambient Water Quality Criteria for the Protection of Aquatic Life in Freshwaters.

Type/Media	Acute Water Column (CMC)[1,4]	Chronic Water Column (CCC)[1,5]	Chronic Invertebrate Whole-Body[1,2]	Chronic Fish Whole-Body[1,2]	Chronic Fish Muscle[1,2]
Magnitude	0.071 mg/L	0.00025 mg/L	0.028 mg/kg ww	0.201 mg/kg ww	0.087 mg/kg ww
Duration	One-hour average	Four-day average	Instantaneous[3]		
Frequency	Not to be exceeded more than once in three years on average	Not to be exceeded more than once in three years on average	Not to be exceeded[6]		

[1] All five of these water column and tissue criteria are intended to be independently applicable and no one criterion takes primacy. All of the above recommended criteria (acute and chronic water column and tissue criteria) are intended to be protective of aquatic life. These criteria are applicable throughout the year.
[2] Tissue criteria are derived from the chronic water-column criterion magnitude (CCC) with the use of bioaccumulation factors and are expressed as wet weight (ww) concentrations.
[3] Tissue data provide instantaneous point measurements that reflect integrative accumulation of PFOS over time and space in aquatic life population(s) at a given site.
[4] Criterion Maximum Concentration; applicable throughout the water column.
[5] Criterion Continuous Concentration; applicable throughout the water column.
[6] PFOS chronic freshwater tissue-based criteria should not be exceeded, based on measured tissue concentrations representing the central tendency of samples collected at a given site and time.

This Aquatic Life Ambient Water Quality Criteria and Acute Saltwater Benchmark for PFOS document includes a water column based acute benchmark for estuarine/marine waters. The derivation of this benchmark is described in detail in Appendix L. The saltwater acute benchmark 0.55 mg/L (magnitude component), expressed as a one-hour average (duration component), that is not to be exceeded more than once in three years on average (Table 3-13).

Aquatic life benchmarks, developed under 304(a)(2) of the CWA, are informational values that the EPA generates when there are limited high quality toxicity data available and data gaps exist for several aquatic organism families. The EPA develops aquatic life benchmarks to provide information that states and Tribes may consider in their water quality protection

programs. In developing aquatic life benchmarks, data gaps may be filled using new approach methods (NAMs), such as computer-based toxicity estimation tools (e.g., EPA's Web-ICE) or other new approach methods intended to reduce reliance on additional animal testing (https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-vertebrate-animals-chemical), including the use of read-across estimates based on other chemicals with similar structures. The EPA's aquatic life benchmark values are not regulatory, nor do they automatically become part of a state's water quality standards.

Table 3-13. Acute Perfluorooctane Sulfonate (PFOS) Benchmark for the Protection of Aquatic Life in Estuarine/Marine Waters.

Type/Media	Acute Water Column Benchmark
Magnitude	0.55 mg/L
Duration	One hour on average
Frequency	Not to be exceeded more than once in three years on average

4 EFFECTS CHARACTERIZATION FOR AQUATIC LIFE

The purpose of this section was to describe the supporting information for the derivation of the PFOS aquatic life AWQC that contributed to the weight-of-evidence for the derivation. This section includes: (1) additional analyses supporting the criteria that were used as part of the lines-of-evidence discussion to better understand the influence of using less certain toxicity data (Section 4.1); (2) an assessment of the influence of including non-North American resident species in water column criteria derivation (i.e., species not resident to North America removed from dataset; Section 4.2); (3) summaries of the toxicity studies with apical endpoints (e.g., effects on survival, growth, or reproduction) that were not used directly to derive the water column criteria, but were used qualitatively to support them (Section 4.3); (4) a discussion of acute to chronic ratios (Section 4.4); (5) a comparison of empirical tissue concentrations to translated tissue criteria (Section 4.5); (6) a discussion of the effects of PFOS on aquatic plants (Section 4.6); and (7) a discussion of the effects of PFOS on threatened and endangered species (Section 4.7). The EPA derived the final national recommended PFOS aquatic life AWQC described in the Effects Analysis Section (see Section 3 above). The additional analyses presented here are solely intended to support the PFOS criteria through a weight-of-evidence approach that evaluated the influence of data variation and uncertainties on the PFOS criteria.

4.1 Additional Analyses Supporting the Derivation of the Chronic Water Column Criterion for Freshwater

In addition to the EPA's recommended freshwater chronic water column criterion of 0.00025 mg/L PFOS described above in Section 3.2.1.3, eight additional analyses supporting the derivation of the chronic criterion were examined as part of a line-of-evidence evaluation to consider the effect of including less certain toxicity data (i.e., the chronic toxicity values for damselfly, fatmucket, and mayfly) on the magnitude of the freshwater chronic water column

criterion. The data considered to be less certain generally centered around two specific areas: (1) the difficultly in reliably estimating a chronic toxicity value given the wide spacing (up to 15-fold difference) of the treatment concentrations (e.g., for fatmucket in Hazelton et al. (2012) and damselfly in Bots et al. (2010) (see Section 3.1.1.3.3 and 3.1.1.3.4, respectively) and (2) the uncertainty in the chronic toxicity values given the level of data presented in the papers associated with the mayfly (*N. triangulifer*), fatmucket (*L. siliquoidea*), and damselfly (*E. cyathigerum*) – see study summaries in Appendices C.2.1, C.2.3, and C.2.4, respectively).

The eight additional analyses presented below involved either changing or excluding toxicity values from the three toxicity studies (Table 4-1). The additional analyses presented here are solely intended to support the PFOS chronic criterion through a weight-of-evidence approach that evaluated the influence of data variation on the criterion derivation process. Based on these additional analyses, the EPA decided to retain the mayfly, fatmucket, and damselfly values as presented in Section 3.1.1.3, to ensure protection of these sensitive taxa as well as the many untested species for which these species may serve as representative taxonomic surrogate species. The availability of additional toxicity data for these particular taxa would reduce the uncertainty in the analysis. The criteria presented in Section 3.3 are the EPA's best estimate of the maximum concentrations of PFOS that will support protection of sensitive aquatic life from unacceptable chronic exposures.

Table 4-1. Additional Analyses Supporting the Derivation of the Freshwater Chronic Water Column Criterion.

*Presented in the order that is summarized in the text below.**

Order of Additional Analyses	Purpose of Additional Analysis	Details of Additional Analysis	Chronic Water Column Concentration for Additional Analysis (mg/L)	Study
1	To explore the impact of using the various author reported toxicity values for damselfly	Used 10-day MATC of 0.3162 mg/L for damselfly instead of 150-day MATC of 0.03162 mg/L	0.00025	Bots et al. (2010)
2		Used 60-day NOEC of 0.1 mg/L for damselfly instead of 150-day MATC of 0.03162 mg/L	0.00025	
3		Used 320-day NOEC of 0.01 mg/L for damselfly instead of 150-day MATC of 0.03162 mg/L	0.00027	
4	To explore the impact of using the MATC for fatmucket	Removed MATC of 0.01768 mg/L for fatmucket	0.00021	Hazelton et al. (2012)
5	To explore the impact of using both the EC_{10} for fatmucket and the 150-day MATC for damselfly	Removed both MATC of 0.01768 mg/L for fatmucket and 150-day MATC of 0.03162 mg/L for damselfly	0.00016	Hazelton et al. (2012) and Bots et al. (2010), respectively
6	To explore the impact of using the EC_{10} for fatmucket	Use estimated EC_{10} of 0.0123 mg/L for fatmucket instead of MATC of 0.01768 mg/L	0.00025	Hazelton et al. (2012)
7	To explore the impact of using the various author-reported toxicity values for mayfly	Use EC_{10} of 0.000272 mg/L for mayfly calculated with linear regression without control	0.00029	Soucek et al. (2023)
8		Use EC_{10} of 0.000232 mg/L for mayfly calculated with linear regression without control	0.00026	

*Final derived freshwater chronic water column criterion was 0.00025 mg/L PFOS.

In the first additional analysis, instead of using the 150-day MATC of 0.03162 mg/L for *Enallagma cyathigerum* as described in the calculation of the final freshwater chronic water column criterion described above in Section 3.2.1.3, the 10-day MATC of 0.3162 mg/L was used (Table 4-2) (Bots et al. 2010), yielding a freshwater FCV for PFOS of 0.0002486 mg/L. This chronic water column concentration of 0.00025 mg/L (rounded to two significant figures) is the same as the final chronic value of 0.00025 mg/L derived above. This first additional analysis indicated that there is little difference in the calculated chronic criterion based either on the 150-

day or 10-day MATC for *E. cyathigerum.* However, as the 150-day MATC was more comparable to the other aquatic insect data and more representative of life-cycle effects than the 10-day MATC, the EPA has concluded that the 150-day MATC should be used quantitatively to derive the freshwater chronic criterion.

In the second analysis, instead of using the 150-day MATC of 0.03162 mg/L for *E. cyathigerum*, the 60-day NOEC of 0.1 mg/L from the same test was used (Table 4-2) (Bots et al. 2010), also yielding an FCV of 0.0002486 mg/L. Similar to the first analysis, there is no difference in the calculated chronic criterion based either on the 150-day or 60-day NOEC for *E. cyathigerum.* However, since the 150-day MATC was more comparable to the other aquatic insect data and representative of life-cycle effects than the 10-day MATC, the EPA has concluded that the 150-day MATC should be used quantitatively to derive the freshwater chronic criterion.

In the third analysis, instead of using the 150-day MATC of 0.03162 mg/L for *E. cyathigerum*, the 320-day NOEC of 0.01 mg/L from the same test was used (Table 4-2) (Bots et al. 2010), yielding an FCV of 0.0002661 mg/L, or 0.00027 mg/L (rounded to two significant figures). This analysis indicated that there is a slightly higher FCV (less stringent) in the calculated chronic criterion if the 320-day NOEC for *E. cyathigerum* is used. However, as there were concerns with the control survival of test organisms (reported as roughly 60% in the first 60 days), the EPA has determined that the 150-day MATC should be used quantitatively to derive the freshwater chronic water column criterion since this toxicity value still represents a life-cycle effect and control survival of test organisms was determined to be acceptable at this time point in the test.

In the fourth analysis, the MATC for fatmucket (*L. siliquoidea)* of 0.01768 mg/L was removed from the chronic dataset to understand the influence of this toxicity value on the criterion magnitude (Table 4-2). This additional analysis placed the GMCV of 0.03217 mg/L for *Danio* among the four most sensitive genera, and yielded an FCV of 0.0002097 mg/L or 0.00021 mg/L (rounded to two significant figures) (Section 3.2.1.3; U.S. EPA 1985). The removal of the chronic toxicity value for *L. siliquoidea* has only a modest influence on the calculated chronic criterion magnitude (criteria became more stringent) but would eliminate mollusks from the chronic PFOS dataset. The EPA decided to retain the fatmucket value to ensure representation and protection of this sensitive taxon.

In the fifth analysis, the 150-day MATC of 0.03162 mg/L for damselfly (*E. cyathigerum*) and MATC for fatmucket (*L. siliquoidea*) of 0.01768 mg/L were removed since these values are less certain compared to other quantitative studies in the chronic criterion dataset (Table 4-2). As noted above, these toxicity values were considered to be less certain due to (1) the difficultly in reliably estimating a chronic toxicity value given the wide spacing (15-fold difference in Hazelton et al. (2012) for *L. siliquoidea* and 10-fold difference in Bots et al. (2010) for *E. cyathigerum*) of the treatment concentrations, and (2) the uncertainty in the chronic toxicity values given the level of data presented in the papers. This fifth analysis yielded a freshwater FCV for PFOS of 0.0001621 mg/L, or 0.00016 mg/L (rounded to two significant figures). The calculated chronic criterion magnitude was reduced 1.6-fold. The EPA decided to retain the damselfly and fatmucket values as presented in Section 3.1.1.3 in the criterion derivation to ensure representation and protection of these sensitive taxa.

In the sixth analysis, the estimated EC_{10} for fatmucket of 0.0123 mg/L was used in the chronic dataset to understand the influence of this estimated toxicity value on the criterion

derivation (Table 4-2), particularly since the EPA was not able to fit a curve to estimate an EC_{10} given that there were only two PFOS treatment groups and the gap in these exposure concentrations is large (about 15-fold). This additional analysis yielded an FCV of 0.0002476 mg/L, or 0.00025 mg/L. This additional analysis indicated that the estimated toxicity value from *L. siliquoidea* has no influence on the calculated chronic criterion. Since the estimated toxicity value had no influence on the recommended CCC value, the author-reported MATC was used instead.

In the seventh analysis, another author-reported EC_{10} for mayfly of 0.000272 mg/L (based on log-linear regression without controls) was used in the chronic dataset to understand the influence of this alternate toxicity value on the criterion derivation (Table 4-2). This additional analysis yielded a FCV was 0.0002938 mg/L, or 0.00029 mg/L, indicating that this other toxicity value for *N. triangulifer* has little influence on the final calculated chronic criterion. Since this other toxicity value had limited influence on the recommended CCC value, the author-reported EC_{10} (0.000226 mg/L, two parameter, threshold sigmoidal curve) described in Section 3.1.1.3.1 in the criterion derivation was used instead.

Lastly, in the eighth analysis, a second author-reported EC_{10} for mayfly of 0.000232 mg/L (based on log-linear regression with controls) was used in the chronic dataset to understand the influence of this third toxicity value on the chronic criterion derivation (Table 4-2). This additional analysis yielded a FCV was 0.0002550 mg/L, or 0.00026 mg/L, indicating that this third possible toxicity value for *N. triangulifer* has limited influence on the calculated chronic criterion. Since this alternate toxicity value had limited influence on the recommended CCC value, the author-reported EC_{10} (0.000226 mg/L, two parameter, threshold sigmoidal curve) described in Section 3.1.1.3.1 in the criterion derivation was used instead.

Table 4-2. GMCVs Used in Derivation of Chronic Criterion and Additional Analyses Supporting the Chronic Criterion for Freshwater.

MDR Group[1]	Genus	Species	Chronic Criterion	Additional Analysis							
			GMCV (mg/L PFOS)[2]	First[3]	Second[3]	Third[3]	Fourth[4]	Fifth[5]	Sixth[6]	Seventh[7]	Eighth[7]
F	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	0.000226	0.000226	0.000226	0.000226	0.000226	0.000226	0.000226	**0.000272**	**0.000232**
F	*Chironomus*	Midge, *Chironomus dilutus*	0.005198	0.005198	0.005198	0.005198	0.005198	0.005198	0.005198	0.005198	0.005198
G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	0.01768	0.01768	0.01768	0.01768	-	-	**0.0123**	0.01768	0.01768
F	*Enallagma*	Blue damselfly, *Enallagma cyathigerum*	0.03162	**0.3162**	**0.1**	**0.01**	0.03162	-	0.03162	0.03162	0.03162
B	*Danio*	Zebrafish *Danio rerio*	0.03217	0.03217	0.03217	0.03217	0.03217	0.03217	0.03217	0.03217	0.03217
D	*Daphnia*	Cladoceran, *Daphnia carinata*	0.06519	0.06519	0.06519	0.06519	0.06519	0.06519	0.06519	0.06519	0.06519
		Cladoceran, *Daphnia magna*									
A	*Salmo*	Atlantic salmon, *Salmo salar*	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1
B	*Pimephales*	Fathead minnow, *Pimephales promelas*	0.1098	0.1098	0.1098	0.1098	0.1098	0.1098	0.1098	0.1098	0.1098
E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167
D	*Moina*	Cladoceran, *Moina macrocopa*	0.1789	0.1789	0.1789	0.1789	0.1789	0.1789	0.1789	0.1789	0.1789
H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
C	*Xiphophorus*	Swordtail fish, *Xiphophorus helleri*	0.5997	0.5997	0.5997	0.5997	0.5997	0.5997	0.5997	0.5997	0.5997
C	*Xenopus*	African clawed frog, *Xenopus laevis*	0.7507	0.7507	0.7507	0.7507	0.7507	0.7507	0.7507	0.7507	0.7507
		Clawed frog, *Xenopus tropicalis*									
C	*Lithobates*	Northern leopard frog, *Lithobates pipiens*	1.316	1.316	1.316	1.316	1.316	1.316	1.316	1.316	1.316
E	*Hyalella*	Amphipod, *Hyalella azteca*	2.899	2.899	2.899	2.899	2.899	2.899	2.899	2.899	2.899

MDR Group[1]	Genus	Species	Chronic Criterion	Additional Analysis							
			GMCV (mg/L PFOS)[2]	First[3]	Second[3]	Third[3]	Fourth[4]	Fifth[5]	Sixth[6]	Seventh[7]	Eighth[7]
G	*Physella*	Snail, *Physella heterostropha pomilia*	8.527	8.527	8.527	8.527	8.527	8.527	8.527	8.527	8.527
D	*Ceriodaphnia*	Cladoceran, *Ceriodaphnia dubia*	10.69	10.69	10.69	10.69	10.69	10.69	10.69	10.69	10.69
Chronic Water Column Concentration			**0.00025**	**0.00025**	**0.00025**	**0.00027**	**0.00021**	**0.00016**	**0.00025**	**0.00029**	**0.00026**

[1] MDR Groups identified according to the list provided in Section 2.10.1 above.
[2] GMCVs as presented in Table 3-7 in Section 3.1.1.3. Genera presented in order of rank according to the chronic criterion derivation. The rank order of GMCVs was not changed for the additional analyses.
[3] Additional analysis with changes to toxicity value for *E. cyathigerum.*
[4] Additional analysis with the exclusion of *L. siliquoidea.*
[5] Additional analysis with the exclusion of *L. siliquoidea* and *E. cyathigerum.*
[6] Additional analysis with the changes to toxicity value for *L. siliquoidea*.
[7] Additional analysis with the changes to toxicity value for *N. triangulifer*.

4.2 Influence of Using Non-North American Resident Species on PFOS Criteria

The EPA conducted two additional analyses of the freshwater criteria by analyzing the effect of reducing the limited toxicity datasets to only organisms that are resident to, or have been introduced and have established populations in the conterminous U.S. These analyses were conducted for illustrative purposes, to indicate the effects on the criteria magnitude of the inclusion of data for taxa that are not resident species to North America but serve as surrogates for other sensitive organisms. This analysis was conducted for both the acute and chronic freshwater datasets only, since the estuarine/marine datasets are limited even when all species are included.

4.2.1 Freshwater Acute Water Column Criterion with Native and Established Organisms (Species Not Resident to North America removed from dataset)

For the purpose of illustrating the effect of including non-resident species in the acute criterion calculation, additional analyses were made. For this illustrative analysis, four species were removed from the freshwater acute water column criterion calculation to ensure that only native, reproducing, or established organism in the conterminous U.S. were included: Japanese swamp shrimp (*Neocaridina denticulata*), planarian (*Dugesia japonica*), crayfish (*Pontastacus leptodactylus*) and cladoceran (*Daphnia carinata*). Removal of these species truncated the freshwater acute dataset to 25 species (Table 4-3). None of the non-resident species were among the four most sensitive, with the cladoceran (*Daphnia carinata*), being the most sensitive SMAV of the four (Table 3-3). The acute water column concentration was 0.048 mg/L PFOS (Table 4-4) when using the reduced dataset which was slightly lower than the recommended CMC of 0.071 mg/L. This value is lower than all of the GMAVs in Table 3-3. The EPA decided to retain the full acute dataset and associated acute criterion for PFOS of 0.071 mg/L in order to have the

largest, high-quality dataset to serve as surrogate species for the broad range of the thousands of untested species present in the freshwater environment in the U.S.

Table 4-3. Ranked Freshwater Genus Mean Acute Values with Native and Established Organisms, excluding Species Not Resident to North America.

Rank[a]	GMAV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOS)
1	0.07617	F	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	0.07617
2	3.075	D	*Moina*	Cladoceran, *Moina macrocopa*	17.20
				Cladoceran, *Moina micrura*	0.5496
3	6.950	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	6.950
4	7.515	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	7.515
5	13.5	G	*Ligumia*	Black sandshell, *Ligumia recta*	13.5
6	15.99	C	*Xenopus*	African clawed frog, *Xenopus laevis*	15.99
7	16.5	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	16.5
8	19.88	C	*Hyla*	Gray treefrog, *Hyla versicolor*	19.88
9	27.86	B	*Danio*	Zebrafish, *Danio rerio*	27.86
10	47.40	C	*Ambystoma*	Jefferson salamander, *Ambystoma jeffersonianum*	51.71
				Small-mouthed salamander, *Ambystoma texanum*	30.00
				Eastern tiger salamander, *Ambystoma tigrinum*	68.63
11	56.49	C	*Anaxyrus*	American toad, *Anaxyrus americanus*	56.49
12	59.87	E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	59.87
13	61.8	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	61.8
14	64.35	G	*Elliptio*	Eastern elliptio, *Elliptio complanata*	64.35

Rank[a]	GMAV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOS)
15	83.36	D	*Daphnia*	Cladoceran, *Daphnia magna*	51.86
				Cladoceran, *Daphnia pulicaria*	134
16	109.2	C	*Lithobates*	American bullfrog, *Lithobates catesbeiana*	133.3
				Green frog, *Lithobates clamitans*	113
				Northern leopard frog, *Lithobates pipiens*	72.72
				Wood frog, *Lithobates sylvatica*	130
17	172.1	G	*Physella*	Bladder snail, *Physella acuta*	183.0
				Snail, *Physella heterostropha pomilia*	161.8

[a] Ranked from the most sensitive to the most tolerant based on Genus Mean Acute Value.
[b] From Appendix A: Acceptable Freshwater Acute PFOS Toxicity Studies.
[c] MDR Groups identified by list provided in Section 2.10.1 above.

Table 4-4. Calculation of Freshwater Acute Water Column Concentration with Native and Established Organisms (Species Not Resident to North America Removed from Dataset).

Calculated Freshwater FAV based on 4 lowest values: Total Number of GMAVs in Dataset = 17						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Neocloeon*	0.07617	-2.57	6.63	0.056	0.236
2	*Moina*	3.075	1.12	1.26	0.111	0.333
3	*Pimephales*	6.950	1.94	3.76	0.167	0.408
4	*Oncorhynchus*	7.515	2.02	4.07	0.222	0.471
		Σ (Sum):	**2.50**	**15.72**	**0.56**	**1.45**

S^2 = 458.21 **S = slope**
L = -7.127 **L = X-axis intercept**
A = -2.340 **A = lnFAV**
FAV = 0.0963 **P = cumulative probability**

Acute Water Column Concentration = 0.048 mg/L PFOS (rounded to two significant figures)

4.2.2 Freshwater Chronic Water Criterion with Native and Established Organisms (Species Not Resident to North America removed from dataset)

For the purpose of illustrating the effect of including non-resident species in the chronic criterion calculation, additional analyses were made. For this illustrative analysis, two species were removed from the chronic freshwater dataset that are not native or established organism in the conterminous U.S.: the cladoceran (*Daphnia carinata*) and the clawed frog (*Xenopus tropicalis*). Removal of these species truncated the freshwater chronic dataset to 17 species representing 17 genera (Table 4-5). The revised freshwater chronic dataset consisted of all eight MDRs. The cladoceran and clawed frog GMCVs were not among the four most chronically sensitive species. Removal of the species that are not resident to North America had no effect on the chronic water column concentration (Table 4-6) because other species were available from the same genera and neither of the non-resident species were from the four most chronically sensitive genera. The chronic water column concentration was 0.0002491 mg/L PFOS when using the reduced dataset, which was the same as the recommended chronic criterion of 0.00025 mg/L. Therefore, the EPA decided to retain the full chronic dataset and associated chronic water column criterion for PFOS of 0.00025 mg/L in order to have the largest, high quality dataset to serve as surrogate species for the broad range of the thousands of untested species present in the freshwater environment in the U.S.

Table 4-5. Ranked Freshwater Genus Mean Chronic Values with Native and Established Organisms.

Rank[a]	GMCV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMCV[b] (mg/L PFOS)
1	0.000226	F	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	0.000226
2	0.005198	F	*Chironomus*	Midge, *Chironomus dilutus*	0.005198
3	0.01768	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	0.01768
4	0.03162	F	*Enallagma*	Blue damselfly, *Enallagma cyathigerum*	0.03162
5	0.03217	B	*Danio*	Zebrafish, *Danio rerio*	0.03217
6	>0.1	A	*Salmo*	Atlantic salmon, *Salmo salar*	>0.1
7	0.1098	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	0.1098
8	0.167	E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	0.167
9	0.1789	D	*Moina*	Cladoceran, *Moina macrocopa*	0.1789
10	0.25	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	0.25
11	0.5997	C	*Xiphophorus*	Swordtail fish, *Xiphophorus helleri*	0.5997
12	> 0.7610	C	*Xenopus*	African clawed frog, *Xenopus laevis*	> 0.7610
13	1.316	C	*Lithobates*	Northern leopard frog, *Lithobates pipiens*	1.316
14	1.344	D	*Daphnia*	Cladoceran, *Daphnia magna*	1.344
15	2.899	E	*Hyalella*	Amphipod, *Hyalella azteca*	2.899
16	8.527	G	*Physella*	Snail, *Physella heterostropha pomilia*	8.527
17	8.640	D	*Ceriodaphnia*	Cladoceran, *Ceriodaphnia dubia*	8.640

[a] Ranked from the most sensitive to the most tolerant based on Genus Mean Chronic Value.
[b] From Appendix C: Acceptable Freshwater Chronic PFOS Toxicity Studies
[c] MDR Groups identified by list provided in Section 2.10.1 above.

Table 4-6. Calculation of Freshwater Chronic Water Column Concentration with Native and Established Organisms.

Calculated Freshwater FCV based on 4 lowest values: Total Number of GMCVs in Dataset = 17						
Rank	**Genus**	**GMCV (mg/L)**	**ln(GMCV)**	**ln(GMCV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Neocloeon*	0.000226	-8.39	70.48	0.056	0.236
2	*Chironomus*	0.005198	-5.26	27.66	0.111	0.333
3	*Lampsilis*	0.01768	-4.04	16.28	0.167	0.408
4	*Enallagma*	0.03162	-3.45	11.93	0.222	0.471
		Σ (Sum):	**-21.14**	**126.35**	**0.56**	**1.45**

S^2 = 472.36 **S = slope**
L = -13.157 **L = X-axis intercept**
A = -8.297 **A = lnFCV**
FCV = 0.0002491 **P = cumulative probability**

Chronic Water Column Concentration = 0.00025 mg/L PFOS (rounded to two significant figures)

4.3 Qualitatively Acceptable Water Column-Based Toxicity Data

Several studies were identified as either not meeting the EPA's data quality guidelines for inclusion in the criteria derivation or did not have data available to support the independent calculation of a toxicity value (e.g., LC_{50} and/or EC_{10}). However, these studies were used qualitatively as supporting information to the PFOS criterion derived to protect aquatic life and provide additional evidence of the observed toxicity and effects of PFOS, including the relative sensitivities of surrogate, untested species. The key studies with apical endpoints (e.g., effects on survival, growth, or reproduction) that were used qualitatively in the derivation of the PFOS water column criteria are summarized below, grouped as either acute or chronic exposures and sorted by relative sensitivity of genera following the previous study summaries included in the Effects Analysis (Section 3). Qualitative study summaries within a factor of two of the final acute and chronic values were also included and arranged according to taxonomic relatedness. NOEC and LOEC values were provided in several of the study summaries for comparison to the toxicity values summarized in the Effects Analysis section. The toxicity values summarized

below as part of this Effects Characterization were not used quantitatively to derive the acute or chronic PFOS freshwater criteria. Results of each individual study (as well as the rationale why a study was not quantitatively acceptable) were considered relative to the corresponding freshwater acute or chronic criterion magnitude to ensure the water column-based PFOS criteria were not underproductive and to provide additional supporting evidence of the potential toxicity of PFOS to aquatic organisms. Tabulated data for the studies summarized below, as well as additional qualitative studies of less sensitive taxa, were listed in Appendix G.

4.3.1 Consideration of Qualitatively-Acceptable Acute Data

4.3.1.1 Qualitatively Acceptable Acute Data for Species Among the Four Most Sensitive Genera Used to Derive the Acute Water Column Criterion

4.3.1.1.1 Most acutely sensitive genus, Neocloeon

There were no qualitatively-acceptable acute tests with the genus, *Neocloeon*.

4.3.1.1.2 Second most acutely sensitive genus, Moina

There were no qualitatively-acceptable acute tests with the genus, *Moina*.

4.3.1.1.3 Third most acutely sensitive genus, Pimephales

There were no qualitatively-acceptable acute tests with the genus, *Pimephales*.

4.3.1.1.4 Fourth most acutely sensitive genus, Oncorhynchus

Raine et al. (2021) exposed unfertilized rainbow trout (*Oncorhynchus mykiss*) oocytes for three hours to PFOS (perfluorooctanesulfonic acid, > 97% pure, CAS No. 1763-23-1, obtained from SynQuest Laboratories). The authors reported a residue accumulation NOEC of 0.87 mg/L and LOEC of 7.47 mg/L PFOS. This test is considered for qualitative use since the exposure duration was too short for both acute and chronic test exposure according to the test guidelines. Also, no apical endpoints were reported.

4.3.1.2 Consideration of Relatively Sensitive Tests with Freshwater Species based on Qualitatively-Acceptable Acute Data

4.3.1.2.1 Genus: Danio (zebrafish)

Cormier et al. (2019) evaluated the acute effects of (1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonic acid (PFOS, purity ≥ 98%, CAS No. 2785-37-3, purchased from Sigma-Aldrich in St. Louis, MO) on zebrafish (*Danio rerio*) via a 96-hour measured, static-renewal study. Zebrafish embryos were collected and tested according to OECD TG 236. The authors reported a 96 hour NOEC value of 700 ng/L PFOS (or 0.0007 mg/L) for hatching success, embryo mortality, and developmental deformations. Since the value represents a greater than low value (see description of decision rule in Section 2.10.3.2) (U.S. EPA 2013), the study is only used qualitatively in the acute criterion.

Haimbaugh et al. (2022) evaluated the acute toxic effects of low-level (≤2,400 ng/L) PFOS on zebrafish from zero to five days post fertilization. Unmeasured test concentrations (24, 240, or 2,400 ng/L PFOA) were renewed daily. At test termination, the highest test concentration (2,400 ng/L or 0.0024 mg/L) had no effects on mortality or abnormal development. This test was not used quantitatively and retained for qualitative use only because the exposure durations were too long for an acute test and too short for a chronic test with no effects observed. This study also represents a greater than low value.

The noted toxicity values provided above (>0.0007 mg/L and >0.0024 mg/L), indicated that this genus might be more sensitive to acute exposures of PFOS than the quantitative data for the genus (with a GMAV of 27.86 mg/L). However, this non-definitive qualitative value does not provide any clarity on true sensitivity. All eight of the quantitatively-acceptable acute tests for this species reported LC_{50} values (range = 3.502 – 71.12 mg/L; geometric mean = 27.86

mg/L; n = 8) that were more than an order of magnitude greater than the FAV, leading the EPA to conclude that *D. rerio* is not a sensitive species to acute PFOS exposures.

4.3.2 Consideration of Qualitatively-Acceptable Chronic Data

4.3.2.1 Qualitatively Acceptable Chronic Data for Species Among the Four Most Sensitive Genera Used to Derive the Chronic Water Column Criterion

4.3.2.1.1 Most chronically sensitive genus, Neocloeon

There were no qualitatively-acceptable chronic tests with the genus, *Neocloeon*.

4.3.2.1.2 Second most chronically sensitive genus, Chironomus

Zhai et al. (2016) exposed *Chironomus plumosus* larvae to PFOS (perfluorooctane sulfonate, obtained from Tokyo Chemical Industries, Tokyo, Japan, 98% pure) spiked in sediment for 10.3 days. The sediment was collected from the upstream region of the Yongding River in Beijing, China. Spiking involved adding 1 mL of PFOS methanol solution (20 mg/L) to the sediment to obtain a concentration of 100 ng/g PFOS and thoroughly mixing in a fume hood. The midge larvae used in this study were collected from the uncontaminated upstream area of the Yangliuqing River in the outer suburbs of Tianjin, China. At the end of the experiment, the surviving larvae were counted. The 10-day mortality NOEC was 0.00985 mg/L PFOS, the only concentration tested. This study was considered for qualitative use for the following reasons: (1) the exposure duration was relatively short when comparing to the test guidelines for aquatic invertebrates and considered a sub-chronic exposure, (2) the sediment and test organisms used appear to have been previously exposed to low levels of PFAS (albeit low exposures) based on the measured concentrations reported in the paper, and (3) the apical endpoint for mortality results in a > NOEC that is a low value, which provides little information to the relative sensitivity of midge and was not used to derive the chronic PFOS criterion based on the data use rules established for these criteria (see Section 2.10.3.2).

Stefani et al. (2014) conducted a chronic (10 generation) test of PFOS (form and purity not reported) with the midge, *Chironomus riparius*. The NOEC and LOEC were 0.0035 and > 0.0035 mg/L (as time-weighted average) as there were no effects on emergence, reproduction, or sex ratio at this concentration. The results from this study were not acceptable for quantitative use because only a single test concentration was used, the chronic value is a greater than low value and not informative for criterion development, and there was a lack of details pertaining to the characteristics of the sediment used in the exposure, including details regarding any differences in measured concentrations over the duration of the exposure. Since this study was focused on the chronic effects of PFOS to a relatively sensitive species, however, consideration of the greater than chronic value from this study (> 0.0035 mg/L) in the context of other values for the midge was prudent. The EC_{10}s for *Chironomus dilutus* of 0.05896 mg/L, 0.001588 mg/L, and 0.0015 mg/L from MacDonald et al. (2004), McCarthy et al. (2021), and Krupa et al. (2022), respectively, that were used quantitatively in the chronic criterion derivation are more robust values than the toxicity value reported in Stefani et al. (2014), and likely a better estimation of the sensitivity of *C. riparius*. The chronic value reported by Stefani et al. (2014), although slightly lower than the *Chironomus* GMCV, is higher than the final recommended chronic criterion, and was expressed as a NOEC, as no effects were observed at 0.0035 mg/L (as time-weighted average).

In a companion paper to Stefani et al. (2014), Marziali et al. (2019) similarly conducted a chronic (10 generation) test of PFOS (form and purity not reported) with *C. riparius*. The LOEC based on F1 developmental time and F1 adult weight was < 0.004 mg/L (time-weighted average). The were no effects on F1 exuvia length at this concentration. The results from this study were not considered for quantitative use because only a single test concentration was used,

there was a lack of consistent observed effects in both the control and the treatment groups across the generations, and details pertaining to the characteristics of the sediment used in the exposure were lacking, including details regarding any differences in measured concentrations over the duration of the exposure. Again, it is prudent to consider the less than chronic value from this study (< 0.004 mg/L) in the context of the more robust and definitive values for midge. Similar to the determination above, the chronic values established for the related midge, *C. dilutus*, from MacDonald et al. (2004) and McCarthy et al. (2021) are deemed more reliable and definitive values representing the sensitivity of the genus in the freshwater chronic water column criterion dataset.

4.3.2.1.3 Third most chronically sensitive genus, Lampsilis

There were no qualitatively-acceptable chronic tests with the genus, *Lampsilis*.

4.3.2.1.4 Fourth most chronically sensitive genus, Enallagma

Van Gossum et al. (2009) conducted a chronic, approximately 4-month renewal test of PFOS (tetraethylammonium salt, 98% purity) with damselfly, *Enallagma cyathigerum.* The test organisms were larvae that had reached the F2 instar stage. Dilution water was dechlorinated tap water. Photoperiod was 16 hours light and 8 hours dark. Light intensity was not reported. A primary stock solution was prepared and proportionally diluted with dilution water to prepare the test concentrations. Exposure vessels were plastic containers (15 cm x 10 cm x 11 cm) with a 2 cm depth of test solution. The test employed 19-20 larvae each in two test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 0.01, 0.1, 1, and 10 mg/L. All larvae were housed (and presumably tested) in temperature-controlled rooms at 21±1.3°C. No other water quality parameters were reported as having been measured in test solutions. Negative control mortality was said to be much lower than the 100% mortality that occurred at 1

and 10 mg/L but was not reported. The 4-month NOEC (behavioral – including general activity, swimming performance, foraging success) was 0.010 mg/L. The 4-month LOEC was 0.100 mg/L. The calculated MATC was 0.03163 mg/L. The chronic value was acceptable for qualitative use because only non-apical endpoints were reported.

4.3.2.2 Consideration of Relatively Sensitive Tests with Freshwater Species based on Qualitatively-Acceptable Chronic Data

4.3.2.2.1 Genus: Lithobates (leopard frog)

Flynn et al. (2021) evaluated the chronic effects of perfluorooctane sulfonic acid (PFOS, CAS# 1763-23-1, ≥ 96% purity, purchased from Sigma-Aldrich) on Northern Leopard frogs, *Lithobates pipiens* (formerly, *Rana pipiens*), via a 30-day sediment-spiked measured, static mesocosm study. The study authors reported a 30-day NOEC of 0.016 mg/L for weight, snout-vent length and mortality and a 30-day LOEC of 0.00006 mg/L for developmental stage (measured as Gosner stage). Independently-calculated EC_{10}s could not be calculated as the EPA was unable to fit a model with significant parameters. Therefore, given this was an outdoor mesocosm with spiked sediment that included the addition of algal and zooplankton communities, and the EPA was unable to independently calculate toxicity values based on the replicate level data provided by the study authors, this study was used qualitatively to derive the final recommended chronic water column criterion.

Hoskins et al. (2022) evaluated the chronic effects of PFOS alone and in mixture with PFHxS on northern leopard frogs (*Lithobates pipiens*) from Gosner Stage 25 tadpoles through metamorphosis (Gosner Stage 46) in a static-renewal measured exposure. No effects were observed on survival, mass index and snout-vent length at 0.000934 mg/L PFOS (the highest test concentration at test termination (day 120). The test results in a greater than NOECs (>0.000934 mg/L) and provides little information to the relative sensitivity of the species. Therefore, this

study was not used to derive the chronic PFOS criterion based on prior data use rules (U.S. EPA 2013).

4.4 Acute-to-Chronic Ratios

The 1985 Guidelines allow the use of a final acute-to-chronic ratio (FACR) to convert the FAV to the FCV as an alternative approach to derive the chronic criterion instead of the direct calculation to determine the FCV (as described in Section 2.10.1) when the eight MDRs are not met (U.S. EPA 1985). While this alternative approach was not needed for the derivation of the chronic PFOS criterion, which was derived from empirical chronic data with all of the eight MDRs met, the possibility of calculating a scientifically-defensible FACR is as follows, for illustrative purposes only. Seventeen ACRs for eight invertebrate species and two fish species can be calculated from the quantitative acute and chronic toxicity data (Appendix A and Appendix C). Appendix I includes the ACRs for freshwater aquatic species with quantitative chronic values for which comparable quantitative acute values were reported from the same study or same investigator and laboratory combination. For each species where more than a single ACR was calculated, species mean acute-to-chronic ratios (SMACRs) were also calculated as the geometric mean value of individual ACRs for a species. In the case of a single ACR within a species, that ACR was the SMACR.

The ACRs ranged from 4.110 to 12,877 across all tests (a factor of 3,133), which occurs within the *Daphnia magna* SMACR. There was little explanation for the extreme range in ACRs among paired tests with *D. magna*. However, the ACR of 12,877 from paired tests conducted by Lu et al. (2015) appears to be an outlier. Excluding the 12,877 outlier ACR from the paired tests with *D. magna* reported by Lu et al. (2015) and from the paired test with *Daphnia carinata* (Logeshwaran et al. 2021) produced an SMACR range of 16.32 to 1,030. This range was greater

than a factor of 10 with no relationship between SMACR and SMAV apparent. The 1985 Guidelines do not provide for calculation of a FACR under these circumstances.

4.5 Comparison of Empirical Tissue Concentrations to Translated Tissue Criteria

Measured PFOS tissue data were reported in 14 publications focused on freshwater species, six of which were quantitatively acceptable and eight of which were qualitatively acceptable (Table 4-7). The six quantitatively acceptable studies included data for one invertebrate, two fish, and one amphibian species, and the eight qualitatively acceptable studies included data for two invertebrate and four fish species. Results of these studies are summarized in Section 4.5.1 and Section 4.5.2, below.

Tissue concentration data from these toxicity studies were compared to the translated tissue criteria (fish whole body, fish muscle, invertebrate whole body) and supplemental fish tissue values (blood, liver, reproductive tissue) to better understand the protectiveness of the chronic aquatic life tissue criteria. Although tissue concentrations from the toxicity literature were limited, translated tissue criteria and supplemental fish tissue values were lower than tissue-based PFOS concentrations from chronic toxicity studies where toxic effects were observed, suggesting that the tissue criteria (and supplemental fish tissue values) are protective. Finally, while no amphibian tissue criteria are available, tissue concentrations from two amphibian toxicity tests suggest that the fish tissue criteria are protective of amphibians.

Table 4-7. Comparison of Empirical Tissue Concentrations to Chronic Tissue Criteria and Additional Tissue Values.

Species	Endpoint	Percent Effect Observed	Measured Tissue Concentration (mg/kg ww)[1]	Calculated Chronic Tissue Values[2] (mg/kg ww)	Tissue Type	Reference
Quantitative Studies						
Fatmucket (*Lampsilis siliquoidea*)	Probability of successful metamorphosis of glochidia	33.5%	LOEC: 0.248	0.028	Invertebrate Whole-body (adult)	Hazelton et al. (2012)
Zebrafish (*Danio rerio)*	F1 survival	21%	LOEC: 4.8	0.201	Whole-body (adult female)	Wang et al. (2011)
			LOEC: 6.0	0.201	Whole-body (adult male)	
Fathead minnows (*Pimephales promelas*)	Fecundity	49% (LOEC)	NOEC: 7.1 – LOEC 19.4	0.616	Liver concentrations (adult male)[3]	Ankley et al. (2005)
			NOEC: 31.8 – LOEC 82.9	0.616	Liver concentrations (adult female)	
			NOEC: 8.8 – LOEC 19.9	1.29[3]	Gonad concentrations (adult male)[3]	
			NOEC: 33.1 – LOEC 81.6	1.29	Gonad concentrations (adult female)	
	Growth (weight in F1)	18%	LOEC: 37.9	1.29[3]	Gonad concentrations (adult F0 male)[3]	Suski et al. (2021)
			LOEC: 37.4	1.29	Gonad concentrations (adult F0 female)	
			LOEC: 84.5	0.616	Liver (adult F0 male)	
			LOEC: 68.2	0.616	Liver (adult F0 female)	
Northern leopard frog (*Lithobates pipiens*)	Length at metamorphosis (GS 42[4])	17%	LOEC: 66.6	None Available	Whole-body (before metamorphosis, day 54)	Ankley et al. (2004)
	Gosner stage after 40 days	5%	LOEC: 14.36		Whole-body	Hoover et al. (2017)

Species	Endpoint	Percent Effect Observed	Measured Tissue Concentration (mg/kg ww)[1]	Calculated Chronic Tissue Values[2] (mg/kg ww)	Tissue Type	Reference
Qualitative Studies						
Red worms (*Limnodrilus hoffmeisteri*)	Reduction in superoxide dismutase[5]	13%	LOEC: 1,757 (dw)	0.028	Invertebrate Whole-body	Liu et al. (2016)
Great pond snails (*Lymnaea stagnalis*)	Survival	31%	LOEC: 2,877	0.028	Invertebrate Whole-body	Olson (2017)
European eels (*Anguilla anguilla*)	Survival	0%	NOEC: > 5.037	0.616	Liver	Roland et al. (2014)
Goldfish (*Carassius auratus*)	Survival	0%	NOEC: > 39.91 (dw)	0.087	Muscle	Feng et al. (2015)
Common carp (*Cyprinus carpio*)	Condition factor	3%	LOEC: 168.4	0.616	Liver	Hagenaars et al. (2008)
Zebrafish (*Danio rerio*)	Swimming distance[5]	18%	LOEC: 21.6	0.201	Whole-body	Spulber et al. (2014)

[1] Measured tissue concentrations are author-reported values. The EPA did not independently calculate toxicity values for tissue concentrations.

[2] Chronic tissue value concentrations represent chronic tissue criteria (invertebrates, fish muscle, fish whole body) or additional tissue values (fish blood, fish liver, fish reproductive tissue) calculated from BAFs for a given tissue type. See Section 3.2.3 and Appendix P for details.

[3] Fish reproductive tissue value based on female reproductive tissue.

[4] Gosner stage (GS) associated with this endpoint is not specifically reported by the study authors. However, the authors define complete metamorphosis as emergence of the forelimbs, which is GS 42 according to Taylor and Kollros (1946).

[5] Non-apical endpoint.

4.5.1 Comparison of Quantitative Studies and Tissue-Based Criteria

Tissue concentration data from these toxicity studies were compared to the translated tissue values for invertebrates and fish to better understand the protectiveness of the aquatic life tissue criteria. Hazelton et al. (2012) exposed adult fatmucket (*Lampsilis siliquoidea*) to aqueous PFOS for 36 days. Measured PFOS water concentrations in the control and exposure treatments averaged 0.0021, 0.0045, and 0.0695 mg/L, respectively. Corresponding tissue concentrations were 0.009, 0.015 and 0.248 mg/kg wet weight. A statistically significant decrease in the probability of successful metamorphosis of glochidia to the juvenile stage was observed in the highest PFOS exposure concentration.

Wang et al. (2011) exposed larval (8 hpf) zebrafish (*Danio rerio*) to aqueous PFOS for five months. Fish were exposed to three nominal PFOS concentrations (0.005, 0.05, and 0.25 mg/L, respectively). Whole-body PFOS tissue concentrations measured after five months in the two highest exposure concentrations averaged 6.0 and 11.2 mg/kg wet weight, respectively, in males, and 4.8 and 7.8 mg/kg wet weight, respectively, in females. PFOS was also measured in embryos produced from exposed parents and averaged 5.69 and 11.35 ng/embryo wet weight in the two highest exposure concentrations. Weights of embryos were not reported by the study authors, so concentrations could not be calculated to compare embryo tissue concentrations to the translated tissue criteria. However, given the study design included tissue measurements in the parental (F0) generation and the exposure to the offspring generation (F1) was via maternal transfer, the tissue concentration in the F0 generation associated with the F1 survival LOEC of 0.05 mg/L was a whole-body tissue concentration of 6.2 and 4.0 mg/kg wet weight (ww) in male and females, respectively.

Ankley et al. (2005) exposed sexually mature adult fathead minnows (*Pimephales promelas*) to aqueous PFOS for 21 days during which time they were allowed to reproduce, and

then the resulting offspring were held for an additional 24 days in the same exposure concentrations. Aqueous measured PFOS concentrations in the control and exposure treatments averaged <0.001, 0.0276, 0.101, 0.281, and 0.818 mg/L, respectively. PFOS was measured in the plasma, livers, and gonads of adult males and females after 21 days, in embryos, and in whole-body larval samples after 12 and 24 days. Tissue measurements were not made in organisms from the highest exposure concentration, where exposed adults were either dead or listless after 14 days. Plasma PFOS concentrations in adult organisms exposed to 0.0276, 0.101, and 0.281 mg/L PFOS averaged 26.9, 135, and 354 mg/L in males, and 47.1, 177, and 471 mg/L in females. Liver PFOS concentrations in adults averaged 7.1, 19.4, and 109 mg/kg wet weight in males, and 31.8, 82.9, and 261 mg/kg wet weight in females, respectively. Similarly, gonad PFOS concentrations averaged 8.8, 19.9, and 108 mg/kg wet weight in males, and 33.1, 81.6, and 263 mg/kg wet weight in females. PFOS concentrations in embryos from parents exposed to 0.0276, 0.101, and 0.281 mg/L PFOS were 9.3, 11.5, and 28.6 mg/kg, respectively. Larval PFOS concentrations measured after 12 and 24 days of exposure were similar, with whole-body concentrations corresponding to the 0.0276, 0.101, and 0.281 mg/L exposures were 19.8, 48.0, and 57.5 mg/kg wet weight after 12 days, and 17.8, 49.0, and 83.5 mg/kg wet weight after 24 days. The most sensitive apical endpoint was fecundity, with an aqueous EC_{10} of 0.051 mg/L. No corresponding tissue-based EC_{10} was calculated, but the corresponding liver concentrations would be expected to fall between 7.1 and 19.4 mg/kg in males and 31.8 and 82.9 mg/kg in females, and the corresponding gonad concentrations would be expected to fall between 8.8 and 19.9 mg/kg in males and 33.1 and 81.6 mg/kg in females. No muscle or whole-body measurements in adults are available to perform a direct comparison to the tissue criteria.

Suski et al. (2021) reported the chronic toxicity of PFOS-K (PFOS potassium salt, CAS# 2795-39-3, ≥ 98%,) on the fathead minnow, *Pimephales promelas*. Measured PFOS concentrations in water were 0.00014 (control), 0.044, 0.088, 0.14, and 0.231 mg/L. The most sensitive endpoint from the study was a significant decrease in the mean mass of individuals in the larval F1 generation with the author-reported NOEC and LOEC, based on growth in the F1 generation, being 0.044 (6% reduction in growth compared to controls) and 0.088 mg/L PFOS (associated with an 18% reduction in growth), respectively. The calculated MATC based on mean mass of individuals in the larval F1 generation is 0.06222 mg/L. The F1 larval LOEC was associated with measured gonad and liver concentrations in F0 male and females of 37.9, 37.4, 84.5, and 68.2 mg/kg ww, respectively. No corresponding tissue-based EC_{10} was calculated, but the corresponding gonad and liver EC_{10} concentrations would be expected to be greater than the translated reproductive tissue concentration of 1.29 mg/kg ww and the translated liver tissue concentration of 0.616 mg/kg ww. No muscle or whole-body measurements in adults are available to perform a direct comparison to the fish tissue criteria.

Ankley et al. (2004) exposed Northern leopard frogs (*Lithobates pipiens*) to PFOS from Gosner stage 8/9 embryos through metamorphosis. The time to metamorphosis ranged from 60-112 days. All frogs in the highest exposure concentration died before metamorphosis. The most sensitive apical endpoint was length at metamorphosis, which was significantly lower ($p < 0.05$) in the second highest exposure relative to the control. The measured aqueous PFOS concentrations in the NOEC and LOEC exposure concentrations averaged 0.957 and 3.42 mg/L over the full exposure duration. PFOS in whole body tissue was analyzed as dry weight but reported by the authors as wet weight normalized. Corresponding whole-body tissue NOEC and LOEC concentrations measured in tadpoles exposed for 54 days (before metamorphosis) were

16.9 and 66.6 mg/kg wet weight normalized. Whole body concentrations were also measured after 35 days and were higher than the 54-day measurements at the 0.957 mg/L exposure (22.1 mg/kg wet weight normalized) and 3.42 mg/L exposures (117.0 mg/kg wet weight normalized). Tadpole moisture content was not reported.

In a separate study with *L. pipiens*, Hoover et al. (2017), exposed juvenile (Gosner stage 26) northern leopard frogs to three PFOS concentrations (0.008, 0.078, and 0.884 mg/L measured PFOS, respectively) for 40 days. Survival, growth (snout-vent length), and developmental time (Gosner stage after 40 days) were measured, and the most sensitive apical endpoint was developmental time (Gosner stage after 40 days), with a NOEC of 0.008 mg/L and a LOEC of 0.078 mg/L. Whole body PFOS concentrations in frogs exposed to 0.008 mg/L PFOS in solution averaged 10.45 mg/kg dry weight after 40 days, and concentrations in frogs exposed to 0.078 mg/L averaged 51.46 mg/kg dry weight after 40 days. Tadpole moisture content was not reported in this study. In order to convert the reported dry weight concentrations to wet weight concentrations, so that they would be more directly comparable to the whole-body fish tissue criteria, a whole-body moisture content of 72.1% was applied, calculated as the average for all fish collected as part of the USGS National Contaminant Biomonitoring Program (NCBP Fish Database (usgs.gov)). Corresponding 40-day NOEC and LOEC wet weight PFOS tissue concentrations were 2.92 and 14.36 mg/kg wet weight, respectively.

In all of the studies described above, the translated tissue criteria and supplemental fish tissue concentrations were lower than the measured tissue concentrations where toxicity was observed, suggesting that the tissue criteria are protective. As noted above, tissue concentrations associated with the LOEC in both Ankley et al. (2004) and Hoover et al. (2017) are higher than

the fish whole-body tissue criterion, suggesting that the fish tissue criteria is protective of amphibians.

4.5.2 Comparison of Qualitative Studies and Tissue-Based Criteria

Like the comparison with the quantitative studies, tissue concentration data from these qualitative toxicity studies were compared to the translated tissue values for invertebrates and fish to better understand the protectiveness of the aquatic life tissue criteria. Liu et al. (2016) exposed 4-5 cm body length red worms (*Limnodrilus hoffmeisteri*) to two aqueous concentrations of PFOS for 10 days at pH 8.0 and measured oxidative stress biomarker activity. Measured exposure concentrations were 0.567 and 5.494 mg/L PFOS, and corresponding whole-body tissue PFOS concentrations were 89.5 and 1,757 mg/kg dry weight. Moisture content was not reported. A significant ($P < 0.05$) reduction in superoxide dismutase was observed in the highest treatment concentration after 10 days. Apical endpoints were not reported for this exposure. In a separate study with *L. hoffmeisteri*, Qu et al. (2016) calculated 48-hour EC_{50}s in response to PFOS at three pH values (6.2, 7.0, 8.0). PFOS was not measured in water. However, whole body tissue concentrations were measured after 48-hours in the control, 0.2 mg/L, and 2.0 mg/L nominal PFOS exposures. Whole-body tissue concentrations in the 2.0 mg/L exposure were 23.41 mg/kg dry weight at pH 6.2 and 12.61 mg/kg dry weight at pH 8.0. After 48 hours, a significant ($P < 0.05$) increase in superoxide dismutase was observed in both the 0.2 mg/L and 2.0 mg/L PFOS treatments; however, no significant differences were observed at pH 8.0 for either treatment level.

Olson (2017) exposed adult great pond snails (*Lymnaea stagnalis*) to PFOS for 21 days. The most sensitive apical endpoint was survival, with a NOEC of 3 mg/L PFOS nominal, and a LOEC of 6 mg/L PFOS nominal. Whole-body PFOS tissue concentrations at the NOEC and LOEC after 21 days were 8,969 mg/kg dry weight and 9,820 mg/kg dry weight, respectively.

Percent moisture was not reported by the study authors, so dry weights were converted to wet weights using the average whole soft body % moisture content of 70.7% for the snail species *Achatina achatina* (Achaglinkame et al. 2020) in order to more directly compare *L. stagnalis* tissue concentrations from this study to the invertebrate chronic whole body tissue criterion. Resulting wet weight PFOS concentrations at the NOEC and LOEC were 2,628 and 2,877 mg/kg, respectively.

Roland et al. (2014) exposed juvenile European eels (*Anguilla anguilla*) to PFOS for 28 days. Measured PFOS water concentrations were 0.00001 mg/L in the control and 0.00081 and 0.011 mg/L in the two aqueous exposure concentrations. Corresponding liver tissue PFOS concentrations after 28 days were 0.0338 and 5.037 mg/kg wet weight, respectively. The study authors noted that during the study, there was no mortality, and no significant differences in growth across either PFOS treatment. Significant ($p < 0.05$) changes in protein expression were reported for both exposure concentrations.

Feng et al. (2015) conducted a 96-hour study with juvenile goldfish (*Carassius auratus*) and measured the effects of PFOS on mortality or antioxidant enzyme activity. Measured PFOS in the two exposure concentrations were 1.04 µmol/L (0.520 mg/L) and 10.18 µmol/L (5.09 mg/L). Liver, gill, and muscle PFOS concentrations were 32.81, 42.13, and 33.08 mg/L dry weight, respectively, at the lower exposure level, and 58.37, 69.02, and 39.91 mg/L dry weight, respectively, at the higher exposure level. No mortality occurred during the test. Among the antioxidant enzyme activity endpoints, glutathione peroxidase activity was significantly ($p < 0.05$) lower in the highest exposure concentration than the control.

Hagenaars et al. (2008) exposed juvenile common carp (*Cyprinus carpio*) to three exposure concentrations of PFOS plus a control for 14 days and measured relative condition

factor and several non-apical endpoints related to liver function. Nominal PFOS exposure concentrations were control, 0.1, 0.5, and 1 mg/L. Corresponding liver PFOS concentrations after the 14-day exposure were 0.97, 35.97, 168.4, and 283.0 mg/kg wet weight. The most sensitive endpoint was condition factor, which was significantly ($p < 0.0001$) lower than controls at the 0.5 mg/L nominal aqueous exposure concentration. Hepatosomatic index was also significantly ($P < 0.05$) lower at the 0.5 mg/L concentration compared to the control. The corresponding liver tissue PFOS concentration at this effect concentration (LOEC) was 168.4 mg/kg wet weight.

Spulber et al. (2014) exposed *Danio rerio* embryos (2 hpf) to 0.1 mg/L and 1.0 mg/L nominal PFOS concentrations for seven days. Corresponding whole-body PFOS concentrations in 7-day-old larvae were 21.6 and 213.5 mg/kg wet weight, respectively. Spulber et al. (2014) reported no effects of PFOS on viability, time to hatch, or deformities. The most sensitive endpoint was swimming distance, where fish exposed to the 0.1 and 1.0 mg/L PFOS treatments exhibited lower levels of activity ($p < 0.05$) in response to a pulse of darkness.

The translated tissue criteria and supplemental fish tissue concentrations were lower than the measured tissue concentrations where toxicity was observed for all of the qualitative studies. Although tissue concentrations from the toxicity literature were limited, available data suggest that the tissue criteria are protective.

4.6 Effects on Aquatic Plants

Available data for aquatic plants and algae were reviewed to determine if aquatic plants were likely to be more sensitive than aquatic animals to aqueous PFOS exposure (see Appendix E). Toxicity values for freshwater plants were well above the freshwater chronic water column criterion. Effect concentrations for freshwater plants and algae ranged from 0.19 to 252 mg/L

compared to the range in animal chronic values of 0.000226 to 16.35 mg/L (Appendix C: Acceptable Freshwater Chronic PFOS Toxicity Studies). Therefore, it was not necessary to develop a criterion based on the toxicity of PFOS to aquatic plants. The PFOS freshwater acute and chronic criteria are expected to be protective of freshwater plants.

4.7 Protection of Threatened and Endangered Species

The PFOS acute and chronic datasets are include some data representing species that are listed as threatened or endangered by the U.S. Fish and Wildlife Service and/or National Oceanic and Atmospheric Administration (NOAA) Fisheries. Summaries are provided here describing the available PFOS toxicity data for listed species indicating that the 2024 PFOS criteria are protective of these listed species, based on available scientific data.

4.7.1 Quantitatively Acceptable Acute Toxicity Data for Listed Species

Quantitatively acceptable acute toxicity test data evaluating the effects of PFOS on threatened and endangered freshwater species were available for rainbow trout (*Oncorhynchus mykiss*) with a SMAV of 7.515 mg/L PFOS (Palmer et al. 2002a; Sharpe et al. 2010). The SMAV is over 100 times higher than the recommended acute criterion (CMC) of 0.071 mg/L, indicating the acute criterion is protective of rainbow trout and is expected to be protective of other listed salmonid species.

Quantitatively acceptable acute data were also available for the Eastern tiger salamander (*A. tigrinum*). While the species is not considered to be a federally listed species, it is considered endangered in Delaware, Maryland, New Jersey, New York, and Virginia (Smith 2003), threatened in North Carolina (Smith 2003), and critically imperiled in Louisiana (2024). The Eastern tiger salamander is also closely related to the endangered California tiger salamander (U.S. FWS 2016a; U.S. FWS 2016b; U.S. FWS 2017). The *A. tigrinum* SMAV of 68.63 (Tornabene et al. 2021) is almost an order of magnitude above the recommended acute criterion

(CMC) of 0.071 mg/L, indicating the acute criterion is protective of the Eastern tiger salamander and the federally-listed California tiger salamander. There were no acceptable acute toxicity data for endangered or threatened estuarine/marine aquatic species.

4.7.2 Quantitatively Acceptable Chronic Toxicity Data for Listed Species

Quantitatively acceptable chronic toxicity test data evaluating the effects of PFOS on threatened and endangered freshwater species were available for the listed Atlantic salmon (*Salmo salar*) with a SMCV of >0.1 mg/L PFOS (Spachmo and Arukwe 2012). The SMCV is 400 times higher than the recommended chronic criterion (CCC) of 0.00025 mg/L, indicating the acute criterion is protective of Atlantic salmon and other listed salmonid species. There were no acceptable chronic toxicity data for endangered or threatened estuarine/marine aquatic species.

4.7.3 Qualitatively Acceptable Toxicity Data for Listed Species

Focusing on qualitatively acceptable tests with apical endpoints and water column exposures, there were toxicity data available for two fish species, rainbow trout and Atlantic salmon. For rainbow trout, the NOEC for mortality was 1 mg/L in a 12-day microcosm exposure and 3 mg/L in a 14-day early life stage static laboratory test (Oakes et al. 2005). For Atlantic salmon (*Salmo salar*), no adverse effects for growth were observed at the highest treatment concentration (0.1 mg/L PFOS) following a 49-day exposure (Arukwe et al. 2013). For both species, the qualitative NOECs were orders of magnitude greater than the recommended acute criterion (CCC) of 0.00025 mg/L, which further indicates that the chronic criteria are protective of listed salmonid species. There were no qualitative acute or chronic toxicity data for endangered or threatened estuarine/marine aquatic species.

4.8 Summary of the PFOS Aquatic Life Criterion and the Supporting Information

The PFOS aquatic life AWQC were developed to protect aquatic life against adverse effects, such as mortality, altered growth, and reproductive impairments, associated with acute and chronic exposure to PFOS. The national recommended criteria include water column-based acute and chronic criteria for fresh waters. The freshwater acute water column-based criterion magnitude is 0.071 mg/L, and the chronic water column-based criterion magnitude is 0.00025 mg/L (0.25 µg/L). The chronic freshwater criterion also contains tissue-based criteria expressed as 0.201 mg/kg wet weight (ww) for fish whole-body, 0.087 mg/kg ww for fish muscle tissue, and 0.028 mg/kg ww for invertebrate whole-body tissue. These PFOS aquatic life criteria are expected to be protective of all freshwater aquatic life on a national basis. Although empirical PFOS toxicity data for estuarine/marine species were not available to fulfill the eight MDRs directly, the EPA included an acute aquatic life benchmark for estuarine/marine environments using available estuarine/marine species toxicity data and a NAMs application of the EPA ORD's peer-reviewed web-ICE tool (see Appendix L). The estuarine/marine acute water column-based benchmark magnitude is 0.55 mg/L and is expected to protect estuarine/marine aquatic life from acute aqueous PFOS exposures. The EPA conducted additional analyses supporting the derivation of the water column criteria for PFOS (as summarized above in Sections 4.1 and 4.2) and confirmed that the criteria and benchmark calculations presented in this document accurately reflect the latest and best available scientific knowledge.

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Appendix A Acceptable Freshwater Acute PFOS Toxicity Studies

A.1 Summary Table of Acceptable Quantitative Freshwater Acute PFOS Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	EPA Calculated Effect Conc. (mg/L)	Final Effect Conc. (mg/L)[e]	Species Mean Acute Value (mg/L)	Reference
Planaria (0.9 cm), *Dugesia japonica*	S, U	96 hr	PFOS-K >98%	-	25	LC50	17	-	**17**	-	Li (2008)
Planaria (0.9 ±0.1 cm), *Dugesia japonica*	S, U	96 hr	PFOS-K >98%	-	25	LC50	23	22.68	**22.68**	-	Li (2009)
Planaria (10-12 mm), *Dugesia japonica*	R, U	96 hr	PFOS-K >99%	-	20	LC50	29.46	-	**29.46**	22.48	Yuan et al. (2014)
Eastern elliptio (76.5 g, 48.7 mm), *Elliptio complanata* (formerly, *Unio complamatus*)	R, M	96 hr	PFOS-K 90.49%	7.9-8.5	21.8-23.7	LC50	59	64.35	**64.35**	64.35	Drottar and Krueger (2000f)
Fatmucket (glochidia, <24 hr), *Lampsilis siliquoidea*	S, M	24 hr	PFOS >98%	8.46	20	EC50 (viability)	16.5	-	**16.5**	-	Hazelton (2013); Hazelton et al. (2012)
Fatmucket (juvenile, 4-6 wks), *Lampsilis siliquoidea*	R, M	96 hr	PFOS >98%	8.46	20	LC50	158.1	-	158.1[d]	16.5	Hazelton (2013); Hazelton et al. (2012)
Black sandshell (glochidia, <24 hr), *Ligumia recta*	S, M	24 hr	PFOS >98%	8.46	20	EC50 (viability)	13.5	-	**13.5**	-	Hazelton (2013); Hazelton et al. (2012)
Black sandshell (juvenile, 4-6 wk), *Ligumia recta*	R, M	96 hr	PFOS >98%	8.46	20	LC50	141.7	-	141.7[d]	13.5	Hazelton (2013); Hazelton et al. (2012)
Bladder snail (mixed age), *Physella acuta* (formerly, *Physa acuta*)	S, U	96 hr	PFOS-K >98%	-	25	LC50	178	183.0	**183.0**	183.0	Li (2009)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	EPA Calculated Effect Conc. (mg/L)	Final Effect Conc. (mg/L)[e]	Species Mean Acute Value (mg/L)	Reference
Snail (adult, 4 mo.), *Physella heterostropha pomilia* (formerly, *Physa pomilia*)	S, M	96 hr	PFOS-K ≥98%	-	25	LC50	161.77	-	**161.8**	161.8	Funkhouser (2014)
Rotifer (<2 hr old neonates), *Brachionus calyciflorus*	S, U[b]	24 hr	PFOS-K ≥98%	-	20	LC50	61.8	-	**61.8**	61.8	Zhang et al. (2013)
Cladoceran (6-12 hr), *Daphnia carinata*	S, U	48 hr	PFOS-K ≥98%	-	21	LC50	8.8	11.56	**11.56**	11.56	Logeshwaran et al. (2021)
Cladoceran (<24 hr), *Daphnia magna*	S, M	48 hr	PFOS-K 90.49%	8.2-8.6	19.3-20.2	EC50	61	58.51	**58.51**	-	Drottar and Krueger (2000g)
Cladoceran (<24 hr), *Daphnia magna*	S, U	48 hr	PFOS-K 95%	-	21	EC50 (immobility)	67.2	-	**67.2**	-	Boudreau (2002); Boudreau et al. (2003a)
Cladoceran (<24 hr), *Daphnia magna*	S, U	48 hr	PFOS Unreported	-	21	EC50 (immobility)	37.36	35.46	**35.46**	-	Ji et al. (2008)
Cladoceran (<24 hr), *Daphnia magna*	S, U	48 hr	PFOS-K >98%	7.82-7.91	25	EC50	63	55.40	**55.40**	-	Li (2009)
Cladoceran (<24 hr), *Daphnia magna*	S, U	48 hr	PFOS-K >98%	7.82-7.91	25	EC50	63	72.70	**72.70**	-	Li (2009)
Cladoceran (<24 hr), *Daphnia magna*	S, U	48 hr	PFOS-K >98%	7.82-7.91	25	EC50	63	64.60	**64.60**	-	Li (2009)
Cladoceran (<24 hr), *Daphnia magna*	S, M	48 hr	PFOS-K 99%	7	22	LC50	78.09	-	**78.09**	-	Yang et al. (2014)
Cladoceran (<24 hr), *Daphnia magna*	S, U	48 hr	PFOS 98%	7.2	20	EC50 (death/immobility)	23.41	-	**23.41**	-	Lu et al. (2015)
Cladoceran (<24 hr), *Daphnia magna*	S, U	48 hr	PFOS-K ≥98%	7	20	EC50 (death/immobility)	79.35	94.58	**94.58**	-	Liang et al. (2017)
Cladoceran (12-24 hr), *Daphnia magna*	S, U	48 hr	PFOS-K 98%	-	-	LC50	22.77	22.43	**22.43**	51.86	Yang et al. (2019)
Cladoceran (<24 hr), *Daphnia pulicaria*	S, U	48 hr	PFOS-K 95%	-	21	EC50 (immobility)	134	-	**134**	134	Boudreau (2002); Boudreau et al. (2003a)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	EPA Calculated Effect Conc. (mg/L)	Final Effect Conc. (mg/L)[e]	Species Mean Acute Value (mg/L)	Reference
Cladoceran (<24 hr), *Moina macrocopa*	S, U	48 hr	PFOS Unreported	-	25	EC50 (immobility)	17.95	17.20	**17.20**	17.20	Ji et al. (2008)
Cladoceran (<48 hr), *Moina micrura*	S, M	48 hr	PFOS ≥98%	-	27	LC50	0.5496	-	**0.5496**	0.5496	Razak et al. (2023)
Crayfish (intermolt), *Pontastacus leptodactylus* (formerly, *Astacus leptodactylus*)	R, M	96 hr	PFOS-K ≥98%	6.79	21	LC50	48.81	-	**48.81**	48.81	Belek et al. (2022)
Crayfish (juvenile, 2 wks, 0.041 g), *Procambarus fallax f. virginalis*	S, M	96 hr	PFOS-K ≥98%	-	25	LC50	59.87	59.87	**59.87**	59.87	Funkhouser (2014)
Japanese swamp shrimp, *Neocaridina denticulata*	S, U	96 hr	PFOS-K >98%	-	25	LC50	10[g]	12.91	**12.91**	-	Li (2009)
Japanese swamp shrimp, *Neocaridina denticulata*	S, U	96 hr	PFOS-K >98%	-	25	LC50	10[g]	28.55	**28.55**	-	Li (2009)
Japanese swamp shrimp, *Neocaridina denticulata*	S, U	96 hr	PFOS-K >98%	-	25	LC50	10[g]	10.32	**10.32**	15.61	Li (2009)
Mayfly (<24 hr larva), *Neocloeon triangulifer*	S, M	96 hr	PFOS-K 98%	-	23	LC50	0.08	0.07617	**0.07617**	0.07617	Soucek et al. (2023)
Rainbow trout (juvenile), *Oncorhynchus mykiss*	S, M	96 hr	PFOS-K 86.9%	-	11.3-12.9	LC50	22	22.59	**22.59**	-	Palmer et al. (2002a)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	EPA Calculated Effect Conc. (mg/L)	Final Effect Conc. (mg/L)[e]	Species Mean Acute Value (mg/L)	Reference
Rainbow trout (parr), *Oncorhynchus mykiss*	R, M	96 hr	PFOS-K 98%	-	10	LC50	2.5	-	**2.5**	7.515	Sharpe et al. (2010)
Zebrafish (embryo), *Danio rerio*	R, U	96 hr	PFOS Unreported	7-8.5	26	LC50	71	-	**71**	-	Ye et al. (2007)
Zebrafish (embryo), *Danio rerio*	S, U	96 hr	PFOS-K ≥97%	7.2-7.5	26	LC50	58.47	-	**58.47**	-	Hagenaars et al. (2011a)
Zebrafish (adult), *Danio rerio*	R, M	96 hr	PFOS-K 98%	-	26	LC50	22.2	-	**22.2**	-	Sharpe et al. (2010)
Zebrafish (3 mo., 2.2 cm), *Danio rerio*	R, U	96 hr	PFOS-K Unreported	-	23	LC50	17.0	-	**17.0**	-	Wang et al. (2013)
Zebrafish (embryo), *Danio rerio*	S, U	96 hr	PFOS-K 98%	8.3	28.5	LC50	68	71.12	**71.12**	-	Li et al. (2015)
Zebrafish (embryo), *Danio rerio*	S, U	96 hr	PFOS-K 98%	-	28	LC50	3.502	-	**3.502**		Du et al. (2016a); Du et al. (2017)
Zebrafish (embryo, 1 hpf), *Danio rerio*	R, U	96 hr	PFOS Unreported	-	26	LC50	34.2	38.82	**38.82**	-	Stengel et al. (2017b)
Zebrafish (embryo), *Danio rerio*	R, M	96 hr	PFOS-K ≥98%	-	26	LC50	23.99[c]	-	**23.99**	27.86	Nilén et al. (2022)
Fathead minnow (juvenile), *Pimephales promelas*	S, M	96 hr	PFOS-K 90.49%	8.2-8.5	22	LC50	9.5	9.020	**9.020**	-	Drottar and Krueger (2000c)
Fathead minnow (79 d), *Pimephales promelas*	S, U	96 hr	PFOS-Li 24.5%	8.0-8.4	19.2-19.5	LC50	4.655[f]	5.356[f]	**5.356**	6.950	3M Company (2000)
American toad (larva, Gosner stage 26), *Anaxyrus americanus*	S, U	96 hr	PFOS Unreported	-	21	LC50	62[g]	63.41	63.41[d]	-	Tornabene et al. (2021)
American toad (larva, Gosner stage 41), *Anaxyrus americanus*	S, U	96 hr	PFOS Unreported	-	21	LC50	62[g]	56.49	**56.49**	56.49	Tornabene et al. (2021)
Gray treefrog (larva, Gosner stage 26), *Hyla versicolor*	S, U	96 hr	PFOS Unreported	-	21	LC50	79	78.33	78.33[d]	-	Tornabene et al. (2021)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	EPA Calculated Effect Conc. (mg/L)	Final Effect Conc. (mg/L)[e]	Species Mean Acute Value (mg/L)	Reference
Gray treefrog (larva, Gosner stage 40), *Hyla versicolor*	S, U	96 hr	PFOS Unreported	-	21	LC50	24	19.88	**19.88**	19.88	Tornabene et al. (2021)
American bullfrog (tadpole, Gosner stage 25), *Lithobates catesbeiana* (formerly, *Rana catesbeiana*)	S, U	96 hr	PFOS Unreported	-	21	LC50	144	154.8	154.8[d]	-	Flynn et al. (2019)
American bullfrog (larva, Gosner stage 26), *Lithobates catesbeiana*	S, U	96 hr	PFOS Unreported	-	21	LC50	163	133.3	**133.3**	133.3	Tornabene et al. (2021)
Green frog (larva, Gosner stage 26), *Lithobates clamitans* (formerly, *Rana clamitans*)	S, U	96 hr	PFOS Unreported	-	21	LC50	113	-	**113**	113	Tornabene et al. (2021)
Northern leopard frog (larva, Gosner stage 26), *Lithobates pipiens* (formerly, *Rana pipiens*)	S, U	96 hr	PFOS Unreported	-	21	LC50	73	72.72	**72.72**	72.72	Tornabene et al. (2021)
Wood frog (larva, Gosner stage 26), *Lithobates sylvatica* (formerly, *Rana sylvatica*)	S, U	96 hr	PFOS Unreported	-	21	LC50	130	-	**130**	130	Tornabene et al. (2021)
African clawed frog (embryos), *Xenopus laevis*	R, M	96 hr	PFOS-K 86.9%	7.3	24	LC50	13.8	15.53	**15.53**	-	Palmer and Krueger (2001)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	EPA Calculated Effect Conc. (mg/L)	Final Effect Conc. (mg/L)[e]	Species Mean Acute Value (mg/L)	Reference
African clawed frog (embryos), *Xenopus laevis*	R, M	96 hr	PFOS-K 86.9%	7.27	24	LC50	17.6	18.04	**18.04**	-	Palmer and Krueger (2001)
African clawed frog (embryos), *Xenopus laevis*	R, M	96 hr	PFOS-K 86.9%	7.26	24	LC50	15.3	14.6	**14.60**	15.99	Palmer and Krueger (2001)
Jefferson salamander (larva, Harrison stage 40), *Ambystoma jeffersonianum*	S, U	96 hr	PFOS Unreported	-	21	LC50	64	51.71	**51.71**	51.71	Tornabene et al. (2021)
Small-mouthed salamander (larva, Harrison stage 40), *Ambystoma texanum*	S, U	96 hr	PFOS Unreported	-	21	LC50	41[g]	46.71	46.71[d]	-	Tornabene et al. (2021)
Small-mouthed salamander (larva, Harrison stage 46), *Ambystoma texanum*	S, U	96 hr	PFOS Unreported	-	21	LC50	41[g]	30.00	**30.00**	30.00	Tornabene et al. (2021)
Eastern tiger salamander (larva, Harrison stage 40), *Ambystoma tigrinum*	S, U	96 hr	PFOS Unreported	-	21	LC50	73	68.63	**68.63**	68.63	Tornabene et al. (2021)

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

[b] Chemical concentrations made in a side-test representative of exposure and verified stability of concentrations of PFOS in the range of concentrations tested under similar conditions. Daily renewal of test solutions.

[c] Reported in moles converted to milligram based on a molecular weight of 500.13 mg/mmol or 538.22 mg/mmol PFOS-K.

[d] Not used in SMAV calculation; only the most sensitive life-stage used.

[e] Values in bold used the in the SMAV calculation.

[f] Author-reported LC_{50} of 19 mg/L x 24.5% PFOS = 4.655 mg/L PFOS; EPA-calculated LC_{50} of 21.86 mg/L x 24.5% PFOS = 5.356 mg/L PFOS.

[g] Author pooled tests or life-stages.

A.2 Detailed PFOS Acute Freshwater Toxicity Study Summaries and Corresponding Concentration-Response Curves (when calculated for the most sensitive genera)

The purpose of this section was to present detailed study summaries for tests that were considered quantitatively acceptable for criteria derivation, with summaries grouped and ordered by genus sensitivity. Concentration-response (C-R) models developed by the EPA that were used to determine acute toxicity values used for water column criterion derivation are also presented for the most sensitive genera when available. C-R models included here with study summaries were those for the five most sensitive genera (consistent with Section 3.1.1.1). When required, the EPA also included models for non-resident species that were more sensitive than the fourth most sensitive North American resident genus. In many cases, authors did not report C-R data in the publication/supplemental materials and/or did not provide C-R data upon the EPA request. In such cases, the EPA did not independently calculate a toxicity value and the author-reported effect concentrations were used in the derivation of the criterion.

A.2.1 Most Sensitive Freshwater Genus for Acute Toxicity: *Neocloeon* (mayfly)

Soucek et al. (2023) conducted a 96-hour acute toxicity test to determine the effects of PFOS-K (PFOS potassium salt, CAS # 2795-39-3, 98% purity) on the parthenogenetic mayfly, *Neocloeon triangulifer*. The test was performed under static, nonrenewal conditions beginning with < 24-hour old nymphs. Exposures consisted of five mayfly nymphs per 30 mL polypropylene cup filled with 20 mL test solution. The control and each of six PFOS test concentrations were replicated five times for a total of 25 test organisms per treatment. Nominal test concentrations were 0 (control), 0.0156, 0.0313, 0.0625, 0.125, 0.250, and 0.500 mg/L PFOS. Mean measured PFOS concentrations (EPA Analytical Method 1633; LC-MC/MS) were 0.0002 (control), 0.017, 0.046, 0.052, 0.103, 0.253, and 0.358 mg/L PFOS, respectively. Animals were exposed at 23 ± 1°C under a 16:8 hour light (˜110 – 300 lux):dark cycle and fed live diatom

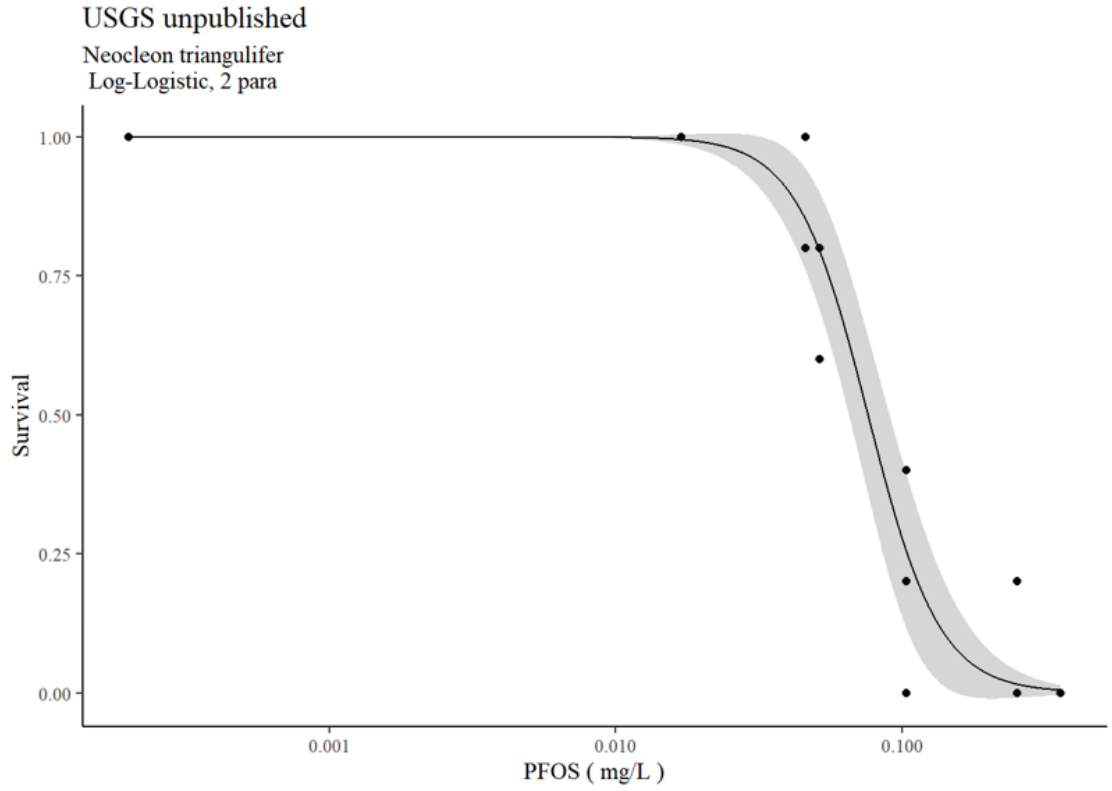
biofilm scraping beginning on Day 0. Percent survival in the control treatment after 96 hours was 100%. A uniform C-R pattern for percent survival was observed decreasing from 100 and 96% in the lowest treatments to 4 and 0% in the highest test treatments. The EPA was able to independently calculate a 96-hour LC_{50} of 0.07617 mg/L (0.06546 – 0.08688 mg/L; 95% CI) for this study. The EPA's independently-calculated LC_{50} is in line with the author-reported LC_{50} of 0.08 mg/L. Therefore, the independently-calculated LC_{50} of 0.07617 mg/L was acceptable for quantitative use in the derivation of the freshwater acute water column criterion for PFOS.

A.2.1.1 Soucek et al. (2023) Concentration Response Curve – Neocloeon (mayfly)

Publication: Soucek et al. (2023)
Species: Mayfly, *Neocloeon triangulifer*
Genus: *Neocloeon*
EPA-Calculated LC_{50}: 0.07617 (0.06546 – 0.08688) mg/L
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	3.4747	0.5921	58679	$4.414\ e^{-9}$
e	0.07617	0.0063135	12.0644	$< 2.2\ e^{-16}$

Concentration-Response Model Fit:



A.2.2 Second Most Sensitive Freshwater Genus for Acute Toxicity: *Moina* (cladoceran)

Ji et al. (2008) performed a 48-hour static, unmeasured acute test of PFOS (acid form, CAS # 1763-23-1, purity unreported) with *Moina macrocopa*. The test followed the EPA's Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms [U.S. EPA/600/4-90/027F; (U.S. EPA 2002)]. *M. macrocopa* used for testing were obtained from brood stock cultured at the Environmental Toxicology Laboratory at Seoul

National University (South Korea). Test organisms were less than 24 hours old at test initiation. Dilution water was moderately hard reconstituted water (hardness typically 80-100 mg/L as $CaCO_3$). Experiments were conducted in glass jars of unspecified size and fill volume. The photoperiod for the test was assumed by the reviewers to have been 16:8-hours light:dark, as was used for daphnid culture in tests by the same authors. Preparation of test solutions was not described. The test involved four replicates of five neonates each in five nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 6.25, 12.5, 25, 50 and 100 mg/L. Test temperature was maintained at 25 ± 1°C. Authors note water quality parameters (pH, temperature, conductivity, and D.O.) were measured 48 hours after exposure, but the information was not reported. Survival of organisms in the negative control was not reported in the paper. However, raw data were obtained by the EPA from the study authors and control survival was 100% in the acute test, meeting the EPA/600/4-90/027F requirement of at least 90% survival for test acceptability. The study authors reported a 48-hour EC_{50} value of 17.95 mg/L (C.I. 14.72 - 21.18) for *M. macrocopa*. The 48-hour EC_{50} value was independently-calculated by the EPA as 17.20 (13.73 – 20.66) mg/L. The independently-calculated acute toxicity value was quantitatively used in the derivation of the freshwater acute water column criterion.

Razak et al. (2023) tested the acute toxicity of perfluorooctanesulfonate (PFOS) on *Moina micrura* for 48 hours in a measured, static experiment. PFOS (≥98% purity) analytical standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and PFOA and solvents for making test solutions were purchased from Fisher Scientific (New Jersey, USA). Organisms were obtained from the Aquatic Animal Health and Therapeutics Laboratory (Aquahealth) at the Institute of Bioscience, Universiti Putra Malaysia. Culturing procedures

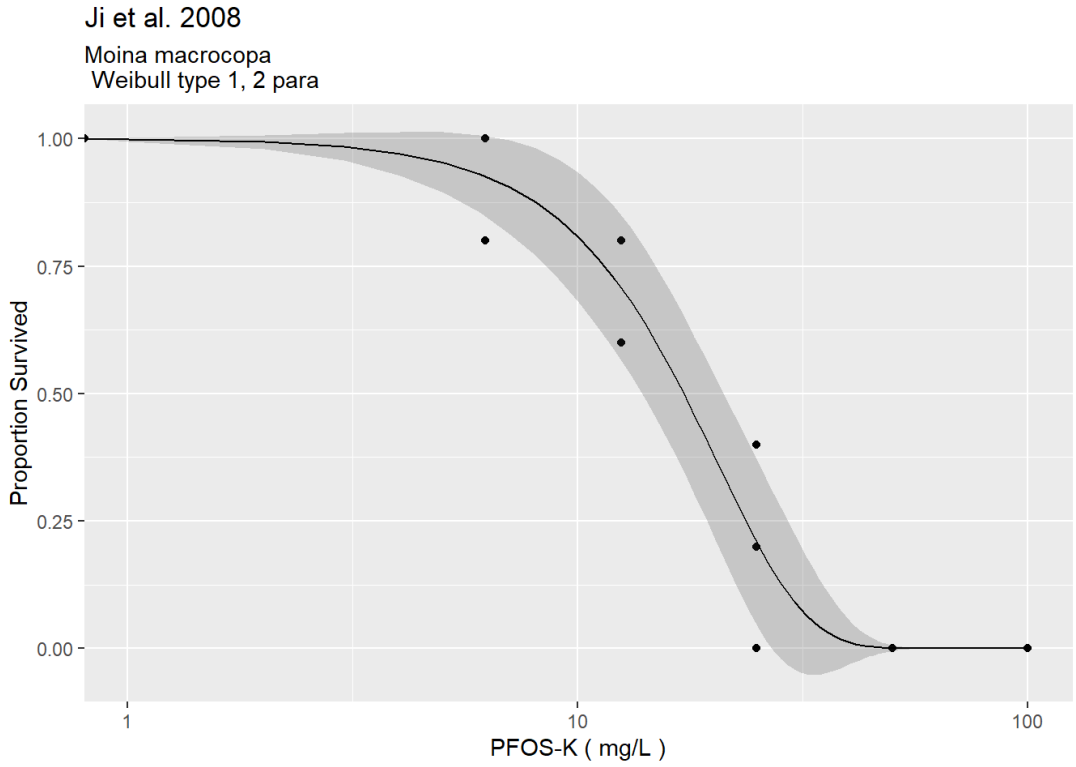
followed International Organisation for Standardization (ISO) procedure 6341:2012. Cultures were kept under a 12:12 light:dark cycle at 27±1°C. Culture water was renewed every two weeks, and culture organisms were fed green algae (*Chlorella vulgaris*) three times weekly. Both culture and test water was filtered (0.2 µm) surface lake water. A stock solution of 100 mg/L PFOS with filtered surface lake water was made just before testing began. Testing methods followed OECD 202 (OECD 2004) with nominal testing concentrations of 10, 25, 50, 75, 100, 250, 500, 750, 1,000, 2,500, 5,000, 7,500, and 10,000 µg/L, plus a control, with four replicates per treatment. Each replicate consisted of 10 neonates (<48 hours old) in 50 mL of solution in a 100 mL beaker, and organisms were not fed during the study. Nonparametric Kruskal-Wallace tests followed by post-hoc tests were used to calculate significant ($P<0.05$) differences between controls and treatment concentrations for all endpoints. The lethal effect concentrations (LC_{10}, LC_{50}, LC_{75}, LC_{90}) were calculated using Probit analysis, and the 48-hour LC_{50} value of 549.6 µg/L, or 0.5496 mg/L was determined to be acceptable for quantitative use. C-R data could not be obtained for this test (beyond the visual presentation in the Razak et al. (2023), so the EPA was unable to perform independent C-R analysis.

A.2.2.1 Ji et al. (2008) Concentration Response Curve – Moina (cladoceran)

Publication: Ji et al. (2008)
Species: Cladoceran, *Moina macrocropa*
Genus: *Moina*
EPA-Calculated LC_{50}: 17.20 (13.73 – 20.66) mg/L
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	2.18	0.47	4.60	$4.16\ e^{-06}$
e	20.34	2.06	9.87	$<2.2\ e^{-16}$

Concentration-Response Model Fit:



A.2.3 Third Most Sensitive Freshwater Genus for Acute Toxicity: *Pimephales* (fathead minnow)

3M Company (2000) provides the results of a 96-hour static, unmeasured acute toxicity test with the fathead minnow, *Pimephales promelas*, and PFOS-Li (perfluorooctanesulfonate lithium salt, CAS # 29457-72-5). A stock solution was made with carbon-filtered well water at a test sample concentration of 400 mg/L and where the test sample was reported as a mixture of PFOS-Li (24.5%) in water (75.5%). Fish were obtained from a commercial supplier (Aquatic Biosystems, Fort Collins, CO) and were 79 days old at test initiation with an average length of 2.1 cm and weight of 0.069 g. Exposure vessels were 2 L glass beakers containing 1 L of solution and 10 fish per beaker (0.69 g fish/L). Each test treatment was replicated twice with nominal test concentrations (control, 3.2, 5.6, 10.0, 18.0, 32.0 and 56.0 mg/L test sample). Throughout the experiment the dissolved oxygen (D.O.) ranged from 4.8 - 7.9 mg/L, pH 8.0 - 8.4 and a test temperature of 19.2 - 19.5°C. The low D.O. of 4.8 mg/L was only observed in one

replicate of the highest test concentration at 96 hours; D.O. was ≥6.0 mg/L for all other treatments and replicates. No mortality occurred in the control treatment and 100% was observed in the highest treatment (56 mg/L). The study authors reported that the test sample containing 24.5% PFOS-Li exhibited a 96-hour LC_{50} of 19 mg/L, which equates to 4.655 mg/L as PFOS. The independently-calculated 96-hour LC_{50} value was 21.86 (17.63 – 26.08) mg/L, which equates to 5.356 mg/L as PFOS and is acceptable for quantitative use in the derivation of the acute freshwater criterion for PFOS.

Drottar and Krueger (2000c) evaluated the acute effects of PFOS-K (CAS# 2795-39-3, Lot # 217 (T-6295) obtained from the 3M Company, 90.49% purity, stored at ambient room temperature) on juvenile fathead minnows (*Pimephales promelas*) during a 96-hour measured, static study. Researchers stated they followed protocols by U.S. EPA Series 850 (OPPTS 850.1075), OECD Guideline 203, and ASTM E729-88a. A primary stock solution was prepared at 27 mg/L and mixed with an electric mixer for 22 hours prior to use in testing to ensure solubilization of the test substance. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare the four additional test concentrations. Test fish were obtained from cultures at Wildlife International Ltd. in Easton, Maryland. The minnows were held for approximately 126 days prior to testing and were acclimated to test conditions for 48 hours prior to test initiation. Fish were fed a commercially-prepared diet prior to the 48-hour acclimation period. All fish used in the test were from the same source and year class, and the total length of the longest fish was no more than twice the length of the shortest. Fathead minnows were randomly distributed among mean measured test concentrations of 0 (control), 3.3, 5.6, 9.5, 17 and 28 mg/L, with 10 fish per 25-L polyethylene aquarium provided in duplicate. Aquaria were filled with 15 L of test solution with an observed D.O. of 7.7 - 8.4 mg/L,

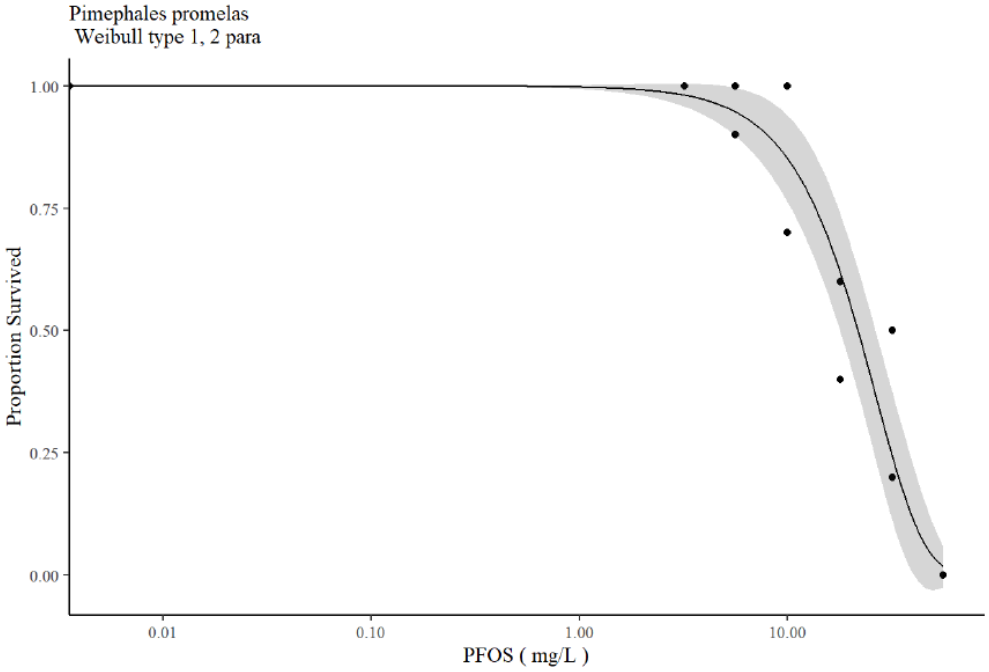
temperature of 22 ± 2ºC, pH of 8.2 - 8.5, and a total hardness of 131 mg/L as $CaCO_3$. Fathead minnows were subjected to a 16:8-hour light:dark photoperiod at 391 lux. Sand and 0.45 um filtered well water from a 40 m deep well on site served as both the culture water and the testing media. The authors reported an LC_{50} of 9.5 mg/L PFOS. The EPA's independently-calculated 96-hour LC_{50} was 9.020 (7.146 - 10.89) mg/L, rounded to four significant figures, and was used quantitatively to derive the freshwater acute water column criterion.

A.2.3.1 3M Company (2000) Concentration Response Curve – Pimephales (fathead minnow)

Publication: 3M Company (2000)
Species: Fathead minnow, *Pimephales promelas*
Genus: *Pimephales*
EPA-Calculated LC_{50}: 21.86 (95% C.I. 17.63 – 26.08) mg/L
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.8756	0.3016	6.2191	$4.999\ e^{-10}$
e	26.5720	2.5673	10.3501	$< 2.2\ e^{-16}$

Concentration-Response Model Fit:

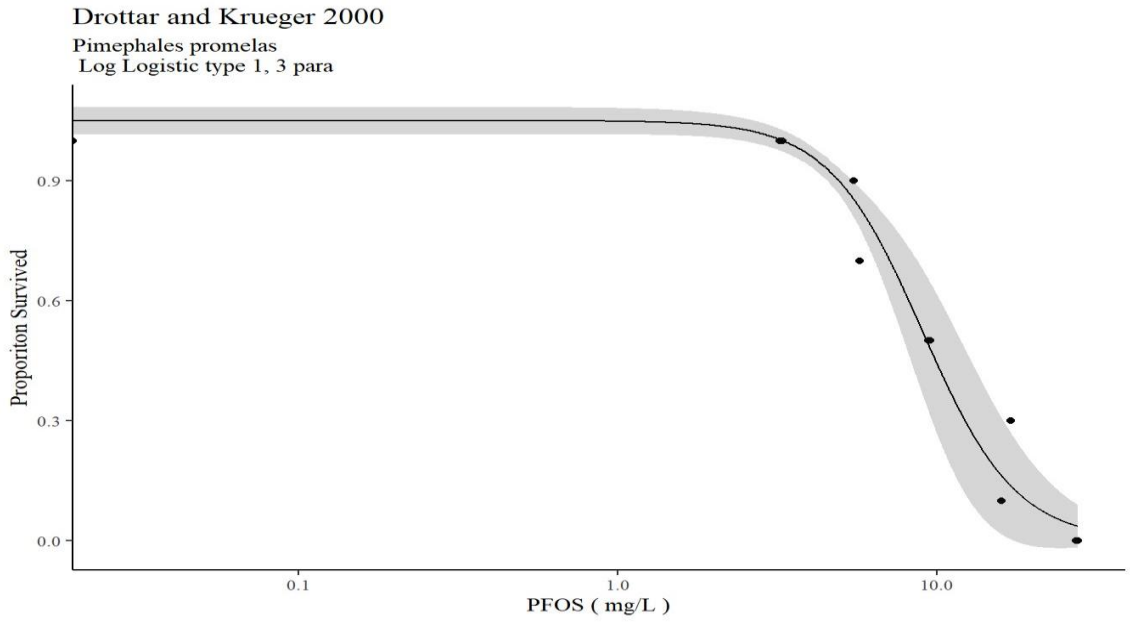


A.2.3.2 Drottar and Krueger (2000c) Concentration Response Curve – Pimephales (fathead minnow)

Publication: Drottar and Krueger (2000c)
Species: Fathead minnow, *Pimephales promelas*
Genus: *Pimephales*
EPA-Calculated LC_{50}: 9.020 (95% C.I. 7.146 - 10.89) mg/L
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	2.9683	0.4433	6.6965	$2.134\ e^{-11}$
d	1.0503	0.0174	60.4403	$< 2.2\ e^{-16}$
e	9.0196	0.8923	10.1084	$< 2.2\ e^{-16}$

Concentration-Response Model Fit:



A.2.4 Fourth Most Sensitive Freshwater Genus for Acute Toxicity: *Oncorhynchus* (trout)

Sharpe et al. (2010) evaluated the acute effects of perfluorooctane sulfonate (PFOS, potassium salt, CAS #2795-39-3, 98% purity) to *Oncorhynchus mykiss*, rainbow trout, via a 96-hour renewal measured exposure (renewal not stated in paper, but assumed based on other information provided, including the test Guideline protocol). Limited details about the test protocol were provided in the publication, but the authors noted they followed OECD Guideline 203, and did not identify any deviations from these test guidelines. Trout eggs were obtained

from Raven Trout Hatchery, transported immediately postfertilization to the University of Alberta aquatics facility, and kept in dechlorinated City of Edmonton water. The eggs were reared until hatching in Heath trays in a recirculating, temperature-controlled system at 10°C with a 12:12-hour light:dark photoperiod (the same conditions are assumed for the toxicity test). The rainbow trout used in the study were parr (2-3 g, the fourth stage of the salmon life cycle) at test initiation. The dilution water was dechlorinated City of Edmonton water, and dissolved oxygen content and temperature were monitored daily, but physico-chemical results were not reported. PFOS was dissolved in MeOH, and all vehicle controls received a volume of MeOH equal to that present in the highest PFOS dose of that experiment (final MeOH content 0.2% v/v). The concentration of PFOS in any experiment was always well below its reported solubility in water (≈500 mg/L). Trout toxicity tests were performed using food-grade 2 L plastic tanks with four fish per tank, and two tanks per dose. The EPA obtained clarification from the study authors regarding the experimental set-up pertaining to the biomass loading rate, which was 1 to 1.5 g/L (based on four fish weighing a total of 2 to 3 g per 2 L tank (personal communication with Greg Goss and Rainie Sharpe, March 2021). This biomass loading rate was nearly two-fold higher than that stated in OECD Guidelines of 0.8 g/L (OECD 1992). The trout were randomly assigned to doses defined as control (0 mg/L PFOS); vehicle control (0 mg/L PFOS, 0.2% MeOH v/v); and 0.78, 1.56, 3.12, 6.25, and 12.5 mg/L PFOS. The authors indicated that measured PFOS concentrations averaged 88% of nominal but did not indicate whether LC_{50}s were based on measured or nominal concentrations. Given the clarifications regarding the biomass loading, this study was considered for quantitative use in the derivation of the acute PFOS freshwater criterion. The author-reported 96-hour LC_{50} for the study of 2.5 mg/L (authors did not specify if this concentration was nominal or measured) was acceptable for quantitative

use and was among other toxicity values used for this species to calculate the SMAV/GMAV (see further details at the end of the study summaries in this section) that was utilized to derive the freshwater acute water column criterion.

Palmer et al. (2002a) evaluated the acute effects of PFOS (PFOS, potassium salt, identified as FC-95 obtained from 3M Company) to *Oncorhynchus mykiss*, rainbow trout, via a 96-hour static exposure with measured concentrations. The test organisms were obtained from Thomas Fish Company in Anderson, California and were reported as juveniles with a mean weight of 0.34 g and mean total length of 3.6 cm. All test organisms were from the same source and year class, and the length of the longest fish was no more than twice the length of the shortest. The fish were held for approximately five weeks prior to the initiation of the test. This acclimation was done in water from the same source and at the same temperature as the test. During the acclimatation period, no mortalities or signs of disease were observed. Test organisms were only fed a commercially-prepared diet (reported from Zeigler Brothers Inc.) during a 14-day holding period after which point fish were no longer fed through the acclimation period (at least 48 hours prior to the test) or during the test. The test water was obtained from a well located near the testing facility and was characterized as moderately-hard water. The target test temperature was 12 ± 1°C and a 16:8-hour light:dark photoperiod was maintained through the holding, acclimatation, and testing periods. Dissolved oxygen and pH measurements were made on water samples collected at test initiation followed by 24-hour intervals for each replicate test chamber of each treatment and control. Test chambers were 25-L polyethylene aquaria containing 15 L of test solution. At the initiation of the test, rainbow trout were indiscriminately moved from the acclimation tank and distributed two at a time to the test chambers until each contained ten fish. The resulting biomass loading rate was 0.23 g fish/L of

test water. A 40-L stock solution was prepared in dilution water at a concentration of 150 mg PFOS/L. Nominal concentrations were 3.1, 6.3, 13, 25, and 50 mg/L. Two replicates of each test solution were prepared at nominal concentrations by adding the appropriate volume of stock solution to dilution water in the test aquaria to achieve the final volume of 15 L. Measured test concentrations at the end of the test ranged from 97 to 100% of nominal with concentrations of 3.0, 6.3, 13, 25, and 50 mg/L. Results from this study were based on measured concentrations. Mortality and other signs of toxicity were observed daily. Trout in the control group appeared normal and healthy throughout the test period. Additionally, test organisms in the lowest treatment groups (3.0 and 6.3 mg/L) appeared healthy with no mortalities or other signs of toxicity. After 96-hours of exposure, mortality in the 13, 25, and 50 mg/L treatment groups was 20, 50, and 100%, respectively. The author-reported 96-hour LC_{50} for the study was 22 mg/L. This study was considered acceptable for quantitative use in the derivation of the acute PFOS freshwater water column criterion. The independently-calculated 96-hour LC_{50} value was 22.59 mg/L.

A.2.4.1 Sharpe et al. (2010) Concentration Response Curve - Oncorhynchus (trout)

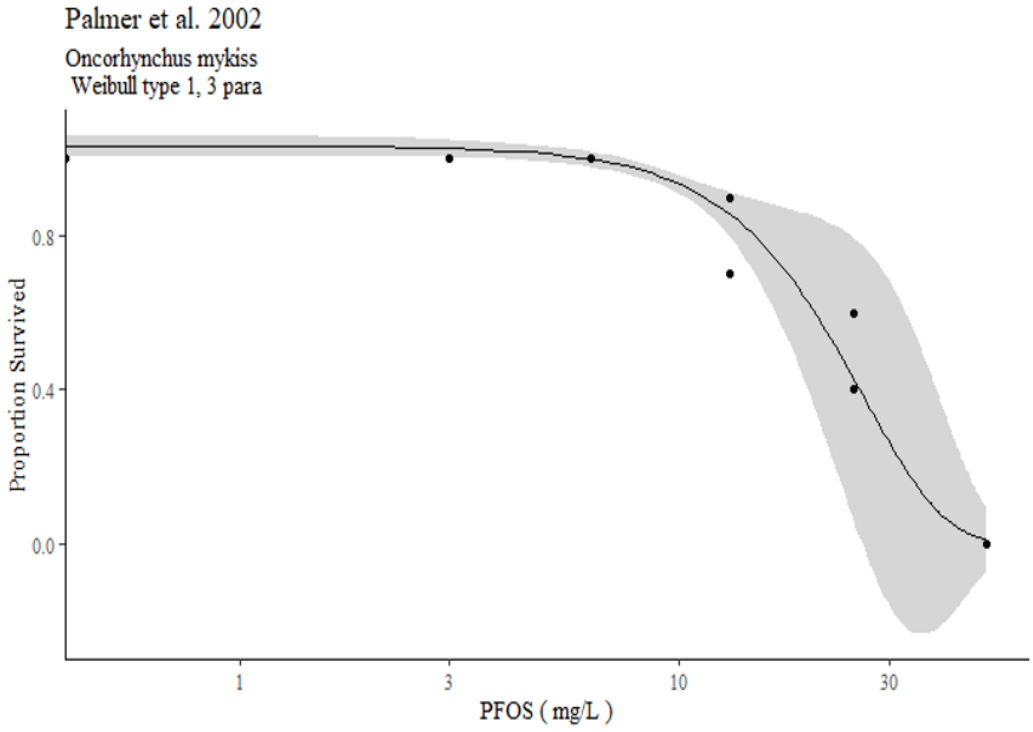
Publication: Sharpe et al. (2010)
Species: Rainbow trout, *Oncorhynchus mykiss*
Genus: *Oncorhynchus*
EPA-Calculated LC_{50}: Not calculable, concentration-response data not available

A.2.4.2 Palmer et al. (2002a) Concentration Response Curve - Oncorhynchus (trout)

Publication: Palmer et al. (2002a)
Species: Rainbow trout, *Oncorhynchus mykiss*
Genus: *Oncorhynchus*
EPA-Calculated LC_{50}: 22.59 (95% C.I. 14.53 – 30.65) mg/L
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	2.3775	0.5634	4.2189	$2.455\ e^{-5}$
d	1.0339	0.0140	73.6910	$< 2.2\ e^{-16}$
e	26.3557	5.7449	4.5877	$4.482\ e^{-6}$

Concentration-Response Model Fit:



A.2.5 Fifth Most Sensitive Freshwater Genus for Acute Toxicity: *Ligumia* (mussel)

Hazelton (2013); Hazelton et al. (2012) evaluated the acute effects of PFOS (acid form, > 98% purity) on two freshwater mussels*: Ligumia recta* and *Lampsilis siliquoidea.* The tests yielded the 4th and 7th most sensitive genera values (respectively) in the PFOS freshwater acute criterion dataset (the *L. siliquoidea* results are reported below). Acute toxicity was observed under either static or renewal conditions over a 24-hour period (< 24-hour old glochidia; static exposure) or a 96-hour period (4–6-week-old juveniles; renewal exposure). The tests followed the (ASTM 2006) test method. Dilution water was hard reconstituted water (total hardness typically 160-180 mg/L as $CaCO_3$). Photoperiod and light intensity were not reported. No details were provided regarding primary stock solution and test solution preparation. Experiments were conducted in 3.8 L glass jars of unspecified fill volume. The test employed three replicates of 150 glochidia or seven juvenile mussels each in six measured test concentrations plus a negative control (10 juveniles for the control treatment). Nominal concentrations were 0 (negative control), 0.005, 0.05, 0.5, 5, 50, and 500 mg/L; respective measured concentrations were < limit of quantitation (LOQ; specifics not provided), 0.0054, 0.0514, 0.456, 4.68, 47.2, and 490 mg/L. Recovery of PFOS standards ranged from 85.3-123% over all experiments. For all acute tests, alkalinity ranged from 97 to 110 mg $CaCO_3$/L with a mean of 104.4 mg $CaCO_3$/L; total hardness ranged from 132 to 162 mg $CaCO_3$/L with a mean of 149.6 mg $CaCO_3$/L; conductivity ranged from 514 to 643 µS/cm with a mean of 556.5 µs/cm; pH ranged from 8.05 to 8.56 with a mean of 8.46; and D.O. ranged from 8.16 to 9.46 mg/L with a mean of 8.62 mg/L (n = 12 for alkalinity and total hardness, n = 55 for all other parameters). Exposures were conducted in environmental chambers set at a temperature of 20°C (glochidia tests), or in dilution water maintained at 20°C (juvenile tests). Survival of mussels in the negative control was > 90% in all exposures. The 24-hour EC_{50} reported by the study authors for glochidia of *L. recta* was 13.5 mg/L (C.I. 5.7-31.8).

The 96-hour LC_{50} reported by the study authors for juvenile *L. recta* was 141.7 mg/L (C.I. 80.4-249.6). The 24-hour EC_{50} for *L. recta* glochidia represented an acute value acceptable for quantitative use. The juvenile life stage was less sensitive, such that its LC_{50} is not used quantitatively in the SMAV for the species. An independently-calculated toxicity value could not be calculated at this time given the lack of data presented in the paper. The study author reported values are currently used quantitatively to derive the freshwater acute water column criterion.

A.2.5.1 Hazelton et al. (2012) Concentration Response Curve – Ligumia (mussel)

Publication: Hazelton et al. (2012)
Species: Black sandshell, *Ligumia recta*
Genus: *Ligumia*
EPA-Calculated LC_{50}: Not calculable, concentration-response data not available

A.2.6 Sixth Most Sensitive Freshwater Genus for Acute Toxicity: *Neocaridina* (shrimp)

Li (2009) conducted three independent repeats of a 96-hour static test on PFOS (potassium salt, >98% purity) with the freshwater shrimp species, *Neocaridina denticulata* (a non-North American species). Test organisms were obtained from an unspecified local supplier and acclimated in the laboratory for at least seven days prior to the experiments. *N. denticulata* of unspecified age were used at test initiation. Dilution water was dechlorinated tap water. The photoperiod consisted of 12 hours of illumination at an unreported light intensity. A primary stock solution was prepared in dilution water. Exposure vessels were polypropylene beakers of unreported dimensions and 1 L fill volume. The test employed five replicates of six shrimp each in at least five test concentrations (the first repeated experiment had one additional PFOS treatment group at 10 mg/L compared to the other two experimental repeats) plus a negative control. Each treatment was tested three independent times. Nominal test concentrations were in the range of 5-200 mg/L PFOS. The test temperature was maintained at 25±2°C. Water quality parameters including pH, conductivity, and D.O. were reported as having been measured at the

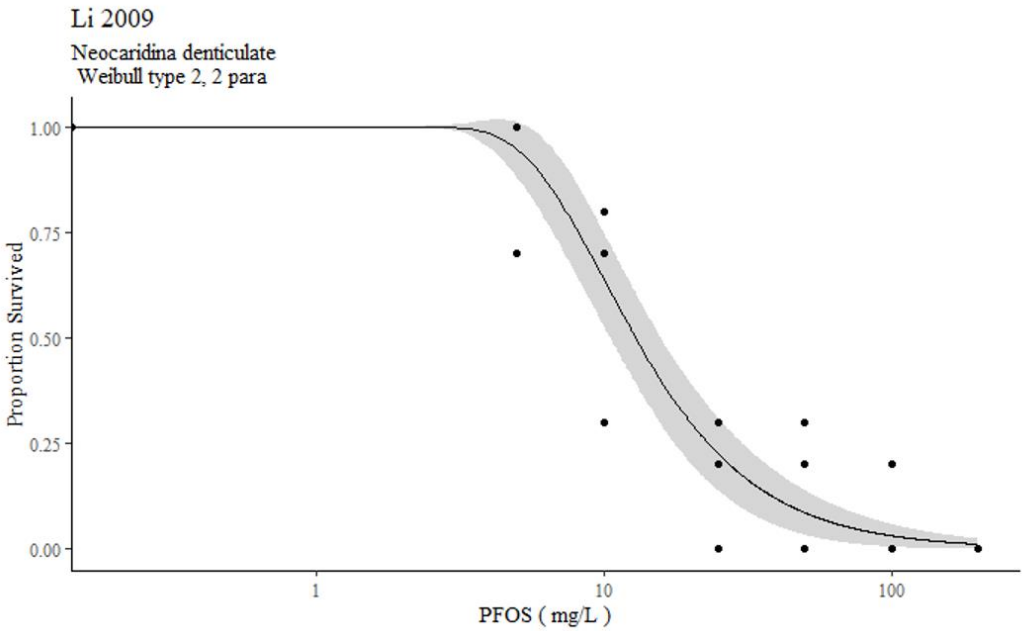
beginning and end of each test, but the information was not reported. Survival of negative control animals was 90%. The study reported 96-hour LC_{50} was 10 mg/L (C.I. 9-12). The toxicity test was acceptable for quantitative use. The independently-calculated LC_{50} values for the three independent experimental repeats were 12.91 (10.29 – 15.53), 28.55 (15.05 – 42.05), 10.32 (7.788 – 12.85) mg/L, respectively. These independently-calculated LC_{50} values were used to calculate the SMAV/GMAV value (as the geometric mean of the three LC_{50} values previously mentioned) of 15.61 mg/L and was used to derive the freshwater acute water column criterion.

A.2.6.1 Li (2009) Concentration Response Curve – Neocaridina (shrimp)

Publication: Li (2009)
Species: Japanese Swamp Shrimp, *Neocaridina denticulata*
Genus: *Neocaridina*
EPA-Calculated LC_{50}s: 12.91 (95% C.I. 10.29 – 15.53), 28.55 (95% C.I. 15.05 – 42.05), 10.32 (95% C.I. 7.788 – 12.85) mg/L
Concentration-Response Model Estimates:

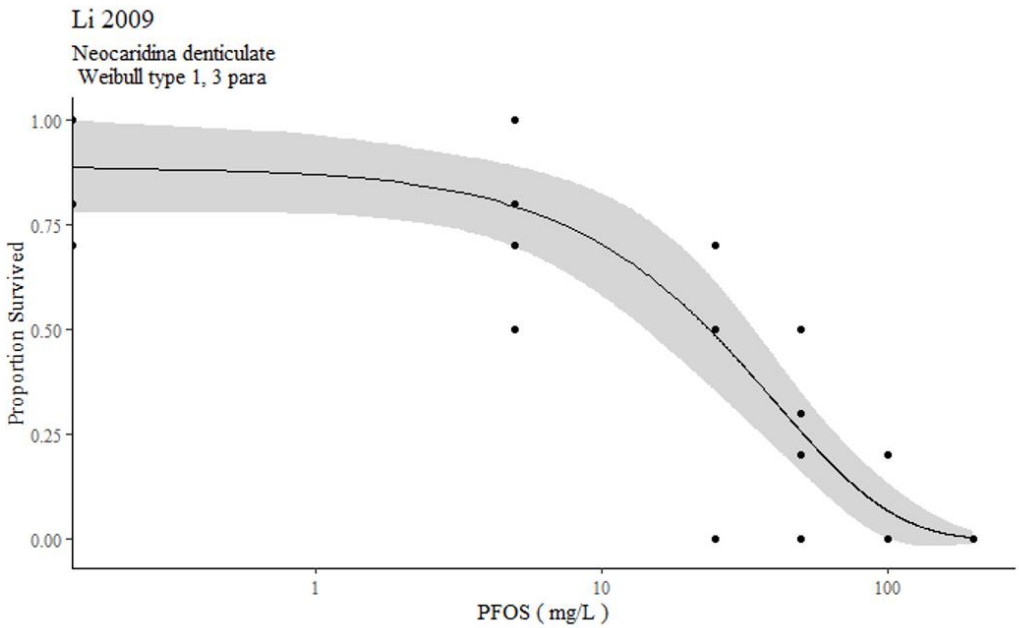
Parameter	Estimate	Std. Error	t-stat	p-value
b	-1.5141	0.1920	-7.8879	$3.091\ e^{-15}$
e	10.1360	1.0252	9.8865	$< 2.2\ e^{-16}$

Concentration-Response Model Fit: *In order of LC_{50}s listed immediately above*



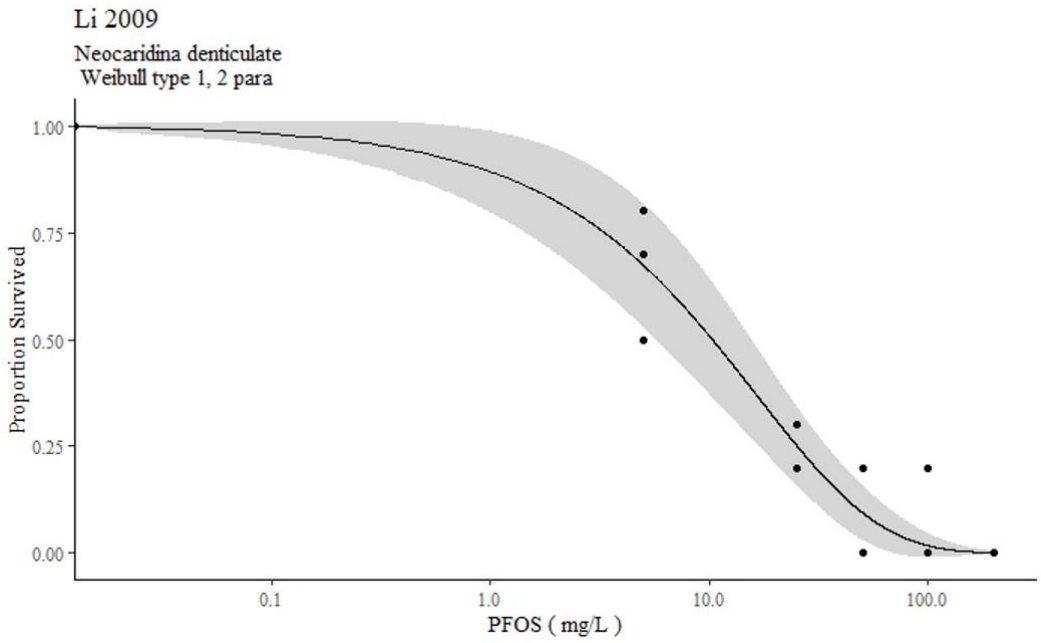
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.0404	0.2369	4.3919	$1.124\ e^{-5}$
d	0.8880	0.0571	15.5493	$< 2.2\ e^{-16}$
e	40.6105	7.5714	5.3637	$8.156\ e^{-8}$



Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	0.7749	0.1332	5.8195	5.903 e^{-9}
e	16.5563	3.3654	4.9196	8.672 e^{-7}



A.2.7 Seventh Most Sensitive Freshwater Genus for Acute Toxicity: *Xenopus* (frog)

Palmer and Krueger (2001), associated with Wildlife International, conducted three good laboratory practice (GLP) renewal definitive assays with the potassium salt of PFOS (86.9% purity) using the frog embryo teratogenesis assay - *Xenopus* (FETAX) with *Xenopus laevis.* A primary PFOS stock solution was prepared in FETAX solution at a concentration of 48 mg/L, and subsequently diluted with FETAX solution to prepare the six nominal test concentrations (1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg PFOS/L). Eggs were obtained from breeding colonies of *X. laevis* at the University of Maryland Wye Research and Education Center. Adults were held in flow-through polyethylene aquaria with 10 cm of dechlorinated tap water (23.5±0.5°C) and a maximum of 10 adults/chamber and photoperiod of 16:8-hours light:dark. They were bred in the dark following injection of human chorionic gonadotropin to

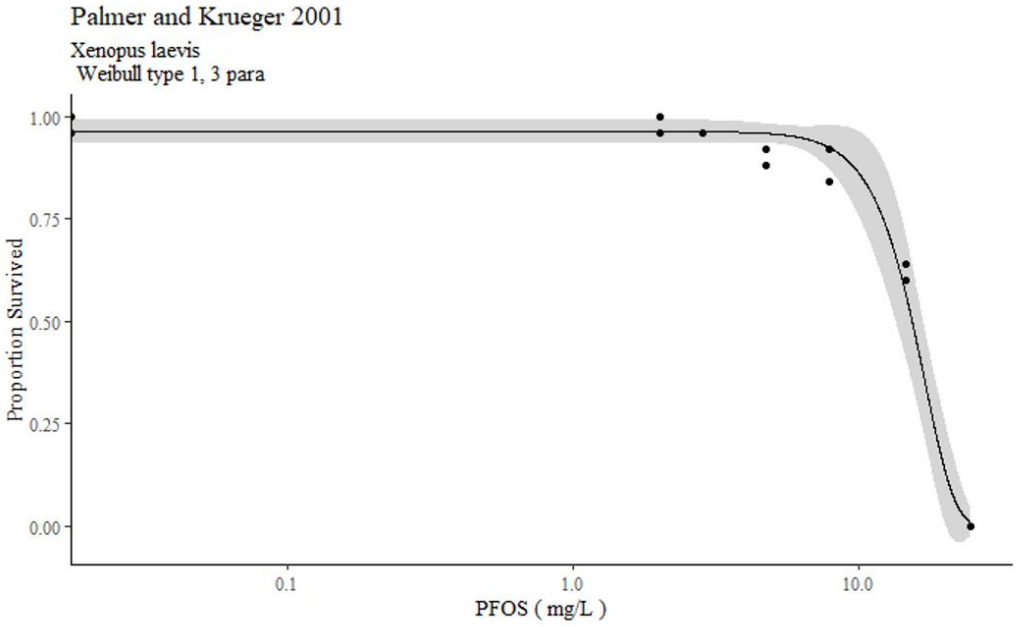
dorsal lymph sac of males and females. During each assay, *X. laevis* embryos (between NF stages 8-11) were exposed to PFOS for 96 hours. Two replicate test chambers were maintained in each treatment group and four replicates were maintained in each control group from the three separate assays. Each test chamber contained 25 embryos for a total of 50 embryos per treatment group and 100 embryos per control group. Tests were conducted at 24°C, pH of 7.26-7.30, estimated total hardness of 75 mg/L as $CaCO_3$, D.O. of 7.8-8.1 mg/L and a 12:12-hour light:dark photoperiod (60-85 foot candles). PFOS concentrations were measured at the initiation and termination of all three assays. The authors reported 96-hour LC_{50} values for mortality of 13.8, 17.6 and 15.3 mg/L PFOS, teratogenesis EC_{50}s of 12.1, 17.6 and 16.8 mg/L PFOS, and minimum concentration to inhibit growth values (effectively a LOEC) of >14.7, 7.97 and 8.26 mg/L for the same three tests, respectively. Independently-calculated 96-hour LC_{50} values for mortality were 15.53 (13.86 – 17.21), 18.04 (15.33 – 20.74), and 14.60 (12.65 – 16.55) mg/L for the three assays, respectively. No additional quantitative, acute toxicity data were available for this species. Therefore, these independently-calculated LC_{50} values were used to calculate the SMAV/GMAV value (as the geometric mean of the three LC_{50} values) of 15.99 mg/L that was used to derive the freshwater acute water column criterion.

A.2.7.1 Palmer and Krueger (2001) Concentration Response Curve – Xenopus (frog)

Publication: Palmer and Krueger (2001)
Species: Frog, *Xenopus laevis*
Genus: *Xenopus*
EPA-Calculated LC_{50}: 15.53 (95% C.I. 13.86 – 17.21), 18.04 (95% C.I. 15.33 – 20.74), 14.60 (95% C.I. 12.65 – 16.55) mg/L
Concentration-Response Model Estimates:

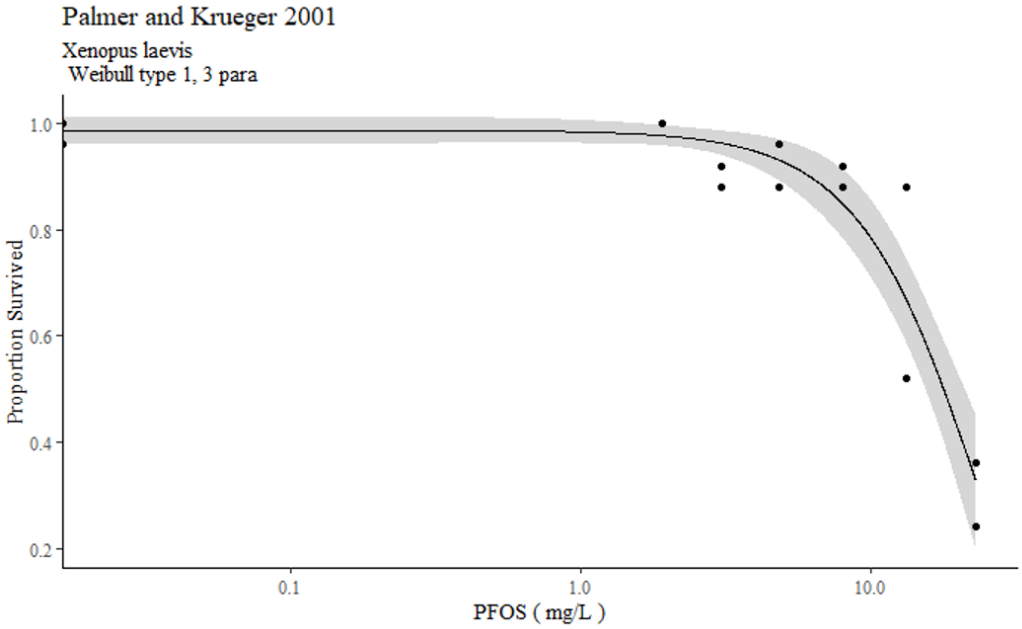
Parameter	Estimate	Std. Error	t-stat	p-value
b	4.1306	1.0464	3.9475	$7.897\ e^{-5}$
d	0.9633	0.0149	64.7242	$< 2.2\ e^{-16}$
e	16.9770	0.7991	21.2452	$< 2.2\ e^{-16}$

Concentration-Response Model Fit: *In order of LC_{50}s listed immediately above*



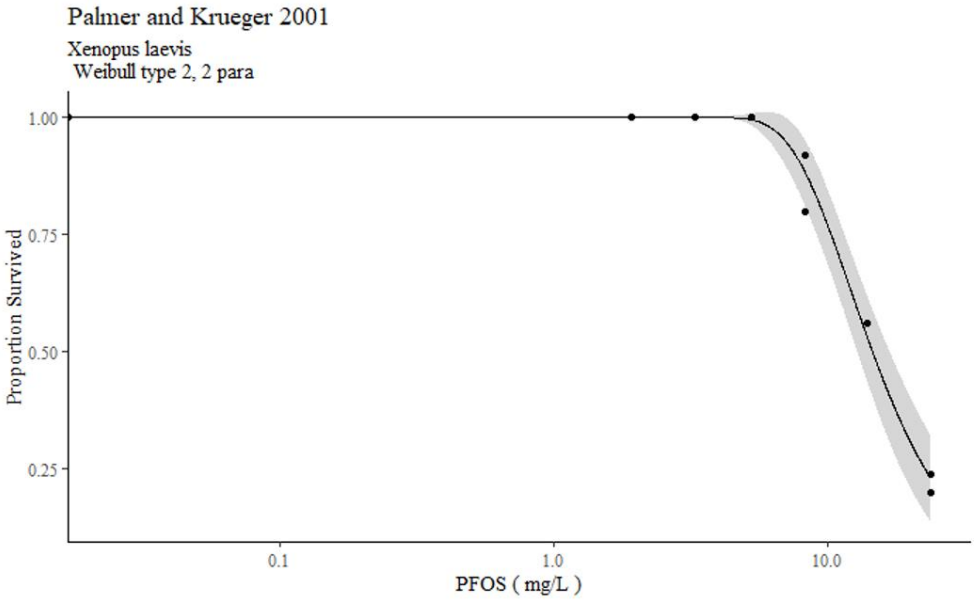
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.8800	0.3458	5.4366	$5.431\ e^{-8}$
d	0.9868	0.0127	77.7694	$< 2.2\ e^{-16}$
e	21.9190	1.9259	11.3812	$< 2.2\ e^{-16}$



Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	-1.9934	0.2667	-7.4757	$7.681\ e^{-14}$
e	12.1461	0.7197	16.8763	$< 2.2\ e^{-16}$



A.2.8 Eighth Most Sensitive Freshwater Genus for Acute Toxicity: *Lampsilis* (mussel)

Hazelton (2013); Hazelton et al. (2012) evaluated the acute effects of PFOS (acid form, > 98% purity) on two freshwater mussels, as noted above*: Lampsilis siliquoidea* and *Ligumia recta.* The *L. siliquoidea* studies yielded the 7th most sensitive genus value in the PFOS freshwater acute criterion dataset. Hazelton et al.'s experimental design and study conditions for *L. siliquoidea* were reported above under the description of the fourth most sensitive taxa, *Ligumia*. The 24-hour EC_{50} reported by the study authors for glochidia of *L. siliquoidea* was 16.5 mg/L (C.I. 8.0-33.9). The 96-hour LC_{50} reported by the study authors for juvenile *L. siliquoidea* was 158.1 mg/L (C.I. not calculable). The 24-hour EC_{50} for *L. siliquoidea* glochidia represented an acute value acceptable for quantitative use for the mussel species. Because the juvenile life stage was less sensitive, only the glochidia LC_{50} was used to calculate the SMAV. An

independently-calculated toxicity value could not be calculated at this time given the data presented in the paper. The study author reported value was used quantitatively to derive the freshwater acute water column criterion.

A.2.9 Ninth most Sensitive Freshwater Genus for Acute Toxicity: *Hyla* (frog)

Tornabene et al. (2021) conducted acute toxicity tests with the gray treefrog, *Hyla versicolor*, and PFOS (purchased from Sigma Aldrich, Catalog # 77282-10G; purity not provided). The acute tests followed standard 96-hour acute toxicity test guidance (ASTM 2017; U.S. EPA 2002). The frog was collected from the field in the wetlands of Indiana near the campus of Purdue University. Collected egg masses were raised outdoors in 200 L polyethylene tanks filled with well water. Experiments began when frogs reached Gosner stage 26, defined as when larvae are free swimming and feeding. An additional acute test with Gosner stage (GS) 40 was run to determine if toxicity varied between life stages. Before test initiation larvae were acclimated to test conditions (21°C and 12:12-hour light:dark photoperiod) for 24 hours. A stock solution of PFOS (500 mg/L) was made in UV-filtered well water and diluted with well water to reach test concentrations (ranged from 0 - 500 mg/L PFOS). Test concentrations were not measured in test solutions, based on previous research that demonstrated limited degradation under similar conditions. Larva were transferred individually to 250 mL plastic cups with 200 mL of test solutions and were not fed during the exposure period. The number of replicates varied by life stage and treatment; 10 replicates for each treatment for GS 26 larva, and five to six replicates for each treatment for GS 40 frogs. No mortality occurred in the controls of the GS 26 test and two of the six frogs died in the controls of the GS 40 test. The author reported 96-hour LC_{50}s were 79 and 24 mg/L PFOS for GS 26 and 40, respectively. The independently-calculated 96-hr LC_{50} values were 78.33 and 19.88 mg/L and are acceptable for quantitative use.

Given that GS 40 frogs appear to be a more sensitive life-stage the LC_{50} of 19.88 (13.80 – 25.95) mg/L was utilized in the derivation of the freshwater acute water column criterion.

A.2.10 Tenth Most Sensitive Freshwater Genus for Acute Toxicity: *Dugesia* (planarian)

Li (2008) conducted three independent repeats of a 96-hour static, unmeasured acute toxicity test on the potassium salt of PFOS (CAS # 2795-39-3, > 98% purity) with the planarian, *Dugesia japonica* (a non-North American species). The test organisms were originally collected from Nan-shi stream located in Wu-lai, Taipei County, Taiwan in 2004 and maintained in the laboratory in dechlorinated tap water. The planarians had a body length of 0.9 ±0.1 cm at test initiation. Dilution water was dechlorinated tap water. The photoperiod consisted of 12 hours of illumination at an unreported intensity. A primary stock solution was prepared in dilution water. Exposure vessels were polypropylene beakers of unreported dimensions and 50 mL fill volume. The test employed five replicates of five planarians each in at least five test concentrations plus a negative control. Nominal test concentrations were in the range of 10-200 mg/L PFOS. The test temperature was maintained at 25 ±1°C. No other water quality parameters were reported for test solutions. Survival of negative control animals was not reported. The author-reported 96-hour LC_{50} was 17 mg/L (C.I. 16-18). The toxicity value could not be independently calculated at this time given the level of data that was presented in the paper. The author-reported value was used quantitatively to derive the freshwater acute water column criterion.

Li (2009) conducted three independent repeats of a second 96-hour static, unmeasured acute test of PFOS (potassium salt, > 98% purity) with *Dugesia japonica.* The tested individuals were also originally collected from Nan-shi stream located in Wu-lai, Taipei County, Taiwan in 2004 and maintained in the laboratory in dechlorinated tap water. The planarians had a body length of 0.9±0.1 cm at test initiation. Dilution water was dechlorinated tap water. The photoperiod consisted of 12 hours of illumination at an unreported intensity. A primary stock

solution was prepared in dilution water. Exposure vessels were polypropylene beakers of unreported dimensions and 50 mL fill volume. Each of the three independent repeats employed three replicates of 10 planarians each in at least five test concentrations plus a negative control. Nominal test concentrations were in the range of 5-200 mg/L PFOS. The test temperature was maintained at 25±1°C. Water quality parameters including pH, conductivity, and D.O. were reported as having been measured at the beginning and end of each test, but the information is not reported. Survival of negative control animals was also not reported. The author-reported 96-hour LC_{50} was 23 mg/L (C.I. 20-25). An independently-calculated LC_{50} could not be estimated for the first and second independent tests (as EPA was unable to fit a model with significant parameters), but was estimated for the third independent test as 22.68 (18.27 – 27.10) mg/L. This acute value was acceptable for quantitative use and was used to derive the freshwater acute water column criterion.

Yuan et al. (2014) also conducted a 96-hour unmeasured acute test on PFOS (potassium salt, > 99% purity) with *Dugesia japonica*, under daily renewal conditions. *D. japonica* used for testing were originally collected from a fountain in Quan HetouBoshan, China, and cultivated in the laboratory for an unspecified time period before use. The planarians had a body length of 10-12 mm at test initiation. Dilution water was aerated tap water. No details were provided regarding photoperiod or light intensity. A primary stock solution was prepared by dissolving the salt in DMSO. The control and exposed planarians received 0.005% DMSO (v/v). Exposure vessels were beakers of unreported material type and dimensions with 50 mL fill volume. The test employed three replicates of 10 planarians each in six test concentrations plus a solvent control. Nominal test concentrations were 0 (solvent control), 10, 30, 35, 37.5, 40 and 45 mg/L PFOS. The test temperature was reported as 20°C. No other water quality parameters were

reported. Survival of solvent control animals was not reported. The author-reported 96-hour LC_{50} was 29.46 mg/L (C.I. 25.80 - 33.12). An independently-calculated toxicity value could not be calculated at this time given the level of data that was presented in the paper. The author-reported value was used quantitatively to derive the freshwater acute water column criterion.

The noted toxicity values provided in each study summary above (17.00, 22.68, and 29.46 mg/L), comprising of both author-reported and independently-calculated LC_{50} values, were used to calculate the SMAV/GMAV value (as the geometric mean of the three LC_{50} values previously mentioned) of 22.48 mg/L, which was used to derive the freshwater acute aquatic life criterion.

A.2.11 Eleventh Most Sensitive Freshwater Genus for Acute Toxicity: *Danio* (zebrafish)

Ye et al. (2007) evaluated the acute effects of perfluorooctanesulfonic acid (PFOS, purity not reported) to *Danio rerio* via a 96-hour static-renewal unmeasured exposure. The PFOS stock solution of 480 mg/L was maintained at a pH of 8.2 with phosphate buffer and test substances were agitated in the reconstituted water by ultrasonification. The solutions were stored at 4 ± 1°C. Each test solution, at the selected concentration, was prepared by diluting the stock solution with reconstituted water. No added solvents were used. The fish (AB strain) used in this experiment were obtained from the School of Life Sciences at Fandan University, Shanghai, China. Breeding fish (1.5 years old) were fed live brine shrimp twice daily and kept with a 14:10-hour light:dark period in aquaria containing aerated natural water. The pH ranged from 7 to 8.5 and water temperature was maintained at 26 ± 1°C. Embryos were obtained from spawning adults, usually 5 male and 3 female. For each toxicant concentration, 48 embryos were randomly distributed into each well of 24-well polystyrene multi-well plates, with four eggs per well. Each well was filled with 2.5 mL test solution which were completely renewed daily by transferring embryos into newly cleaned wells. Nominal exposure concentrations were 0

(control), 10, 85, 100, 110, 160, 200 and 240 mg/L PFOS. The multi-well plates were kept at 26 ± 1°C, with a photoperiod of 16:8-hour light:dark. The observations of embryos were made at distinct stages, which represent important steps of zebrafish development. The author-reported 96-hour LC_{50} was 71 mg/L PFOS. The EPA was unable to independently calculate a 96-hour LC_{50} value based on the level of data provided by the study authors in the paper. Therefore, the author-reported LC_{50} value was used quantitatively to derive the freshwater acute water column criterion.

Hagenaars et al. (2011b) exposed *D. rerio* embryos to the potassium salt of PFOS (CAS # 2795-39-3, purity ≥97%) under static unmeasured conditions for 96 hours. The PFOS was dissolved in medium-hard reconstituted laboratory water, which was aerated and kept at 26°C until use (no solvent). Adult wild-type zebrafish (breeding stock) were obtained from a commercial supplier (Aqua hobby, Heist-op-den-berg, Belgium) and kept in aerated and biologically filtered medium-hard reconstituted freshwater. Four males and four females were used for egg production. Fertilized eggs were collected in egg traps within 30 minutes of spawning. Eggs were transferred to the test solutions (nominal PFOS concentrations of 0.1, 0.5, 1, 5, 10 mg/L in the definitive ELS test and 1, 5, 10, 25, 50 and 100 mg/L in the 96-hour range-finding test) within 60 minutes after spawning. Eggs with anomalies or damaged membranes were discarded and fertilized eggs were separated from the non-fertilized eggs using a stereomicroscope. Twenty normally shaped fertilized eggs per exposure concentration were divided over a 24-well plastic plate and each egg was placed individually in 2 mL of the test solution. The remaining four wells were filled with clean water and used for the control eggs. Two replicate plates were used for each exposure concentration resulting in 40 embryos per treatment at the beginning of the experiment. The 24-well plates were covered with a self-

adhesive foil, placed in an incubation chamber at 26 ± 0.3°C and subjected to a 14:10-hour light:dark cycle. A test was considered valid if more than 90% of the controls successfully hatched and showed neither sublethal nor lethal effects. The authors reported a 96-hour LC_{50} of 58.47 mg/L PFOS, based on the results of the range-finding test. The author-reported value was used quantitatively to derive the freshwater acute water column criterion.

Sharpe et al. (2010) examined the toxicity of PFOS-K (potassium salt, CAS # 2795-39-3, 98% purity) and bioaccumulation of PFOS and its isomers on *Danio rerio* through three different tests, a 96-hour renewal toxicity test on adults, a 48-hour renewal toxicity test on embryos, and a chronic exposure test that evaluated maternal transfer and fecundity of PFOS isomers. The 96-hour test is described in this appendix, as these results were used quantitatively to derive the freshwater acute water column criterion. The 48-hour and chronic toxicity tests were used qualitatively (Appendix G). The authors provided little detail about the test protocol, other than following OECD Guideline 203. Adult zebrafish were obtained from a local pet store. They were acclimated and held in 70 L glass aquaria in an environmental chamber set to 26°C under a 14:10-hour light:dark photoperiod for six to 10 months prior to use in experiments. Conditioned zebrafish water (ZF water) was obtained from the Biological Sciences Zebrafish Facility at the University of Alberta, where an automated reverse osmosis system regulated both the salinity and hardness (160 mg/L total hardness and 20 mg/L calcium carbonate hardness) of the water. A stock solution of 25 mg/ml PFOS in MeOH was used for dosing in all experiments. All vehicle controls received a volume of MeOH equal to that present in the highest PFOS treatment of that experiment (final MeOH content 0.65% v/v). The concentration of PFOS in any experiment was always well below its reported solubility in water (approximately 500 mg/L). Zebrafish toxicity tests were performed using food grade 2 L plastic tanks with four fish per

tank, and two tanks per concentration. Fish were randomly assigned to nominal concentrations defined as control (0 mg/L PFOS); vehicle control (0 mg/L PFOS, 0.4% MeOH v/v); and 6.25, 12.5, 2, 50 and 100 mg/L PFOS. Authors indicated that measured PFOS concentrations averaged 88% of nominal but did not indicate whether the LC_{50} was measured or nominal. The adult 96-hour acute test followed OECD 203 protocol and was acceptable for quantitative use. The author-reported LC_{50} was 22.2 ± 4.6 mg/L for PFOS. The author-reported value was used quantitatively to derive the freshwater acute water column criterion.

Evaluated the acute effects of perfluorooctane sulfonate, potassium salt (PFOS-K, CAS# 2795-39-3 purchased from Wellington Laboratories Inc., Ontario, Canada) on zebrafish (*Danio rerio*) during a 96-hour unmeasured, static-renewal study. Zebrafish were purchased from a local market at approximately three months in age and 2.2 cm in length. Fish were allowed to acclimate for seven days and were fed three times per week until 24 hours before the test was started. Water used for the testing was aerated for 48 hours before testing began, and testing followed OECD Guideline 203. Observed exposure water characteristics were total hardness of 180-220 mg/L as $CaCO_3$, temperature of 23 ± 1ºC, D.O. of 7.0 – 8.6 mg/L and a photoperiod of 12:12-hours light:dark. Each 2-L beaker was filled with 1,500 mL of test solution at nominal concentrations of 0 (control), 2.87, 5, 8.7, 15.14, 26.34, 45.83 and 79.74 mg/L PFOS. There were three replicates per concentration, and seven fish per beaker. Test solutions were renewed at 48 hours. The author-reported 96-hour LC_{50} was 17.0 mg/L PFOS based on a sigmoidal three-parameter regression. The EPA was unable to independently calculate a 96-hour LC_{50} value based on the level data provided in the paper. Therefore, the author-reported LC_{50} value of 17.0 mg/L PFOS was used quantitatively to derive the freshwater acute water column criterion.

Li et al. (2015) evaluated the acute effects of PFOS (CAS # 2795-39-3, 98% purity) to *Danio rerio* via a 96-hour static unmeasured exposure. Solutions for waterborne exposure were formulated with medium used to rear embryos (reconstituted laboratory water). Adult, wild-type zebrafish were obtained from the Institute of Hydrobiology, at the Chinese Academy of Sciences (Wuhan, China), and kept in treated tap water at 26 – 29°C. Fish were reared with a female/male ratio of 1:2 under 14:10-hour light:dark photoperiod, with 1/3 of the water exchanged daily. Spawning and fertilization took place within 30 minutes after the lights were turned on in the morning, with fertilized embryos collected and cleaned with embryo rearing water. Immediately after fertilization, embryos were examined, and damaged or unfertilized embryos were discarded. Zebrafish embryos were exposed in 24-well cell culture plates (assume plastic) to a series of PFOS concentrations (6.25, 12.5, 25.0, 50.0, 100.0 and 200.0 mg/L). Twenty, normally shaped, fertilized embryos were assigned to each treatment (three replicates) and 2 mL of test solution per well; the four remaining wells were assigned with control embryos. Embryos were exposed in an incubator at 28.5°C, pH of 8.3 and a 14:10-hour light:dark photoperiod. Toxicological endpoints included whether embryos were clear or opaque, exhibited edema at 4, 8, 24, 48, 72, or 96 hpf, or developed structural malformations at 72 or 96 hpf until hatching. Coagulated embryos before hatching are opaque, milky white, and appear dark under the microscope. Coagulation of embryos was recorded and used for the calculation of an LC_{50} value. The author reported 96-hour LC_{50} was 68.0 mg/L PFOS. The independently-calculated LC_{50} value was 71.12 (56.82 – 85.42) mg/L PFOS. This toxicity value is acceptable for quantitative use and was used to derive the freshwater acute water column criterion.

Du et al. (2017); Du et al. (2016b) exposed *Danio rerio* to heptadecafluorooctanesulfonic acid (PFOS, potassium salt, CAS# 2795-39-3, 98% purity) using

static unmeasured procedures. Although the study focused on the protective effects of zinc nanoparticles (ZnO-NPs) on PFOS toxicity (development and damage to DNA), data were also reported for PFOS-only exposures. Adult AB strain zebrafish were purchased from State Key Laboratory of Freshwater Ecology and Biotechnology, Chinese Academy of Sciences (Wuhan, China). Fish were maintained and tested at 28°C under a 14:10-hour light:dark cycle. Male and female fish were paired in spawning boxes overnight in rearing water and spawning was completed at the beginning of the light cycle. Eggs were collected from the spawn traps and transferred to clean rearing water prior to testing. The embryos were exposed to PFOS (1, 2, 4, 8 and 16 mg/L in a preliminary test to determine the LC_{50}, and at 0.4, 0.8 and 1.6 mg/L in a later test with ZnO-NPs solutions to evaluate mortality (at 96 hours), body length (at 96 hours), hatch rate (at 72 hours), heart rate (at 48 hours), and malformation rate (at 96 hours). Embryos were kept in 24-well multi-plates at two embryos/well, in which 20 wells contained 2 mL reconstituted water test solution and four wells contained 2 mL of culture medium as the control. Each plate contained 40 embryos for exposure testing and eight embryos as internal water controls. For each concentration and water control, three 24-well plates (replicates) were included. The study authors reported a 96-hour LC_{50} of 3.502 mg/L for PFOS. The EPA was unable to independently calculate a 96-hour LC_{50} value based on the level data provided in the paper. Therefore, the author-reported LC_{50} value of 3.502 mg/L PFOS was used quantitatively to derive the freshwater acute water column criterion.

Stengel et al. (2017b) exposed 1 hpf *Danio rerio* embryos to PFOS for 96 hours using renewal unmeasured procedures as specified in (OECD 2013) guidelines. PFOS stock and exposure solutions were prepared in reconstituted laboratory water. All adult zebrafish used for breeding were wild-type descendants of the “Westaquarium” strain and obtained from the

Aquatic Ecology and Toxicology breeding facilities at the University of Heidelberg. Details of zebrafish maintenance, egg production and embryo rearing were as described previously (Kimmel et al. 1995; Kimmel et al. 1988; Nagel 2002; Spence et al. 2006; Wixon 2000) but included updates for the purpose of the zebrafish embryo toxicity test by Lammer et al. (2009). Embryos no older than 1 hpf were exposed in glass vessels, which had been preincubated (saturated) for at least 24 hours, to a series of PFOS dilutions (0, 3.125, 6.25, 12.5, 25, 50 mg/L). After confirmation of fertilization success, embryos were individually transferred to the wells of 24-well plates, which had been pre-incubated with 2 mL of the test solutions per well for 24 hours prior to the test start and kept in an incubator at 26.0 ±1.0°C under a 14:10-hour light:dark regime. In order to prevent evaporation or cross-contamination between the wells, the plates were sealed with self-adhesive foil. Embryo tests were classified as valid if the mortality in the negative control was ≤ 10%, and if the positive control (3,4-dichloroaniline) showed mortalities between 20 and 80%. All fish embryo tests were run in three independent replicates. Both lethal and sublethal effects were used for the determination of EC values. The author-reported 96-hour LC_{50} and EC_{50} (combination of lethal and sublethal effects) values were 34.2 and 21.4 mg/L PFOS, respectively. The independently-calculated LC_{50} was 38.82 (36.67 – 40.98) mg/L PFOS. The independently-calculated LC_{50} was considered quantitative and used to derive the freshwater acute water column criterion.

Nilén et al. (2022) evaluated the acute effects of perfluorooctanesulfonate, potassium salt (PFOS-K, CAS No. 2795-39-3, ≥ 98% purity, purchased from Sigma-Aldrich, Stockholm, Sweden) on zebrafish (*Danio rerio*) during a 96-hour measured, static-renewal test. PFOS stock solutions (464 mM) were prepared in DMSO. Adult zebrafish (AB strain) were purchased from Karolinska Institute (Stockholm, Sweden). Fish were kept at a 14:10-hour light:dark cycle in a

recirculating system. Fish embryo toxicity tests were performed following the OECD Technical Guideline No. 236 (OECD 2013). Prior to testing, 96-well plates were pre-incubated for 24 hours with the chemical solutions to limit sorption to the well plates. On the day of exposure, five different exposure concentrations were prepared in glass beakers through a serial dilution (1:2) using ISO water. The DMSO content was adjusted to 0.09% (v/v) in all solutions and all tests included three control groups: negative control (ISO-water), solvent control (0.09 % DMSO in ISO-water) and positive control (4 mg/L DCA). Test concentrations were selected based on previous studies about lethality and sub-lethality. One embryo (64-cell stage) with 250 µL of test solution was added to each well on a 96-well plate. For each concentration, 24 embryos were used, and each test contained a total of 192 embryos. The plates were covered with self-adhesive film and incubated at 26 ± 1°C in a 14:10-hour light:dark cycle. An experiment was considered valid by the study authors if the mortality in the negative control and the solvent control was less than 10%. In addition, the mortality of the positive control had to be ≥ 30%. Each experiment was repeated three to five times representing independent replicates and the embryos were exposed for 96 hpf. The exposure solutions were sampled before the start and at the end of the exposure period. Nominal exposure concentrations were 0 (control), 0 (solvent control), 7, 14, 28, 56 and 111.5 µM PFOS-K. Equivalent PFOS concentrations were 3.768, 7.535, 15.07, 30.14 and 60.01 mg/L PFOS-K, respectively, based on a molecular weight of 538.22 g/mol PFOS-K. Measured concentrations were between 1.3 and 23% of nominal at 96 hours. The author-reported 96-hour LC_{50} was 44.57 µM PFOS-K, or 23.99 mg/L PFOS-K (based on a molecular weight of 538.22 g/mol; 44.57 x 538.22 ÷ 1,000). The EPA was unable to independently calculate a 96-hour LC_{50} value based on the level of data provided in the paper by the study authors. Therefore,

the author-reported LC_{50} value of 23.99 mg/L PFOS was used quantitatively to derive the freshwater acute water column criterion.

A.2.12 Twelfth Most Sensitive Freshwater Genus for Acute Toxicity: *Daphnia* (cladoceran)

Logeshwaran et al. (2021) conducted acute and chronic toxicity tests with the cladoceran, *Daphnia carinata*, and PFOS-K (perfluorooctanesulfonate potassium salt, ≥ 98% purity, purchased from Sigma-Aldrich Australia). In-house cultures of daphnids were maintained in 2 L glass bottles with 30% natural spring water in deionized water, 21℃ and a 16:8-hour light:dark photoperiod. The acute test protocol followed OECD (2000) with slight modifications. A PFOS stock solution (20 mg/mL) was prepared in dimethylformamide and diluted with deionized water to achieve a concentration of 200 mg/L PFOS. Cladoceran culture medium was used to prepare the PFOS stock and test solutions. Ten daphnids (6-12 hours old) were transferred to polypropylene containers containing one of 14 nominal test concentrations (0, 0.5, 1, 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg/L PFOS). Each test treatment was replicated three times and held under the same conditions as culturing. At test termination (48 hours) immobility was determined after 15 seconds of gentle stirring. No mortality occurred in the controls. The author-reported 48-hour EC_{50} was 8.8 mg/L PFOS. The independently-calculated 48-hour EC_{50} value was 11.56 (10.06 – 13.07) mg/L and is acceptable for quantitative use in the derivation of the freshwater acute water column criterion.

Drottar and Krueger (2000g) reported the results of a 48-hour static, measured acute toxicity test on PFOS (potassium salt, CAS # 2795-39-3, 90.49% purity) with *Daphnia magna*. The GLP test was conducted at Wildlife International, Ltd. In Easton, MD in February, 1999. The test followed OECD (2004); (U.S. EPA 1996d). The test organisms were less than 24 hours old at test initiation. Dilution water was 0.45 µm filtered well water [hardness: 132 (128-136) mg/L as $CaCO_3$; alkalinity: 178 (176-178) mg/L as $CaCO_3$; pH: 8.3 (8.2-8.3); TOC: < 1.0 mg/L;

conductivity: 313 (310-315) µmhos/cm]. Photoperiod was 16:8-hours light:dark with a 30 minute transition period. Light was provided at an intensity of approximately 359 lux. A primary stock solution was prepared in dilution water at 91 mg/L. It was mixed for ~19.5 hours prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the four additional test concentrations. Exposure vessels were 250 mL plastic beakers containing 240 mL of test solution. The test employed two replicates of 10 daphnids each in five measured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 12, 20, 33, 55, 91 mg/L. Mean measured concentrations were <LOQ (4.58 mg/L), 11, 20, 33, 56 and 91 mg/L. Analyses of test solutions were performed at Wildlife International Ltd. Using HPLC/MS. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 96.2%. Samples collected at test initiation had measured values from 85.5 to 112% of nominal. Measured values for samples taken at 24 hours ranged from 92.2 to 115% of nominal, and at 48 hours from 91.6 to 106% of nominal. Dissolved oxygen in control and highest test concentration (91 mg/L) ranged from 8.6-8.9 mg/L and 8.6-9.1 mg/L; pH ranged from 8.2-8.5 and 8.5-8.6, respectively. Test temperature ranged from 19.5-20.2°C and 19.3-20.1°C, respectively. At 24 hours, daphnids in the negative control, and the 11, 20 and 33 mg/L treatments appeared healthy and normal with no mortality, immobility, or overt clinical signs of toxicity. However, five percent mortality was observed at 48 hours in the negative control. The author-reported 48-hour EC_{50} was 61 mg/L (C.I. 33-91). The independently-calculated toxicity value was 58.51 (53.59 – 63.43) mg/L and was used quantitatively to derive the freshwater acute water column criterion.

Boudreau (2002); Boudreau et al. (2003a) performed a 48-hour static test on PFOS (potassium salt, CAS # 2795-39-3, 95% purity) with *Daphnia magna* and *Daphnia pulicaria* as

part of a Master's thesis at the University of Guelph, Ontario, Canada. The results were subsequently published in the open literature (Boudreau et al. 2003a). The test followed ASTM (1999a). Daphnids used for testing were less than 24 hours old at test initiation. *D. magna* were obtained from a brood stock (Dm99- 23) at ESG International (Guelph, ON, Canada). *D. pulicaria* were acquired from a brood stock maintained in the Department of Zoology at the University of Guelph. Dilution water was clean well water obtained from ESG International. Hardness was softened by addition of distilled deionized water to achieve a range of 200-225 mg/L of $CaCO_3$. Photoperiod was 16:8-hours light:dark under cool-white fluorescent light between 380 and 480 lux. Laboratory-grade distilled water was used for all solutions with maximum concentrations derived from stock solutions no greater than 450 mg/L. Test vessels consisted of 225 mL polypropylene disposable containers filled with 150 mL of test solution. All toxicity testing involved four replicates of 10 daphnids each in five nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 31, 63, 125, 250 and 450 mg/L. Experiments were conducted in environmental chambers at a test temperature of 21 ±1°C. Authors note temperature and pH were measured at beginning and end of study, but the information was not reported. Survival of daphnids in the negative control was also not reported, although ASTM E729-96 requires at least 90% survival for test acceptability. The author-reported 48-hour EC_{50} for *D. magna* was 67.2 mg/L (C.I. 31.3-88.5). The author-reported 48-hour EC_{50} for *D. pulicaria* was 134 mg/L (C.I. 103-175). Independently-calculated toxicity values could not be calculated given the level of data that was presented in the paper. The study author reported values were used quantitatively to derive the freshwater acute water column criterion.

Ji et al. (2008) performed a 48-hour static, unmeasured acute test of PFOS (acid form, CAS # 1763-23-1, purity unreported) with *Daphnia magna*. The test followed the EPA's Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (U.S. EPA 2002). *D. magna* used for testing were obtained from brood stock cultured at the Environmental Toxicology Laboratory at Seoul National University (South Korea). Test organisms were less than 24 hours old at test initiation. Dilution water was moderately hard reconstituted water (hardness typically 80-100 mg/L as $CaCO_3$). Experiments were conducted in glass jars of unspecified size and fill volume. Photoperiod was assumed by the reviewers to have been 16:8-hours light:dark. Preparation of test solutions was not described. The test involved four replicates of five daphnids each in five nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 6.25, 12.5, 25, 50 and 100 mg/L. Test temperature was maintained at 21 ± 1°C. Authors note water quality parameters (pH, temperature, conductivity, and D.O.) were measured 48 hours after exposure, but the information is not reported. Survival of daphnids in the negative control was not reported in the paper. However, raw data were obtained by the EPA from the study authors and control survival was 100% and therefore meets the EPA/600/4-90/027F requirement of at least 90% survival for test acceptability. The author-reported 48-hour EC_{50} value for the study was 37.36 mg/L (C.I. 30.72-43.99) for *D. magna*. The 48-hour EC_{50} value was independently-calculated as 35.46 (28.26 – 42.66) mg/L. The independently-calculated acute toxicity value was included in the derivation of the freshwater acute water column criterion.

Li (2009) conducted three independent repeats of a 48-hour static acute test on PFOS (potassium salt, > 98% purity) with *Daphnia magna*. The test followed OECD (1984). *D. magna* used for the test were less than 24 hours old at test initiation. Dilution water was dechlorinated

tap water. The photoperiod consisted of 12 hours of illumination at an unreported light intensity. A primary stock solution was prepared in dilution water and did not exceed 400 mg/L. Exposure vessels were polypropylene of unreported dimensions and 50 mL fill volume. The test employed five replicates of six daphnids each in at least five test concentrations plus a negative control. Each treatment was tested three independent times. Based on water solubility of test chemicals and preliminary toxicity results, nominal test concentrations were in the range of 10 - 400 mg/L for PFOS. Water quality parameters including water pH, conductivity and D.O. were measured at the beginning and at the end of each test. Initial values of pH were 7.82±0.12 and 7.91±0.03 after 48 hours. At the start of the bioassays, D.O. and specific conductivity were 67.7±6.8% and 101.8±6.8 µS/cm. After the 48-hour testing period, D.O. and specific conductivity were 55.6±1.26% and 109.1±3.5 µS/cm. The D.O. after 48-hours of testing was lower than the test guideline recommendation of >60% (ASTM 2002; U.S. EPA 2016a; U.S. EPA 2016b); however, it was not low enough to change the use of the study. The test was conducted in a temperature incubator at 25 ±2°C. None of the control animals became immobile at the end of the test. The author-reported 48-hour EC_{50} was 63 mg/L (C.I. 58-69) which was an average LC_{50} of the three tests. The independently-calculated LC_{50} values for the three independent experimental repeats were 55.40 (45.97), 72.70 (61.63 – 83.77) and 64.60 (49.53 – 79.66) mg/L, respectively. The three independently-calculated LC_{50} values were used to calculate the SMAV for *D. magna* and derive the freshwater acute water column criterion.

Yang et al. (2014) conducted a 48-hour static acute test of PFOS (potassium salt, CAS # 2795-39-3, 99% purity) with *Daphnia magna,* following ASTM (1993). *D. magna* used for the test were donated by the Chinese Research Academy of Environmental Sciences. The daphnids were less than 24 hours old at test initiation. Dilution water was dechlorinated tap water (pH,

7.0±0.5; D.O., 7.0±0.5 mg/L; total organic carbon, 0.02 mg/L; and hardness, 190.0±0.1 mg/L as $CaCO_3$). Photoperiod was 12:12-hours light:dark at an unreported light intensity. A primary stock solution was prepared by dissolving PFOS in deionized water and cosolvent DMSO. The primary stock was proportionally diluted (0.56x) with dilution water to prepare the test concentrations. Exposure vessels were 200 mL beakers of unreported material type containing 200 mL of test solution. The test employed three replicates of 10 daphnids each in six test concentrations (measured in low and high treatments) plus a negative and solvent control. Nominal concentrations were 0 (negative and solvent controls), 20.00, 36.00, 64.80, 116.64, 209.95 and 377.91 mg/L. Mean measured concentrations before and after renewal were respectively 18.43 and 19.80 and mg/L (lowest concentration) and 341.74 and 372.35 mg/L (highest concentration). Analyses of test solutions were performed using HPLC/MS and negative electrospray ionization. The concentration of PFOS was calculated from standard curves (linear in the concentration range of 1-800 ng/mL), and the average extraction efficiency was in the range of 70-83%. The concentrations and chromatographic peak areas exhibited a significant positive correlation ($r = 0.9987$, $p < 0.01$), and the water sample-spiked recovery was 105%. The temperature, D.O., and pH were reported has having been measured every day during the acute test, but results are not reported. Negative control survival was > 96%. Solvent control survival was 100%. The author reported 48-hour LC_{50} was 78.09 mg/L (C.I. 54.38-112.13) and was used quantitatively to derive the freshwater acute water column criterion.

Lu et al. (2015) conducted a 48-hour static test on PFOS (purity 98%) with *Daphnia magna*, following OECD (2004). *D. magna* used for the test were originally obtained from the Chinese Center for Disease Control and Prevention (Beijing, China) and cultured in the laboratory according to the International Organization for Standardization (ISO 1996). Daphnids

were less than 24 hours old at test initiation. Dilution water was the same used for daphnid culture and was reconstituted according to OECD (2004) with a hardness of 250 mg/L as $CaCO_3$, as calculated based on the recipe provided, and pH ranging from 7.7 to 8.4. Photoperiod was 16:8-hours light:dark at an unreported light intensity. The test solution was prepared immediately prior to use by diluting the stock solution with a daphnia culture medium. Exposure vessels were 100 mL glass beakers containing 45 mL of test solution. The test employed three replicates of 10 daphnids each in six nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 1, 3, 10, 30, 100 and 300 mg/L. Exposure water quality was checked daily and maintained at a temperature of 20 ± 1°C, pH of 7.2 ± 0.3, and D.O. of 6.5 ± 0.5 mg/L. One hundred percent survival was observed at 48 hours in the negative control. The author-reported 48-hour EC_{50} was 23.41 mg/L (LC_{50} = 49.27) and used quantitatively to derive the freshwater acute water column criterion.

Liang et al. (2017) conducted a 48-hour static test on PFOS (potassium salt, CAS # 2795-39-3, ≥ 98% purity) with *Daphnia magna*. The test followed OECD (2004). *D. magna* used for the test were originally obtained from State Key Laboratory of Environmental Aquatic Chemistry (Eco-Environmental Sciences of Chinese Academy of Sciences, Beijing) and cultured in the laboratory according to Revel et al. (2015). Daphnids were less than 24 hours old at test initiation. Dilution water was artificial medium (M4) at 20°C and pH 7 (Revel et al. 2015). Photoperiod was 16:8-hours light:dark at an unreported light intensity. The test solution was prepared immediately prior to use by diluting the stock solution with M4 medium. Exposure vessels were 80 mL beakers of unreported material type containing an unspecified volume of test solution. The test employed five replicates of five daphnids each in six nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 30, 44,

66, 100 and 150 mg/L. No mention was made of water quality being checked during the exposure. 100% survival was observed at 48 hours in the negative control. The author reported 48-hour EC_{50} was 79.35 mg/L. The independently-calculated toxicity value was 94.58 (94.20 – 94.96) mg/L and was used quantitatively to derive the freshwater acute water column criterion.

Yang et al. (2019) evaluated the acute effects of perfluorooctane sulfonate, potassium salt (PFOS-K, CAS# 2795-39-3, 98% purity, purchased from Sigma-Aldrich in St. Louis, MO) on *Daphnia magna* via a 48-hour unmeasured, static mortality test. *D. magna* cultures were obtained from the Institute of Hydrobiology of Chinese Academy of Science in Wuhan, China. Organisms were cultured in Daphnia Culture Medium according to the parameters laid out in OECD Guideline 202, and all testing followed the guideline. Cultures were fed green algae daily and were acclimated for two to three weeks before testing. Acute test concentrations included 0 (control), 0.0000156, 0.0000234, 0.0000349, 0.0000788 and 0.000118 mol/L (or 0 (control), 8.396, 12.59, 18.78, 28.31, 42.41, and 63.51 mg/L given the molecular weight of the form of PFOS used in the study, CAS # 2795-39-3, of 538.22 g/mol). Five neonates (12-24 hours old) were placed randomly in 100 mL glass beakers filled with 60 mL test solution, with four replicates per concentration. Organisms were observed for mortality at 48 hours, and the authors reported a LC_{50} of 22.77 mg/L. The EPA's independently-calculated 48-hour LC_{50} was 22.43 (15.74 – 29.12) mg/L PFOS and was used quantitatively to derive the freshwater acute water column criterion.

A.2.13 Thirteenth Most Sensitive Freshwater Genus for Acute Toxicity: *Ambystoma* (salamander)

Tornabene et al. (2021) conducted acute toxicity tests with three species of salamanders in the genus *Ambystoma* and PFOS (purchased from Sigma Aldrich, Catalog # 77282-10G; purity not provided). Acute tests followed standard 96-hour acute toxicity test guidance (ASTM

2017; U.S. EPA 2002). The three test species (Jefferson salamander, *Ambystoma jeffersonianum*; small-mouthed salamander, *A. texanum*; eastern tiger salamander, *A. tigrinum*) were collected from a field in the wetlands of Indiana near the campus of Purdue University. Collected egg masses were raised outdoors in 200 L polyethylene tanks filled with well water. Experiments began when salamanders reached Harrison stage 40, defined as when larvae are free swimming and feeding. Before test initiation larvae were acclimated to test conditions (21°C and 12:12-hour light:dark photoperiod) for 24 hours. An additional acute test with Harrison stage 46 small-mouthed salamanders was run to determine if toxicity varied between life stages. A stock solution of PFOS (500 mg/L) was made in UV-filtered well water and diluted with well water to reach test concentrations (ranged from 0-500 mg/L PFOS). Test concentrations were not measured in test solutions, based on previous research that demonstrated limited degradation under similar conditions. Larva were transferred individually to 250 mL plastic cups with 200 mL of test solutions and were not fed during the exposure period. The number of replicates varied by species, life stage and treatment; five replicates per treatment for Jefferson salamander and Harrison stage 46 small-mouthed salamander, seven replicates per treatment for Harrison stage 40 small-mouthed salamander, and 20 replicates in the control and 10 replicates in each treatment for eastern tiger salamander. No mortality occurred in any of the control groups. Author-reported 96-hour LC_{50}s were 64, 41 and 73 mg/L PFOS for the Jefferson salamander, small-mouthed salamander and eastern tiger salamander, respectively. The authors did not find a significant difference between the life stages of small-mouthed salamander so results of the two tests were pooled. The independently-calculated 96-hour LC_{50} values were 51.71 (40.84 – 62.58) for Jefferson salamander; 46.71 (34.33 – 59.09) and 30.00 (27.14 – 32.86) for small-mouthed salamander, Harrison stage 40 and 46; and 68.63 (61.90 – 75.37) mg/L for eastern tiger

salamander, respectively. In general, the independently-calculated toxicity values were acceptable for quantitative use and used to derive the freshwater acute water column criterion for PFOS. However, only the LC_{50} value of 30.00 mg/L for Harrison stage 46 small-mouthed salamander was used for this species as this life stage was determined to be the most sensitive.

A.2.14 Fourteenth Most Sensitive Freshwater Genus for Acute Toxicity: *Pontastacus* (crayfish)

Belek et al. (2022) tested heptadecafluorooctanesulfonic acid potassium salt (PFOS-K) on narrow-clawed crayfish (*Pontastacus leptodactylus*; formerly, *Astacus leptodactylus*) for 96 hours in an unmeasured, static experiment. Heptadecafluorooctanesulfonic acid potassium salt (≥98% purity) was obtained from Sigma-Aldrich (USA). Crayfish were obtained from a local breeder in Lake Egirdir, Turkey during the inter-molt stage, and averaged 29.1±0.39 g weight and 10.27±0.05 cm length. Crayfish were acclimated in the laboratory at the Biology Department of Gazi University for two weeks in glass aquariums filled with aerated, dechlorinated tap water for two weeks at a temperature of 21°C, and were fed a daily ration of raw trout. Testing parameters followed animal care guidance from NRC (1996). A 96-hour acute test was conducted to determine the LC_{50}, and a subsequent 21-day chronic test was conducted with a water only control, a DMSO solvent control, and two treatments set to 0.5 and 5 mg/L, which approximated 1% and 10% of the acute LC_{50}. The chronic test measured biochemical and enzymatic responses to PFOS. Mean water quality parameters for test water were as follows: 21±1°C temperature, 6.79±0.31 pH, 117.38±17.20 µS/cm specific conductivity, 0.01±0.001 mg/L total ammonia nitrogen, and 6.72±0.05 mg/L dissolved oxygen. Organisms were kept under a 16:8 light:dark cycle in 15 L of aerated, dechlorinated tap water during testing. The LC_{50} was calculated using Probit analysis using the U.S. EPA LC_{50} Software Program version 1.00. The author reported 96-hour LC_{50} value of 48.81 mg/L was determined to be acceptable for quantitative use.

A.2.15 Fifteenth Most Sensitive Freshwater Genus for Acute Toxicity: *Anaxyrus* (toad)

Tornabene et al. (2021) conducted acute toxicity tests with the American toad, *Anaxyrus americanus*, and PFOS (purchased from Sigma Aldrich, Catalog # 77282-10G; purity not provided). The acute tests followed standard 96-hour acute toxicity test guidance (ASTM 2017; U.S. EPA 2002). The frog was collected from a field in the wetlands of Indiana near the campus of Purdue University. Collected egg masses were raised outdoors in 200 L polyethylene tanks filled with well water. Experiments began when toads reached Gosner stage (GS) 26, defined as when larvae are free swimming and feeding. An additional acute test with GS 41 was run to determine if toxicity varied between life stages. Before test initiation larvae were acclimated to test conditions (21°C and 12:12-hour light:dark photoperiod) for 24 hours. A stock solution of PFOS (500 mg/L) was made in UV-filtered well water and diluted with well water to achieve test concentrations ranging from 0 – 500 mg/L PFOS. Test concentrations were not measured in test solutions, based on previous research that demonstrated limited degradation under similar conditions. Larva were transferred individually to 250 mL plastic cups with 200 mL of test solutions and were not fed during the exposure period. The number of replicates varied by life stage, and treatment; 10 replicates for each treatment for GS 26 toads, and six to 10 replicates for each treatment for GS 41 toads. No mortality occurred in any of the control groups. The author-reported 96-hour LC_{50} was 62 mg/L PFOS. The authors did not find a significant difference between the life stages of the American toad, so results of the two tests were pooled. The independently-calculated 96-hour LC_{50} values were 63.41 (62.32 – 64.51) mg/L for the GS 26 toads and 56.49 (49.10 – 63.90) mg/L for GS 41 toads. Given that the GS 41 toads appear to be a more sensitive life-stage the LC_{50} of 56.49 mg/L was considered acceptable for quantitative use and used in the derivation of the freshwater acute water column criterion.

A.2.16 Sixteenth Most Sensitive Freshwater Genus for Acute Toxicity: *Procambarus* (crayfish)

Funkhouser (2014) conducted a 7-day static acute test on PFOS (potassium salt, ≥98% purity) with the crayfish species, *Procambarus fallax* (f. *virginalis*), as part of a Master's thesis at the Texas Tech University, Lubbock, TX. Juvenile *P. fallax* (2-week old, 0.041 g) used for the test were originally purchased from a private collector. The crayfish reproduced for several generations before being used for experiments. Based on an average reproductive age of 141-255 days, an interclutch period of 50-85 days, and a brooding time of 22-42 days, the author estimated the experimental animals to be stage F4-F6 (Seitz et al. 2005). Dilution water was moderately hard reconstituted laboratory water (3.0 g $CaSO_4$, 3.0 g $MgSO_4$, 0.2 g KCl, and 4.9 g $NaHCO_3$ added to 50 L deionized water). Photoperiod was 14:10-hours light:dark at an unreported light intensity. PFOS was dissolved in dilution water to prepare the test concentrations. Exposure vessels were 1 L polypropylene containers containing 500 mL of test solution. The test employed two replicates of three snails each in five test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 40, 80, 120, 160, and 200 mg/L. Exposure concentrations were reportedly measured, but concentrations were not reported. Analyses of test solutions were performed using LC-MS/MS. Standards were used as part of the analytical method, but details were not reported. The reporting limit was 0.010 mg/L. Experiments were conducted in an incubator at 25 ±1°C and covered with plastic opaque sheeting to limit evaporation. No other water quality parameters were reported as having been measured in test solutions. Negative control survival was 100% after seven days. The author reported 96-hour LC_{50} was reported as 59.87 mg/L. For comparison, the 7-day LC_{50} was 39.71 mg/L. The independently-calculated 96-hour LC_{50} value was also 59.87 mg/L (C.I. 54.29-65.45). This independently-calculated LC_{50} value of 59.7 mg/L was used quantitatively to derive the freshwater acute water column criterion.

A.2.17 Seventeenth Most Sensitive Freshwater Genus for Acute Toxicity: *Brachionus* (rotifer)

Zhang et al. (2013) performed a 24-hour static test of PFOS (potassium salt, CAS # 2795-39-3, ≥98% purity) with *Brachionus calyciflorus*. Organisms were less than two hours old at test initiation. All animals were parthenogenetically-produced offspring of one individual from a single resting egg collected from a natural lake in Houhai Park (Beijing, China). The rotifers were cultured in an artificial inorganic medium at 20°C (16:8-hours light:dark; 3,000 lux) for more than six months before toxicity testing to acclimate to the experimental conditions. All toxicity tests were carried out in the same medium and under the same conditions as during culture (i.e., pH, temperature, illumination). Solvent-free stock solutions of PFOS (1,000 mg/L) were prepared by dissolving the solid in deionized water via sonication. After mixing, the primary stock was proportionally diluted with dilution water to prepare the test concentrations. Exposures were in 15 mL, 6-well cell culture plates (assumed plastic) each containing at total of 10 mL of test solution. The test employed seven measured test concentrations plus a negative control. Each treatment consisted of one replicate plate of 10 rotifers each in individual cells and repeated six times. Nominal concentrations were 0 (negative control), 40, 50, 60, 70, 80, 90, and 100 mg/L. PFOS concentrations were not measured in the rotifer exposures, but rather, in a side experiment using HPLC/MS. The side experiment showed that the concentration of PFOS measured every eight hours over a 24-hour period in rotifer medium with green algae incurs minimal change in the concentration range 0.25 to 2.0 mg/L. The acute test was conducted without green algae added to the exposure medium. One hundred percent survival was observed at 24 hours in the negative control. The author-reported 24-hour LC_{50} was 61.8 mg/L. The author-reported value was used quantitatively to derive the freshwater acute water column criterion.

A.2.18 Eighteenth Most Sensitive Freshwater Genus for Acute Toxicity: *Elliptio* (mussel)

Drottar and Krueger (2000f) reported the results of a 96-hour renewal, measured test on the effects of PFOS (potassium salt, CAS # 2795-39-3, 90.49% purity) on *Elliptio complanata* (formerly known as *Unio complanatus)*. The good laboratory practice (GLP) test was conducted at Wildlife International, Ltd. In Easton, MD in August, 1999, using a protocol based on procedures outlined in U.S. EPA (1996b). *E. complanata* (76.5 g and 48.7 mm) used for the test were purchased from Carolina Biological Supply Company in Burlington, NC, after being caught in the wild. They were of an unspecified age at test initiation. Dilution water was 0.45 µm filtered well water [total hardness: 126 (120-132) mg/L as $CaCO_3$; alkalinity: 174 (170-178) mg/L as $CaCO_3$; pH: 8.3 (8.1-8.5); TOC: < 1.0 mg/L; conductivity: 21 (310-330) µmhos/cm]. Photoperiod was 16:8-hours light:dark with a 30-minute transition period. Light was provided at an intensity of approximately 369 lux. A primary stock solution was prepared in dilution water at 91 mg/L. It was mixed for ~24 hours prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the four additional test concentrations. Exposure vessels were 25 L polyethylene aquaria containing 20 L of test solution. The test employed two replicates of 10 mussels each in five measured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 5.7, 11, 23, 46, and 91 mg/L. Mean measured concentrations were less < 0.115 mg/L, 5.3, 12, 20, 41, and 79 mg/L, respectively. Analyses of test solutions were performed at Wildlife International, Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7%. Concentrations measured at test initiation averaged 86% of nominal. Concentrations measured prior to renewal at 48 hours averaged 89% of nominal. Concentrations measured at 96 hours averaged 100% of nominal. Dissolved oxygen in control and the high-test concentration (79

mg/L) respectively ranged from 5.8-8.5 mg/L and 5.0-8.6 mg/L; pH ranged from 8.0-8.4 and 7.9-8.5. Test temperature ranged from 21.4-21.8°C and 21.8-23.7°C. Mussels in the negative control, the 5.3, 12, and 20 mg/L treatments appeared healthy and normal throughout the test with no mortality, immobility or overt clinical signs of toxicity. The author-reported 96-hour LC_{50} was 59 mg/L (C.I. 51-68). The independently-calculated LC_{50} value was 64.35 (56.22 – 72.48) mg/L and used to derive the freshwater acute water column criterion.

A.2.19 Nineteenth Most Sensitive Freshwater Genus for Acute Toxicity: *Lithobates* (frog)

Flynn et al. (2019) evaluated the acute effects of perfluorooctanesulfonic acid (PFOS, CAS# 1763-23-1, purchased from Sigma-Aldrich) on the American bullfrog (*Lithobates catesbeiana*, formerly, *Rana catesbeiana*) during a 96-hour unmeasured, static study. Testing followed Purdue University's Institutional Animal Care and Use Committee Guidelines Protocol #16010013551. American bullfrog eggs were taken from a permanent pond in the Martell Forest outside of West Lafayette, Indiana. The eggs from a single egg mass were acclimated in 100-L outdoor tanks filled with 70 L of aged well water and covered with a 70% shade cloth. Once hatched, tadpoles were fed rabbit chow and TetraMin® *ad libitum* and were acclimated to laboratory conditions for 24 hours before testing. A 500 mg/L PFOS stock solution was prepared with RO water to create nominal test concentrations of 0 (control), 10, 25, 50, 75, 100, 150, 300 and 500 mg/L. Each treatment contained 10 replicates with one Gosner Stage 25 tadpole in each 250 mL plastic tub maintained at 21ºC and a 12:12-hour light:dark photoperiod. Mortality was monitored twice daily. The author-reported LC_{50} value was 144 mg/L PFOS. The EPA's independently-calculated 96-hour LC_{50} was 154.8 (108.7 – 200.9) mg/L PFOS and used quantitatively to derive the freshwater acute water column criterion. However, this value was not used in the SMAV calculation because a more sensitive life stage was available for the species.

Tornabene et al. (2021) conducted acute toxicity tests with four species of frogs in the genus *Lithobates* (formerly, *Rana*) and PFOS (purchased from Sigma Aldrich, Catalog # 77282-10G; purity not provided). Acute tests followed standard 96-hour guidance (ASTM 2017; U.S. EPA 2002). The four test species (American bullfrog, *Lithobates catesbaeiana*; green frog, *L. clamitans*; northern leopard frog, *L. pipiens*; wood frog, *L. sylvatica*) were collected from a field in the wetlands of Indiana near the campus of Purdue University. Collected egg masses were raised outdoors in 200 L polyethylene tanks filled with well water. Experiments began when frogs reached Gosner stage 26, defined as when larvae are free swimming and feeding. Before test initiation larvae were acclimated to test conditions (21℃ and 12:12-hour light:dark photoperiod) for 24 hours. A stock solution of PFOS (500 mg/L) was made in UV-filtered well water and diluted with well water to reach test concentrations ranging from 0 – 500 mg/L PFOS. Test concentrations were not measured in test solutions, based on previous research that demonstrated limited degradation under similar conditions. Larva were transferred individually to 250 mL plastic cups with 200 mL of test solutions and were not fed during the exposure period. The number of replicates varied by species, and treatment; 20 replicates in the control and five to 10 replicates in each treatment for American bullfrog, 10 replicates for each treatment for green frog, northern leopard frog and wood frog. No mortality occurred in any of the control groups. Author-reported 96-hour LC_{50}s were 163, 113, 73 and 130 mg/L PFOS for the American bullfrog, green frog, northern leopard frog, and wood frog, respectively. The independently-calculated 96-hr LC_{50} values for American bullfrog and northern leopard frog were 133.23 (95.75 – 170.8), and 72.72 (63.88 – 81.55) mg/L, respectively. The EPA was unable to independently calculate LC_{50} values for green frog and wood frog as a curve could not be fit with significant parameters. Therefore, the independently-calculated LC_{50} values for American

bullfrog (133.3 mg/L) and northern leopard frog (72.72 mg/L) were used quantitatively to derive the freshwater acute water column criterion for PFOS, while the author-reported LC_{50} values for green frog (113 mg/L) and wood frog (130 mg/L) were used. The author-reported toxicity values were consistent with the independently-calculated LC_{50} values for other species included in the study.

A.2.20 Twentieth Most Sensitive Freshwater Genus for Acute Toxicity: *Physella* (snail)

Li (2009) conducted three independent repeats of a 96-hour static acute test on PFOS (potassium salt, > 98% purity) with the bladder snail species, *Physella acuta* (Note: formerly known as *Physa acuta*). The test organisms were collected from a ditch located in Shilin of Taipei City in June 2005. Snails were fed with lettuce and half of the culture medium was changed with dechlorinated water every two weeks, implying a holding time of greater than two weeks. *P. acuta* of mixed ages were used at test initiation. Dilution water was dechlorinated tap water. The photoperiod consisted of 12 hours of illumination at an unreported light intensity. A primary stock solution was prepared in dilution water. Exposure vessels were polypropylene beakers of unreported dimensions and 1 L fill volume. The test employed 5-6 replicates of six snails each in at least five test concentrations plus a negative control. Each treatment was tested three independent times. Nominal test concentrations were in the range of 25-300 mg/L PFOS. The test temperature was maintained at 25±2°C. Water quality parameters including pH, conductivity, and D.O. were reported as having been measured at the beginning and end of each test, but the information was not reported. Survival of negative control animals was also not reported. The author-reported 96-hour LC_{50} was 178 mg/L (C.I. 167-189) and represented an average of the LC_{50}s for each test. Only one of three independent experiments could be fitted. The independently-calculated LC_{50} value was 183.0 (161.4 – 204.7) mg/L and was used quantitatively to derive the freshwater acute water column criterion.

Funkhouser (2014) conducted a 96-hour static test on PFOS (potassium salt, ≥98% purity) with the physid snail, *Physella heterostropha pomilia* (Note: formerly known as *Physa pomilia*), as part of a Master's thesis at the Texas Tech University, Lubbock, TX. Adult *P. pomilia* (4 month old) used for the test were field collected from two different collections from the North Fork of the Double Mountain Fork of the Brazos River near Lubbock, TX. Offspring from both collections were reared in 12, 10-gallon aquaria with lab water for several generations prior to use in the test. Dilution water was moderately hard reconstituted laboratory water (3.0 g $CaSO_4$, 3.0 g $MgSO_4$, 0.2 g KCl, and 4.9 g $NaHCO_3$ added to 50 L deionized water). Photoperiod was 12:12-hours light:dark at an unreported light intensity. PFOS was dissolved in dilution water to prepare the test concentrations. Exposure vessels were 400 mL polypropylene containers containing 200 mL of test solution. The test employed two replicates of four snails each in six test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 100, 150, 200, 250, 300, and 375 mg/L. Exposure concentrations were reportedly measured, but concentrations were not reported. Analyses of test solutions were performed using liquid chromatography/ tandem mass spectrometry (LC-MS/MS). Standards were used as part of the analytical method, but details were not reported. The reporting limit was 0.010 mg/L. Experiments were conducted in incubators set to 25°C, which did not vary more than 1°C during the course of the test. No other water quality parameters were reported as having been measured in test solutions. Negative control survival was not reported specifically for the test but was reported to be 85-100% across all experiments. The author-reported 96-hour LC_{50} was reported as 161.77 mg/L. An independently-calculated toxicity value could not be calculated given the level of data that was presented in the paper. The study author reported value was used quantitatively to derive the freshwater acute water column criterion.

Appendix B Acceptable Estuarine/Marine Acute PFOS Toxicity Studies

B.1 Summary Table of Acceptable Quantitative Estuarine/Marine Acute PFOS Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp (°C)	Salinity (ppt)	Effect	Author Reported Effect Conc. (mg/L)	EPA Calculated Effect Conc. (mg/L)	Final Effect Conc. (mg/L)[b]	Species Mean Acute Value (mg/L)	Reference
Sea urchin (larvae), *Paracentrotus lividus*	S, U	72 hr	PFOS Unreported	-	18	35	EC50 (malformation)	1.795	-	**1.795**	1.795	Gunduz et al. (2013)
Purple sea urchin (embryo), *Strongylocentrotus purpuratus*	S, M	96 hr	PFOS-K 98%	-	15	30	EC50 (normal development)	1.7	-	**1.7**	1.7	Hayman et al. (2021)
Mediterranean mussel (larva), *Mytilus galloprovincialis*	S, U	48 hr	PFOS Unreported	7.9-8.1	16	36	EC50 (malformation)	>1	-	>1[c]	-	Fabbri et al. (2014)
Mediterranean mussel (embryo), *Mytilus galloprovincialis*	S, M	48 hr	PFOS-K 98%	-	15	30	EC50 (normal development)	1.1	-	**1.1**	1.1	Hayman et al. (2021)
Mysid (3 d), *Americamysis bahia*	S, M	96 hr	PFOS-K 98%	-	20	30	LC50	5.1	4.914	**4.914**	4.914	Hayman et al. (2021)
Mysid (neonate, <24 hr), *Siriella armata*	S, U	96 hr	PFOS 98%	-	20	-	LC50	6.9	-	**6.9**	6.9	Mhadhbi et al. (2012)
Sheepshead minnow (3.0 cm, 0.44 g), *Cyprinodon variegatus*	R, M	96 hr	PFOS-K 86.9%	-	22	20	LC50	>15	-	**>15**	>15	Palmer et al. (2002b)

a S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

[b] Values in bold used the in the SMAV calculation

[c] Not used in SMAV calculations, because a definitive value is available

B.2 Detailed PFOS Acute Saltwater Toxicity Study Summaries and Corresponding Concentration-Response Curves (when calculated for the most sensitive genera)

The purpose of this section was to present detailed study summaries for acute saltwater tests that were considered quantitatively acceptable for criterion derivation, with summaries grouped and ordered by genus sensitivity. The data available for saltwater invertebrates fulfilled three of the eight MDRs. The EPA could not, therefore, develop acute estuarine/marine criteria following the 1985 Guidelines methods. In the interest of providing informations to states/Tribes on protective values, the EPA developed an estuarine/marine acute benchmark using the available empirical data supplemented with toxicity values generated through the use of New Approach Methods (NAMs), specifically through the use of the EPA Office of Research and Development's peer-reviewed publicly-available web-ICE tool (Raimondo et al. 2010). These benchmarks are provided in Appendix L.

B.2.1 Most Sensitive Estuarine/Marine Genus for Acute Toxicity: *Mytilus* (mussel)

The acute toxicity of perfluorooctane sulfonate (PFOS, purity not provided) on the Mediterranean mussel, *Mytilus galloprovincialis* was evaluated by **Fabbri et al. (2014)**. This species is not resident to North America, but is a surrogate for North American mussel species, including the widespread, commercially and ecologically important blue mussel, *Mytilus edulis.* Sexually mature mussels were purchased from an aquaculture farm in the Ligurian Sea (La Spezia, Italy) and held for two days for gamete collection. Gametes were held in artificial sea water (ASW) made of analytical grade salts and at a constant temperature of 16 ±1°C. It was assumed that the gametes were held at the same environmental conditions as the adults, so test salinity was assumed to be 36 ppt with a pH of 7.9-8.1. Embryos were transferred to 96-well microplates with a minimum of 40 embryos/well. Each treatment had six replicates. Embryos were incubated with a 16:8-hour light:dark photoperiod for 48 hours and exposed to one of six

nominal PFOS concentrations (0.00001, 0.0001, 0.001, 0.01, 0.1, and 1 mg/L) or control (ASW) water. The PFOS stock was made with ethanol, and ASW control samples run in parallel included ethanol at the maximal final ethanol concentration of 0.01%. Each experiment was repeated four times. At test termination (48 hours), the endpoint was the percentage of normal D-larvae in each well, including malformed larvae and pre-D stages. The acceptability of test results was based on the control group exhibiting >75% of normal D-shell stage larvae (ASTM 2004b). Authors noted that controls had ≥80% normal D-larvae across all tests. PFOS was only measured once in one treatment which was similar to the nominal concentration; that is, 0.000085 mg/L versus the nominal concentration of 0.0001 mg/L. PFOS was below the limit of detection (LOD) in the control ASW (0.06 ng/L or 0.00000006 mg/L). The percentage of normal D-larva decreased with increasing test concentrations. The NOEC and LOEC reported for the study were 0.00001 and 0.0001 mg/L, respectively. However, the test concentrations failed to elicit a 50% reduction in malformations in the highest test concentration, and an EC_{50} was not determined. Therefore, the EC_{50} for the study was greater than the highest test concentration (1 mg/L). The 48-hour EC_{50} based on malformation of >1 mg/L was acceptable for quantitative use.

Hayman et al. (2021) report the results of a 48-hour static, measured test on the effects of PFOS-K (potassium salt, CAS # 2795-39-3, 98% purity, purchased from Sigma-Aldrich, St. Louis, MO) on the Mediterranean mussel, *Mytilus galloprovincialis*. Authors note tests followed U.S. EPA (1995) and ASTM (2004b) protocols. Mussels were collected in the field (San Diego Bay, CA) and conditioned in a flow-through system at 15°C. Mussels were induced to spawn by heat-shock and approximately 250 embryos (2-cell stage) were added to 20 mL borosilicate glass scintillation vials with 10 mL of test solution. There were five replicates per test concentration. Test conditions were 30 ppt, 15°C and a 16:8-hour light:dark photoperiod. Six test solutions were

made in 0.45 µm filtered seawater (North San Diego Bay, CA) with PFOS-K dissolved in methanol. The highest concentration of methanol was 0.1% (v/v). Measured test concentrations ranged from 0.52 – 2.50 mg/L. Control mussel embryos were exposed to 100% filtered seawater and the acute test also included a solvent control. At test termination (48 hours), larvae were enumerated for total number of larvae that were alive at the end of the test (normally or abnormally developed), as well as number of normally-developed (in the prodissoconch "D-shaped" stage) larvae. There were no significant differences between solvent and negative (100% filtered seawater) control groups, suggesting no adverse effects of methanol. The author-reported 48-hr EC_{50}, based on normal development, was 1.1 mg/L PFOS. The EPA was not able to independently calculate a 48-hour EC_{50} value as the curve fitted model did not result in a good fit. Therefore, the author-reported EC_{50} of 1.1 mg/L mg/L was considered for quantitative use.

B.2.2 Second Most Sensitive Estuarine/Marine Genus for Acute Toxicity: *Strongylocentrotus* (sea urchin)

Hayman et al. (2021) report the results of a 96-hour static, measured test on the effects of PFOS-K (potassium salt, CAS # 2795-39-3, 98% purity, purchased from Sigma-Aldrich, St. Louis, MO) on the purple sea urchin, *Strongylocentrotus purpuratus*. Authors note that tests followed U.S. EPA (1995) and ASTM (2004b) protocols. Sea urchins were collected in the field (San Diego Bay, CA) and conditioned in flow-through system at 15°C. They were induced to spawn by KCl injection and approximately 250 embryos (2-cell stage) were added to 20 mL borosilicate glass scintillation vials with 10 mL of test solution. There were five replicates per test concentration. Test conditions were 30 ppt, 15°C and a 16:8-hour light:dark photoperiod. Seven test solutions were made in 0.45 µm filtered seawater (North San Diego Bay, CA) with PFOS dissolved in methanol. The highest concentration of methanol was 0.1% (v/v). Measured test concentrations ranged from 0.52 – 10.0 mg/L. Control urchin embryos were exposed to

100% filtered seawater and the acute test also included a solvent control. At test termination (96 hours), the first 100 larvae were enumerated and observed for normal development (4-arm pluteus stage). There were no significant differences between solvent and negative (100% filtered seawater) control groups, suggesting no adverse effects of methanol. The author-reported 96-hour EC_{50}, based on normal development, was 1.7 mg/L PFOS. The EPA was not able to independently calculate a 96-hour EC_{50} value as the curve fitted model did not result in a good fit. Therefore, the author-reported EC_{50} of 1.7 mg/L mg/L was considered for quantitative use.

B.2.3 Third Most Sensitive Estuarine/Marine Genus for Acute Toxicity: *Paracentrotus* (sea urchin)

A 72-hour static, unmeasured PFOS (purity not provided) toxicity test with the sea urchin, *Paracentrotus lividus* (a non-North American species) was conducted by **Gunduz et al. (2013)**. Adult sea urchins were collected from the Aegean coast of Turkey, in an area the authors noted as clean and lacking domestic and industrial wastewater inputs. Filtered natural seawater from the same area was used as the dilution water. Adult sea urchins were cultivated in the same filtered natural sea water with a salinity of 35 ppt and 18°C. Zygote suspensions (1 mL) were added to the controls or 9 mL of the various PFOS treatments. This ensured that there were about 30 fertilized embryos/mL or approximately 300 embryos per treatment. The experiments were conducted in six-well TPP culture plates with six replicates per treatment. PFOS stock solutions were made with dimethyl sulfoxide (DMSO) and diluted with seawater to obtain five nominal treatments (0.5, 1.0, 3.0, 5.0 and 10 mg/L PFOS). In addition to a natural seawater control, experiments also included a DMSO solvent control equal to the amount in the highest test concentration. The embryos were incubated in a growth chamber at 18 ±2°C from 10 minutes after fertilization to up to the 72-hour pluteus larval stage. At test termination, 100 individuals were selected randomly from each treatment and evaluated for normal plutei, retarded plutei,

pathologic malformed plutei, pathologic embryos unable to differentiate up to the pluteus larval stages, and dead embryos/larvae. There was 97.75% and 91% frequency of normal larvae in the control and solvent control, respectively with no deaths reported in the controls or any PFOS treatments. The 72-hour EC_{50} based on normal development to the pluteus stage was 1.795 mg/L PFOS and is acceptable for quantitative use; however, additional consideration needs to be given to the short test duration.

B.2.4 Fourth Most Sensitive Estuarine/Marine Genus for Acute Toxicity: *Americamysis* (mysid)

Hayman et al. (2021) report the results of a 96-hour static, measured test on the effects of PFOS (potassium salt, CAS # 2795-39-3, 98% purity, purchased from Sigma-Aldrich, St. Louis, MO) on the mysid, *Americamysis bahia*. Authors note that tests followed U.S. EPA (1995); U.S. EPA (2002); and ASTM (2004b) protocols. Mysids were purchased from a commercial supplier (Aquatic Research Organisms, Hampton, NH) and acclimated to test conditions (30 ppt, 20°C and a 16:8-hour light:dark photoperiod). Five test solutions were made in 0.45 µm filtered seawater (North San Diego Bay, CA) with PFOS-K dissolved in methanol. The highest concentration of methanol was 0.1% (v/v). Measured test concentrations ranged from 0.95 – 16 mg/L. Control mysids were exposed to 100% filtered seawater and the acute test also included a solvent control. Five mysids (3 days old, which is older than the typical age of < 24 hours at test initiation) were added to 120 mL polypropylene cups and 100 mL of test solutions with six replicates per treatment. Living mysids were counted and dead organisms were removed daily. There were no significant differences between solvent and negative (100% filtered seawater) control groups, suggesting no adverse effects of methanol. Only two organisms were found dead in the controls at test termination. The author-reported 96-hour LC_{50} was 5.1

mg/L PFOS. The independently-calculated 96-hr LC_{50} value was 4.914 (3.578 – 6.250) mg/L and is acceptable for quantitative use.

B.2.5 Fifth Most Sensitive Estuarine/Marine Genus for Acute Toxicity: *Siriella* (mysid)

Mhadhbi et al. (2012) performed a 96-hour static, unmeasured acute test with PFOS (98% purity) and the mysid, *Siriella armata*. Mysids were collected from the same source as the dilution water [filtered sea water from the Ria of Vigo (Iberian Peninsula)] and quarantined before use in 100 L plastic tanks with circulating sand-filtered seawater. The adult stock was fed daily and maintained at laboratory conditions (17-18°C, salinity between 34.4-35.9 ppt, and D.O. 6 mg/L). A stock solution of PFOS was made either with filtered sea water from the Ria of Vigo for low exposure concentrations, or with DMSO for high PFOS concentrations (a final maximum DMSO concentration of 0.01% (v/v) in the test medium). However, the authors did not indicate what was considered a high-test concentration. If DMSO was used, a solvent control was also included. Twenty neonates (<24 hours old) were used per each treatment. Mysids were exposed to one of five nominal PFOS treatments (1.25, 2.5, 5, 10 and 20 mg/L). To prevent cannibalism, a single individual was added to each glass vial with 2-4 mL of test solution. Vials were incubated at 20°C with a 16-hour light period. Neonates were fed 10-15 *Artemia salina* nauplii daily and mortality was recorded after 96 hours. The 96-hour LC_{50} was 6.9 mg/L PFOS and was acceptable for quantitative use.

B.2.6 Sixth Most Sensitive Estuarine/Marine Genus for Acute Toxicity: *Cyprinodon* (sheepshead minnow)

Palmer et al. (2002b) conducted a 96-hour static-renewal measured acute test with PFOS-K (perfluorooctanesulfonate potassium salt, 86.9% purity from the 3M Company) on the sheepshead minnow, *Cyprinodon variegatus*. The test followed standard guidance for acute toxicity tests outlined in U.S. EPA (1985) and ASTM (1994). Sheepshead minnows were purchased from a commercial supplier (Aquatic Biosystems, Fort Collins, CO) and held for

several weeks prior to testing. Fifty-one hours before testing fish were acclimated to test conditions (16:8-hour light:dark photoperiod, salinity of 20 ppt and 22°C). Natural seawater (Indian River Inlet, Delaware) was filtered and diluted with well water to 20 ppt and was used for culturing and testing. A nominal PFOS stock solution (40 mg/L) was made by dissolving PFOS in methanol and diluting it with seawater to achieve the nominal test concentration (20 mg/L). A solvent control (0.5 mL/L methanol) and a sea water control were also included. Ten minnows (3.0 cm, 0.44 g) were added to 25 L polyethylene aquaria with 15 L of test solution (loading was 0.30 g fish/L of test water). Test treatments were replicated three times. PFOS concentrations were measured daily at test solution renewal with averaged measured concentrations in the control and solvent control less than the LOQ (5 mg/L) and PFOS-spiked seawater, 15 mg/L. At test termination (96 hours) none of the minnows died in the controls or single PFOS test treatment, therefore the author-reported LC_{50} was >15 mg/L. The EPA was unable to independently calculate the LC_{50} value as this test only consisted of one treatment group. The author-reported LC_{50} >15 mg/L is being used quantitatively for the acute saltwater benchmark because it is a greater than high value which adds value to the assessment of potential sensitivity of this species to acute PFOS exposure (see Section 2.10.3.2).

Appendix C Acceptable Freshwater Chronic PFOS Toxicity Studies

C.1 Summary Table of Acceptable Quantitative Freshwater Chronic PFOS Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Chronic Value Endpoint	Author Reported Chronic Value (mg/L)	EPA Calculated Chronic Value (mg/L)	Final Chronic Value (mg/L)[c]	Species Mean Chronic Value (mg/L)	Reference
Fatmucket (adult), *Lampsilis siliquoidea*	R, M	36 d	PFOS >98%	7.6-8.5	14.6-16.1	MATC (metamorphosis success)	0.01768	0.0123	**0.01768**	0.01768	Hazelton et al. (2012); Hazelton (2013)
Snail (egg), *Physella heterostropha pomilia* (formerly, *Physa pomilia*)	R, M	44 d	PFOS-K ≥98%	-	25	EC10 (clutch size)	14.14	8.527	**8.527**	8.527	Funkhouser (2014)
Rotifer (<2 hr old neonates), *Brachionus calyciflorus*	R, U[b]	Up to 158 hr	PFOS ≥98%	-	20	LOEC (reduced net reproductive rate)	<0.25	-	0.25	0.2500	Zhang et al. (2013)
Cladoceran (<24 hr), *Ceriodaphnia dubia*	R, M	6 d	PFOS-K 98%	6.91-8.02	24.0-25.9	EC10 (neonates / female)	6.9	10.69	**10.69**	-	Krupa et al. (2022)
Cladoceran (<8 hr), *Ceriodaphnia dubia*	R, M	7 d	PFOS-K 97.5%	7.73 (7.64-7.86)	24.5 (23.8-25.2)	EC10 (neonates / female)	10.0	8.371	**8.371**	-	Kadlec et al. (2024)
Cladoceran (<8 hr), *Ceriodaphnia dubia*	R, M	7 d	PFOS-K 97.5%	7.76 (7.71-7.81)	24.4 (24.1-24.8)	EC10 (neonates / female)	14.5	9.205	**9.205**	-	Kadlec et al. (2024)
Cladoceran (<8 hr), *Ceriodaphnia dubia*	R, M	7 d	PFOS-K 97.5%	7.58 (7.49-7.67)	24.9 (24.3-25.7)	EC10 (neonates / female)	9.8	6.766	**6.766**	8.640	Kadlec et al. (2024)
Cladoceran (6-12 hr), *Daphnia carinata*	R, U	21 d	PFOS-K ≥98%	-	21	MATC (days to first brood)	0.003162	-	**0.003162**	0.003162	Logeshwaran et al. (2021)
Cladoceran (<24 hr), *Daphnia magna*	R, M	21 d	PFOS-K 90.49%	8.1-8.5	19.4-20.1	EC10 (cumulative young)	16.97	11.19	**11.19**	-	Drottar and Krueger (2000e)
Cladoceran (<24 hr), *Daphnia magna*	R, U	21 d	PFOS-K 95%	-	21	EC10 (survival)	35.36	16.35	**16.35**	-	Boudreau (2002);

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Chronic Value Endpoint	Author Reported Chronic Value (mg/L)	EPA Calculated Chronic Value (mg/L)	Final Chronic Value (mg/L)[c]	Species Mean Chronic Value (mg/L)	Reference
											Boudreau et al. (2003a)
Cladoceran (<24 hr), *Daphnia magna*	R, U	21 d	PFOS Unreported	-	21	EC10 (number of young / brood)	1.768	1.051	**1.051**	-	Ji et al. (2008)
Cladoceran (<24 hr), *Daphnia magna*	R, U	21 d	PFOS-K >98%	-	20	EC10 (total neonates/female)	2.236	3.030	**3.030**	-	Li (2010)
Cladoceran (<24 hr), *Daphnia magna*	R, M	21 d	PFOS-K 99%	7	22	EC10 (survival)	4.17	2.610	**2.610**	-	Yang et al. (2014)
Cladoceran (<24 hr), *Daphnia magna*	R, U	21 d	PFOS 98%	7.2	20	EC10 (number of offspring / brood / female)	0.0179	0.001818	**0.001818**	-	Lu et al. (2015)
Cladoceran (<24 hr), *Daphnia magna*	R, U	21 d	PFOS-K ≥98%	7	20	EC10 (survival)	5.657	3.596	**3.596**	-	Liang et al. (2017)
Cladoceran (12-24 hr), *Daphnia magna*	R, U	21 d	PFOS-K 98%	-	20	EC10 (growth-length)	0.8218	0.9093	**0.9093**	-	Yang et al. (2019)
Cladoceran (<24 hr), *Daphnia magna*	R, U	21 d	PFOS >99%	7.5	23	MATC (number of young)	1.581	-	**1.5815**	1.344	Seyoum et al. (2020)
Cladoceran (<24 hr), *Moina macrocopa*	R, U	7 d	PFOS Unreported	-	25	EC10 (number of young / starting adult)	<0.3125	0.1789	**0.1789**	0.1789	Ji et al. (2008)
Amphipod (7-8 d, juvenile), *Hyalella azteca*	R, M	42 d	PFOS-K 98%	7.77-8.10	22.1-22.8	EC10 (survival)	<4.8	**2.899**	**2.899**	2.899	Krupa et al. (2022)
Crayfish (4 wk juvenile, 0.056 g), *Procambarus fallax f. virginalis*	R, M	28 d	PFOS-K ≥98%	-	25	LC20	0.1670	-	**0.1670**	0.1670	Funkhouser (2014)
Blue damselfly (nymph), *Enallagma cyathigerum*	R, U	320 d	Perfluorooctanes ulfonic acid tetraethylammonium >98%	≥7.5	21	MATC (survival at 150 days)	0.03162	-	**0.03162**	0.03162	Bots et al. (2010)
Midge (newly hatched larva), *Chironomus dilutus*	R, M	10 d	PFOS-K 95%	-	21-23	EC10 (growth at 10 days)	0.04920	0.05896	**0.05896**	-	MacDonald et al. (2004)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Chronic Value Endpoint	Author Reported Chronic Value (mg/L)	EPA Calculated Chronic Value (mg/L)	Final Chronic Value (mg/L)[c]	Species Mean Chronic Value (mg/L)	Reference
Midge (4-day old larvae), *Chironomus dilutus*	R, M	16 d	PFOS 98%	6.8-8.7	20.0-23.2	EC10 (mean biomass)	0.001620	0.001588	**0.001588**	-	McCarthy et al. (2021)
Midge (4-day old larvae), *Chironomus dilutus*	R, M	16 d	PFOS-K 98%	7.28-7.75	22.0-22.9	EC10 (growth)	0.0015	-	**0.0015**	0.005198	Krupa et al. (2022)
Mayfly (<24 hr larva), *Neocloeon triangulifer*	R, M	14 d	PFOS-K 98%	-	23	EC10 (dry weight at day 14)	0.000226	-	**0.000226**	0.000226	Soucek et al. (2023)
Atlantic salmon (embryo-larval), *Salmo salar*	F, U	49 d	PFOS 98%	-	5.0-7.0	LOEC (growth – weight and length)	>0.1	-	**>0.1**	>0.1	Spachmo and Arukwe (2012)
Zebrafish (8 hpf), *Danio rerio*	R, U	Life-cycle	PFOS >96%	7.0-7.5	28	EC10 (F1 offspring: % survival)	0.01581[d]	0.01650	**0.01650**	-	Wang et al. (2011)
Zebrafish (male, 3.5 mo), *Danio reio*	R, U	21 d	PFOS Unknown	7.0-7.4	28	EC10 (mean body length)	0.05657	0.06274	**0.06274**	0.03217	Guo et al. (2019)
Fathead minnow (embryo, <24 hpf), *Pimephales promelas*	F, M	47 d	PFOS-K 90.49%	8.2	24.5	EC10 (survival)	0.4243	0.4732	**0.4732**	-	Drottar and Krueger (2000d)
Fathead minnow (adult), *Pimephales promelas*	F, M	21 d	PFOS >98%	7.3	25	EC10 (fecundity)	0.4794	0.05101	**0.05101**	-	Ankley et al. (2005)
Fathead minnow (adult, 5 mo.), *Pimephales promelas*	R, M	42 d	PFOS-K ≥98%	7.9	24.96	EC10 (F1 larval growth – weight)	0.06223	0.0549	**0.0549**	0.1098	Suski et al. (2021)
Swordtail fish (adult female 6-7 mo), *Xiphophorus helleri*	R, U	42 d	PFOS-K >98%	-	27	EC10 (female survival)	1.118	0.5997	**0.5997**	0.5997	Han and Fang (2010)
Northern leopard frog (stage 8/9 embryo), *Lithobates pipiens*	F, M	35 d	PFOS-K 98%	-	20	LC50	6.210	-	6.21		Ankley et al. (2004)
Northern leopard frog (stage 8/9 embryo), *Lithobates pipiens*	F, M	112 d	PFOS-K 98%	-	20	MATC (growth – length)	1.732	-	**1.732**	-	Ankley et al. (2004)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Chronic Value Endpoint	Author Reported Chronic Value (mg/L)	EPA Calculated Chronic Value (mg/L)	Final Chronic Value (mg/L)[c]	Species Mean Chronic Value (mg/L)	Reference
Northern leopard frog (larva, Gosner stage 26), *Lithobates pipiens*	R, M	40 d	PFOS ≥98%	-	20	MATC (Gosner stage at 40 d)	0.0316	-	0.03162	-	Hoover et al. (2017)
Northern leopard frog (larva, Gosner stage 26), *Lithobates pipiens*	R, M	40 d	PFOS ≥98%	-	20	LOEC (growth – snout-vent length)	>1	-	**>1**	1.3161	Hoover et al. (2017)
African clawed frog (larvae, NF 46/47 – 5 dpf), *Xenopus laevis*	R, M	4 mo.	PFOS 98%	6.5-7.0	22	LOEC (survival, weight, sex ratio/intersex)	> 0.7160	-	> **0.7160**	> 0.7160	Lou et al. (2013)
Clawed frog (embryo, NF 10), *Xenopus tropicalis* (formerly, *Silurana tropicalis*)	F, M	150 d post metamorphosis	PFOS ≥98%	7.5	26	MATC (weight at metamorphosis)	0.7871	-	**0.7871**	0.7871	Fort et al. (2019)

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

[b] Chemical concentrations made in a side-test representative of exposure and verified stability of concentrations of PFOS in the range of concentrations tested under similar conditions. Daily renewal of test solutions.

[c] Values in bold used in SMCV calculation. SMCVs are calculated as the geometric mean of all bold-faced values for a species. See section 2.10.3.2 (Chronic Measures of Effect) for decision rules regarding use of greater (>) and less than (<) values in SMCV calculations.

[d] Author-reported value based on a different test endpoint than the EPA-calculated value.

C.2 Detailed PFOS Chronic Freshwater Toxicity Study Summaries and Corresponding Concentration-Response Curves (when calculated for the most sensitive genera)

The purpose of this section was to present detailed study summaries for tests that were considered quantitatively acceptable for freshwater chronic water column criterion derivation, with summaries grouped and ordered by genus sensitivity. C-R models developed by the EPA that were used to determine chronic toxicity values used for criterion derivation are also presented for the most sensitive genera when available. The C-R models included with the study summaries were those for the four most sensitive genera (consistent with Section 3.1.1.3). When required, the EPA also included models for non-resident species that were more sensitive than the fourth most sensitive North American resident genus. In many cases, authors did not report C-R data in the publication/supplemental materials and/or did not provide C-R data upon the EPA request. In such cases, the EPA did not independently calculate a toxicity value and the author-reported effect concentrations were used in the derivation of the criterion.

C.2.1 Most Sensitive Freshwater Genus for Chronic Toxicity: *Neocloeon* (mayfly)

Soucek et al. (2023) conducted a chronic life-cycle test to determine the effects of PFOS-K (PFOS potassium salt, CAS # 2795-39-3, 98% purity) on the parthenogenetic mayfly, *Neocloeon triangulifer*. The test was performed under renewal conditions over 27 days beginning with < 24 hour old nymphs. Single mayfly exposures were static without renewal for the first four days due to the small size of starting organisms and then water was renewed three times per week thereafter by transferring organisms to new exposure chambers. From Day 0 to 14, mayflies were exposed in 30 mL polypropylene cups with 20 mL exposure water. Organisms were transferred after 14 days into 250 mL glass beakers with 100 or 150 mL of test water (or control water) and to 300 mL tall form glass beakers for emergence. There were sixteen replicates (with one mayfly per replicate) per test concentration and control. Replicates one

through eight were destructively sampled on day 14 and replicates nine through sixteen continued until the end of the test (when all mayflies either molted into imago stage or died). The endpoints that were evaluated included survival for all replicates, 14-d length and calculated dry weight (using a previously published body length dry weight equation; Besser et al. 2021) for replicates 1 through 8, and percent survival to pre-emergent nymph (PEN) stage, number of days until PEN stage, percent emergence (to imago stage), and pre-egg laying live weight of imago for replicates 9 through 16. Nominal test concentrations were 0 (control), 0.00016, 0.00031, 0.00063, 0.00125, 0.0025, 0.005, and 0.010 mg/L PFOS. Mean measured PFOS concentrations (EPA Analytical Method 1633; LC-MC/MS) were 0.000056 (control), 0.000205, 0.000418, 0.000764, 0.001143, 0.002057, 0.003892, and 0.006789 mg/L PFOS, respectively. Mayflies were exposed at 23 ± 1°C under a 16:8-hour light:dark cycle and fed 0.2 mL diatom slurry plus small scraping on test Days 0 and 4 followed by live diatom biofilm scraping after Day 4 on solution renewal days. Percent survival in the control after 14 days was 100%. Percent survival of mayflies after 14 days in the remaining seven test concentrations ranged from 79 to 100%. The most sensitive endpoint was 14-day dry weight. The study authors reported three different 14-day dry weight EC_{10} values that were calculated using various point-estimation approaches. The author-reported 14-day dry weight EC_{10} values produced by the various approaches were relatively similar to one another, ranging from 0.000226 (using TRAP [2 parameter, threshold sigmoidal curve]) to 0.000272 mg/L (using log-linear regression, controls excluded). The EPA was not able to fit a reliable model with significant model parameters to the 14-day dry weight C-R dataset and, therefore, relied on the author-reported EC_{10} of 0.000226 mg/L (based on TRAP) as the primary effect concentration. The EPA selected the TRAP-based EC_{10} preferentially over the EC_{10} values based on the two other point estimation approaches (i.e., log-

linear regression with and without controls) because the TRAP-based model (1) considered control responses; (2) was more fundamentally consistent with the maximum likelihood regression approaches used by the EPA to assess the C-R datasets evaluated in this document, and; (3) relied on replicate-level data, which the EPA used preferentially over treatment-mean data in assessing the C-R datasets. The author-reported EC_{10} of 0.000226 mg/L (TRAP-based) was used in the derivation of the freshwater PFOS chronic water column criterion.

Current toxicity literature indicates that aquatic insects, specifically mayfly, midge, and odonates, are sensitive to PFOS exposures. Further, given that recent research has led to the development of successful culturing methods for mayflies that are now being used in laboratory-based toxicity studies (Soucek and Dickinson 2015; Soucek et al. 2023), new toxicity studies indicate that mayflies (*Neocloeon triangulifer*) are the most sensitive taxa to acute and chronic PFOS exposures. This finding is consistent with other chemical exposures, including major geochemical ions, pesticides, and heavy metals (Johnson et al. 2015; Kim et al. 2012; Raby et al. 2018; Soucek and Dickinson 2015; Soucek et al. 2020; Soucek et al. 2023; Wesner et al. 2014). The high sensitivity of mayflies to contaminant exposures has also been observed in mesocosm-based experiments (Mebane et al. 2020) and field-based surveys (Cormier et al. 2018; U.S. EPA 2011). Many of these laboratory-based toxicity tests used mayflies capable of adapting to laboratory settings, therefore it is hypothesized that mayfly species unable to survive in laboratory settings may also be even more sensitive to contaminant exposures than the relatively hardy mayfly species (e.g., *N. triangulifer*) now commonly being used to toxicity testing. Thus, inclusion of the mayfly toxicity data (Soucek et al. 2023) is important to ensuring the protectiveness of the PFOS aquatic life AWQC.

Publication: Soucek et al (2023)
Species: Mayfly, *Neocloeon triangulifer*
Genus: *Neocloeon*
EPA-Calculated EC_{10}: unable to fit a reliable model with significant model parameters; author-reported TRAP-based EC_{10} used

C.2.2 Second Most Sensitive Freshwater Genus for Chronic Toxicity: *Chironomus* (midge)

MacDonald et al. (2004) conducted larval sub-chronic partial-life cycle and chronic life-cycle tests to determine the effects of PFOS (potassium salt, 95% purity) on the midge, *Chironomus dilutus* (formally known as *Chironomus tentans*). The test was performed under renewal conditions over 10 days for the larval sub-chronic partial-life cycle test and 60 days for the chronic life-cycle test, with four of the twelve replicates terminated following 20 days of exposure to evaluate survival and growth. The tests followed the general guidance given by EPA-600-R99-064 (U.S. EPA 2000b) and ASTM E 1706-00 (ASTM 2002). These methods are for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates and have different exposure durations than those typically considered for invertebrate aqueous exposures, as well as different control survival requirements and recommendations. *C. dilutus* used for the tests were 10-day old larvae for the 10-day exposure (larval sub -chronic partial-life cycle test) and newly-hatched larvae at test initiation for the chronic life-cycle test (both 60-d and 20-day exposures). Dilution water was reconstituted hard water consistent with ASTM (2002) with unspecified total hardness, but typically 160-180 mg/L as $CaCO_3$ (based on ASTM 2002), with alkalinity 110-120 mg/L as $CaCO_3$, and pH 7.6-8.0. The photoperiod was 16:8-hours light:dark. Light intensity was not reported. A primary stock solution was proportionally diluted with dilution water to prepare the test concentrations. Exposure vessels were 250 mL polypropylene beakers containing 240 mL of test solution and a sediment substrate. The 10-day exposure test employed at least two replicates with 10

individuals all of which were obtained from four large C-shaped egg cases that were distributed among seven test solutions plus a negative control. The life-cycle test (60-day exposure) employed 12 replicates of 12 midges each in five measured test solutions plus a negative control. From these 12 replicates, four were randomly terminated following a 20-day exposure to measured survival and growth endpoints (thereby referred to as the 20-day exposure henceforth) The remaining eight replicates were monitored over the test duration for emergence and reproduction. Nominal test concentrations for the 10-day test were 0 (negative control), 0.001, 0.005, 0.010, 0.020, 0.040, 0.080, and 0.150 mg/L. The nominal test concentrations for the 20-day exposure were 0 (negative control), 0.001, 0.005, 0.010, 0.050, and 0.100 mg/L. Mean measured concentrations for the 10-day test were 0 (LOQ not reported), 0.0008, 0.00460, 0.0115, 0.241, 0.0491, 0.0962, and 0.1501 mg/L, respectively. Mean measured concentrations for the 20-day exposure were 0 (LOQ not reported), 0.0023, 0.0144, 0.0217, 0.0949, and 0.149 mg/L, respectively. Analyses of test solutions were performed using LC-MS. The mean percent recovery and detection limits were not reported. Measured values of test concentrations in the 20-day exposure were 2 to 2.5-fold higher than nominal concentrations. Temperature and D.O. concentrations were measured in at least two replicates per treatment on a daily basis for the 10-day test and up to day 20 in the life-cycle test. Afterwards they were measured every other day (on alternate days from test solution renewal) from days 21 to 60 for the life-cycle test. The frequency of monitoring was reduced during this period, because both parameters consistently remained within acceptable ranges (21.0-23.0°C; D.O. >5.0 mg/L). Survival of negative control animals was >75%, which was considered acceptable for a full life-cycle exposure per ASTM (2002). The study authors reported EC_{10}s and NOECs; however, specific details pertaining to the

curve fitting process (including statistical output from the models and the curves) were not provided in the paper and therefore, limit independent interpretation of the toxicity values.

The observed effects of PFOS on *C. dilutus* reported in the paper by the study authors include survival and growth as weight (measured as mg of ash-free dry mass per individual) for both the 10-day and 20-day exposure durations and emergence and reproduction over the 60-day exposure duration. Significant reductions in larval weight were observed after 10 days of exposure to PFOS in the 0.0962 and 0.1501 mg/L treatment groups (roughly 0.38 and 0.19 mg, respectively) compared to the control group (roughly 0.88 mg). These differences resulted in roughly a 56.8 and 78.4% decline in midge weight in these treatment groups compared to those observed in the control. In contrast, there were no significant differences reported for survival between any of the PFOS treatments (with percent survival ranging between roughly 69.7% in the highest treatment group and 100% in the lowest) and the control (with roughly 100% survival). However, the authors noted that there was a 30% decline of midge survival in the highest PFOS treatment group with a measured concentration 0.1501 mg/L. The author-reported 10-day growth and survival EC_{10}s for the study were 0.0492 and 0.1079 mg/L, respectively. The study authors also reported NOECs of 0.0491 mg/L, LOECs of 0.0962 mg/L, and MATCs of 0.0687 mg/L for both endpoints.

Similar to the 10-day exposure results summarized above, there was a general decline in growth (as ash-free dry mass per individual) across the PFOS treatment groups (ranging roughly between 29.2 and 47.2% reduction compared to controls) in the 20-day exposure (chronic life-cycle test). However, only the decline in the 0.0949 mg/L treatment group was significantly different (roughly 0.29 mg) compared to the control (roughly 0.89 mg) and there was not a C-R relationship across the PFOS treatment groups. Additionally, midge survival was reduced after

20 days of exposure to PFOS in the 0.0949 and 0.149 mg/L treatment groups (29.2 and 0% survival, respectively) compared to the control (75% survival). Survival was determined to be not significantly different across the rest of the PFOS treatment groups (ranging roughly between 56.5 and 75% survival) compared to the control. However, it should be noted that there was a 25% decline in survival in the 0.0217 mg/L PFOS treatment group compared to the control that was determined not to be significantly different. The author-reported 20-day EC_{10}s for growth, survival, and total emergence were 0.0882, 0.0864, and 0.0893 mg/L, respectively, and the study authors also reported NOECs of 0.0217 mg/L for growth and survival and < 0.0023 mg/L for emergence, LOECs of 0.0949 mg/L for growth and survival and 0.0217 mg/L for emergence, and MATCs of 0.0454 mg/L for growth and survival and 0.0071 mg/L for emergence. Also, it should be noted, the paper reports contrasting NOECs for 20-day survival. The text in the paper stated that the NOEC was 0.0217 mg/L for growth and survival and Table 2 of the paper stated 0.0949 mg/L. The EPA assumed the NOEC in Table 2 of the paper was not correct and that 0.0217 mg/L was the correct NOEC based on the data presented in Figure 3A of the paper. This assumption was applied to the summary of the study results presented in this document.

Independent statistical analyses were conducted for both the 10-day (larval sub-chronic partial-life cycle test) and 60- and 20-day (chronic life-cycle test) exposure durations using data that were estimated (using Web plot digitizer) from the figures presented in the paper. The EPA could not fit a curve to independently verify the 10-day survival (due to a lack of a specific sample size for this endpoint as the number of replicates was not stated in the paper; however, the number of replicates was between two and four and the EPA sought to obtain clarification and treatment level data from the study authors) or the 20-day growth toxicity values (due to a lack of an observed C-R for this endpoint). However, the EPA-calculated 10-day EC_{10} for

growth was 0.05896 mg/L, which was slightly higher than the growth-based EC_{10} of 0.0492 mg/L reported in the paper. The chronic life-cycle EC_{10}s for larval survival (following 20 days of exposure) and emergence (with 60 days of exposure) were 0.0171 and 0.0102 mg/L, respectively. These chronic life-cycle EC_{10}s were much lower than those reported in the paper of 0.0864 and 0.0893 mg/L, respectively. The EC_{10}s for survival and emergence endpoints from the chronic life-cycle test were not considered to be reliable endpoints at this time given the disparities in the calculated EC_{10}s and the level of data that was presented in the paper, which made independent verification of the toxicity values less accurate. Specifically, for the 20-day survival endpoint, there appeared to be overdispersion (i.e., observed data display a larger variability than would be expected given an assumed statistical distribution about the mean response) in the data as it was presented in the paper (in Figure 3A of the paper), which adds uncertainty around the independently-calculated EC_{10} of 0.0171 mg/L and may explain the disparity between the reported EC_{10} and the EPA's independently-calculated value. As for the 60-day emergence endpoint, there was a lack of a C-R relationship and there were very similar levels of observed effects (which ranged between 42.6 and 50.1%) despite the more than nine-fold increase in the mid-range treatment concentrations (0.0023, 0.0144, 0.0217 mg/L, respectively). Lastly, the toxicity values from the observed effects from the chronic life-cycle exposure were considered to be less certain given the relatively large difference between the nominal and measured concentrations for this test. The dosing of the chronic life-cycle test (20- and 60-day exposure) was more of a concern than the larval sub-chronic partial-life cycle test (10-day exposure), which had measured concentrations that were much more in line with the expected nominal concentrations. Thus, the survival and emergence endpoints from the chronic life-cycle test were not considered for quantitative use in the derivation of the freshwater chronic

water column criterion. Instead, these endpoints were considered as supporting information until detailed replicate level data can be obtained from the study authors.

The most sensitive endpoint from the remaining toxicity values that could be independently calculated was for 10-day growth with an EC_{10} of 0.05896 mg/L. As mentioned in the Bots et al. (2010) summary in Section C.2.4 below, the observed effects of PFOS on aquatic insects appears to be consistent across the available data for chironomids and odonates. However, Bots et al. (2010) did not measure the effects of PFOS on nymph growth and therefore, the observed effects in MacDonald et al. (2004) on larval weight cannot be compared across the two studies. The EC_{10} of 0.05896 (0.05769 – 0.06023) mg/L for 10-day growth was used quantitatively to derive the freshwater chronic water column criterion. The remainder of the toxicity values were used as supporting information to corroborate the toxicity value used and to better understand the effects of PFOS on aquatic insects.

McCarthy et al. (2021) conducted a 10-day range-finding toxicity test and a separate 20-day (note, based on age of starting organisms, this test was actually 16 or 19 days of exposure) "abbreviated partial-life cycle" toxicity test with PFOS (98% purity, purchased from Sigma-Aldrich) on the midge, *Chironomus dilutus*. PFOS stock solution was dissolved in reconstituted moderately hard water without the use of a solvent and stored in polyethylene at room temperature until use. Two chronic exposures with PFOS were run, a 10-day and a 20-day exposure, following standard protocols (U.S. EPA 2000b) with slight modifications. The 10-day exposure was considered a range-finding test, with concentrations spaced by ~100x and only mortality measured, whereas the 20-day exposure measured both survival and growth. The 20-day exposure is less than the recommended 65-day full-life cycle method outlined in U.S. EPA (2000b) and since exposures of midges started on day two or four the actual exposure duration is

only 16 or 19 days long; therefore, the study authors referred to this test as an "abbreviated partial-life cycle test". Exposure vessels for both experiments were 1 L high-density polyethylene beakers containing natural-field collected sediment. The 10-day exposure had 60 mL of sediment and 105 mL of test solution and the 20-day exposure had 100 mL of sediment and 175 mL of test solution. PFOS in test solutions was added via pipette to the beakers with the tip just above the sediment substrate. Nominal test concentrations for the 10-day and 20-day exposure were 0, 0.0004086, 0.33, 33, 100 and 350 mg/L PFOS and 0, 0.001, 0.005, 0.01, 0.05 and 0.1 mg/L PFOS, respectively. Egg cases were obtained from outside suppliers (Aquatic Biosystems or USGS Columbia Environmental Research Center) and held for 10 days in the 10-day test or held for four days before testing in the 20-day exposure (in test vessels). In the 20-day exposure the test organism age (four-day old larvae) was greater than the protocol recommendation (< 24 hour) because earlier experiments had control survival issues (< 70%). In both tests each beaker held 12 organisms with five replicates per exposure treatment. Solutions were renewed every 48 – 72 hours in the 10-day exposure and daily for the 20-day exposure. Water samples of test concentrations were measured on day one and day 10 in the 10-day exposure and day 10, 15 and 20 in the 20-day exposure. In the 10-day exposure measured test concentrations ranged from 7 – 62% of nominal. In the 10-day exposure, the author-reported LOEC, based on mortality, of 0.4086 µg/L (0.0004086 mg/L PFOS) is reported as a nominal concentration. Mean PFOS concentrations in the 20-day exposure were 0 (control), 0.000447, 0.00209, 0.0042, 0.0231 and 0.0463 mg/L PFOS. Results of the *C. tetans* 16- to 19-day "abbreviated full life cycle test" were used preferentially over the results of the 10-day range-finding test to inform the chronic sensitivity of *C. tetans* because: (1) the 10-day range-finding test only measured survival, (2) the 10-day range-finding test had exposure concentrations that

differed by up to a factor of 100, which make C-R modeling more difficult than the dilution series in the16- to 19-day test, and (3) the 16- to 19-day test was a longer exposure duration that was more akin to a full life-cycle test. Consequently, the results of the 10-day range finding test were not used quantitatively to derive the freshwater chronic water column criterion, but they were retained for qualitative use.

From the 16- to 19-day "abbreviated full life cycle test" percent survival in the control and lowest test concentration were 77% with no survivors reported in the highest two test concentrations. The most sensitive endpoint appeared to be survival with an author-reported 16-day reported EC_{10} of 1.36 µg/L (0.00136 mg/L PFOS). Additionally, the authors reported EC_{10}s of 1.62 µg/L (0.00162 mg/L PFOS) and 3.23 µg/L (0.00323 mg/L PFOS) for growth as mean biomass and mean weight, respectively. The EPA was unable to independently calculate EC_{10}s for survival and mean weight. However, the 16- to 19-day independently-calculated EC_{10} value for mean biomass was 0.001588 (0.00118 – 0.00200) mg/L PFOS. This independently-calculated EC_{10} was acceptable for quantitative use and was used in the derivation of the freshwater chronic water column criterion.

Krupa et al. (2022) conducted a partial-life cycle chronic toxicity test with the midge, *Chironomus dilutus*, and PFOS-K (perfluorooctanesulfonate potassium salt, > 98% purity, CAS No. 2795-39-3, purchased from Sigma-Aldrich). *C. dilutus* egg masses obtained from Aquatic Biosystems (Fort Collins, Colorado, USA) were placed in 12-inch glass culture bowls (2 – 3 egg mases per dish) containing carbon-filtered municipal tap water and examined daily for viability and hatch. Hatching began after 2 days and larvae typically left the egg case 24-hours after the first hatch. The larvae were fed daily finely ground TetraMin® fish food flakes (~150 mg/dish as a slurry) with partial water changes as necessary until the larvae were the appropriate age for

initiating the test (4-days old). The larvae were exposed to PFOS for 16 days. A 100 mg/L PFOS-K stock solution was prepared by dissolving PFOS-K salt into ultrapure water using a stir bar and stir plate for >20-hours. Controls and PFOS solutions were then prepared using carbon filtered dechlorinated tap water. Each test concentration was individually spiked rather than serially diluted to reduce PFOS waste generated. All test solutions for water renewals were prepared the day before test initiation. The measured exposure concentrations were < LOD, 0.001, 0.0025, 0.004, 0.0075, 0.016 and 0.03 mg/L, with 5 replicates of 12 animals per replicate for each concentration. Tests were conducted in 300 mL polycarbonate beakers containing approximately 200 mL of exposure solution under a 16:8-hour light:dark cycle in an environmental chamber maintained at 22.5 ± 1°C. Each beaker received 50 mL of clean silica sand (250 – 500 µm) as substrate. Aeration was provided and larvae were fed finely ground TetraMin® (6 mg/day, added as a slurry in dechlorinated water). Partial water renewals (150 mL of solution was exchanged) were conducted three times per week, after 48 or 72-hour periods. At test termination, larval survival was assessed, and ash-free dry weight (AFDW) was determined following ASTM (2019). The AFDW of five groups of 12 larvae was measured at test initiation to establish a baseline for growth. The temperature, D.O., pH, and total hardness test values ranged from 22.0 - 22.9°C, 7.29 - 8.05 mg/L, 7.28 - 7.75 SU and 52 - 62 mg/L as $CaCO_3$, respectively. Water samples for verification of PFOS concentrations were collected at test initiation (day 0) and termination (day 16), in addition to before (out-water) and after (in-water) every renewal of test solution. Water samples from days 0, 6, 10, and 16 were analyzed to verify PFOS concentrations. The author-reported 16-day growth EC_{10} was 0.0015 mg/L PFOS-K. The EPA was unable to fit a reliable model for any of the chronic endpoints from this test. Therefore,

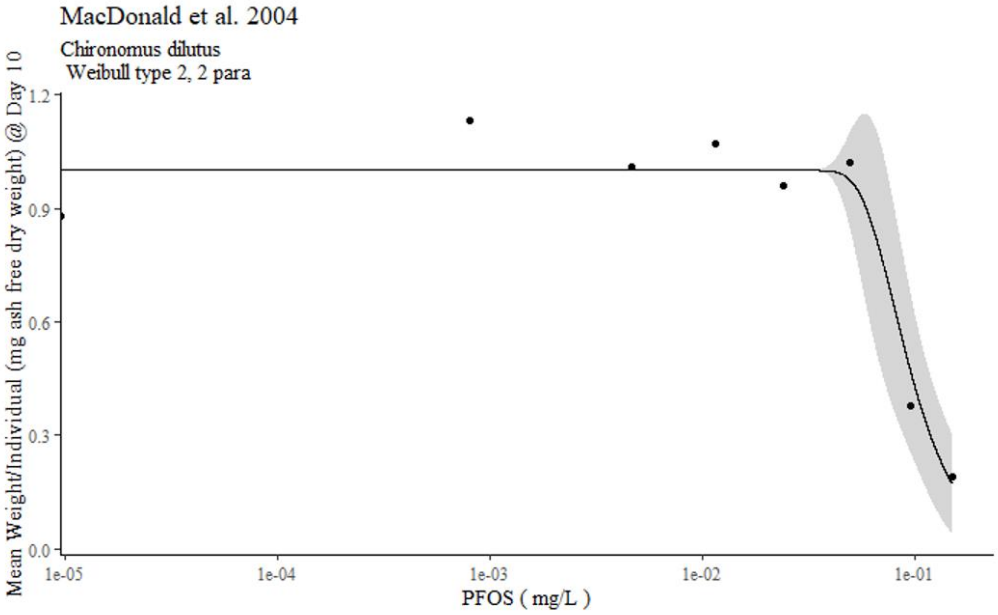
the author-reported EC_{10} value of 0.0015 mg/L for growth was used to derive the freshwater chronic water column criterion.

C.2.2.1 MacDonald et al. (2004) Concentration Response Curve – Chironomus (midge)

Publication: MacDonald et al. (2004)
Species: Midge (*Chironomus dilutus*)
Genus: *Chironomus*
EPA-Calculated EC_{10}: 0.05896 (95% C.I. 0.05769 – 0.06023) mg/L
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	-2.6770	0.6384	-4.1933	0.0057
e	0.0805	0.0090	8.9243	0.0001

Concentration-Response Model Fit:

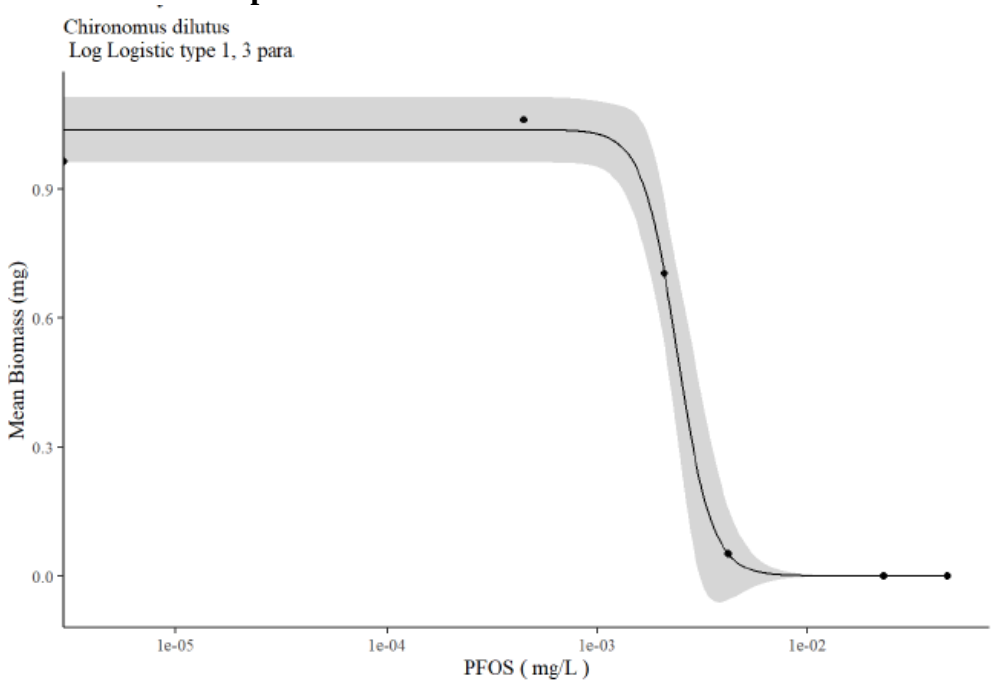


C.2.2.2 McCarthy et al. (2021) Concentration Response Curve – Chironomus (midge)

Publication: McCarthy et al. (2021)
Species: Midge *Chironomus dilutus*
Genus: *Chironomus*
EPA-Calculated EC_{10}: 0.001588 (95% C.I. 0.00118 – 0.00200) mg/L
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	5.2881	1.0432	5.0693	0.0148
d	1.0372	0.0238	43.4942	$2.675\ e^{-5}$
e	0.0024	0.0001	21.9936	0.0002

Concentration-Response Model Fit:



C.2.2.3 Krupa et al. (2022) Concentration Response Curve – Chironomus (midge)

Publication: Krupa et al. (2022)
Species: Midge, *Chironomus dilutus*
Genus: *Chironomu*
EPA-Calculated EC_{10}: unable to fit a reliable model with significant model parameters; author-reported EC_{10} used

C.2.3 Third Most Sensitive Freshwater Genus for Chronic Toxicity: *Lampsilis* (mussel)

Hazelton (2013); Hazelton et al. (2012) conducted a test of the long-term effects of PFOS (acid form, > 98% purity) on glochidia and juvenile life stages from the mussel *Lampsilis siliquoidea*. To initiate the PFOS partial life-cycle test, brooding females were collected from Perche Creek, Missouri and shipped over night to the test laboratory. The length of time between collection from Perche Creek and shipment was not reported, and authors were unable to recall such details (R. Bringolf, personal comm.); however, the EPA did not believe storage, shipping, and handling compromised test results since study authors only relied on those mussels with >70% glochidia viability. Dilution water was dechlorinated tap water. Mean total hardness (47.5 ± 9.2 mg $CaCO_3$/L) and alkalinity (34.8 ± 4.1 mg $CaCO_3$/L) were measured by titration twice weekly (n = 8) prior to water changes. Replicates used for water quality measurements were changed daily to allow measurements from all four replicates every four days. For all treatments, water temperature ranged from 14.6 to 16.1°C, D.O. ranged from 6.1 to 7.3 mg/L, and pH ranged from 7.6 to 8.5, but did not differ across treatments. Photoperiod and light intensity were not reported. No details were provided regarding primary stock solution and test solution preparation. The test exposed brooding glochidia (in marsupia) for 36 days followed by a 24-hour exposure of free glochidia. Experiments were conducted in 3.8 L glass jars of unspecified fill volume. The 36-day *in marsupia* exposure test employed four replicates individually containing single brooding females for each of the two PFOS treatment groups plus the control. The *in marsupia* exposure was followed by a 24-hour free glochidia exposure consisting of a factorial design, such that free glochidia from the control group of the *in marsupia* exposure were divided between a control and the two PFOS treatments and the PFOS treatments were split into control and the same PFOS treatment group as the *in marsupia* exposure. This factorial design allowed for the comparison of PFOS effects in two different life stages. However, it

should be noted that glochidia were pooled from females within each *in marsupia* treatment group, and thus the influence of parental effects could be a confounding factor that cannot be separated from the PFOS effects. Nevertheless, the influence of the potential parental confounding factor was likely to be minimal compared to the effects of the PFOS exposures.

Nominal concentrations throughout the exposures were 0 (negative control), 0.001 and 0.100 mg/L. Mean measured concentrations were 0.00211 (negative control), 0.00452 and 0.0695 mg/L. Analyses of test solutions were performed at the U.S. EPA National Exposure Research Laboratory in Research Triangle Park, NC using HPLC/MS. Two standard curves were used to quantify PFOS water concentrations during the experiment: low range (0.00005, 0.00025, 0.0005, 0.00075, 0.001, 0.0025, 0.005 mg/L) and high range (0.001, 0.005, 0.010, 0.025, 0.050, 0.100, 0.150 mg/L). Two replicate samples were measured at each standard concentration. Accuracy (recovery) of PFOS in the low-range standard curve ranged from 89.5 to 123% (n = 7) and for the high-range standard curve accuracy was 85.3 to 123% (n = 7). Adult mussel and glochidia survival in the negative control was 100% and > 90%, respectively. The study authors determined that the *in marsupia* exposure held the greatest weight of evidence and explained 78% of the variability in the glochidia viability (AIC = 22843, w_i = 0.78) and 83% of the metamorphosis success (AIC = 21955, w_i = 0.83), and therefore it appeared that the data presented in the study are in terms of the *in marsupia* exposure alone and there are no data presented in terms of the factorial design during the 24-hour free glochidia exposure. Additionally, the specific treatment groups of the data presented in the paper are unclear in terms of the factorial design during the 24-hour free glochidia exposure (e.g., it is unclear if the data presented in Figure 2 of the paper are lumped according to marsupial exposure, reducing seven

treatments to three, or if only the data in which the *in marsupia* and free glochidia exposures were the same are presented).

The test resulted in an author-reported NOEC of 0.0695 mg/L, which was associated with a 38% reduction in the viability of free glochidia at 24 hours post removal from females, a point when control viability of free glochidia was > 80% (author reported LOEC and MATC > 0.0695 mg/L). While a 38% reduction was observed at the NOEC (0.0695 mg/L) treatment group compared to controls, the authors reported this reduction was not statistically different from the control. Over time, the study authors reported significant reductions in free glochidia survival between three- and seven-days post removal from females, indicating a potential LOEC < 0.0045 mg/L. However, it should be noted that the observed level of effect between the two PFOS treatment groups (0.0045 and 0.0695 mg/L) were extremely similar despite the 15-fold difference between treatment groups. Additionally, in accordance with the decision rule described in Section 2.10.3.2 and a study by Bringolf et al. (2013), only glochidia toxicity data within 24 hours and with survival of at least 80% in the control treatment would be considered (U.S. EPA 2013). These specific data requirements ensured that the related effects of PFOS exposure to the viability of glochidia were consistent with environmental exposures during this short life stage and also take the unique life cycle of mussels into account. Therefore, the chronic toxicity value for viability of free glochidia at 24 hours following removal from females resulted in a NOEC of >0.0695 mg/L, which is an uncertain value and indicated that viability of free glochidia at 24 hours was a less sensitive endpoint.

In contrast, the data presented in the paper for metamorphosis success suggest a NOEC of 0.0045 mg/L and a LOEC of 0.0695 mg/L, or MATC = 0.01768 mg/L. The reduction in metamorphosis success at the LOEC was estimated to be 35.4%. However, as there were only

two PFOS treatment groups and the gap in these exposure concentrations is large (about 15-fold), the EPA was not able to fit a curve to estimate an EC_{10} in a manner similar to the other toxicity studies used to derive the freshwater chronic water column criterion. Instead, both the use of an MATC and an estimated EC_{10} were considered for the chronic value. An EC_{10} was estimated by assuming the 0.0695 mg/L treatment represents an $EC_{35.4}$ and estimating the EC_{10} using the exposure response slope from another chronic PFOS toxicity study focused on another mussel species (*Perna viridis*). Specifically, the chronic exposure of *Perna viridis* reported by Liu et al. (2013), which is summarized in Section 3.1.1.4.1 and D.2.1, was used to derive a ratio of $EC_{10}/EC_{35.4}$ values from that study equal to: $EC_{10}/EC_{35.4} = 0.0033/0.0186 = 0.1774$. Applying this ratio to Hazelton et al. (2012) yields an estimated EC_{10} of 0.0123 mg/L. Given the similarity between this EC_{10} and the author-reported MATC for metamorphosis success of 0.01768 mg/L, the latter was used to derive the freshwater chronic water column criterion. While this MATC is currently used quantitatively to derive the chronic water column criterion, the EPA hopes to further refine this estimated EC_{10} by obtaining the treatment level data from the study authors and exploring additional exposure response slopes from the study-specific dataset.

C.2.3.1 Hazelton et al. (2012) Concentration Response Curve – Lampsilis (mussel)

Publication: Hazelton et al. (2012)
Species: Fatmucket, *Lampsilis siliquoidea*
Genus: *Lampsilis*
EPA-Calculated EC_{10}: 0.0123 mg/L
Concentration-Response Model Fit: Concentration-response data not available
Value used Quantitatively in Criterion: Author-reported MATC of 0.01768 mg/L

C.2.4 Fourth Most Sensitive Freshwater Genus for Chronic Toxicity: *Enallagma* (damselfly)

Bots et al. (2010) conducted a 320-day partial life-cycle study under renewal test conditions to assess the effects of PFOS (tetraethylammonium salt, 98% purity) on the damselfly

Enallagma cyathigerum. Test organisms were obtained by collecting mature female *E. cyathigerum* all from the same location near the edge of a fen (a groundwater fed wetland) in northern Belgium. After collection, females were transported to the laboratory in small cages and housed in oviposition chambers for 24 hours before eggs were collected. *E. cyathigerum* used for the test were newly-hatched nymphs at test initiation. Dilution water was dechlorinated tap water that contained only a negligible concentration of PFOS (2.64 ng/L) and no other water quality parameters from the tap water were provided other than pH ≥7.5. Photoperiod was 16:8-hours light:dark in a climate room. Light intensity was not reported. Test solutions were prepared taking purity into account. To start the test, a total of 18,552 eggs were distributed amongst 150 exposure chambers (i.e., petri dishes of unreported size and material type). The distribution of the total number of eggs consisted of the entire clutch from each of the 30 females being divided into five subsamples, which were then randomly allotted to the various test treatments; thereby ensuring that each treatment group consisted of an even distribution of test organisms from the 30 females. After hatching, a total of 7,938 nymphs continued to be exposed (10 individuals per cup of unreported size and material type). After 10 days, seven nymphs for every female and treatment were monitored (resulting in a total of 741 nymphs). Nominal concentrations were 0 (negative control), 0.01, 0.1, 1.0, and 10 mg/L. Actual test concentrations were not measured. All nymphs were housed (and presumably tested) in a climate room at 21°C. Water quality (pH, carbonate and total water hardness, O_2, NO_2, and NO_3 levels) was checked weekly using standard aquarium tests, but values are not reported. Approximately 40% of the nymphs in the control treatment died during the first 60 days and similar mortality levels were observed in the other treatments. Additionally, it appears that control survival plateaued between 60 and 200 days, with 82.57% of the remaining nymphs in the control treatment surviving during this time,

indicating that survival settled out during this phase of the experiment. The initial drop in nymph survival can likely be attributed to the handling of the test organisms between the various phases of the experiment. This would explain the observed plateau between 60 and 200 days, as the nymphs were not handled during this time. The observed control mortality in this test was consistent with other odonate tests and excessive mortality of nymphs is typically expected within the first 200 days given the difficulty in maintaining odonates in a lab setting (Abbott and Svensson 2007; Rice 2008). Therefore, the observed control survival for this study was considered within the acceptable range for this species up to the 200-day exposure duration. Further, the control survival observed in this study was largely consistent with the toxicity testing guidelines for chironomids (requiring 70% control survival)(ASTM 2002; U.S. EPA 2002), which represent the only test guidelines for an emergent aquatic insect species as similar test guidelines for odonates are not available. Therefore, considerations regarding the use of these data for chronic water column criterion derivation was based on best scientific judgement and were restricted to the first 200 days of the experiment. After 200 days, nymph survival in the control and the PFOS treatments decreased. This drop in survival likely coincided with metamorphosis. However, control survival at the end of the exposure duration was only roughly 40% of the starting nymphs and therefore, survival after 200 days of exposure was not considered a viable test endpoint for this particular study.

The other possible observed effects of PFOS on *E. cyathigerum* reported by the authors included decreased survival over the exposure duration and decreased metamorphosis success. Nymph survival after five days did not differ between the control, 0.01 and 0.100 mg/L treatments and was significantly lower in the 1.0 and the 10.0 mg/L treatments. After 10 days of exposure, 80% of the nymphs in the 1.0 mg/L treatment and all nymphs in the 10 mg/L treatment

died. After 20 days of exposure, all nymphs in the 1.0 mg/L treatment died. However, there was no observed statistical difference between the control and any of the other treatment groups during this exposure time through 120 days. Between 120 and 250 days of exposure there was not an observed difference in survival between the control and the lowest treatment group (0.01 mg/L). In contrast, nymph survival in the 0.100 mg/L treatment group started to decrease compared to the control and the 0.01 mg/L treatment group, with 60% survival in the control compared to 48.5% survival in the 0.100 mg/L treatment after 150 days of exposure. This decrease was statistically significantly different from controls. All nymphs in the 0.100 mg/L treatment group died within 250 days of exposure. While nymph survival in the control was roughly 40% at the end of the 320-day exposure duration, there was no observed difference between the control and the lowest treatment group of 0.01 mg/L. Lastly, the paper also reported observed effects of PFOS on metamorphosis success stating that metamorphosis success was lower with 75.5% success in the 0.01 mg/L treatment (the only treatment group to have nymphs survive to this life stage) compared to the control with 92.5%. However, data for this observed endpoint was not provided in the paper beyond the percentages observed in the control and 0.01 mg/L PFOS treatment group. The specific sample sizes for this endpoint were difficult to ascertain from the paper as only the total number of test organisms across all test treatments was provided.

As indicated in the summary of the results above, toxicity values through the experiment decline with exposure duration. The EPA took all of the author-reported toxicity values between 10 (which was considered to be the start of the chronic exposure) and 200 days of exposure into account. Independently-calculated EC_{10} values could not be determined given the level of data that were presented in the paper. Author-reported toxicity values after 10 days of exposure were

a NOEC of 0.1 mg/L and a LOEC of 1.0 mg/L. The LOEC was associated with a 79% decrease in nymph survival compared to the control at this time. This NOEC and LOEC resulted in a MATC of 0.3162 mg/L. Author-reported toxicity values after 150 days of exposure were a NOEC of 0.01 mg/L and a LOEC of 0.1 mg/L. The LOEC was associated with a 19% decrease in nymph survival compared to the control at this time. This NOEC and LOEC resulted in a MATC of 0.03162 mg/L. Lastly, the authors also reported a NOEC of 0.01 mg/L for survival and an LOEC of < 0.01 mg/L for metamorphosis success after 320 days of exposure. Both of these toxicity values fell outside the 200-day exposure duration and were not considered for use in the freshwater chronic criterion calculation since control survival at this point was low (40%) and considered unacceptable for quantitative use. Additionally, there was insufficient data provided in the paper to evaluate the reported results for the endpoints at 320 days of exposure. Therefore, these toxicity values were considered as supporting information and only the toxicity values from 10 to 200 days of exposure range were considered further for chronic water column criterion derivation.

The 150-day MATC of 0.03162 mg/L for nymph survival was similar to the author-reported 10-day and 20-day survival and growth MATCs of 0.0687 and 0.0454 mg/L for chironomid (MacDonald et al. 2004), and these later toxicity values were therefore more comparable than the 10-day MATC of 0.3162 mg/L for nymph survival, which was focused on the effects of PFOS on a much earlier instar of odonate (which has a much longer development time and life span) in relation to the 20-day MATC of 0.0454 mg/L for chironomid. These results indicated that PFOS effects to the two aquatic insects was likely similar; however additional data are needed to fully understand the effects of PFOS to odonates. The MATC for nymph survival at 150-day reported above was used quantitatively to derive the chronic water column criterion.

Additionally, the EPA ran additional analyses with some of the other toxicity values for *E. cyathigerum* to understand the influence of this study on the overall chronic criterion (see Section 4.1).

C.2.4.1 Bots et al. (2010) Concentration Response Curve – Enallagma (damselfly)

Publication: Bots et al. (2010)
Species: Damselfly, *Enallagma cyathigerum*
Genus: *Enallagma*
EPA-Calculated EC_{10}: Not calculable, concentration-response data not available

C.2.5 Fifth Most Sensitive Freshwater Genus for Chronic Toxicity: *Danio* (zebrafsh)

Wang et al. (2011) evaluated the full life-cycle effects of PFOS (> 96% purity) on *Danio rerio* via a static-renewal study that reported nominal exposure concentrations. This test evaluated the effects of PFOS on a parental (F0) generation and included breeding trials to assess the effects of PFOS on an offspring (F1) generation exposed via maternal transfer. PFOS stock solutions were prepared in 100% dimethylsulfoxide (DMSO). Adult zebrafish (wild-type strain AB) were raised and kept at standard laboratory conditions of 28°C with a 14:10-hour light:dark cycle in a recirculation system according to standard zebrafish culture protocols. Water supplied to the system was filtered by reverse osmosis (pH 7.0-7.5), and Instant Ocean salt was added to the water to raise the conductivity to a range of 450 to 1,000 µS/cm (system water). Zebrafish embryos were obtained from spawning adults in tanks overnight with a sex ratio of 1:1. Embryos were collected within one hour after spawning and rinsed in embryo medium. High-quality 8-hpf embryos were divided into four treatment groups: DMSO vehicle control (0.01% v/v), and PFOS concentrations of 0.005, 0.050, and 0.250 mg/L. Embryos were first exposed to PFOS in a petri dish (100 embryos/treatment) for five days without media change, and all embryos hatched and survived in this stage. After five days, the fish were transferred into 2 L tanks for the period of 5-dpf to 30 dpf, and after that were raised in 9 L tanks (30 fish/tank) until the end of the

experiment, 150 dpf. Fish were kept in a static system, and 50% water was renewed with freshly prepared solutions every five days. Each tank was checked for morbid fish on a daily basis, and water quality was monitored on a weekly basis. Feeding was initiated at day five. Between five and 14 dpf, fish were fed three times daily with zebrafish larval diet (Aquatic Habitats), and after 14 dpf they were fed twice daily with freshly hatched live *Artemia*. The experiment was repeated three times with embryos derived from different parental stocks. At the end of exposure period (150 dpf or five months), all fish were checked for their sex. However, the method used for determining sex, as either external morphology or genetic testing, was not stated in the paper. The EPA assumed external morphology was used and concluded that the effects on sex ratio may not be reliable since determining sex through external morphology in zebrafish is difficult. A subsample of 10 male and 10 female fish from each batch were also measured for standard body length and wet weight. Condition factor (K) was tabulated to determine their overall fitness, and sperm motility in male F0 fish was also determined after chronic PFOS exposure. The most sensitive endpoint was F0 parental male sperm density with a chronic value of <0.005 mg/L PFOS. However, as sperm density was not typically considered an apical endpoint and the reported effects of PFOS on sperm density did not translate to other reproductive effects (i.e., fertilization), this endpoint was not considered further. Instead, the most sensitive apical endpoint for the F0 generation was considered to be male growth (length and weight) with an author-reported MATC of 0.01581 mg/L PFOS. However, the EPA was unable to fit a C-R curve with significant model parameters for the male growth endpoints; and therefore, was unable to independently verify the reported toxicity value for the F0 generation.

Breeding trials were also carried out to produce F1 offspring. Six different crosses were employed between F0 females and males to incorporate both the exposure of the same treatment

groups throughout and crosses between the control and highest treatment group. Specifically, for the groups exposed to the same treatment throughout the experiment, females were paired with males in the same treatment group (DMSO control or PFOS-exposed concentrations of 0.005, 0.050, and 0.250 mg/L). For the crosses between the control and the highest treatment group, some females from the 0.250 mg/L PFOS treatment group were paired with males from the DMSO controls, and some females from the controls were paired with males from the 0.250 mg/L PFOS treatment group. For each of these crosses, eight randomly selected female fish were paired with four male fish in two separate spawning tanks with four females and two males per tank. Spawning was induced every other day for five days, and embryos were used for monitoring their developmental progress. All eggs from each spawn were evaluated for fertilization success. Percent fertilization was expressed as the number of fertilized eggs divided by total number of eggs. Fifty fertilized embryos from each spawn were further monitored for continuous development. Percent hatch was calculated at 72 hpf. Larvae were also assessed for their morphological appearance. Percent survival was monitored until 8 dpf. Surviving larvae at 5 dpf with normal morphology were further subjected to behavior assessment (larval swimming speeds were recorded when they responded to a 70-minute dark to light, 10-minute for each period, transition stimulation). Following the receipt of treatment level data from the study authors, the EPA independently calculated an EC_{10} value of 0.0165 (0.01267 – 0.02033) mg/L for F1 survival. While this EC_{10} has some uncertainty given the wide spacing (10x) of the treatment concentrations, this toxicity value was supported by others in the PFOS toxicity literature (see Section 4.3.2.1.1 and Appendix G).The independently-calculated EC_{10} value of 0.01650 mg/L value for F1 survival from this study was used to derive the freshwater chronic water column criterion.

Guo et al. (2019) evaluated the chronic effects of perfluorooctane sulfonate (PFOS solution of ~40% in water purchased from Sigma-Aldrich) to AB strain zebrafish (*Danio rerio*) males in a 21-day static-renewal, unmeasured study. Use of official test guidelines were not cited by the authors. Approximately 3.5-month-old male adult zebrafish were purchased from Taiyuan fish hatcheries in Shanxi Province, PR China. Prior to exposure, fish were acclimated for 15 days in a flow-through dechlorinated tap water system (<1% mortality during the holding period) with the following water quality characteristics and conditions: pH: 7.0-7.4, temperature: 28 ± 1°C and a 14:10-hour light:dark photoperiod. The fish were fed a commercially available adult zebrafish compound feed during both acclimation and exposure. Nominal concentrations of PFOS dissolved in dechlorinated tap water were reported to be 0 (control), 0.02, 0.04 and 0.08 mg/L. Three replicates were tested at each concentration. A total of 660 fish were divided equally among the four concentration groups. Water quality was maintained the same throughout the experiment as during acclimation, as well as to meet the following conditions: D.O. between 5 - 6 mg/L and total hardness 20.0 mg/L as $CaCO_3$. Exposure media was changed every three days and aquaria were cleaned during testing. On days 7, 14 and 21, 50 fish from each group were sacrificed, with 30 fish measured for length and body weight, while the other 20 dissected on ice to evaluate PFOS concentrations in the liver. The test fish had a mean body weight of 0.19 ± 0.03 g and a mean length of 2.5 ± 0.3 cm at test initiation. On day seven fish lengths ranged from >2 cm to <3 cm for all groups, and weights were >0.3 to <0.4 g for the control and 0.02 mg/L exposure. Mean fish weight measured for the 0.04 and 0.08 mg/L treatment groups were significantly different from the control group after 7 days. At days 14 and 21, the length of fish in the highest concentration (0.08 mg/L PFOS) was significantly different from the control group, and the same effect of PFOS on mean fish weight was observed at 14 and 21 days as

reported at seven days. Therefore, weight was the most sensitive endpoint at 21 days, with a NOEC and LOEC of 0.02 and 0.04 mg/L PFOS, respectively. No mortality was observed in any treatment. An independently-calculated EC_{10} could not reliably be estimated for mean fish weight as the data were sparse, was inconsistent with the author-reported toxicity values, and the confidence bands were wide. Instead, the EPA's independently-calculated EC_{10} based on mean body length (in cm) at 21 days was 0.06274 (0.06229 – 0.06318) mg/L PFOS and used quantitatively to derive the freshwater chronic water column criterion.

C.2.6 Sixth Most Sensitive Freshwater Genus for Chronic Toxicity: *Daphnia* (cladoceran)

Logeshwaran et al. (2021) conducted acute and chronic toxicity tests with the cladoceran, *Daphnia carinata*, and PFOS-K (perfluorooctancesulfonate potassium salt, ≥ 98% purity, purchased from Sigma-Aldrich Australia). In-house cultures of daphnids were maintained in 2 L glass bottles with 30% natural spring water in deionized water, 21°C and a 16:8-hour light:dark photoperiod. The chronic test protocol followed OECD (2012). A PFOS stock solution (20 mg/mL) was prepared in dimethylformamide and diluted with deionized water to achieve a concentration of 200 mg/L PFOS. Cladoceran culture medium was used to prepare the PFOS stock and test solutions. One daphnid (6-12 hours old) was transferred to 100 mL polypropylene containers containing 50 mL of nominal test solutions (0, 0.001, 0.01, 0.1, 1.0 and 10 mg/L PFOS). Each test treatment was replicated ten times with test solutions renewed and daphnids fed daily. At test termination (21 days) test endpoints included survival, days to first brood, average offspring in each brood and total live offspring. At the higher test concentrations (1 and 10 mg/L) reproduction was completely inhibited. No mortality occurred in the controls and lowest test concentration. However, reproduction was inhibited at the lowest test concentration. The author-reported 21-day NOEC and LOEC, based on average offspring in each brood and total live offspring, was < 0.001 and 0.001 mg/L PFOS, respectively. Additionally, the author-

reported 21-day NOEC and LOEC based on the days to first brood was 0.001 and 0.01 mg/L, respectively, resulting in an MATC of 0.003162 mg/L. The EPA could not independently calculate 21-day EC_{10} values for any of the endpoints given the level of data provided in the paper by the study authors. And while the endpoints of mean offspring per each brood and total living offspring appear to be more sensitive than the days to first brood, they result in less than LOECs of 0.001 mg/L and are not consistent with other chronic toxicity values for this species. Therefore, the author-reported MATC of 0.003162 mg/L for the days to first brood was used to derive the freshwater chronic water column criterion.

Exclusion of the *D. carinata* SMCV under the basis of being an overly sensitive outlier (relative to *D. magna* and the chronic data overall except for aquatic insects [*N. triangulifer* and *C. dilutus*]) had the possibility that the *Daphnia* GMCV could be underproductive. Conversely, excluding the *D. magna* SMCV under the basis of being a tolerant outlier (relative to *D. carinata*) would result in the *Daphnia* GMCV being highly influenced by a single test/species with a relatively sensitive chronic value. The *D. carinata* chronic value was also an MATC, calculated as the geometric mean of the NOEC (0.001 mg/L) and LOEC (0.01 mg/L) from a 10X dilution series, meaning the MATC was influenced by a relatively low NOEC. However, offspring-based endpoints reported by (Logeshwaran et al. 2021) suggest a LOEC of <0.001 mg/L. Using both the *D. magna* and *D. carinata* SMAVs resulted in protective a *Daphnia* GMCV based on the *Daphnia* data as a whole. Finally, the chronic PFOS freshwater criterion (i.e., 0.00025 mg/L) is four times lower than the offspring-based LOECs (i.e., 0.001 mg/L) reported by (Logeshwaran et al. 2021) and should be protective of *D. carinata* based on all endpoints measured by (Logeshwaran et al. 2021).

Drottar and Krueger (2000e) reported the results of a life-cycle, 21-day renewal, measured test of PFOS (potassium salt, CAS # 2795-39-3, 90.49% purity) with *Daphnia magna*. The GLP test was conducted at Wildlife International, Ltd. in Easton, MD in February, 1999. The test followed (U.S. EPA 1996c). *D. magna* used for the test were less than 24 hours old at test initiation. Dilution water was 0.45 µm filtered and UV sterilized well water [total hardness: 124 (120-128) mg/L as $CaCO_3$; alkalinity: 169 (164-172) mg/L as $CaCO_3$; pH: 8.2 (8.0-8.3); TOC: <1.0 mg/L; and conductivity: 329 (315-340) µmhos/cm]. Photoperiod was 16:8-hours light:dark with a 30 minute transition period. Light was provided at an intensity of 329-383 lux. A primary stock solution was prepared in dilution water at 46 mg/L. It was mixed until all test substance was dissolved prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the five additional test concentrations. Exposure vessels were 250 mL plastic beakers containing 200 mL of test solution. The test employed 10 replicates of one daphnid each in six measured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 1.4, 2.9, 5.7, 11, 23, and 46 mg/L. Mean measured concentrations were < 0.458 mg/L (the LOQ), 1.5, 2.9, 5.6, 12, 24, and 48 mg/L, respectively. Analyses of test solutions were performed at Wildlife International Ltd. using HPLC/MS. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 104%. Measured values of new samples ranged from 94 to 121% of nominal. Measured values from the old solutions ranged from 90 to 108% of nominal. PFOS was stable throughout the renewal periods. Dissolved oxygen in new and old test concentrations ranged from 8.3-8.9 mg/L in the negative controls and 8.3-9.0 mg/L at the NOEC of 12 mg/L. Similarly, pH ranged from 8.1-8.4 and 8.2-8.5, respectively, and test temperature from 19.4-20.1°C (negative control and at the NOEC). Cumulative young in the 1.5, 2.9, 5.6, 12, and 24 mg/L treatment groups was 100, 100,

100, 90, and 0%, respectively. After 48 hours, cumulative young of the second generation in the negative control was 95%. The 21-day NOEC (survival, growth, and reproduction) was 12 mg/L. The 21-day LOEC was 24 mg/L and the calculated MATC is 16.97 mg/L. No second-generation *D. magna* survived the 24 mg/L treatment. The independently-calculated EC_{10} based on cumulative young was 11.19 (10.50 – 11.89) mg/L and used quantitatively to derive the freshwater chronic water column criterion.

Boudreau (2002) also conducted a chronic life-cycle 21-day renewal, unmeasured test of PFOS (potassium salt, CAS # 2795-39-3, 95% purity) with *Daphnia magna* as part of a Master's thesis at the University of Guelph, Ontario, Canada. The results were subsequently published in the open literature **Boudreau et al. (2003a)**. The test followed ASTM (1999a). *D. magna* used for testing were less than 24 hours old at test initiation. *D. magna* were obtained from a brood stock (Dm99-23) at ESG International (Guelph, ON, Canada). Dilution water was clean well water. Hardness was softened by addition of distilled deionized water to achieve a range of 200-225 mg/L of $CaCO_3$. Photoperiod was 16:8-hours light:dark under cool-white fluorescent light between 380 and 480 lux. Laboratory-grade distilled water was used for all solutions with maximum concentrations derived from stock solutions no greater than 450 mg/L. Test vessels consisted of 225 mL polypropylene disposable containers containing 120 mL of test solution. All toxicity testing involved four replicates of three daphnids each in five nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 6, 13, 25, 50, and 100 mg/L. The test was conducted in environmental chambers at 21 ±1°C. Authors noted that temperature and pH were measured at the beginning and end of study, but the information was not reported. Survival of daphnids in the negative control was 100%. The 21-day NOEC (survival and reproduction) was 25 mg/L. The 21-day LOEC was 50 mg/L and the

calculated MATC is 35.36 mg/L. The independently-calculated EC_{10} based on survival was 16.35 (7.377 – 25.33) mg/L and was used quantitatively to derive the freshwater chronic water column criterion.

Ji et al. (2008) conducted chronic life-cycle tests of the effects of PFOS (acid form, CAS # 1763-23-1, purity unreported) on *Daphnia magna*. Tests were done under renewal conditions over a 21-day period. The test followed OECD (1998). *D. magna* used for testing were obtained from brood stock cultured at the Environmental Toxicology Laboratory at Seoul National University (in South Korea). Organisms were less than 24 hours old at test initiation. Dilution water was moderately-hard reconstituted water (total hardness typically 80-100 mg/L as $CaCO_3$). Experiments were conducted in glass jars of unspecified size and fill volume. Photoperiod was assumed to be 16:8-hours light:dark as was used for daphnid culture. Preparation of test solutions was not described. The test involved 10 replicates of one daphnid each in five nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 0.3125, 0.625, 1.25, 2.5, and 5 mg/L. Test temperature was 21 ±1°C. Authors noted water quality parameters (pH, temperature, conductivity, and D.O.) were measured after changing the medium, but the information was not reported. Survival of daphnids in the negative control was 100%. The author reported *D. magna* 21-day NOEC for the reproductive endpoint of number of young per brood was 1.25 mg/L. The author reported 21-day LOEC for the same endpoint was 2.5 mg/L. The calculated MATC was 1.768 mg/L. In the independent verification of the toxicity values, the EPA recalculated the reproductive endpoint noted to be the number of young per brood. This recalculated reproductive endpoint took the full effects of PFOS into account as it was representative of the full life cycle. The calculated EC_{10} for *D. magna* was 1.051 (0.2680 –

1.834) mg/L. The independently-calculated EC_{10} of 1.051 mg/L was used quantitatively to derive the freshwater chronic water column criterion.

Li (2010) conducted a chronic life-cycle 21-day test on the effects of PFOS (potassium salt, >98% purity) on *Daphnia magna*. The test followed OECD (1998). *D magna* used for the test were maintained in the laboratory for more than one year and were less than 24 hours old at test initiation. Dilution water was distilled water with ASTM medium (0.12 g/L $CaSO_4.2H_2O$, 0.12 g/L $MgSO_4$, 0.192 g/L $NaHCO_3$, and 0.008 g/L KCl – calculated total hardness 169 mg/L as $CaCO_3$). Photoperiod was 16:8-hours light:dark at an unreported light intensity. A primary stock solution was prepared in dilution water and did not exceed 400 mg/L. Exposure vessels were 50 mL polypropylene culture tubes with 50 mL fill volume. The test involved 10 replicates of one daphnid each in five nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 0.5, 1, 5, 10, and 20 mg/L. Test temperature was maintained at 20 ±1°C. Water quality parameters measured in test solutions were not reported. Survival of daphnids in the negative control was 96.7%. The *D. magna* 21-day NOEC (reproduction – no. young per female) was 1 mg/L. The 21-day LOEC was 5 mg/L and the calculated MATC was 2.236 mg/L. The independently-calculated toxicity value (EC_{10}) based on total neonates per female was 3.030 (-1.280 – 7.340) mg/L and was used quantitatively to derive the freshwater chronic water column criterion.

Yang et al. (2014) evaluated the chronic 21-day renewal, measured test of PFOS (potassium salt, CAS # 2795-39-3, 99% purity) with *Daphnia magna*. The test followed (ASTM 1993). *D. magna* used for the test were donated by the Chinese Research Academy of Environmental Sciences, and less than 24 hours old at test initiation. Dilution water was dechlorinated tap water (pH, 7.0 ± 0.5; D.O., 7.0 ± 0.5 mg/L; total organic carbon, 0.02 mg/L;

and total hardness, 190.0 ± 0.1 mg/L as $CaCO_3$). Photoperiod was 12:12-hours light:dark at an unreported light intensity. A primary stock solution was prepared by dissolving PFOS in deionized water and cosolvent DMSO. The primary stock was proportionally diluted with dilution water to prepare the test concentrations. Exposure vessels were 200 mL beakers of unreported material type containing 100 mL of test solution. The test employed 10 replicates of one daphnid each in six test concentrations (measured in low and high treatments) plus a negative and solvent control. Nominal concentrations were 0 (negative and solvent controls), 2.00, 2.60, 3.38, 4.39, 5.71 and 7.43 mg/L. Mean measured concentrations before and after renewal were 1.74 and 1.98 mg/L (lowest concentration) and 6.78 and 7.54 mg/L (highest concentration). Analyses of test solutions were performed using HPLC/MS and negative electrospray ionization. The concentration of PFOS was calculated from standard curves (linear in the concentration range of 1-800 ng/mL), and the average extraction efficiency was in the range of 70-83%. The concentrations and chromatographic peak areas exhibited a significant positive correlation ($r=0.9987$, $p<0.01$), and the water sample-spiked recovery was 105%. Test temperature was maintained at 22 ±2°C. The D.O. and pH were reported as having been measured, but results are not reported. Negative and solvent control survival was 100%. The *D. magna* 21-day EC_{10} for reproduction was reported to be 2.26 mg/L from the study authors and 4.17 mg/L for survival. The independently-calculated EC_{10} based on survival was 2.610 (1.291 – 3.929) mg/L and was used quantitatively to derive the freshwater chronic water column criterion.

Lu et al. (2015) conducted a chronic life-cycle 21-day renewal, unmeasured test of PFOS (purity 98%) with *Daphnia magna*. The test followed OECD (2012). *D. magna* used for the test were originally obtained from the Chinese Center for Disease Control and Prevention (Beijing, China) and cultured in the laboratory according to the International Organization for

Standardization (ISO 1996). Daphnids were less than 24 hours old at test initiation. Dilution water was the same used for daphnid culture and was reconstituted according to OECD (2004) with a total hardness of 250 mg/L as $CaCO_3$, as calculated based on the recipe provided, and pH ranging from 7.7 to 8.4. Photoperiod was 16:8-hours light:dark at an unreported light intensity. The test solution was prepared immediately prior to use by diluting the stock solution with culture medium. Exposure vessels were 100 mL glass beakers containing 45 mL of test solution. The test employed 20 replicates of one daphnid each in six nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 0.008, 0.04, 0.2, 1, and 5 mg/L. Exposure water quality was checked daily and maintained at 20 ±1°C, pH of 7.2 ±0.3, and D.O. of 5.3 mg/L. Negative control survival was 100%. The author reported *D. magna* 21-day NOEC (no. offspring per brood per female) was 0.008 mg/L and the 21-day LOEC was 0.04 mg/L. The calculated MATC was 0.0179 mg/L and the independently-calculated EC_{10} was 0.001818 (-0.0000395 – 0.003675) mg/L for the same endpoint. Other endpoints, including growth and other reproductive endpoints, could not be independently-calculated by the EPA. The independently-calculated EC_{10} from this study was acceptable for quantitative use to derive the freshwater chronic water column criterion.

Liang et al. (2017) conducted a chronic life-cycle 21-day renewal, unmeasured test of PFOS (potassium salt, CAS # 2795-39-3, ≥98% purity) with *Daphnia magna*. The test organisms were originally obtained from State Key Laboratory of Environmental Aquatic Chemistry (Eco-Environmental Sciences of Chinese Academy of Sciences, Beijing) and cultured in the laboratory according to Revel et al. (2015). Daphnids were less than 24 hours old at test initiation. Dilution water was artificial medium "M4 (Elendt)" at 20°C and pH 7. Photoperiod was 16:8-hours light:dark at an unreported light intensity. The test solution was prepared

immediately prior to use by diluting the stock solution with M4 medium. Exposure vessels were 80 mL glass beakers containing an unspecified volume of test solution. The test employed 10 replicates of one daphnid each in six nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 1, 2, 4, 8, and 16 mg/L. No mention was made of water quality being checked during the exposure. Negative control survival was 100%. The *D. magna* 21-day NOEC (days to 1st brood, intrinsic rate of natural increase, r) was 4 mg/L. The 21-day LOEC was 8 mg/L and the calculated MATC was 5.657 mg/L. The independently-calculated EC_{10} based on survival was 3.596 (2.1207 – 5.0704) mg/L and used quantitatively to derive the freshwater chronic water column criterion.

Yang et al. (2019) evaluated the chronic effects of perfluorooctane sulfonate, potassium salt (PFOS-K, CAS# 2795-39-3, 98% purity, purchased from Sigma-Aldrich in St. Louis, MO) on *Daphnia magna* via a 21-day unmeasured, static-renewal test that evaluated growth and reproductive effects. *D. magna* cultures were obtained from the Institute of Hydrobiology of Chinese Academy of Science in Wuhan, China. Organisms were cultured in Daphnia Culture Medium according to the parameters laid out in OECD Guideline 202 and all testing followed OECD Guideline 211. Cultures were fed green algae daily and were acclimated for two to three weeks before testing. The 21-day chronic study had nominal concentrations of 0 (control), 0.00000124, 0.00000188, 0.0000281 and 0.00000420 mol/L (or 0 (control), 0.6674, 1.012, 1.512, and 2.261 mg/L given the molecular weight of the form of PFOS used in the study, CAS # 2795-39-3, of 538.22 g/mol). Each neonate (12–24 hours old) was placed in a 100 mL glass beaker, in which there were 10 replicates, each filled with 80 mL of test solution maintained at 20 ±1°C and a 16:8-hour light:dark photoperiod with a light intensity maintained at 1000 - 1500 lux. *D. magna* were fed green algae and test solutions were renewed every 72 hours. Test

organisms were counted daily, with any young also removed. The author-reported NOEC and LOEC for reproduction (measured as mean offspring proportion relative to control at 21 days) was <0.6674 and 0.6674 mg/L PFOS, respectively. The author-reported NOEC and LOEC for growth (measured as length) was 0.6674 and 1.012 mg/L PFOS (MATC = 0.8218 mg/L). The independently-calculated EC_{10} values for reproduction and growth are 0.3773 and 0.9093 mg/L, respectively. However, the reproduction EC_{10} of 0.3773 mg/L was determined to be less statistically robust as the independently-calculated toxicity values were control normalized and could not be weighted given the level of data provided by the study authors in the paper. Therefore, the independently-calculated EC_{10} for growth of 0.9093 (0.7423 – 1.076) mg/L was used quantitatively to derive the freshwater chronic water column criterion freshwater.

Seyoum et al. (2020) evaluated the chronic effects of perfluorooctane sulfonic acid (PFOS, CAS# 1763-23-1, > 99%, purchased from Sigma) on *Daphnia magna* neonates via a 21-day unmeasured, static-renewal study. The study authors did not report following any specific protocol. *D. magna* ephippia were purchased from MicroBioTests Inc. (Belgium) and were activated by rinsing in tap water. Ephippia were hatched by incubating at 20-22 ºC for 72 to 90 hours in standard freshwater under a continuous light intensity (6,000 lux). Newly hatched neonates (<24-hour old) were fed a suspension of Spirulina micro-algae two hours before testing. Nominal concentrations of 0 (control), 1, 10 and 25 µM (or 0 (control), 0.5001, 5.001, and 12.50 mg/L given the molecular weight of the form of PFOS used in the study, CAS # 176-23-1, of 500.13 g/mol) were prepared by mixing the respective amounts of PFOS in dimethyl sulfoxide (DMSO). Ten <24-hour old neonates, exposed in triplicate, were placed into 250 mL crystallization dishes with 100 mL of test solution. A mean temperature of 23°C, D.O. of 8 to 9 mg/L, total hardness of 250 mg/L as $CaCO_3$, pH of 7.5 ±0.25 and salinity of 0.02% were

reported in the exposure water. *D. magna* were fed a mixture of *Spirulina* microalgae and yeast (*Saccharomyces cerevisiae*) daily during the test, and 50% of the test solution was changed every other day. Neonates were counted daily and removed. At day 21, neonate counts were reported to be highest in the control with >40 to < 60 neonates, and >20 to <40 neonates were reported at the 0.5001 and 5.001 mg/L (or 1 and 10 µM) concentrations, respectively. Neonate counts for the 12.50 mg/L (or 25 µM) concentration were not reported. A reproductive NOEC of 0.5001 mg/L and a LOEC of 5.001 mg/L were reported by the study authors, resulting in an MATC of 1.581 mg/L. This LOEC of 5.001 mg/L was associated with a 42.95% decrease in reproduction (measured as the mean number of daphnids at 21 days) compared to control. An independently-calculated EC_{10} value could not be determined as the EPA was unable to fit a model with significant parameters. Instead, the author-reported MATC of 1.581 mg/L PFOS was used quantitatively to derive the chronic water column criterion for freshwater.

C.2.7 Seventh Most Sensitive Freshwater Genus for Chronic Toxicity: *Salmo* (salmon)

Atlantic salmon, *Salmo salar*, embryos were evaluated by **Spachmo and Arukwe (2012)** via a 56-day unmeasured exposure to PFOS (98% purity). Eggs were obtained from Lundamo Hatcheries, Norway (Aquagen) and transported to the Norwegian University of Science and Technology Centre of Fisheries and Aquaculture in Trondheim, Norway. The eggs were kept in plastic tanks (25 L) at 5-7°C with filtered, re-circulating and aerated water. Approximately one-third of the water volume was changed once per week. The eggs and larvae were exposed to PFOS (100 µg/L) for 49 days representing the developmental period from 404 to 679-degree days. PFOS was dissolved in methanol (carrier solvent: 0.01%) and control group was exposed to the carrier solvent only. Hatching occurred at 20 calendar days after start of exposure, at an effective developmental age of 504-degree days, after which riverbed environment was simulated by tank bed gravel and continuous water flow. Fish sampling was performed at 21, 35,

49 and 56 calendar days after exposure, or at respective developmental ages of 549, 597, 679 and 721 degree days. The exposure was terminated at 679-degree days, and 712-degree days represents the end of a one-week exposure-free recovery period. Thus, day 49 sampling was performed 24 hours after terminating the exposure and no exposure related differences in hatching rate were observed. The 49-day growth NOEC and LOEC were 0.10 and >0.10 mg/L PFOS, respectively. These data are deemed quantitative and used to derive the freshwater chronic water column criterion.

C.2.8 Eighth Most Sensitive Freshwater Genus for Chronic Toxicity: *Pimephales* (minnow)

Drottar and Krueger (2000d), associated with Wildlife International, conducted a good laboratory practice (GLP) 47-day flow-through measured early life-stage toxicity test with <24-hour old *P. promelas* embryos. A primary stock solution was prepared by dissolving PFOS (90.49% purity) in dilution water at a concentration of 88.4 mg a.i./L, then proportionally diluted with dilution water to prepare five secondary stock solutions at concentrations of 44.2, 22.1, 11.0, 5.52 and 2.76 mg a.i./L. Stock solutions were prepared every three to four days during the test. The five stocks were injected into the diluter mixing chambers (at a rate of 6.0 mL/minute) where they were mixed with dilution water (at a rate of 116 mL/minute) to achieve the desired test concentrations. The water used for culturing and testing was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International Ltd. site. The well water was characterized as moderately-hard water. The well water was passed through a sand filter to remove particles greater than approximately 25 μm and then pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to delivery to the diluter system, the water again was filtered (0.45 μm), then passed through a UV sterilizer to remove microorganisms and particles. Fathead minnow embryos used in this test originated from cultures maintained by Wildlife International Ltd., Easton, MD. The embryos were removed

from spawning substrates and examined under the dissecting microscope to select healthy specimens at approximately the same stage of development. Embryos collected for use in the test were from six individual spawns. Embryos were exposed to a geometric series of six test concentrations and a negative (dilution water) control under flow-through conditions at 24.5°C, pH of 8.2, total hardness of 140 mg/L as $CaCO_3$ and a photoperiod of 16:8-hours light:dark. Four replicate test chambers (9 L glass aquaria) were maintained in each treatment and the control group. Each test chamber contained one incubation cup with 20 embryos, resulting in a total of 80 embryos per treatment. The exposure period included a five-day embryo hatching period, and a 42-day post-hatch juvenile growth period. Nominal test concentrations were 0.14, 0.29, 0.57, 1.1, 2.3 and 4.6 mg/L a.i. Mean measured test concentrations (0, 0.15, 0.30, 0.60, 1.2, 2.4 and 4.6 mg/L) were determined from samples of test water collected from each treatment and the control group at the beginning of the test, on day four, at weekly intervals during the test, and at test termination. To start the test, embryos less than 24 hours old were collected from cultures and groups of one and two individuals were impartially distributed among incubation cups until each cup contained 20 embryos. One cup was then placed in each treatment and control test chamber. Twice during the next twenty-four hours and daily thereafter, all dead embryos were counted and removed from the cups to avoid contaminating viable embryos. All eggs that remained were considered viable. Dead embryos continued to be removed daily. After hatching, the larvae were counted and released into the test chambers, where exposure continued until test termination. Observations of mortality and other clinical signs were made daily during the test. Time to hatch, hatching success, growth, and survival were monitored in each treatment and control group. The most sensitive 47-day chronic value (MATC) of 0.4243 mg/L PFOS was based on post-hatch survival as reported by the study authors. The independently-calculated EC_{10} based on survival

was 0.4732 (0.3308 – 0.6156) mg/L and used quantitatively to derive the freshwater chronic water column criterion.

Ankley et al. (2005) also exposed *Pimephales promelas* to PFOS (potassium salt, > 98% pure) under flow-through measured conditions for 21 days. Stock solutions were prepared by dissolving crystals in Lake Superior control water with stirring (mean measured test conditions: 25°C, pH of 7.3, total hardness of 46 mg/L as $CaCO_3$, alkalinity of 40 mg/L as $CaCO_3$ and D.O. of 6.2 mg/L). Two stock solutions of approximately 9.7 and 97 mg/L were used to span the desired range of target concentrations in test water. Final test concentrations were generated by appropriate dilution of the PFOS stocks with Lake Superior water and were supplied to the test tanks at a flow rate of approximately 45 mL/min. Sexually mature fathead minnows (six to seven months old) obtained from the on-site culture facility were used for the toxicity test. Eight pairs of fish (one male and one female) were exposed at each treatment level, 0 (control), 0.03, 0.1, 0.3, and 1.0 mg PFOS/L. Assays were conducted using glass aquaria containing 10 L of test solution, with two pairs of fish separated by perforated nylon screening in each tank. Reproductive viability of the fish used for the test was documented during a 27-day acclimation phase in the same tanks in which the tests were conducted. The number of eggs spawned by each pair was evaluated daily by inspecting the underside of a polyvinyl chloride spawning tile placed on the bottom of the test chambers. Egg fertility was assessed using an optical microscope. The animals were held at 25 ±1°C under 16:8-hour light:dark photoperiod and fed frozen brine shrimp to satiation twice daily. Conditions during the 21-day reproduction phase of the PFOS exposure were the same as during the acclimation phase. To evaluate possible early developmental toxicity of PFOS, 50 to 75 eggs from single viable spawns were collected during the final 7-day of the reproduction phase of the test. A subset of eggs was reserved for the

determination of PFOS concentrations. Embryos were held in 300 mL Pyrex beakers in the same aquaria as the parental fish. Embryos hatched within four to five days and thereafter were fed live brine shrimp twice daily. After 12 days, fry were randomly sampled for PFOS analysis and to reduce the number of animals per chamber to ≤30. Remaining fry were maintained in a larger chamber (1 L plastic container) within the original tank. Developing fish were inspected daily to assess survival. After 24 days, they were anesthetized and weighed. A subset of the fry was collected for PFOS measurements, while others were preserved in Bouin's fixative for histological analyses. The authors reported a 21-day EC_{50} (fecundity) of 0.23 mg/L PFOS, and a chronic value of 0.4794 mg/L PFOS for percent hatch (21-day), probability of survival, and larval weight endpoints (21-day (F0) + 24-day (F1)). The independently-calculated EC_{10} was 0.05101 (0.0408 – 0.0613) mg/L based on fecundity and used quantitatively to derive the freshwater chronic water column criterion.

Suski et al. (2021) reported the chronic toxicity of PFOS-K (perfluorooctancesulfonate potassium salt, CAS # 2795-39-3, ≥ 98%, purchased from Sigma-Aldrich) on the fathead minnow, *Pimephales promelas*. Adult (5-month old) fathead minnows were purchased from a commercial supplier (Aquatic Biosystems) and were sexually mature when the test was initiated. Fish were fed twice a day and held in dechlorinated tap water at test conditions (mean conditions: 24.96°C, D.O. of 7.68 mg/L, pH 7.9 and conductivity of 347.3 µS/cm). Stock solutions of PFOS (150 mg/L) were made without a solvent and prepared weekly with stock solutions shaken at 80 rpm for 24 hours to ensure mixing. Test solutions were made by diluting the stock with dechlorinated tap water and shaking the solutions for 10 minutes prior to water exchanges. Half of the total volume (10 L) in each exposure 5-gallon polycarbonate tank was renewed three times per week. Measured PFOS concentrations were 0.14 (control), 44, 88, 140

and 231 µg/L PFOS (or 0.00014 (control), 0.044, 0.088, 0.14, and 0.231 mg/L PFOS). Each test treatment was replicated six times for each treatment and consisted of two females and one male per tank with exposures lasting 42 days. Tanks were expected daily for eggs and all eggs collected were assumed to be per single female regardless of the number of females per tank. On the last week of testing, eggs were carried through hatching in their respective test treatments, and 20 larval fish per concentration were exposed for an additional 21 days to investigate developmental effects. One liter polypropylene beakers were used for the F1 generation exposure with solutions renewed daily. Survival of adult fathead minnows in the control and two lowest test concentrations was ≥80% at test termination. Survival of male fish in the highest test treatment was significantly less than male control fish, and while female survival was also less compared to control fish, the effects were not significant. The mean number of spawning events per female was also reduced in the two high test treatments, but the effect was only significant in the 140 µg/L (0.14 mg/L) treatment. Larval survival in the F1 generation was significantly reduced in the highest test treatment. The most sensitive endpoint from the study was a significant decrease in the mean mass of individuals in the larval F1 generation with reported values of 3.76, 3.53, 3.09, 2.64 and 2.00 mg for the test treatments of control, 0.044, 0.088, 0.14, and 0.231 mg/L PFOS, respectively. The author-reported NOEC and LOEC, based on growth in the F1 generation, were 0.044 (6% reduction in growth compared to controls) and 0.088 mg/L PFOS (associated with an 18% reduction in growth), respectively, with a MATC of 0.06223 mg/L. The independently-calculated EC_{10} value was 0.0549 (0.0396 – 0.0701) mg/L and used in the derivation of the freshwater chronic column criterion.

C.2.9 Ninth Most Sensitive Freshwater Genus for Chronic Toxicity: *Procambarus* (crayfish)

Funkhouser (2014) conducted a chronic 28-day renewal test of PFOS (potassium salt, ≥98% purity) with a crayfish species, *Procambarus fallax* (f. *virginalis*). The study was

conducted as part of a Master's thesis at Texas Tech University, Lubbock, TX. Juvenile *P. fallax* (4-weeks old, 0.056 g) used for the test were originally purchased from a private collector. The crayfish reproduced for several generations before being used for experiments. Based on an average reproductive age of 141-255 days, an interclutch period of 50-85 days, and a brooding time of 22-42 days, the author estimated the experimental animals to be stage F4-F6 (Seitz et al. 2005). Dilution water was moderately hard reconstituted laboratory water (3.0 g $CaSO_4$, 3.0 g $MgSO_4$, 0.2 g KCl, and 4.9 g $NaHCO_3$ added to 50 L deionized water). Photoperiod was 14:10-hours light:dark at an unreported light intensity. PFOS was dissolved in dilution water to prepare the test concentrations. Exposure vessels were 1 L polypropylene containers containing 500 mL of test solution. The test employed eight replicates of one crayfish each in five test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 0.2, 0.5, 1.3, 3.2, 8 and 20 mg/L. Exposure concentrations were reportedly measured, but concentrations were not provided. Analyses of test solutions were performed using LC-MS/MS. Standards were used as part of the analytical method, but details were not reported. The reporting limit was 0.010 mg/L. Experiments were conducted in an incubator set at 25 ±1°C and covered with plastic opaque sheeting to limit evaporation. No other water quality parameters were reported as having been measured in test solutions. Negative control survival was 85% after 28 days. The 28-day LC_{20} was reported as 0.167 mg/L. An independently-calculated EC_{10} could not be calculated given the level of data that were presented in the paper. The study author-reported LC_{20} value was used quantitatively to derive the freshwater chronic water column criterion.

C.2.10 Tenth Most Sensitive Freshwater Genus for Chronic Toxicity: *Moina* (cladoceran)

Ji et al. (2008) conducted a chronic life-cycle test of the effects of PFOS (acid form, CAS # 1763-23-1, purity unreported) on *Moina macrocopa*. The test was performed under renewal conditions over a 7-day period. The *M. macrocopa* test followed a protocol developed

and reported by Sutherland and Krueger (2001) that was similar to OECD (1998), but with slight modification. *M. macrocopa* used for testing were obtained from brood stock cultured at the Environmental Toxicology Laboratory at Seoul National University (in South Korea). Test organisms were less than 24 hours old at test initiation. Dilution water was moderately hard reconstituted water (total hardness typically 80-100 mg/L as $CaCO_3$). Experiments were conducted in glass jars of unspecified size and fill volume. Photoperiod was assumed 16:8-hours light:dark as was used for daphnid culture. Preparation of test solutions was not described. The test involved 10 replicates of one daphnid each in five nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 0.3125, 0.625, 1.25, 2.5, and 5 mg/L. Test temperature was 25 ±1°C. Authors noted that water quality parameters (pH, temperature, conductivity, and D.O.) were measured after changing the medium, but the information was not reported. Survival of daphnids in the negative control was 100%. The author reported *M. macrocopa* 7-day LOEC for the reproductive endpoint of number of young per surviving adult was 0.3125 mg/L. In the independent verification of the toxicity value, the EPA recalculated the reproductive endpoint to be the number of young per starting adult (instead of surviving adult). This recalculated reproductive endpoint took the full effects of PFOS into account as it was representative of the full life cycle. The independently-calculated EC_{10} for *M. macrocopa* was 0.1789 (0.041 – 0.399) mg/L and used to derive the freshwater chronic water column criterion.

C.2.11 Eleventh Most Sensitive Freshwater Genus for Chronic Toxicity: *Brachionus* (rotifer)

Zhang et al. (2013) conducted a chronic life-cycle renewal test of PFOS (potassium salt, CAS # 2795-39-3, ≥98% purity) with *Brachionus calyciflorus*. The test duration was five days in a full-life cycle test (primary emphasis) and 28 days in a multi-generation population growth test (secondary emphasis – only two exposure concentrations plus a control). Test organisms were

less than two hours old at test initiation. All animals were parthenogenetically-produced offspring of one individual from a single resting egg collected from a natural lake in Houhai Park (Beijing, China). The rotifers were cultured in an artificial inorganic medium at 20°C (16:8-hours light:dark; 3,000 lux) for more than six months before toxicity testing to acclimate to the experimental conditions. Culture medium was an artificial inorganic medium and all toxicity tests were carried out in the same culture medium and under the same conditions as during culture (i.e., pH, temperature, illumination). Solvent-free stock solutions of PFOS (1,000 mg/L) were prepared by dissolving the solid in deionized water via sonication. After mixing, the primary stock was proportionally mixed with dilution water to prepare the test concentrations. Exposures were carried out in 24-well cell culture plates (assumed plastic) containing 2 mL of test solution per cell. The test employed four measured test concentrations plus a negative control. Each treatment consisted of one replicate plate of 15 rotifers each in individual cells. Treatments were repeated six times. Nominal concentrations were 0 (negative control), 0.25, 0.5, 1.0, and 2.0 mg/L. PFOS concentrations were not measured in the rotifer exposures, but rather, in a side experiment using HPLC/MS. The side experiment showed that the concentration of PFOS measured every eight hours over a 24-hour period in rotifer medium with green algae incurs minimal change in the concentration range 0.25 to 2.0 mg/L. One hundred percent survival was observed at 24 hours in the negative control in the corresponding acute test but was not provided for the life-cycle test. The *B. calyciflorus* 5-day LOEC (net reproductive rate and intrinsic rate of natural increase) was 0.25 mg/L. The author-reported value (<0.25 mg/L) was used quantitatively to derive the freshwater chronic water column criterion.

C.2.12 Twelfth Most Sensitive Freshwater Genus for Chronic Toxicity: *Xiphophorus* (swordtail fish)

The toxicity of PFOS (potassium salt, > 98% purity) to the swordtail fish, *Xiphophorus helleri*, was evaluated by **Han and Fang (2010)**. A PFOS stock solution (250 mg/L) was prepared by dissolving crystals in dechlorinated tap water (from the same water source as that used in fish keeping). Six- to seven-month old adult swordtails were purchased from a local fish farm with no water pollution. The fish were separated by sex into different aquaria. Both the males and females were acclimated for eight weeks under semi-static conditions in charcoal filtered, aerated tap water at 27 ±1°C with a 14:10-hour light:dark photoperiod. The water in each aquarium was completely renewed every 48 hours. The fish were fed once daily in the morning with flake food and once daily at dusk with frozen blood worms. Adult male fish were then randomly distributed into 30 L tanks containing 20 L dechlorinated tap water or a corresponding PFOS solution. Swordtail fish were exposed to 0 (control), 0.1, 0.5 or 2.5 mg/L PFOS for three weeks and then transferred into clean water for one-week recovery. Every day, half of the water in each tank was replaced with fresh water, and the fish were exposed to the appropriate concentrations daily. Exposure conditions were the same as those during the acclimation period. Each aquarium housed 10 swordtails. Three aquaria were used for each exposure concentration and for the controls, resulting in three full biological replicates for each exposure group. Body, liver and testis weights were determined at 7, 14, 21 and 28 days after ice-bath anaesthetization. The livers were weighed immediately, then frozen in liquid nitrogen and stored at -80°C for RNA extraction. The hepatosomatic index (HSI) and gonadal somatic index (GSI) values were also calculated. Nonpregnant adult female fish were housed under the same exposure conditions as the males for the six-week exposure period. At the same time, to ensure impregnation of the females, nine adult females were paired with three adult males and

kept in each aquarium for one week, after which the males were taken out. There were also three biological replicates for each exposure group. One pregnant female per aquarium was isolated and housed until giving birth. Larvae were maintained in clean water for up to 14 days after birth to calculate their survival rate. At the end of the exposure period, the survival rate, HSI and GSI values of all groups were determined. The total number of puerperal females and females with eggs or embryos in each group was recorded to determine their corresponding ratios. More than 100 adult swordtails (with a male:female ratio of about 1:3) were housed together to obtain at least 240 juveniles (20-30 days old). All of the fry were then randomly separated into two exposure groups (0 and 0.1 mg/L) and kept under the same housing conditions as the males. Each tank contained 40 fry. There were also three biological replicates in each group. After a 90-day exposure period, the HSI, GSI, and condition factor (CF) values and the sex ratio of each group were calculated by sex category. Body length from the snout to the end of the caudal fin and sword length from the distal end of the middle rays of the caudal fin to the tip of the sword were measured for each young male. After an extended period of stable breeding, part of the juveniles became young females and some of them were with eggs, embryos or puerperal. So, just like adult females, the total number of puerperal females and females with eggs or embryos in each group were recorded as a single entity to determine their corresponding ratios. The 4-week (adult male), 6-week (adult female) and 90-day (juvenile female and male) survival chronic values were >2.5, 1.118 and >0.1 mg/L PFOS, respectively. The study-author reported survival chronic value for offspring of females exposed for six weeks was 0.2236 mg/L PFOS, and the 90-day growth and percent females with eggs chronic value was <0.1 mg/L PFOS. The independently-calculated EC_{10} for adult female survival was 0.5997 (0.2336 – 0.9658) mg/L, which was acceptable for quantitative use.

C.2.13 Thirteenth Most Sensitive Freshwater Genus for Chronic Toxicity: *Xenopus* (frog)

Lou et al. (2013) evaluated the chronic toxicity of PFOS to the African clawed frog, *Xenopus laevis*. PFOS (98% purity) stock solutions (8 mg/mL) were prepared by dissolving in DMSO every four weeks and stored at 4°C. Stock solutions were diluted by charcoal-filtered tap water to prepare test water. DMSO concentrations were 0.001% (v/v) in all tanks including the solvent control group. The same charcoal-filtered tap water (pH 6.5-7.0, D.O. >5 mg/L, and total water hardness, as $CaCO_3$, of approximately 150 mg/L) was used to raise *X. laevis* frogs and tadpoles. Adult female and male *X. laevis* (3 years old, obtained from Nasco, USA.) were raised separately in glass tanks at 22 ±2°C with a 12:12-hour light:dark cycle and fed with chopped pork liver (commercial amphibian diet three times a week). A pair of *X. laevis* was injected by human chorionic gonadotropin to induce breeding. Fertilized eggs were incubated in the same dechlorinated tap water at 22 ±2°C for six days (and were fed live *Artemia* starting on the 5^{th} day). On the fifth day postfertilization, tadpoles at NF stage 46/47 were exposed to PFOS (nominal: 0.0001, 0.001, 0.100 and 1.0 mg/L; measured: 0, 0.00009, 0.001, 0.1117, 0.7160 mg/L) until two months post-metamorphosis. Each exposure group and control group consisted of three replicated tanks. Each tank with 18 L water was assigned randomly 25 tadpoles. The tadpoles were fed with live *Artemia* three times daily. After metamorphosis, the juvenile frogs were fed with live *Artemia* daily and chopped pork liver every other day. The test water (22 ±2°C) was completely replaced every other day. Fluorescent lighting provided a photoperiod of 12 hours and a light intensity ranging from 600 to 1,000 lux at the water surface. During the exposure, the animals were observed for mortality and growth daily and dead tadpoles were removed. At the end of exposure, the survival rate of the frogs in each tank was recorded. After anaesthetization, the frogs were weighted and dissected. The liver tissue of each frog was weighed and hepatosomatic index (HSI) was calculated. The sex or intersex of each frog was

determined by examining the gross gonadal morphology with a stereo microscope. The survival, weight and sex ratio/intersex chronic value were all > 0.7160 mg/L PFOS (or 1 mg/L PFOS as the nominal concentration). The study-author reported value was used quantitatively to derive the freshwater chronic water column criterion.

Fort et al. (2019) evaluated the chronic effects of perfluorooctane sulfonic acid (PFOS, ≥98% purity, CAS # 1763-23-1, lot # BCBH2834V from Sigma-Aldrich in St. Louis, MO) on clawed frogs (*Xenopus tropicalis*, formerly *Silurana tropicalis*) in a 150-day post-metamorphosis flow-through, measured study. Stock solutions were prepared by dissolving PFOS into filtered, dechlorinated tap water in 18 L glass carboys, which were then pumped into the master mixing cell of the continuous flow diluter. Adult frogs were obtained from Xenopus 1 and fed salmon starter pellets daily for 30 days during acclimation prior to breeding. Temperature during acclimation was maintained at 26 ±0.5ºC. Researchers followed the breeding guidance of Fort et al. (2002), and added human chorionic gonadotropin the day before breeding began. Three pairs of frogs were isolated and allowed to breed, but only a single clutch with a >70% spawn rate was utilized for the experiment. Normal appearing dejellied embryos (Nieuwkoop and Faber Stage 10) were randomly selected, and 20 were placed in each of four aquaria, each 4-L in size, for a total of 80 embryos per concentration. The frogs were subjected to a 12:12-hour light:dark photoperiod with a light intensity of 600 ± 50 lux, and the pH was maintained naturally at 7.5 ±0.3. The diluter system achieved a complete volume change every 6.5 hours, and diluter performance, flow rates, temperature, D.O. and light intensity were measured daily. Test organisms were exposed to mean measured concentrations of <0.03 (control), 0.05, 0.13, 0.31, 0.59 and 1.05 mg/L PFOS until metamorphosis, and liquid chromatography mass-spectrometry was used to verify differences in PFOS concentrations. At metamorphosis (NF Stage 66), weight

and snout-vent lengths were measured. Frogs were kept an additional 150 days past metamorphosis without PFOS to determine weights, lengths, and sex differences amongst the organisms. Mortality data showed a NOEC value >1.05 mg/L while the pre-metamorphosis portion of the study showed a NOEC of 0.59 mg/L and a LOEC of 1.05 mg/L for both snout-vent length and weight (MATC = 0.7871 mg/L). The LOEC of 1.05 mg/L was associated with 5% (snout-vent length) and 14% (weight) decrease compared to controls, respectively. A significant increase in the median metamorphosis time was observed in the 1.05 mg/L PFOS treatment relative to the control. The post-metamorphosis LOEC was reported as 1.05 mg/L. No LC_{50} value was reported in that only 5.2 percent mortality was observed in the highest exposure concentration (1.05 mg/L) at test termination. Independently-calculated EC_{10}s could not be calculated as the EPA was unable to fit a model with significant parameters. Instead, the author-reported MATC of 0.7871 mg/L PFOS based on growth (measured as mean body weight at metamorphosis) was used quantitatively to derive the freshwater chronic water column criterion.

C.2.14 Fourteenth Most Sensitive Freshwater Genus for Chronic Toxicity: *Lithobates* (frog)

The chronic flow-through measured toxicity of PFOS (potassium salt, 98% purity) to the northern leopard frog, *Lithobates pipiens* (formerly, *Rana pipiens*), was investigated by **Ankley et al. (2004)**. Two PFOS stock solutions (708 and 21.7 mg/L) were prepared by dissolving solid PFOS with one liter of Lake Superior water in a glass carboy for 24 hours and then brought to a volume of 18 L for the final stock solutions. Contents were stirred at room temperature (~20°C) for 24 hours prior to being used. Solutions were pumped from the carboys to the glass aquaria through Teflon® tubing using fluid metering pumps equipped with stainless-steel rotary dispensers. Target concentrations were achieved by diluting the high and low stock solutions with an appropriate volume of the Lake Superior (control) water. The PFOS stock solutions were renewed every seven days. Fertilized eggs were collected from Grand Lake (St. Louis County,

MN), near a sandy shoreline with no development. Tests were initiated with stage 8/9 embryos; animals were gently separated with a plastic spatula from the egg mass, inspected under a microscope for viability (evidence of cell division), and randomly allocated to treatment groups. Exposures were conducted in glass aquaria in 10 L of water, which was continually renewed at a flow rate of about 50 mL/minute (72 L/day). Duplicate tanks at target (nominal) PFOS concentrations of 0.03, 0.1, 0.3, 1, 3, and 10 mg/L and four replicate control aquaria were used. Embryos (n=120) were placed in each aquarium; in addition, two of the control tanks and the duplicate tanks at 0.1 and 1 mg PFOS/L received an extra 80 organisms (total of 200) at test initiation to provide animals for determination of PFOS concentrations during the early part of the assay. Although biomass varied between the tanks with 120 versus 200 tadpoles, in both situations total loading to the system was more than two orders of magnitude lower than guidance recommended for a test at this flow rate. Water temperature was maintained at 20 ± 0.5°C, and the photoperiod (provided by fluorescent lights) was a constant 16:8-hour light:dark cycle. On hatching (at approximately six days), animals were fed a mixture of live brine shrimp, ground trout chow, and Tetrafin *ad libitum* two times daily. Dead organisms were removed daily and inspected for gross abnormalities. On test day 6, 10 newly-hatched (<24 hours) animals were randomly removed from each tank, preserved in 10% neutral buffered formalin, and subsequently examined for developmental anomalies. Groups of animals were randomly selected from each treatment (excluding the 10 mg/L group, which had been terminated because of high mortality) on test days 35 (10 tadpoles/tank) and 54 (three tadpoles/tank). The animals were weighed, and developmental stage was recorded, before being processed for PFOS tissue analysis. The first tadpoles to undergo complete metamorphosis (defined as emergence of the forelimbs) were observed on test day 60. Metamorphs were removed from the test tanks,

sacrificed with an overdose of MS-222, weighed, measured (total and snout-vent length), and assessed for gross abnormalities. Metamorphosis of the tadpoles continued over the next 51 days, until the test was terminated, when remaining tadpoles were counted, staged, and weighed. A subset of tadpoles from the control and 3 mg PFOS/L treatments were processed for histological analysis of the thyroid gland when they were sampled at forelimb emergence. The most sensitive apical chronic value was the 112-day growth MATC of 1.732 mg/L PFOS, followed by the 5-week LC_{50} of 6.21 mg/L PFOS. These data are considered quantitative even though the control mortality was >20% at test termination (Note: Excessive mortality of amphibian larvae should be expected within the full duration of this experiment given the life history strategy employed by amphibians. Therefore, the observed control survival for this study was considered within the acceptable range for this species and the toxicity data should be limited to the first 10 weeks of the experiment). The author-reported value (112-d growth MATC of 1.732 mg/L) was used quantitatively to derive the chronic water column criterion.

Hoover et al. (2017) also evaluated the chronic toxicity of PFOS (>98% purity) to *Lithobates pipiens*. Test solutions were renewed every four days and exposure concentrations were measured prior to and after each water change. Stock solutions consisted of 1 g of chemical dissolved in 2 L of Milli-Q water, then vacuum-filtered before storage in polycarbonate bottles. Eight northern leopard frog egg masses were collected during early spring from a temporary pond at the Purdue Wildlife Area in West Lafayette, IN, and randomly assigned to outdoor ~100 L wading pools. After hatching, larvae were checked daily for mortality and fed Purina Rabbit Chow *ad libitum*. Treatments consisting of a control and PFOS at three concentrations (nominally 0.010, 0.100, and 1.0 mg/L) were placed in two replicates on adjacent shelves within an environmental chamber. Experimental units consisted of 15 L plastic aquaria filled with 7.5 L

of filtered, UV-irradiated well water. Tadpoles (n=35 per aquarium) were randomly assigned to the experimental units. Prior to addition to aquaria, a subset of animals was examined to confirm development at Gosner stage 26, when hind limb buds start to develop. Tadpoles with visible irregularities in morphology, coloration, or behavior were excluded. Animals were maintained at 20 ±2°C with a 12:12-hour light:dark photoperiod for 10 days to acclimate to indoor conditions and were fed a TetraMin® slurry *ad libitum*. Water changes (100%) were conducted every four days. Tadpoles were exposed for 40 days and were monitored daily for mortality and abnormalities. A water sample (~5 mL) was taken immediately prior to and after each water change to monitor concentration of test chemicals. Every 10 days, six animals were randomly collected from each aquarium. The animals were euthanized, measured (total length at 10 days, snout-vent length otherwise), and staged (Gosner) prior to storage at -20°C for chemical analyses. After 40 days, the depuration phase was initiated by removing animals, cleaning each aquarium with a methanol-soaked sponge, and rinsing to remove adsorbed compound. Aquaria were refilled with clean water; animals were returned to the same aquarium and monitored as described above. Water changes were carried out every four days with fresh water, and a water sample was taken prior to each water change. Two tadpoles were sampled every 10 days for an additional 30 days. The 40-day chronic value was 0.0316 mg/L PFOS based on Gosner stage reached at test termination. This study was deemed quantitative, even though PFOS was detected in the control organisms. While the concentrations were much lower than any of the PFOS treatment groups (3 orders of magnitude lower), it indicated that some potential contamination may have occurred in the controls. The author-reported value (a developmental-based MATC of 0.0316 mg/L) was used quantitatively to derive the freshwater chronic water column criterion.

C.2.15 Fifteenth Most Sensitive Freshwater Genus for Chronic Toxicity: *Hyalella* (amphipod)

Krupa et al. (2022) conducted a 42-day chronic toxicity test with the amphipod, *Hyalella azteca*, and PFOS-K (perfluorooctanesulfonate potassium salt, > 98% purity, CAS No. 2795-39-3, purchased from Sigma-Aldrich). *H. azteca* were obtained from an in-house culture maintained according to U.S. EPA (2000b). Following published methods for conducting water-only testing with *H. azteca* (Bartlett et al. 2021; Ivey et al. 2016), the methods for this chronic 42-d static-renewal test were modified from standard guidance for sediment testing (U.S. EPA 2000b) to a water-only exposure to avoid sorption of PFOS to sediment. Juvenile amphipods approximately 7- to 8-d old were obtained from mixed-age animals passing through a 425 µm sieve and retained on a 355 µm sieve. Animals were then acclimated to test conditions for 2-days before the start of the exposures. Tests were conducted in 300 mL polycarbonate beakers containing 200 mL of test water, a thin layer of clean silica sand (250 – 500 mm diameter, 5 mL per beaker) and ten amphipods under a 16:8-hour light:dark cycle in an environmental chamber maintained at 23 ± 1°C for 42-days. Aeration was provided at a trickle flow rate via glass pipettes. Mean measured exposure concentrations were 0.0093 (control), 4.8, 9.3, 21, and 45 mg/L PFOS, with eight replicates per concentration. Three liters of a 300 mg/L PFOS stock solution was made by dissolving the PFOS salt into dechlorinated tap and mixing the solution on a stir plate for > 20-hours. A total of 10 L of each test concentration was then prepared by mixing the stock solution into carbon filtered dechlorinated tap water containing added sodium bromide (0.052 mg/L). Amphipods were fed 1 mL of YCT food mixture (1.8 g/L mixture) per beaker daily and a ramped (food increased over time) ration of finely ground TetraMin® (0.25 mg/day during week 1, 0.5 mg/day at week 2, 1 mg/day at week 3, and 1.5 mg/day thereafter). For beakers with ≤ 50 % survival, the feeding ration was halved. The YCT and TetraMin® were mixed together as a slurry. The test solution was renewed every 7 days. On Day 7, a partial water

change removing about 150 mL of solution was performed to avoid disturbing the younger *H. azteca*. Complete renewal of the test solution started on Day 14 and continued weekly for the remainder of the test. For these water changes, the entire content of a beaker was transferred to a 12-inch diameter glass culture bowl. New sand and exposure solution were then placed in the beaker. Surviving adult amphipods were enumerated and transferred back to the beaker. On Day 21, all beakers were replaced with new ones. On days 28, 35 and 42, after the adults were transferred back to the beakers, the former contents of the beaker (all the discarded sand and water) were preserved in 70% ethanol with rose bengal stain for later enumeration of offspring. At test termination, sex and combined replicate dry biomass were determined for each replicate. To determine biomass, animals were loaded onto a pre-weighed pan made of aluminum foil and placed in a 60°C oven for a minimum of 24-hours. The pans were then placed in a desiccator for at least 1-hour before being weighed. Eight pans with ten animals each were loaded with organisms on Day 0 to establish a baseline for growth. Water quality parameters observed during testing ranged from 7.31 - 8.57 mg/L D.O., 7.77 - 8.10 SU pH, 22.1 - 22.8°C and 62 - 72 mg/L as $CaCO_3$ total hardness. Aqueous samples for PFOS concentration verification were collected before test initiation (day 0) and termination (day 42). Analytical samples were also collected during test solution renewals: out-water on days 7, 14, 21, 28 and 35; and in-water on day 21. The author- reported 42-day reproductive EC_{10} was 0.7 mg/L PFOS. The EPA's independently-calculated models for EC_{10} estimation were similar; however, the survival model was the most robust. The EPA's independently-calculated EC_{10}s for the other 2 endpoints (the growth as dry weight and reproduction as neonates per female) were different from the author-reported values of 0.9 and 0.7 mg/L, respectively. The study authors were not able to calculate an EC_{10} for survival and so was reported to be <4.8 mg/L. However, the EC_{50}s for all endpoints were similar

between the author-reported and the EPA's independently-calculated values. Thus, the differences in the EC_{10} values were considered to be a result of the difference in models. Therefore, the EPA's calculated value for the 42-day survival endpoint (EC_{10} = 2.899 mg/L PFOS-K; 1.132 - 4.667, 95% CI) was used to derive the chronic freshwater chronic water column criterion.

C.2.16 Sixteenth Most Sensitive Freshwater Genus for Chronic Toxicity: *Physella* (snail)

Funkhouser (2014) conducted a 44-day renewal test of PFOS (potassium salt, ≥98% purity) with *Physella heterostropha pomilia* as part of a Master's thesis at Texas Tech University, Lubbock, TX. Egg masses from 100 *P. pomilia* adults were collected from Canyon Lake 6, Lubbock Lakes System, Lubbock, TX, in May 2013 and used for testing. Dilution water was moderately hard reconstituted laboratory water (3.0 g $CaSO_4$, 3.0 g $MgSO_4$, 0.2 g KCl, and 4.9 g $NaHCO_3$ added to 50 L deionized water). Photoperiod was 12:12-hours light:dark at an unreported light intensity. PFOS was dissolved in dilution water to prepare the test concentrations. Exposure vessels were 250 mL polypropylene containers containing 200 mL of test solution. The test employed two replicates composed of four egg masses each with an average of 37.25 eggs/egg mass at start, then truncated to just four snails per replicate once snails hatched. The test consisted of seven test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 10, 20, 40, 50, 70, 80, and 90 mg/L. Exposure concentrations were reportedly measured, but concentrations were not provided. Analyses of test solutions were performed using LC-MS/MS. Standards were used as part of the analytical method, but details were not reported. The reporting limit was 0.010 mg/L. Experiments were conducted in incubators set to 25°C, which did not vary more than 1°C during the course of the studies. No other water quality parameters were reported as having been measured in test solutions. Negative control survival was not reported specifically for the test but was reported to

be 85-100% across all experiments. The 44-day life-cycle MATC was 14.14 mg/L (from the author-reported NOEC and LOEC, 10 and 20 mg/L, respectively) for mean number of eggs per egg mass. The independently-calculated EC_{10} for the same endpoint was 8.527 (6.170 – 10.88) mg/L. The independent statistical analysis was conducted using data that was estimated (using Web plot digitizer) from the figures presented in the paper. This chronic value was acceptable for quantitative use and was used to derive the freshwater chronic water column criterion.

C.2.17 Seventeenth Most Sensitive Freshwater Genus for Chronic Toxicity: *Ceriodaphnia* (cladoceran)

Krupa et al. (2022) conducted a 6-day chronic toxicity test with the cladoceran, *Ceriodaphnia dubia*, and PFOS-K (perfluorooctanesulfonate potassium salt, > 98% purity, purchased from Sigma-Aldrich). In-house cultures of daphnids were reared according to U.S. EPA (2002) and maintained in 100 mL glass beakers filled with 80 mL of moderately hard reconstituted water (MHRW) prior to testing so that organisms used in the test were less than 24 hours old and were all released within an 8-hour period. The chronic toxicity test was conducted as a three-brood (6-d) static-renewal test according to standard protocol (U.S. EPA 2002). The measured exposure concentrations were < LOD-0.0003, 1.7, 3.5, 7.1, 13, 27, and 48 mg/L PFOS. There were 10 replicates per concentration with one organism per each replicate. The highest test concentration was prepared by dissolving the appropriate mass of PFOS into MHRW water. Once the compound was fully dissolved, the highest concentration was then serially diluted to the other, lower target concentrations. The tests were conducted in 20 mL glass scintillation vials containing 15 mL of test water under a 16:8-hour light:dark cycle in an environmental chamber maintained at 25 ± 1°C without aeration. *C. dubia* were fed daily with 0.45 mL of 1:1 *P. subcapitata* and YCT (yeast-cerophyll-trout chow). Survival and reproduction were recorded each day and neonates and any dead animals were removed. Complete renewal of the test

solution and count of survivors and neonates was performed daily. Water quality parameters were measured at every water renewal for both the in-water and out-water and ranged from 6.1 - 12.4 mg/L D.O., 6.91 - 8.02 SU pH and 24.0 - 25.9°C. Water samples collected at test initiation (day 0), before (out-water) and after (in-water) test solution renewals, and at test termination (day 6) were analyzed to verify PFOS concentrations. The author-reported 6-day reproductive EC_{10} was 6.9 mg/L PFOS-K. The independently-calculated 6-day EC_{10} value based on reproduction was 10.69 (5.839 – 15.54) mg/L and was considered acceptable for quantitative use to the chronic freshwater chronic water column criterion.

Kadlec et al. (2024) tested the chronic toxicity of potassium perfluorooctanesulfonate (PFOS-K) on *Ceriodaphnia dubia* for 7 days in a measured, renewal experiment. Similar chronic tests were also performed with *Chironomus dilutus* and *Hyallela azteca*, but this summary is limited to the results of the *C. dubia* tests. Test chemicals were obtained from Sigma, Alfa Aesar, Synquest, and Toronto Research Chemical (purity 96-99%). Test organisms were obtained from in-house cultures maintained following ASTM and EPA protocols. Test water was UV-treated and sand-filtered Lake Superior water was supplemented with Na_2SO_4, NaCl, KCl, $CaCl_2$ x $2H_2O$, and $MgCl_2$ x $6H_2O$. Testing protocols followed species-specific ASTM methodologies (ASTM 2002). Three separate tests were conducted, each with a 0.5x dilution series of measured PFOS concentrations, with ten replicates of each concentration, and one organism per replicate. Test 1 mean concentrations were 0.23 (control), 2.3, 4.4, 8.8, 18, and 38 mg/L. Test 2 mean concentrations were 0.0049 (control), 0.049, 0.090, 0.25, 0.42, 0.92, 1.9, 3.9, 8.0, 17, and 35 mg/L. Test 3 mean concentrations were 0.27 (control), 2.7, 5.9, 11, 23, and 46 mg/L. *C. dubia* neonates (<8-hour) were placed in 1 oz polystyrene cups filled with 15 mL of solution. Dissolved oxygen and pH were measured twice in each exposure per treatment and twice in stock solutions.

Tests chambers were placed in a water bath to maintain a steady temperature under a 16:8 light:dark cycle. Study authors reported average water quality measurements of 24.7°C, 8.6 mg/L DO, 7.8 pH, 52 mg/L as $CaCO_3$ hardness, 41 mg/L as $CaCO_3$ alkalinity, and 145 µmhos/cm conductivity. Testing solutions were renewed daily, and organisms were fed daily with 100 µg/L YCT and algae. Control survival was 96.8% with a mean reproduction of 26.8. EC_{20}s and EC_{50}s for survival and young per surviving female were calculated following methods described in Mount et al. (2016), using custom software written with Intel Visual Fortran Compiler Xe and Winteracter 13.0. The author reported EC_{20}s for young per surviving female for tests 1, 2, and 3 were 10.0, 14.5 and 9.8, respectively. Concentration-response data were reported for these tests, allowing the EPA to independently model concentration-response curves using the dose-response curve package in R. The EPA-calculated EC_{10}s for tests 1, 2, and 3 were 8.371, 9.205, and 6.766 mg/L, respectively, which were determined to be acceptable for quantitative use.

Appendix D Acceptable Estuarine/Marine Chronic PFOS Toxicity Studies

D.1 Summary Table of Acceptable Quantitative Estuarine/Marine Chronic PFOS Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Chronic Value Endpoint	Author Reported Chronic Value (mg/L)	EPA Calculated Chronic Value (mg/L)	Final Chronic Value (mg/L)[b]	Species Mean Chronic Value (mg/L)	Reference
Asian green mussel (60-65 mm), *Perna viridis*	R, M	7 d	PFOS-K 98%	-	25	25	EC10 (growth condition index)	0.03190	0.0033	**0.0033**	0.0033	Liu et al. (2013)
Copepod (nauplii), *Tigriopus japonicus*	R, U	20 d	PFOS Unreported	-	25	32	MATC (developmental stage)	0.7071	-	**0.7071**	0.7071	Han et al. (2015)
Amphipod (juvenile, 14 d), *Austrochiltonia subtenuis*	S, U	7 d	PFOS Unreported	8.12-8.3	20.3-21.2	-	MATC (mortality)	**0.01118**	-	**0.01118**	0.01118	Sinclair et al. (2022)
Mysid (< 24 hr) *Americamysis bahia*	F, M	35 d	PFOS-K 90.49%	8.2-8.4	25	19-21	MATC (reproduction, growth)	0.3708	-	**0.3708**	0.3708	Drottar and Krueger (2000h)
Japanese medaka, *Oryzias latipes*	S, U	30 d	PFOS-K 98%	7.87	24.77	34.68	NOEC (growth condition index)	1.0	-	**>1.0**	>1.0	Oh et al. (2013)

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

[b] Values in bold used in SMCV calculation.

D.2 Detailed PFOS Acute Toxicity Study Summaries and Corresponding Concentration-Response Curves (when calculated for the most sensitive genera)

The purpose of this section was to present detailed study summaries for tests that were considered quantitatively acceptable for estuarine/marine chronic criterion derivation, with summaries grouped and ordered by genus sensitivity. Data for chronic PFOS toxicity were available for three saltwater invertebrate species, representing three genera and three families. Chronic PFOS toxicity data was available for one fish species. The data available fulfilled only four of the eight MDRs, therefore the EPA could not develop a chronic estuarine/marine criterion following the 1985 Guideline methods.

D.2.1 Most Sensitive Estuarine/Marine Genus: *Perna* (mussel)

Liu et al. (2013) evaluated the chronic effects of perfluorooctanesulfonate, potassium salt (PFOS-K, CAS# 2795-39-3, 98% purity, purchased from Sigma-Aldrich) on green mussels, *Perna viridis*) via a 7-day measured, static-renewal study. The mussels were obtained from a local farm in Singapore, and subsequently acclimated to laboratory conditions for seven days before testing. Mussels were kept at a salinity of 25 ppt (artificial seawater) and a temperature of 25ºC. Forty mussels (60-65 mm length) per 50-L polypropylene tank in duplicate were exposed to measured PFOS concentrations of 0 (control), 0.00012, 0.0011, 0.0096, 0.106 and 0.968 mg/L. Mussels were fed a commercial marine micro-alga purchased from Reed Mariculture on renewal days, which occurred every two days, two hours before the solution renewal. PFOS concentrations were verified through water and muscle tissue samples via LC/MS. Weights and lengths were determined on days 0 and 7. A NOEC of 0.0096 mg/L and a LOEC of 0.106 mg/L was determined for the growth condition index resulting in an MATC of 0.03190 mg/L. No LC_{50} value was reported. The EPA's independently-calculated EC_{10} is 0.0033 (0.00330 – 0.00332) mg/L and was acceptable for quantitative use.

D.2.2 Second Most Sensitive Estuarine/Marine Genus: *Austrochiltonia* (amphipod)

Sinclair et al. (2022) tested perfluorooctane sulfonic acid (PFOS) on amphipods (*Austrochiltonia subtenuis*) in a 7-day unmeasured, static experiment. PFOS (purity not reported) was purchased from Sigma-Aldrich (Melbourne, VIV, Australia). Test organisms were originally field collected from Deep Creek, Victoria, Australia, but had been maintained in a laboratory culture for over five years at RMIT University in Melbourne, Australia. Organisms were cultured in standard artificial media (SAM) modified from Borgmann (1996) at 21±1°C under a 16:8 light:dark cycle, and they were fed powdered Tetramin fish food and YTC every two days. Two days prior to testing, 14-day old amphipods were selected and held in 2 L glass beakers until test initiation. The 7-day experiment consisted of five controls, one solvent control (methanol 0.25 mL/L), and five nominal PFOS concentrations (0.04, 0.2, 1.0, 5.0, 25 µg/L). Test vessels were 600 mL beakers with 400 mL of test material and a 2x2 cm gauze substrate. Each test vessel included 20 amphipods, and all test media was dissolved in SAM. Seven-day survival was assessed using a series of two-sample Student's t-tests (assuming equal variance) comparing survival in each treatment to its corresponding control. After seven days, PFOS in tissues was measured to calculate bioconcentration factors, and a suite of metabolites were also measured. Based on the results of the t-tests, there was a statistically significant ($p<0.05$) decrease in survival (21% mortality) at the highest concentration compared to its control. Setting the highest concentration 0.025 mg/L as the LOEC and the highest concentration with no adverse effect 0.005 mg/L as the NOEC, the resulting MATC was 0.01118 mg/L, which was determined to be acceptable for quantitative use.

D.2.3 Third Most Sensitive Estuarine/Marine Genus: *Americamysis* (mysid)

Drottar and Krueger (2000h) reported the results of a life-cycle, 35-day flow-through, measured test of PFOS-K (potassium salt, 90.49% purity) with *Americamysis bahia* (formerly

Mysdiopsis bahia). This good laboratory practice (GLP) test was conducted at the Wildlife International, Ltd. toxicology facility in Easton, MD in June, 1999. The test followed U.S. EPA OPPTS 850.1350, and ASTM Standard E 1191-90 test guidelines. Mysids used for the test were neonates less than 24 hours old at test initiation. The dilution water was filtered natural seawater collected at Indian River Inlet, DE diluted to a salinity of approximately 20 ppt with well water [pH: 8.3 (8.2-8.4); TOC: ≥5.8 mg/L; temperature: 25±2°C]. Photoperiod was 16:8-hours, light:dark with a 30-minute transition period. Light was provided at an intensity of 623-815 lux. A primary stock solution was prepared in dilution water at 89.5 mg/L. It was mixed until all of the test substance was dissolved prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the five additional test concentrations. Exposure vessels were glass beakers with nylon mesh screens on each side placed in 9 L glass aquaria with 5 L of test solution. After mysids reached sexual maturity, they were placed in pairs in glass petri dishes to observe reproduction. The test employed four replicates of fifteen mysids each in six measured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 0.086, 0.17, 0.34, 0.69, 1.4, and 2.7 mg/L. Mean measured concentrations were < 0.0458 (LOQ), 0.057, 0.12, 0.25, 0.55, 1.3, and 2.6 mg/L, respectively. Analyses of test solutions were performed at Wildlife International Ltd. using HPLC/MS. Measured values ranged from 66 to 96% of nominal. Mortality from test initiation to pairing (day 20) in the 0.057, 0.12, 0.25, 0.55, 1.3, and 2.6 mg/L treatment groups was 8, 25, 18, 17, 32 and 100%, respectively, and mean control mortality was 22%. From pairing until test termination (day 20 to day 35) survival was greater than 90% in the control and all but the 1.3 mg/L treatment, which had 57% survival during that period. The 35-day NOEC (reproduction and growth) was 0.25 mg/L and corresponding LOEC was 0.55 mg/L. The EPA-calculated MATC was 0.3708 mg/L. The EC_{10}

could not be calculated at this time given the level of data that was presented in the paper. The chronic value was considered acceptable for quantitative use despite the control survival of 78% because it was only slightly below the 80% survival threshold, and because there were no other deficiencies in the study design.

D.2.4 Fourth Most Sensitive Estuarine/Marine Genus: *Tigriopus* (copepod)

A 20-day renewal, unmeasured full life-cycle test with PFOS (analytical grade) was conducted on the copepod, *Tigriopus japonicus* (non-North American species) by **Han et al. (2015)**. Copepods were cultured and maintained in 0.2 µm filtered artificial seawater adjusted to 32 psu salinity and 25°C under a 12-hour photoperiod. *T. japonicus* were fed with green algae, *Tetraselmis suecica*. PFOS (100 mg/L in MeOH) was concentrated by evaporation and re-dissolved in DMSO to obtain a maximum stock concentration (1,000 mg/L). The PFOS stock was diluted with artificial seawater to obtain four nominal test concentrations (0, 0.25, 0.5 and 1 mg/L PFOS). The final concentration of DMSO in seawater was 0.001% (v/v) or less for each treatment. Ten newly-hatched nauplii (<12 hour post hatch) were allocated to each well of a 12-well tissue culture plate with 4 mL of test solution. There were three replicates per each treatment. Organisms were fed algae during testing and 50% of test media was replaced daily. Over the next 20 days, the development of the copepod's growth from nauplii to copepodite and from nauplii to adults was determined daily based on morphological characteristics. Results were presented as the number of days needed to reach the normal development stages. The highest test concentration (1 mg/L PFOS) significantly increased the amount of time it took the copepods to reach the development stage. Additionally, the authors assessed the reproduction of the copepods by counting the nauplii produced by eight ovigerous females for 10 days in each well exposed to PFOS. However, it was unclear if this was a subsampling of the organisms used in the 20-day developmental test or if an independent assay with adult females. Results are presented

graphically as daily nauplii production/individual. There was a statistically significant decrease in production (daily nauplii production/individual) in the 0.25, 0.5 and 1.0 mg/L PFOS concentrations compared to the control. It was decreased by approximately 50% in the highest concentration (1 mg/L). While this endpoint was more sensitive than the growth endpoint, the publication is unclear about the method used for the reproduction test endpoint and whether it was an independently-conducted 10-day test or a subsample of reproducing adults were observed from the 20-day test. The EPA sought but did not receive responses to clarifying questions posed to the authors. Additionally, the authors were asked if control survival for the test was above 80% and if the authors could provide the data. Based on the information presented in the paper without additional information and data provided by the authors to clarify adherence to the EPA data quality objectives and allow independent calculation and verification of point estimates, the developmental stage is considered for quantitative use and the reproductive endpoint for qualitative use. The 20-day MATC (based on time to reach development stage) was 0.7071 mg/L and was acceptable for quantitative use.

D.2.5 Fifth Most Sensitive Estuarine/Marine Genus: *Oryzias* (fish)

Oh et al. (2013) evaluated the 30-day chronic toxicity of PFOS to the Japanese medaka, *Oryzias latipes*, via an unmeasured static exposure. PFOS (98% pure, CAS No. 2795-39-3) was dissolved in filtered seawater with the minimal concentration (< 0.001%) of dimethyl sulfoxide (DMSO) used as a vehicle to prevent cellular damage to the fish. Chemical measurements were not made, and nominal concentrations were used throughout the study. Prior to test initiation, the fish were adapted to a seawater environment by first acclimation in a 50/50 freshwater/saltwater mix, then sequentially increasing the saltwater component by replacing half of the water with the same volume of seawater every day for 15 days. The fish were fed newly-hatched brine shrimp (< 24 hours after hatching) and commercial flake food twice per day. No mortality was observed

during acclimation to the seawater over one month. The fish were maintained at 25°C under a constant photoperiod of 16:8-hour light:dark, and water quality was monitored by measuring the pH, D.O., and temperature. Fish from the third generation of *O. latipes* (n = 7/group) that had adapted to seawater for over one month were used in the series of exposure experiments. Fish were exposed for 30 days to one PFOS concentration (1 mg/L) plus a 0.22 µm filtered seawater control and a DMSO carrier solvent control to examine biological effects (specifically, the condition factor, K, a growth endpoint). Test conditions were maintained at an average temperature of 24.77 ℃, pH of 7.87 SU, D.O. of 5.90 mg/L, and salinity of 34.68 PSU with fish fed daily.

The 30-day NOEC condition factor of 1.0 mg/L PFOS was selected as the primary endpoint from this study. Authors also stated, "*In our preliminary study, fish mortality was altered 30 days after PFC (perfluorinated compounds) exposure, suggesting that repeated exposure to PFCs for 30 days at 1 µg/mL causes adverse effect on O. latipes*." The statement about results from the preliminary test is in direct conflict with the results of condition factor in the final test. Few details are provided about the preliminary test. Results of the final test were retained as quantitatively acceptable because they provide chronic estuarine/marine data that are limited.

Appendix E Acceptable Freshwater Plant PFOS Toxicity Studies

E.1 Summary Table of Acceptable Quantitative Freshwater Plant PFOS Toxicity Studies

Species	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Reported Effect Concentration (mg/L)	Reference
Cyanobacteria, *Microcystis aeruginosa*	S, U	72 hr	PFOS-K 98%	7.4	23	MATC (cell density)	0.3162	Muhammad (2023)
Cyanobacteria, *Microcystis aeruginosa*	S, U	96 hr	PFOS-K 98%	-	25	EC30 (population growth rate)	77.39	Zhang et al. (2023b)
Diatom, *Navicula pelliculosa*	S, M	96 hr	PFOS-K 86.9%	7.5-8.9	24	EC50 (area under growth curve)	252	Sutherland and Krueger (2001)
Green alga, *Chlorella vulgaris*	S, U	96 hr	PFOS-K 95%	-	23	IC50 (cell density)	81.6	Boudreau (2002); Boudreau et al. (2003a)
Green alga, *Chlorella vulgaris*	S, U	96 hr	PFOS-K 98%	-	25	EC20 (population growth rate)	13.42	Zhang et al. (2023b)
Green alga, *Raphidocelis subcapitata* (formerly, *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*)	S, U	96 hr	PFOS-K 24-28%	-	23	EC50 (specific growth rate)	49.28[b]	3M Company (2000)
Green alga, *Raphidocelis subcapitata*	S, U	4 d	PFOS-K Unknown	-	23	EC50 (cell count)	77.19	3M Company (2000)
Green alga, *Raphidocelis subcapitata*	S, U	7 d	PFOS-K Unknown	-	23	EC50 (cell count)	76.68	3M Company (2000)
Green alga, *Raphidocelis subcapitata*	S, U	10 d	PFOS-K Unknown	-	23	EC50 (cell count)	83.92	3M Company (2000)
Green alga, *Raphidocelis subcapitata*	S, U	14 d	PFOS-K Unknown	-	23	EC50 (cell count)	76.78	3M Company (2000)
Green alga, *Raphidocelis subcapitata*	S, M	96 hr	PFOS-K 90.49%	7.4-8.4	24	EC50 (cell density)	71	Drottar and Krueger (2000b)
Green alga, *Raphidocelis subcapitata*	S, U	96 hr	PFOS-K 95%	-	23	IC50 (cell density)	48.2	Boudreau (2002); Boudreau et al. (2003a)
Green alga, *Scenedesmus quadricauda*	S, M	96 hr	PFOS-K 99%	7	22	EC50 (growth inhibition rate)	89.34	Yang et al. (2014)

Species	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Reported Effect Concentration (mg/L)	Reference
Duckweed, *Lemna gibba*	S, M	7 d	PFOS-K 86.9%	7.5	25	IC10 (frond number)	18.06	Desjardins et al. (2001b)
Duckweed, *Lemna minor*	S. M	96 hr	PFOS ≥95.0%	6.5	25	NOEC (population growth rate)	9.859	Wu et al. (2023)
Water milfoil (5 cm apical shoots), *Myriophyllum sibiricum*	S, M	14 d	PFOS-K Unreported	-	-	EC10 (wet weight)	0.7	Hanson et al. (2005)
Water milfoil (5 cm apical shoots), *Myriophyllum sibiricum*	S, M	28 d	PFOS-K Unreported	-	-	EC10 (wet weight)	0.19	Hanson et al. (2005)
Water milfoil (5 cm apical shoots), *Myriophyllum sibiricum*	S, M	42 d	PFOS-K Unreported	-	-	EC10 (wet weight)	0.6	Hanson et al. (2005)
Water milfoil (5 cm apical shoots), *Myriophyllum spicatum*	S, M	14 d	PFOS-K Unreported	-	-	EC10 (plant length)	4.8	Hanson et al. (2005)
Water milfoil (5 cm apical shoots), *Myriophyllum spicatum*	S, M	28 d	PFOS-K Unreported	-	-	EC10 (dry weight)	3.3	Hanson et al. (2005)
Water milfoil (5 cm apical shoots), *Myriophyllum spicatum*	S, M	42 d	PFOS-K Unreported	-	-	EC10 (wet weight)	3.5	Hanson et al. (2005)

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, NR=not reported

[b] The independently-calculated EC_{50} value was 176.0 mg/L as the test substance, or 49.28 mg/L based on the percentage of PFOS-K (active ingredient 28%) in the test substance.

E.2 Summary of Plant PFOS Toxicity Studies Considered in the Aquatic Life Criterion Derivation

E.2.1 Cyanobacteria, *Microcystis aeruginosa*

Muhammad (2023) tested perfluorooctane sulfonic acid potassium salt (PFOS-K) on *Microcystis aeruginosa* for seven days in a static, unmeasured experiment. PFOS potassium salt (98% purity) was obtained from Sigma-Aldrich. Algae were obtained from Golder Laboratory, at the State University of New York, USA. Algae were cultured in 500 mL Erlenmeyer flasks containing 200mL of BG11 medium at the Phycology Laboratory of Ahmadu Bello University's Department of Botany in Zaria. Cultures were maintained at 23°C, pH 7.4, under a 16:8 hour light:dark photoperiod at a light intensity of 40 µmol/m^2/s. The BG11 growth medium was sterilized by autoclaving at 121°C 24 hours before use. Cultures were changed and maintained in a manner to insure they were experiencing exponential growth. *M. aeruginosa* in the exponential growth phase were added to test vessels containing test solution at a density of approximately $1.0x10^6$ cells/mL. The experimental design consisted of nominal concentrations of 0 (control), 0.001, 0.01, 0.1, 1 and 10 mg/L PFOS, diluted from a PFOS stock solution prepared in BG-11 medium, with three replicates per treatment level. Cell density was measured on days 1, 3, and 7. Cellular pigment content was measured on days 1 and 7, biochemical composition, antioxidant enzyme composition, and microcystin analysis was measured on day 7. One-way ANOVA and means comparisons tests were used to assess significant differences ($p<0.05$) between treatments, and repeated measures ANOVA was used to assess significant differences between sampling dates. All analyses were conducted using the R statistical analysis program v3.63. By day three, cell density in the 1 mg/L treatment were significantly lower than in controls, and by day seven, cell density in the 0.1 mg/L treatment were significantly lower than in controls. Chlorophyll *a* also decreased with increasing PFOS concentrations, and biochemical and

enzymatic endpoints also differed among PFOS treatment concentrations. The author-reported 72 hour NOEC and LOEC for cell density were 0.1 and 1 mg/L, and the resulting MATC of 0.3162 mg/L was determined to be acceptable for quantitative use.

Zhang et al. (2023b) tested perfluorooctane sulfonic acid potassium salt (PFOS-K) on *Microcystis aeruginosa* for 12 days in a static, unmeasured experiment. PFOS potassium salt (98% purity) was purchased from Sigma-Aldrich (St. Louis, USA). Algae were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology in Wuhan, China. Algae were cultured in Erlenmeyer flasks within BG-11 media in an illumination incubator at 25±1°C on a 12:12 hour light-dark cycle at a light intensity of 4,000±50 lux. *M. aeruginosa* in the exponential growth phase were added to 100 mL Erlenmeyer flasks containing test solution at a density of approximately $1.0x10^5$ cells/mL. The experimental design consisted of nominal concentrations of 0 (solvent control), 0.01, 1, 20, 50, 100, 250 and 500 mg/L PFOS, diluted from a PFOS stock solution prepared in BG-11 medium and containing 0.05% formaldehyde as a solvent, with three replicates per treatment. Testing methods followed OECD guidelines with modifications (OECD 2006). Algal cell density was measured after 4 and 12 days using a UV-vis spectrophotometer. Algal cell densities were used to calculate growth inhibition at all concentrations relative to controls, and effect concentrations (ECs) were calculated using nonlinear regression modeling. Chlorophyll *a* and optical quantum yield were also measured after 4 and 12 days. ANOVA followed by Tukey's multiple comparison test was used to identify significant ($p<0.05$) differences between treatments and controls, using GraphPad Prism v8.0. EC_{20}s were calculated using a four-parameter nonlinear regression model in GraphPad Prism v8.0. A 96-hour EC_{20} was not reported, so the 96-hour EC_{30} for growth inhibition of 77.39 mg/L was determined to be acceptable for quantitative use.

E.2.2 Diatom, *Navicula pelliculosa*

Sutherland and Krueger (2001) conducted a 96-hour static acute algal growth inhibition test on PFOS (potassium salt, 86.9% purity) with the freshwater diatom, *Navicula pelliculosa*. The good laboratory practice (GLP) test was conducted at the Wildlife International, Ltd. in Easton, Maryland in February-March, 2000. The test followed U.S. EPA (1996a) and ASTM (1990). The freshwater diatom was provided from in-house cultures that had been actively growing in the culture medium for at least two weeks. The test media was prepared by adding the stock nutrient solution to purified well water according to ASTM 1218 and adjusting pH to 7.5. Seven measured concentrations (0, 62.3, 83.2, 111, 150, 206, 266, 335 mg/L PFOS) were tested from one negative control and six nominal concentrations: 61.5, 81.3, 110, 147, 198, 264 and 347 mg/L based on PSOF-K purity. Solutions were stirred for approximately 24 hours before testing. Exposures were conducted in 250 mL plastic Erlenmeyer flasks containing 100 mL solution and plugged with foam stoppers. Each flask contained $1x10^4$ cells/mL and each test concentration had three replicates. Flasks were incubated in environmental chambers at 24±2°C under constant illumination (4,300 lux) and shaken continuously at ~100 rpm. pH in the test solutions ranged from 7.5-8.9 over the exposure period. Samples were collected daily to determine cell density and to calculate area under the curve and growth rates. The cell density of the control replicates increased by greater than two orders of magnitude during the test. The 96-hour EC_{50}, based on area under the growth curve, was 252 mg/L PFOS (NOEC<62.3 mg/L). The plant value was acceptable for quantitative use.

E.2.3 Green alga, *Chlorella vulgaris*

Boudreau (2002) performed a 96-hour static acute algal growth inhibition test on PFOS (potassium salt, 95% purity) with *Chlorella vulgaris* as part of a Master's thesis at the University of Guelph, Ontario, Canada. The same information was subsequently published in the open

literature as **Boudreau et al. (2003a)**. The acute algal growth inhibition tests followed protocols found in ASTM (1999b) and Geis et al. (2000). All treatment concentrations were based on the PFOS anion (without K) and solutions were prepared in laboratory-grade distilled water. *C. vulgaris* (UTCC 266 strain) were obtained as slants from the University of Toronto Culture Collection (UTCC; Toronto, Canada). Toxicity testing consisted of initial range-finder tests (0, 28, 56, 113, 225, and 450 mg/L) followed by at least two definitive tests (0, 12.5, 25, 50, 100, 200, and 400 mg/L). Tests were conducted in 20 mL solution in 60 x 15 mm polyethylene disposable petri dishes. Each dish contained $1.5x10^4$ cells/mL and each test concentration had four replicates. Tests were continuously illuminated with cool-white fluorescent light between 3,800 and 4,200 lux and incubated at 23±1°C. Each dish was manually shaken twice a day during testing. Toxicity test endpoints included cell density and chlorophyll *a* content. The most sensitive endpoint, cell density, had a reported NOEC of 8.2 mg/L and an IC_{50} of 81.6 mg/L. The IC_{50} value was considered quantitatively acceptable for use.

Zhang et al. (2023b) tested perfluorooctane sulfonic acid potassium salt (PFOS-K) on *Chlorella vulgaris* for 12 days in a static, unmeasured experiment. PFOS potassium salt (98% purity) was purchased from Sigma-Aldrich (St. Louis, USA). Algae were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology in Wuhan, China. Algae were cultured in Erlenmeyer flasks within BG-11 media in an illumination incubator at 25±1°C on a 12:12 hour light-dark cycle at a light intensity of 4,000±50 lux. *C. vulgaris* in the exponential growth phase were added to 100 mL Erlenmeyer flasks containing test solution at a density of approximately $1.0x10^5$ cells/mL. The experimental design consisted of nominal concentrations of 0 (solvent control), 0.01, 1, 20, 50, 100, 250 and 500 mg/L PFOS, diluted from a PFOS stock solution prepared in BG-11 medium and containing 0.05% formaldehyde as a

solvent, with three replicates per treatment. Testing methods followed OECD guidelines with modifications (OECD 2006). Algal cell density was measured after 4 and 12 days using a UV-vis spectrophotometer. Algal cell densities were used to calculate growth inhibition at all concentrations relative to controls, and EC_{50}s were calculated using nonlinear regression modeling. Chlorophyll *a* and optical quantum yield were also measured after 4 and 12 days. ANOVA followed by Tukey's multiple comparison test was used to identify significant ($p<0.05$) differences between treatments and controls, using GraphPad Prism v8.0. Effect concentrations (ECs) were calculated using a four-parameter nonlinear regression model in GraphPad Prism v8.0. The 96-hour EC_{20} for growth inhibition was 13.42 mg/L, and was determined to be acceptable for quantitative use.

E.2.4 Green alga, *Raphidocelis subcapitata*

3M Company (2000) provided the results of a 96-hour toxicity test completed in 1991 with the green alga, *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*), and PFOS-K (perfluorooctancesulfonate potassium salt, CAS # 2795-39-3) in a formulated mixture with diethylene glycol butyl ether and water (mixed product FM-3820, with 24-28% PFOS-K). Based on this purity the author made calculations to adjust test concentrations using 28% active ingredient, but the presence of diethylene glycol could also contribute to toxicity. The toxicity test followed OECD test guidelines with five test concentrations and control in a static unmeasured exposure. A stock culture of the alga was obtained from the Culture Collection of Algae at the University of Texas at Austin. Alga were transferred to 250 mL flasks with an initial density of $1x10^4$ cells/mL and 100 mL of test solution. There were three replicates for each of the five nominal test concentrations (62.5, 125, 250, 500 and 1,000 mg/L) and control. Synthetic nutrient medium was used as the dilution media for all test treatments. Alga were grown at 23°C and continuously shaken. The author-reported EC_{50}, based on average specific growth rate, was

255 mg/L as the test substance, or 71 mg/L based on the percentage of PFOS-K (active ingredient 28%) in the test substance. The independently-calculated EC_{50} value was 176.0 mg/L as the test substance, or 49.28 mg/L based on the percentage of PFOS-K (active ingredient 28%) in the test substance. The plant value was acceptable for quantitative use.

3M Company (2000) provides the results of four separate toxicity tests completed in 1981 with the green alga, *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*), and PFOS-K (perfluorooctancesulfonate potassium salt, CAS # 2795-39-3, unknown purity). The toxicity tests followed a protocol modified from OECD (1979). There were four separate exposure regimes: 1) four day exposure + 10 day recovery period; 2) seven day exposure + seven day recovery period; 3) 10 day exposure + four day recovery period; and 4) 14 day continuous exposure. A bacteria-free culture of the alga was obtained from the U.S. EPA (Corvallis, OR) and stored in the dark until testing. Seven-day old stock cultures with an initial density of $1x10^4$ cells/mL were placed in 250 mL flasks with 50 mL of test solution. There were three replicates for each of the six nominal test concentrations (26, 40, 61, 93, 145 and 225 mg/L) and control. Nutrient medium was used as the dilution media for all test treatments and test solutions were not renewed during the exposure. Alga were grown at 23°C and continuously shaken at 100 rpm. The author-reported EC_{50}, based on cell counts, was 82, 99, 98, and 95 mg/L, for the 4-, 7-, 10- and 14-day exposures, respectively. However, it should be noted that the authors do not specify if the EC_{50}s were determined after the exposure period or the post-observation period. The independently-calculated EC_{50} values were 77.19, 76.68, 83.92, 76.78 mg/L and are acceptable for quantitative use.

Drottar and Krueger (2000b) conducted a 96-hour static acute algal growth inhibition test on PFOS (potassium salt, 90.49% purity) with the freshwater alga, *Raphidocelis subcapitata*

(formerly *Selenastrum capricornutum*). The good laboratory practice (GLP) test was conducted at the Wildlife International, Ltd. in Easton, Maryland in April, 1999. The test followed ASTM (1990); OECD (1984); U.S. EPA (1996a) methodologies. The green alga was originally obtained from the culture collection at University of Texas at Austin (or another supplier) and maintained at Wildlife International Ltd. for a minimum of two weeks in culture medium. Algae used in tests were in exponential growth phase. The test media was prepared by adding the stock nutrient solution to purified well water according to ASTM 1218 and adjusting pH to 7.5. Seven measured concentrations (< 0.115, 5.5, 11, 21, 44, 86, 179 mg/L PFOS) were tested from a negative control and six nominal concentrations: 5.7, 11, 23, 46, 91, 183 mg/L based on PFOS-K purity. Test concentrations were measured at test initiation, at 72 hours, and at test termination by HPLC-MS with a mean recovery of 99.1%. Solutions were stirred for approximately 24 hours before testing. Exposures were conducted in 250 mL polycarbonate flasks containing 100 mL solution and plugged with foam stoppers. Each flask contained $1x10^4$ cells/mL and each test concentration had three replicates. Flasks were incubated in environmental chambers at 24±2°C under constant illumination (4,300 lux) and shaken continuously at ~100 rpm. The pH in test solutions ranged from 7.4-8.4 over the exposure period. Samples were collected daily to determine cell density and to calculate area under the curve and growth rates. The 96-hour EC_{50}, based on cell density and area under the growth curve, was 71 mg/L PFOS (NOEC=44 mg/L). The plant value was acceptable for quantitative use.

Boudreau (2002) performed a 96-hour static acute algal growth inhibition test on PFOS (potassium salt, 95% purity) with *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*). The study was part of a Master's thesis at the University of Guelph, Ontario, Canada and subsequently published in the open literature as **Boudreau et al. (2003a)**. The acute

algal growth inhibition tests followed protocols found in ASTM (1999b); Geis et al. (2000) and Geis et al. (2000). All treatment concentrations were based on the PFOS anion (without K) and solutions were prepared in laboratory-grade distilled water. *R. subcapitata* (UTCC 37 strain) were obtained as slants from the University of Toronto Culture Collection (UTCC; Toronto, Canada). Toxicity testing consisted of initial range-finder tests (0, 28, 56, 113, 225, and 450 mg/L) followed by at least two definitive tests (0, 12.5, 25, 50, 100, 200, and 400 mg/L). Tests were conducted in 20 mL solutions in 60 x 15 mm polyethylene disposable petri dishes. Each dish contained $1.5x10^4$ cells/mL and each test concentration had four replicates. Tests were continuously illuminated with cool-white fluorescent light between 3,800 and 4,200 lux and incubated at 23±1°C. Each dish was manually shaken twice a day during testing. Toxicity test endpoints included cell density and chlorophyll *a* content. The reported NOEC and IC_{50} based on most sensitive endpoint, cell density, were 5.3 mg/L and 48.2 mg/L. The IC_{50} from the study was acceptable for quantitative use.

E.2.5 Green alga, *Scenedesmus quadricauda*

Yang et al. (2014) conducted a 96-hour static, measured test on the growth effects of PFOS (potassium salt, CAS # 2795-39-3, 99% purity) with the green alga, *Scenedesmus quadricauda*. Algae were obtained from in-house cultures from the Chinese Research Academy of Environmental Sciences. The algae used for testing were inoculated at a cell density equal to $2.0x10^4$ cells/mL in 50 mL beakers. PFOS was dissolved in deionized water and DMSO (amount not provided) and then diluted with M4 medium. Alga were exposed to 0 (solvent control), 50.00, 65.00, 84.50, 109.85, 142.81 and 185.65 mg/L PFOS. Each treatment was replicated three times. While the text implied the exposures were static, the supplemental information provided the measured test concentrations in the highest and lowest test treatments both before and after renewal. Measured concentrations ranged from 42.56 mg/L (before renewal) to 49.78 mg/L

(after renewal) in the lowest treatment, and from 165.61 (before renewal) to 183.90 mg/L (after renewal) in the highest treatment. The experiments were conducted at 22±2°C with a 12:12-hour light:dark cycle. The initial pH of the test solution was 7.0±0.5, total hardness was 190±0.1 mg/L as $CaCO_3$, and total organic carbon was 0.02 mg/L. Algae concentrations in the beakers were measured daily with a microscope. The 96-hour EC_{50} (based on growth inhibition) was 89.34 mg/L and was acceptable for quantitative use.

E.2.6 Duckweed, *Lemna* sp.

Desjardins et al. (2001b) performed a static, measured 7-day growth inhibition study on the duckweed *Lemna gibba* with PFOS-K (perfluorooctanesulfonate potassium salt, 86.9% purity from 3M Corporation). The test protocol from U.S. EPA, OPPTS Number 850.4400 was followed. Duckweed was cultured and tested at Wildlife International Ltd. in 20X AAP medium and were actively growing for at least two weeks prior to testing. The pH of the medium was adjusted to pH 7.5 with HCl and filtered to sterilize before use. Test chambers were covered 250 mL plastic beakers with 100 mL of culture medium or test concentration and held at 25°C under continuous warm-white lighting with a target intensity of 5,000 lux. Fronds of duckweed were exposed to six test concentrations and a control with three replicates for each treatment. PFOS concentrations in the test medium were measured on day 0, 3, 5 and 7 with mean reported concentrations of <4.39 (LOQ), 7.74, 15.1, 31.9, 62.5, 147 and 230 mg/L PFOS active ingredient. Growth was defined as an increase in the total number of fronds in each replicate and measured by direct count on day 3, 5 and 7. Frond numbers on day seven in the 147 and 230 mg/L test treatments were inhibited by 65 and 81% as compared to the control. The reported 5-day IC_{10} based on frond number was 30.7 mg/L PFOS. The independently-calculated 5-day IC_{10} value was 18.06 mg/L and was acceptable for quantitative use.

Wu et al. (2023) tested perfluorooctane sulfonate (PFOS) on duckweed, *Lemna minor*, for 96 hours in a static, measured experiment. PFOS (≥95.0% purity) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). DMSO solvent (≥ 99.9% purity) was obtained from Merck (Germany). Duckweed was obtained from in house cultures that had been grown in a modified Swedish Standards Institute (SIS) medium. Duckweed cultures were housed in 150 mm petri dishes with 100 mL SIS medium that was changed every two weeks. SIS medium pH was adjusted to 6.5 by using NaOH or HCl. Plants were cultured at 25±1°C and 60% humidity under a 12:12 light dark cycle at 2,000 lux. Duckweed was precultured for 1 week in clean SIS media for seven days prior to testing. Duckweed experiments were conducted in 6-well polypropylene plates to avoid PFOS sorption to container walls. Each replicate well contained 10mL of test material and two colonies approximately the same size of a 3-frond *L. minor*. Nominal test concentrations were 0 (control), 0.001, 0.1, and 10 mg/L PFOS. All test chambers included DMSO solvent, and each treatment had three duplicates. PFOS concentrations measured on day 0 were 1.00±0.02, 90.0±0.73, and 9,859±2.83 µg/L. PFOS in solvent controls were not reported for day 0 but was below detection levels when measured after 96 hours. The number of fronds in each treatment well were counted after 48 and 96 hours. In addition, Fourier-transform infrared spectroscopy (FTIR) was performed on a subset of fronds from each treatment at the end of the 96-hour exposure to examine responses to PFOS at the biochemical level. Statistically significant ($p<0.05$) differences between treatment groups were assessed with one-way ANOVA followed by Dunnett's tests using SPSS Statistics 26. No statistically significant differences in frond number were observed. However, FTIR analysis revealed structural and functional alterations in response to PFOA at the biochemical level. The reported NOEC of 9.859 mg/L for population growth rate was determined to be acceptable for quantitative use.

E.2.7 Watermilfoil, *Myriophyllum* sp.

Hanson et al. (2005) conducted a 42-day toxicity study of PFOS (potassium salt, purity not provided) with two species of submergent watermilfoils, *Myriophyllum spicatum* and *M. sibiricum*. The study was conducted in 12,000 L outdoor microcosms at the University of Guelph Microcosm Facility located in Ontario, Canada. Each microcosm was below ground and was flush with the surface. Plastic trays filled with sediment (1:1:1 mixture of sand, loam and organic matter, mostly manure) were placed in the bottom of each microcosm. The total carbon content of the sediment was 16.3%. Ten apical shoots, 5 cm in length, from in-house cultures using the same sediment were transferred to each microcosm, with three separate microcosms used for each treatment (0, 0.3, 10 and 30 mg/L). Endpoints of toxicity that were monitored on days 1, 14, 28 and 42 of the study included growth in plant length, root number, root length, longest root, node number, wet mass, dry mass, and chlorophyll *a* and *b* content. PFOS treatments were dissolved in the same water (well water) used to supply the microcosms. Measured concentrations in the microcosms were reported in a companion publication (Boudreau et al. 2003b). Results from the companion paper showed that measured concentrations remained similar to nominal concentrations throughout the entire exposure period and did not change appreciably over the course of the study. Water quality (i.e., pH, temperature, D.O., hardness, and alkalinity) and light levels were measured regularly, but were not reported. *M. sibiricum* was more sensitive to PFOS than *M. spicatum*. The 42-day EC_{10} (based on wet weight) was 0.6 mg/L for *M. sibiricum* and 3.5 mg/L for *M. spicatum*. The plant values were acceptable for quantitative use.

Appendix F Acceptable Estuarine/Marine Plant PFOS Toxicity Studies

F.1 Summary Table of Acceptable Quantitative Estuarine/Marine Plant PFOS Toxicity Studies

Species	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Effect	Reported Effect Concentration (mg/L)	Reference
Green alga, *Chlorella sp.*	S, U	96 hr	PFOS >98%	-	23	30[c]	EC50 (population abundance)	77.62	Mao (2023)

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, NR=not reported

[b] Salinity of Erdschreiber's medium

F.2 Summary of Plant PFOS Toxicity Studies Considered in the Aquatic Life Criterion Derivation

F.2.1 Green alga, *Chlorella* sp.

Mao (2023) tested perfluorooctane sulfonate (PFOS) on an estuarine/marine *Chlorella sp.* for seven days in a static, unmeasured experiment. PFOS (>98% purity) was obtained from Tokyo Chemical Industry Co. Ltd. Algae were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology in Wuhan, China. Algae were obtained from the Algae Culture Collection at the Institute of Hydrobiology in Wuhan, China. Algae were cultured in Erdschreiber medium within a conical flask inside an illumination incubator at 23±1°C under a 12:12 light:dark cycle at 5,000 lux. Algae were shaken three times per day to prevent sticking to the sides of the flask, and inoculated once every two weeks to maintain optimal growth. *Chlorella sp.* in the exponential growth phase were added to test vessels containing test solution at a density of approximately $5.0x10^4$ cells/mL. The experimental design consisted of nominal concentrations of 0 (control), 5, 10, 20, 40, 80, and 160 mg/L PFOS. Testing protocols followed OECD guidelines, and all experiments were performed in triplicate. Statistical analysis included one-way ANOVA, using SPSS version 26 software. Algal cell density and size were measured daily, and algal growth inhibition was calculated using the equation provided in the OECD guidelines. Chlorophyll *a*, maximum quantal yield, cell membrane integrity, esterase activity relative to control, relative electron transfer rate, and reactive oxygen species were reported after 1, 3, 5, and 7 days, respectively. Algae exhibited maximum growth at 10 mg/L, but growth significantly declined at 40 mg/L and higher concentrations. Increasing PFOS concentrations also inhibited chlorophyll *a*, and increased oxidative stress. The 96-hour EC_{50} for algal growth inhibition was 77.62 mg/L, and was determined to be acceptable for quantitative use.

Appendix G Other Freshwater PFOS Toxicity Studies

G.1 Summary Table of Acceptable Qualitative Freshwater PFOS Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Unicellular protist, *Paramecium caudatum*	S, U	1 hr	Heptadecafluorooctane sulfonic acid potassium salt >98%	7.2	20-24	LC50	-	12.86[e]	Duration too short for an acute test, single-cell organism	Matsubara et al. (2006)
Protozoa, *Tetrahymena pyriformis*	S, U	2 hr	PFOS Unreported	7.2	25	EC50 (population abundance)	-	51.51[e]	Duration too short for an acute test, single-cell organism	Lim (2022)
Protozoa, *Tetrahymena pyriformis*	S, U	96 hr	PFOS Unreported	7.2	25	EC50 (population abundance)	-	13.2	Single-cell organism	Lim (2022)
Cyanobacteria, *Anabaena sp.*	S, M	24 hr	PFOS 98%	-	-	EC50 (bioluminescence)	-	16.29	Duration too short for a plant test, non-apical endpoint	Rodea-Palomares et al. (2012)
Cyanobacteria, *Anabaena sp.*	S, U	24 hr	PFOS-K 98%	7.8	28	EC50 (bioluminescence)	-	83.51	Duration too short for a plant test, non-apical endpoint	Rodea-Palomares et al. (2015)
Green alga (7.0×10^5 cells/ml), *Chlorella vulgaris*	S, M	96 hr	PFOS-K 98%	-	-	LOEC (chlorophyll a)	-	40	Missing exposure details	Xu et al. (2017)
Green alga, *Raphidocelis subcapitata*	S, M	72 hr	PFOS-K 98%	-	21-24	EC50 (growth)	-	35	Duration too short for a plant test, missing some exposure details	Rosal et al. (2010)
Green alga, *Raphidocelis subcapitata*	S, U	72 hr	PFOS-K 98%	-	22	EC50 (growth inhibition)	-	35	Duration too short for a plant test	Boltes et al. (2012)
Green alga, *Scenedesmus obliquus*	S, U	72 hr	PFOS Unreported	7.5	22	IC50 (growth rate reduction)	-	77.8[e]	Duration too short for a plant test	Liu et al. (2008)
Green alga, *Scenedesmus obliquus*	S, U	72 hr	PFOS ≥98%	7.5	22	NOEC (growth)	-	40	Duration too short for a plant test	Liu et al. (2009)
Green alga, *Scenedesmus obliquus*	S, U	7 d	PFOS-K >98%	7.1	25	EC50 (biomass)	-	136.69	Missing exposure details	Xue et al. (2022)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Duckweed, *Lemna gibba*	S, U	7 d	PFOS-K 95%	-	25	IC50 (wet weight)	-	31.1	Culture water not characterized, missing some exposure details	Boudreau et al. (2003a)
Blue green algae, *Scynechocystis sp.*	S, M	2 d	PFOS-K >98%	7.5	30	NOEC (abundance)	1->1	1	Only one exposure concentration	Marchetto et al. (2021)
Blue green algae, *Scynechocystis sp.*	F, M	12-15 d	PFOS-K >98%	7.5	30	NOEC (biomass)	1->1	1	Only one exposure concentration	Marchetto et al. (2021)
Aquatic microcosm (mixed invertebrate and aquatic plant community)	S, M	35-42 d	PFOS-K 86%	8.3-8.6	15.9-20.5	MATC (zooplankton community abundance)	3.0-10	5.478	Mixed species exposure, static chronic exposure	Boudreau (2002); Boudreau et al. (2003b)
Aquatic microcosm (mixed invertebrate and aquatic plant community)	S, M	35 d	PFOS-K Unreported	8.3	18	MATC (zooplankton abundance; *Cyclops diaptomus* abundance)	1.0-10	3.162	Mixed species exposure	Sanderson et al. (2002)
Tubificid worm (0.03g, 0.8cm), *Limnodrilus hoffmeisteri*	S, M	96 hr	PFOS-K 99%	7	22	LC50	-	120.97	Atypical source of organisms	Yang et al. (2014)
Tubificid worm, *Limnodrilus hoffmeisteri*	S, U	24 hr	PFOS >98%	5.0	23	LC50	-	45.26	Duration too short for an acute test, missing some exposure details	Liu et al. (2016)
Tubificid worm, *Limnodrilus hoffmeisteri*	S, U	24 hr	PFOS >98%	6.0	23	LC50	-	46.23	Duration too short for an acute test, missing some exposure details	Liu et al. (2016)
Tubificid worm, *Limnodrilus hoffmeisteri*	S, U	24 hr	PFOS >98%	7.0	23	LC50	-	60.70	Duration too short for an acute test, missing some exposure details	Liu et al. (2016)
Tubificid worm, *Limnodrilus hoffmeisteri*	S, U	24 hr	PFOS >98%	8.0	23	LC50	-	64.48	Duration too short for an acute test, missing some exposure details	Liu et al. (2016)
Tubificid worm, *Limnodrilus hoffmeisteri*	S, U	24 hr	PFOS >98%	9.0	23	LC50	-	65.74	Duration too short for an acute test, missing some exposure details	Liu et al. (2016)
Tubificid worm (3-4 cm), *Limnodrilus hoffmeisteri*	R, U	48 hr	PFOS-K 98%	6.2	22	LC50	-	23.81	Duration too short for an acute test, missing some exposure details	Qu et al. (2016)
Tubificid worm (3-4 cm), *Limnodrilus hoffmeisteri*	R, U	48 hr	PFOS-K 98%	7.0	22	LC50	-	35.89	Duration too short for an acute test, missing some exposure details	Qu et al. (2016)
Tubificid worm (3-4 cm), *Limnodrilus hoffmeisteri*	R, U	48 hr	PFOS-K 98%	8.0	22	LC50	-	39.80	Duration too short for an acute test, missing some exposure details	Qu et al. (2016)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Planarian (10-12 mm), *Dugesia japonica*	R, U	10 d	PFOS-K >99%	-	20	LOEC (regeneration: decreased appearance of auricles)	< 0.5-0.5	0.5	Duration too long for an acute test and too short for a chronic test	Yuan et al. (2014)
Planarian, *Dugesia japonica*	R, U	10 d	PFOS >99%	-	20	LOEC (enzymatic, gene expression and biochemistry changes)	-	5	Duration too long for an acute exposure and too short for a chronic exposure, atypical endpoints	Zhang et al. (2023a)
Planarian, *Dugesia japonica*	R, U	7 d	PFOS Unreported	-	20	MATC (gene expression)	0.5-1	0.7071	Duration too long for an acute exposure and too short for a chronic exposure, atypical endpoints	Sun et al. (2023a)
Chinese pond mussel (1 year), *Sinanodonta woodiana* (formerly, *Anodonta woodiana*)	S, U	48 hr	PFOS Unreported	7	24	LC50	-	28.39	Duration too short for an acute test	Xia et al. (2018)
Freshwater mussel (6 cm), *Unio ravoisieri*	R, U	96 hr	PFOS-K ≥98%	8	18	LC50	-	65.9	Test species fed from the natural freshwater used	Amraoui et al. (2018)
Asian clam (adult), *Corbicula fluminea*	R, M	28 d	PFOS-K 98%	6.85-7.35	23	LOEC (biochemical, enzyme and genetic markers)	-	0.0005082	Atypical endpoint, only one exposure concentration	Bi et al. (2022)
Mud snail (4.0 g, 2.0 cm) *Cipangopaludina cathayensis*	S, M	96 hr	PFOS-K 99%	7	22	LC50	-	247.14	Source of organisms may be problematic	Yang et al. (2014)
Snail (adult), *Lymnaea stagnalis*	S, M	96 hr	PFOS-K 95%	-	20	LC50	-	196	Test species fed	Olson (2017)
Snail (0-3 week, juvenile), *Lymnaea stagnalis*	S, M	96 hr	PFOS-K 95%	-	20	LC50	-	150	Test species fed	Olson (2017)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Snail (0-3 week, juvenile), *Lymnaea stagnalis*	R, M	21 d	PFOS-K 95%	-	20	NOEC (survival, feeding rate, mass change, length change, carbohydrate concentration)	50->50	>50	Duration too short for a chronic test	Olson (2017)
Snail (3-6 week, juvenile), *Lymnaea stagnalis*	R, M	21 d	PFOS-K 95%	-	20	MATC (mass change, length change)	25-50	35.35	Duration too short for a chronic test	Olson (2017)
Snail (6-9 week, juvenile), *Lymnaea stagnalis*	R, M	21 d	PFOS-K 95%	-	20	NOEC (survival, mass change, length change, carbohydrate and protein concentration)	50->50	>50	Duration too short for a chronic test	Olson (2017)
Snail (9-12 week, juvenile), *Lymnaea stagnalis*	R, M	21 d	PFOS-K 95%	-	20	NOEC (survival, feeding rate, mass change, length change, protein concentration)	50->50	>50	Duration too short for a chronic test	Olson (2017)
Snail (adult), *Lymnaea stagnalis*	R, M	21 d	PFOS-K 95%	-	20	MATC (survival)	3.0-6	4.243	Duration too short for a chronic test	Olson (2017)
Snail (5 mm), *Physella heterostropha pomilia* (formerly, *Physa pomilia)*	S, M	50 hr	PFOS-K ≥98%	-	25	NOEC-LOEC (avoidance)	< 30-30	-	Duration too short for an acute test; atypical endpoint	Funkhouser (2014)
Snail (adult, 4 mo.), *Physella heterostropha pomilia* (formerly, *Physa pomilia*)	R, M	14 d	PFOS-K ≥98%	-	25	LC50	-	94.99	Duration too long for an acute test and too short for a chronic test	Funkhouser (2014)
Rotifer (< 2 hr old neonates), *Brachionus calyciflorus*	R, U[b]	4 d	PFOS-K 98%	-	20	MATC (intrinsic rate of population increase and resting egg production)	0.125-0.25	0.1768	Atypical concentration-response pattern	Zhang et al. (2014)
Cladoceran (<24 hr), *Daphnia magna*	R, U	25 d	PFOS-K 99%	7.8	20	MATC (reproduction F0 generation)	0.01-0.1	0.03162	No consistent concentration-response relationship	Jeong et al. (2016)
Cladoceran (<24 hr), *Daphnia magna*	S, U	48 hr	PFOS-Li 24.5%	8.6	20.1-21.0	EC50 (death/immobility)	-	51.45	Inability to verify author-reported LC50	3M Company (2000)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Cladoceran (0-24 hr), *Daphnia magna*	S, U	48 hr	PFOS-K Unreported	7.6	22	EC50		27	Another test within the same publication had 24.5% purity and this test purity was unknown, could be of low purity.	3M Company (2000)
Cladoceran (0-12 hr), *Daphnia magna*	R, U	28 d	PFOS-K Unreported	7.6	22	MATC (reproduction)	7.0-18.0	11.22	Inability to calculate an EC10 and comments by authors	3M Company (2000)
Cladoceran (adult, ~ 14 d), *Daphnia magna*	S, M	48 hr	PFOS-K ≥98%	-	21	MATC (biochemistry changes)	13.34-27.33	19.09	Non-apical endpoint	Labine et al. (2022)
Amphipod (7 d), *Hyalella azteca*	R, M	7 d	PFOS-K 97.5%	7.11 (6.85-7.46)	22.8 (22.1-23.3)	EC20 (growth - biomass)	-	7.20	Duration too short for a chronic exposure	Kadlec et al. (2024)
Crayfish (3 wk juvenile, 0.048 g), *Procambarus fallax f. virginalis*	R, M	38 d	PFOS-K ≥98%	-	25	MATC (survival/growth)	0.2->0.2	>0.2	Only two organisms per exposure concentration; invasive species	Funkhouser (2014)
Crayfish (juvenile, 2 wk, 0.041 g), *Procambarus fallax f. virginalis*	R, M	7 d	PFOS-K ≥98%	-	25	LC50	-	39.71	Duration too long for an acute test and too short for a chronic test, only six organisms per exposure concentration, test species fed; invasive species	Funkhouser (2014)
Crayfish (intermolt), *Pontastacus leptodactylus* (formerly, *Astacus leptodactylus*)	R, M	21 d	PFOS-K ≥98%	6.79	21	MATC (oxidative status index)	0.5-5	1.581	Non-apical endpoint	Belek et al. (2022)
Oriental river prawn (0.30 g, 4.0 cm), *Macrobrachium nipponense*	S, M	96 hr	PFOS-K 99%	7	22	LC50	-	19.77	Source of organisms may be problematic	Yang et al. (2014)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Yellow fever mosquito (1st instar), *Aedes aegypti*	S, U	48 hr	PFOS Unreported	-	25	LC50	-	1.18	Duration too short for an acute test, missing some exposure details	Olson (2017)
Yellow fever mosquito (1st instar), *Aedes aegypti*	R, U	~42 d	PFOS Unreported	-	25	MATC (average time to emergence)	0.05-0.125	0.079	Missing some exposure details	Olson (2017)
Blue damselfly (larva, F2 instar stage), *Enallagma cyathigerum*	R, U	4 mo	Perfluorooctanesulfonic acid tetraethylammonium 98%	-	21	MATC (general activity, burst swimming, foraging success)	0.01-0.1	0.03163	Sporadic solution renewal, behavioral endpoints	Van Gossum et al. (2009)
Midge (Instar, 3 d), *Chironomus dilutus*	R, M	7 d	PFOS-K 97.5%	7.01 (6.54-7.33)	22.6 (21.7-23.7)	EC20 (growth - biomass)	-	0.018	Duration too short for a chronic exposure	Kadlec et al. (2024)
Midge (Instar, 3 d), *Chironomus dilutus*	R, M	7 d	PFOS-K 97.5%	7.38 (7.32-7.47)	22.9 (20.5-23.9)	EC20 (growth - biomass)	-	0.016	Duration too short for a chronic exposure	Kadlec et al. (2024)
Midge, *Chironomus dilutus*	R, M	10 d	PFOS 98%			LOEC (mortality)	-	0.0004086	Range-finding test	McCarthy et al. (2021)
Midge (0.05 g, 1.2 cm), *Chironomus plumosus*	S, M	96 hr	PFOS-K 99%	7	22	LC50	-	182.12	Source of organisms may be problematic	Yang et al. (2014)
Midge (larva, 3rd-4th instar), *Chironomus plumosus*	S, M	10.33 d	PFOS 98%	-	25	NOEC (mortality)	-	0.00985	Only one exposure concentration, sediment exposure	Zhai et al. (2016)
Midge (larva, 3rd-4th instar), *Chironomus plumosus*	S, M	10.33 d	PFOS 98%	-	25	NOEC (mortality)	-	0.00987	Only one exposure concentration, sediment exposure	Zhai et al. (2016)
Midge (larva, 3rd-4th instar), *Chironomus plumosus*	S, M	10.33 d	PFOS 98%	-	25	NOEC (mortality)	-	0.00987	Only one exposure concentration, sediment exposure	Zhai et al. (2016)
Midge (larva, 3rd-4th instar), *Chironomus plumosus*	S, M	10.33 d	PFOS 98%	-	25	NOEC (mortality)	-	0.00985	Only one exposure concentration, sediment exposure	Zhai et al. (2016)
Midge (larva, 3rd-4th instar), *Chironomus plumosus*	S, M	10.33 d	PFOS 98%	-	25	NOEC (mortality)	-	0.00985	Only one exposure concentration, sediment exposure	Zhai et al. (2016)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Midge (larva, 3rd-4th instar), *Chironomus plumosus*	S, M	10.33 d	PFOS 98%	-	25	NOEC (mortality)	-	0.00985	Only one exposure concentration, sediment exposure	Zhai et al. (2016)
Midge (larva, 3rd-4th instar), *Chironomus plumosus*	S, M	10.33 d	PFOS 98%	-	25	NOEC (mortality)	-	0.00987	Only one exposure concentration, sediment exposure	Zhai et al. (2016)
Midge (larva, 3rd-4th instar), *Chironomus plumosus*	S, M	10.33 d	PFOS 98%	-	25	NOEC (mortality)	-	0.00987	Only one exposure concentration, sediment exposure	Zhai et al. (2016)
Midge (multi-generational), *Chironomus riparius*	S, M	10 generations (~20-28 d ea.)	PFOS Unspecified	7.8-8.2	20	NOEC (emergence, reproduction, sex ratio)	-	0.0035	Only one exposure concentration, static chronic test	Stefani et al. (2014)
Midge (multi-generational), *Chironomus riparius*	S, M	10 generations (~20-28 d ea.)	PFOS Unspecified	7.8-8.2	20	LOEC (increased mutation rate)	-	0.0035	Only one exposure concentration, static chronic test	Stefani et al. (2014)
Midge (1st instar larva), *Chironomus riparius*	S, M	~36 d[d] (1st of 10 generations)	PFOS Unreported	7.5-8.2	20.1	LOEC (F1 developmental time, adult weight, exuvia length)	-	0.004	Only one exposure concentration, static chronic test, significant responses not observed in every generation	Marziali et al. (2019)
European eel (juvenile, 138.3 g), *Anguilla anguilla*	R, M	28 d	PFOS-K >98%	-	20	NOEC (survival, growth)	0.011->0.011	0.011	Not true ELS test (28 days beginning with juvenile)	Roland et al. (2014)
European eel (juvenile, 138.3 g), *Anguilla anguilla*	R, M	28 d	PFOS-K >98%	-	20	LOEC (proteomic growth)	< 0.00081-0.00081	0.00081	Not true ELS test (28 days beginning with juvenile), atypical endpoint	Roland et al. (2014)
Rainbow trout (immature, 16.4 cm, 22.7 g), *Oncorhynchus mykiss*	Microcosm	12 d	PFOS 89%	9.2	6.0-16.5	NOEC (mortality)	-	3	Atypical exposure, not a true ELS test	Oakes et al. (2005)
Rainbow trout (immature, 16.4 cm, 22.7 g), *Oncorhynchus mykiss*	Microcosm	12 d	PFOS 89%	9.2	6.0-16.5	LOEC (decrease LSI and condition index (K) in females)	-	3	Atypical exposure, not a true ELS test	Oakes et al. (2005)
Rainbow trout (female, mature, 34.8 cm, 511.1 g), *Oncorhynchus mykiss*	S, U	14 d	PFOS 89%	-	12	NOEC (mortality)	-	1	Atypical exposure, not a true ELS test	Oakes et al. (2005)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Rainbow trout (female, mature, 34.8 cm, 511.1 g), *Oncorhynchus mykiss*	S, U	14 d	PFOS 89%	-	12	LOEC (decrease LSI)	-	1	Atypical exposure, not a true ELS test	Oakes et al. (2005)
Rainbow trout (11 mo), *Oncorhynchus mykiss*	Diet, U	15 d	PFOS-K Unknown	-	12	NOEC (growth - weight)	-	250 mg/kg bw per day	Dietary exposure	Benninghoff et al. (2011)
Rainbow trout (fry, 15 week), *Oncorhynchus mykiss*	Diet, U	8 mo	PFOS-K Unknown	-	12	LOEC (survival, tumor incidence)	-	2.5 mg/kg bw per day	Dietary exposure, mixture exposure	Benninghoff et al. (2012)
Rainbow trout (oocyte, ova), *Oncorhynchus mykiss*	S, M	3 hr	PFOA >97%	-	6	NOEC (accumulation residue)	-	0.87	Duration too short for an acute test	Raine et al. (2021)
Rainbow trout (oocyte, ova), *Oncorhynchus mykiss*	S, M	3 hr	PFOA >97%	8.5	6	LOEC (accumulation residue)	-	7.47	Duration too short for an acute test	Raine et al. (2021)
Rainbow trout (juvenile, ~7 mo), *Oncorhynchus mykiss*	R, M	10 d	PFOS Unreported	-	12	LOEC (enzymatic changes)	-	0.0008	Non-apical endpoint, duration too short for a chronic exposure	Solan et al. (2022)
Atlantic salmon (embryo), *Salmo salar*	R, U	49 d	Sodium perfluoro-1-octanesulfonate Unreported	-	5-7	NOEC (growth - length and weight)	0.1->0.1	0.1	Only one exposure concentration; greater than low value so less informative	Arukwe et al. (2013)
Goldfish (6.91 g, 6.01 cm), *Carassius curatus*	R, U	48 hr	PFOS-K >99%	-	18	NOEC-LOEC (swimming behavior: motion distance and % of actionless time)	2.0-8	-	Atypical endpoint and source of organisms, duration too short for an acute test	Xia et al. (2013a)
Goldfish (6.0 g, 7.0 cm), *Carassius curatus*	S, M	96 hr	PFOS-K 99%	7	22	LC50	-	81.18	Source of organisms may be problematic	Yang et al. (2014)
Goldfish (juvenile, 27.85 g), *Carassius curatus*	S, M	96 hr	PFOS >98%	7.25	23	Antioxidant enzyme activity	-	5.001[e]	Atypical endpoint, no point estimate	Feng et al. (2015)
Common carp (juvenile, 3.72g, 5.18 cm), *Cyprinus carpio*	R, U[c]	14 d	PFOS >98%	-	-	NOEC (liver protein)	1->1	1	Duration too short for a chronic test, atypical endpoint	Hagenaars et al. (2008)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Common carp (juvenile, 3.72g, 5.18 cm), *Cyprinus carpio*	R, U[c]	14 d	PFOS >98%	-	-	MATC (liver glycogen)	0.5-1	0.7071	Duration too short for a chronic test, atypical endpoint	Hagenaars et al. (2008)
Common carp (juvenile, 3.72g, 5.18 cm), *Cyprinus carpio*	R, U[c]	14 d	Perfluorooctanesulfonic PFOS >98%	-	-	NOEC (liver lipid)	1->1	1	Duration too short for a chronic test, atypical endpoint	Hagenaars et al. (2008)
Common carp (juvenile, 3.72g, 5.18 cm), *Cyprinus carpio*	R, U[c]	14 d	PFOS >98%	-	-	LOEC (relative condition factor)	< 0.1-0.1	0.1	Duration too short for a chronic test	Hagenaars et al. (2008)
Common carp (juvenile, 3.72g, 5.18 cm), *Cyprinus carpio*	R, U[c]	14 d	PFOS >98%	-	-	MATC (HSI)	0.1-0.5	0.2236	Duration too short for a chronic test, atypical endpoint	Hagenaars et al. (2008)
Common carp (juvenile, ~12 cm; ~20 g), *Cyprinus carpio*	F, M	96 hr	PFOS 100.3%	6.9	23	LOEC (DNA damage)	-	5.395	Atypical endpoint	Kim et al. (2010)
Common carp (juvenile), *Cyprinus carpio*	R, M	76 d	PFOS 98%	-	-	NOEC (growth - weight)	-	0.00082	Greater than low value	Shan et al. (2022)
Zebrafish (female fry, 14 dpf), *Danio rerio*	R, U	70 d	PFOS-K >99%	-	27	EC10 (male weight)	0.01-0.05	0.001990	Missing some exposure details	Du et al. (2009)
Zebrafish (embryo - blastula stage), *Danio rerio*	R, U	Fert. up to 15 dpf	PFOS-K 99%	-	-	MATC (body length and average weight)	0.200-0.400	0.2828	Duration too short for a chronic test	Shi et al. (2009)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, M	114 hr	PFOS >96%	7.0-7.5	28	LC50	-	2.20	Duration too long for an acute test	Huang et al. (2010)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, M	114 hr	PFOS >96%	7.0-7.5	28	EC50 (malformation)	-	1.12	Duration too long for an acute test, atypical endpoint	Huang et al. (2010)
Zebrafish (embryo), *Danio rerio*	R, M	21 d	PFOS-K 98%	-	26	LOEC (reduce fecundity)	<0.5-0.5	0.5	Only one exposure concentration, control issues	Sharpe et al. (2010)
Zebrafish (embryo), *Danio rerio*	R, M	48 hr	PFOS-K 98%	-	26	LC50 (range of 3 tests)	-	7.7-38.9	Duration too short for an acute test, results are not reproducible	Sharpe et al. (2010)
Zebrafish (embryo, 4 hpf), *Danio rerio*	S, U	96 hr	PFOS-K >99%	-	28.5	NOEC-LOEC (increased ROS formation)	0.2-0.4	-	Atypical endpoint, missing exposure details	Shi and Zhou (2010)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo), *Danio rerio*	R, U	96 hr	PFOS-K 98%	-	26	LC50	-	54.36[e]	Problems with reported data to be used for LC50 analysis	Ding et al. (2012); Ding et al. (2013)
Zebrafish (F2 embryo, 0 hpf), *Danio rerio*	F, M	300-330 d	PFOS-K ≥98%	8.25-8.75	26	MATC (F2 180 d survival)	0.1-0.3	0.1732	Poor concentration-response, test design complications	Keiter et al. (2012)
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	R, U	6 d	PFOS Unreported	-	26	AC50 (toxicity score: includes survival, hatchability, and malformation index)	-	16.44[e]	Duration too long for an acute test and too short for a chronic test, atypical endpoint	Padilla et al. (2012)
Zebrafish (embryo), *Danio rerio*	S, U	72 hr	PFOS 98%	8.3	28.5	LC50	-	68	Duration too short for an acute test, missing some exposure details	Zheng et al. (2012)
Zebrafish (embryo), *Danio rerio*	S, U	72 hr	PFOS 98%	8.3	28.5	EC50 (malformation)	-	37	Duration too short for an acute test, atypical endpoint, missing exposure details	Zheng et al. (2012)
Zebrafish (embryo, F0 generation), *Danio rerio*	R, U	120 dpf	PFOS >96%	6.8-7.6	28	LOEC Increase mortality and malformations in the F1 generation	<0.250-0.250	0.250[e]	Only one exposure concentration	Chen et al. (2013)
Zebrafish (embryo, 4hpf), *Danio rerio*	R, U	120 hr	PFOS ≥98%	-	28	NOEC-LOEC (suppression of steroidogenic enzyme synthesis)	0.1-0.2	-	Duration too long for an acute test, atypical endpoint, missing exposure details	Du et al. (2013)
Zebrafish (embryo, 4 hpf), *Danio rerio*	R, U	116 hr	PFOS-K Unreported	-	-	NOEC (development, hatch, mortality)	5->5	5	Duration too long for an acute test and too short for a chronic test, only one exposure concentration	Liu et al. (2013)
Zebrafish (embryo – 4 cell stage), *Danio rerio*	S, U	Fert. to 144 hpf	PFOS Unreported	7.2-7.6	26	EC50 (lethal and sublethal effects)	-	1.5	Duration too long for an acute test and too short for a chronic test, static chronic exposure	Ulhaq et al. (2013)
Zebrafish (embryo - 4 cell stage), *Danio rerio*	S, U	Fert. to 144 hpf	PFOS Unreported	7.2-7.6	26	LC50	-	>10	Duration too long for an acute test and too short for a chronic test, static chronic exposure	Ulhaq et al. (2013)
Zebrafish (embryo), *Danio rerio*	S, U	48 hr	PFOS >96%	-	-	Malformation (100%)	-	8.002[e]	Duration too short for an acute test, atypical endpoint, no point estimate	Chen et al. (2014)
Zebrafish (embryo), *Danio rerio*	R, U	6 d	PFOS-K 98%	7.5	28.5	LC50	-	6.25	Duration too long for an acute test and too short for a chronic test	Hagenaars et al. (2014)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo), *Danio rerio*	R, U	6 d	PFOS-K 98%	7.5	28.5	EC50 (uninflated swim bladder)	-	2.29	Duration too long for an acute test and too short for a chronic test, atypical endpoint	Hagenaars et al. (2014)
Zebrafish (embryo, 2 hpf), *Danio rerio*	S, U	6 d	PFOS-K >98%	7.4	28	NOEC-LOEC (behavior: spontaneous swimming activity)	0.1-1.0	-	Duration too long for an acute test and too short for a chronic test, atypical endpoint, only two exposure concentrations	Spulber et al. (2014)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS-K Unknown	-	28	LOEC (mortality)	3.307-33.07	33.07[e]	Duration too long for an acute test and too short for a chronic test	Truong et al. (2014)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unknown	-	28	LOEC (mortality)	0.32-3.2	3.2[e]	Duration too long for an acute test and too short for a chronic test	Truong et al. (2014)
Zebrafish (embryo, 8 hpf), *Danio rerio*	R, U	42 dpf	PFOS >96%	7.0-7.5	28	LOEC (increased condition index)	-	0.25	Only one exposure concentration	Chen et al. (2016)
Zebrafish (embryo, 8 hpf), *Danio rerio*	R, U	150 dpf	PFOS >96%	7.0-7.5	28	LOEC (increased estradiol in male/females and testosterone in males)	-	0.25	Only one exposure concentration	Chen et al. (2016)
Zebrafish (larva, 120 hpf), *Danio rerio*	S, M	24 hr	PFOS ≥98%	7.0-7.5	28	NOEC (various metabolites)	-	9.700	Duration too short for an acute test, atypical endpoint	Huang et al. (2016)
Zebrafish (embryo), *Danio rerio*	R, U	6 d	PFOS Unknown	-	28	LOEC (liver size and gene expression)	<0.0005-0.0005	0.0005	Duration too long for an acute test and too short for a chronic test, atypical endpoint	Tse et al. (2016)
Zebrafish (embryo, 8 hpf), *Danio rerio*	R, U	180 d	PFOS >96%	7.0-7.5	27	MATC (altered sex ratio: female dominance, F1 offspring survival)	0.05-0.25	0.1118[e]	Non-apical endpoint	Cui et al. (2017)
Zebrafish (3 hpf), *Danio rerio*	S, U	5 d + 9 d observation	PFOS Unreported	7.2-7.7	26-28	MATC (growth - total body length)	0.02-0.2	0.06325[e]	Duration too long for an acute test and too short for a chronic test, static chronic exposure	Jantzen et al. (2017)
Zebrafish (3 hpf), *Danio rerio*	S, U	5 d + 9 d observation	PFOS Unreported	7.2-7.7	26-28	LOEC (interoccular distance)	< 0.02-0.02	0.02[e]	Duration too long for an acute test and too short for a chronic test, static chronic exposure	Jantzen et al. (2017)
Zebrafish (3 hpf), *Danio rerio*	S, U	5 d + 9 d observation	PFOS Unreported	7.2-7.7	26-28	MATC (yolk sac area)	0.02-0.2	0.06325[e]	Duration too long for an acute test and too	Jantzen et al. (2017)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
									short for a chronic test, static chronic exposure	
Zebrafish (3 hpf), *Danio rerio*	S, U	5 d + 9 d observation	PFOS Unreported	7.2-7.7	26-28	LOEC - (swimming activity - crossing frequency)	< 0.02-0.02	0.02[e]	Duration too long for an acute test and too short for a chronic test, static chronic exposure	Jantzen et al. (2017)
Zebrafish (embryo), *Danio rerio*	S, M	48 hr	PFOS Unknown	-	27	LC50	-	107.6	Duration too short for an acute test	Rainieri et al. (2017)
Zebrafish (embryo, 3 hpf), *Danio rerio*	R, U	7 d	PFOS Unreported	-	28.5	MATC (islet morphological anomalies)	8.0-16.0[e]	11.31[e]	Duration too long for an acute test and too short for a chronic test	Sant et al. (2017)
Zebrafish (sperm), *Danio rerio*	S, U	20 sec	PFOS-K ≥98%	8	25	NOEC-LOEC (sperm motility)	0.09-0.9	-	Duration too short for an acute test, atypical endpoint	Xia and Niu (2017)
Zebrafish (sperm/egg), *Danio rerio*	S, U	2 min	PFOS-K ≥98%	8	25	NOEC-LOEC (fertilization success)	0.09-0.9	-	Duration too short for an acute test, atypical endpoint	Xia and Niu (2017)
Zebrafish (embryo, 3 hpf), *Danio rerio*	S, U	5 d	PFOS Unknown	7.2-7.7	27	LOEC (gene expression of Leptin A mRNA)	<0.01-0.01	0.01[e]	Duration too long for an acute test and too short for a chronic test, atypical endpoint	Annunziato (2018)
Zebrafish (embryo, 1-2 hpf), *Danio rerio*	R, U	96 hr	PFOS >99%	-	25	NOEC-LOEC (growth: body length)	<0.050-0.050	-	Atypical endpoint, missing exposure details	Dang et al. (2018)
Zebrafish (embro, 2 hpf), *Danio rerio*	R, U	72 hr	PFOS Unknown	-	28.5	LOEC (malformations)	0.5-1.0	1.0[e]	Duration too short for an acute test	Ortiz-Villanueva et al. (2018)
Zebrafish (embro, 2 hpf), *Danio rerio*	R, U	72 hr	PFOS Unknown	-	28.5	LOEC (survival)	5.0-10	10[e]	Duration too short for an acute test	Ortiz-Villanueva et al. (2018)
Zebrafish (embryo, 1 hpf), *Danio rerio*	R, U	96 hr	PFOS Unreported	7.6	28.5	NOEC-LOEC (pericardial area)	8-16[e]	-	Atypical endpoint, missing exposure details	Sant et al. (2018)
Zebrafish (embryo, 1 hpf), *Danio rerio*	S, U	96 hr	PFOS Unreported	-	26	NOEC (enzymes, olfactory cells)	-	6.650	Atypical endpoint, missing exposure details	Stengel et al. (2017a)
Zebrafish (female, 4 mo), *Danio rerio*	R, U	21 d	PFOS Unknown	7.0-7.5	28	NOEC (growth - length and weight)	0.2->0.2	0.2	Inability to independently verify effect values, partial life cycle test	Bao et al. (2019)
Zebrafish (embryo, maximum of 4 hpf), *Danio rerio*	R, M	96 hr	PFOS Unknown	-	26	NOEC (hatching success, embryo mortality, deformation)	0.0007->0.0007	0.0007	Greater than low value	Cormier et al. (2019)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, U	72 hr	PFOS-K ≥98%	-	28	LOEC (growth - total body length)	2.691-5.832	5.382[e]	Duration too short for an acute test	Martinez et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS-K Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	5.732[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS-K Unreported	-	-	Benchmark Dose Value at 10% extra effect (mortality)	-	3.014[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	2.526[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	6.357[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	4.181[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	6.642[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	2.786[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	1.180[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	5.211[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	1.370[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	4.751[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	4.641[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	4.791[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (abnormal development)	-	5.877[g]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	-	Benchmark Dose Value at 10% extra effect (mortality)	-	0.5501[e]	Duration too long for an acute test, atypical test endpoint	Thomas et al. (2019)
Zebrafish (embryo), *Danio rerio*	R, M	6 d	PFOS ≥98%	7.5	28.5	NOEC (growth and survival)	-	1.339	Greater than low value	Tu et al. (2019)
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, M	118 hr	PFOS-K ≥98%	-	28	EC50 (mortality, malformations)	-	2.045[e]	Duration too long for an acute test and too short for a chronic test, mixed test endpoints	Vogs et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	R, M	96 hr	Perfluoroctane sulfonate sodium salt >98%	-	26	NOEC (mortality, hatch)	-	0.4514[e]	Only one exposure concentration	Yi et al. (2019)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	90-94 hr	PFOS-K ≥98%	7-7.5	28	NOEC (mortality, deformity)	-	2.06	Duration too short for an acute test, behavioral focus with secondary reference to no mortality or deformity	Christou et al. (2020)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	66 hr	PFOS 97%	-	28	NOEC (survival)	25->25	25[e]	Duration too short for an acute test	Dasgupta et al. (2020)
Zebrafish (embryo 4-64 stage), *Danio rerio*	S, M	144 hr	PFOS 98%	7.2-7.6	26	MATC (abnormal development)	0.16-2.2	0.5933	Duration too long for an acute test and too short for a chronic test	Menger et al. (2020)
Zebrafish (adult, 4.5 mo), *Danio rerio*	R, U	28 d	PFOS >95%	-	28	LOEC (reproduction - sperm development)	<0.2510-0.2510	0.2501[e]	Atypical endpoint	Xin et al. (2020)
Zebrafish (adult, 4.5 mo), *Danio rerio*	R, U	28 d	PFOS >95%	-	28	NOEC (reproduction - oocyte development)	0.2510->0.2510	0.2501[e]	Atypical endpoint	Xin et al. (2020)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	90 hr exposure + 177 d observation	PFOS-K ≥98%	7.0-7.5	28-28.5	NOEC (mortality)	2.06->2.06	2.06	Atypical exposure duration	Christou et al. (2021)
Zebrafish (embryo, 4 hpf), *Danio rerio*	R, U	92 hr	PFOS ≥98%	-	-	NOEC (mortality)	0.5->0.5	0.5	Only one exposure concentration, duration too short for an acute test	Dong et al. (2021)
Zebrafish (embryo), *Danio rerio*	R, M	120 hr	PFOS-K >95%	-	28.5	NOEC (mortality)	-	>1.803[e]	Duration too long for an acute exposure, too short for a chronic test	Han et al. (2021)
Zebrafish (embryo), *Danio rerio*	R, M	120 hr	PFOS >95%	-	28.5	NOEC (mortality)	-	>1.730[e]	Duration too long for an acute exposure, too short for a chronic test	Han et al. (2021)
Zebrafish (embryo, 6 hpf), *Danio rerio*	R, M	96 hr	PFOS-K ≥98%	-	28.5	LOEC (malformations, locomotive behavior)	<20-20	20	Only one exposure concentration; atypical endpoint	Huang et al. (2021)
Zebrafish (embryo, 6 hpf), *Danio rerio*	R, U	96 hr	PFOS-K ≥98%	-	-	MATC (growth, histology, abnormal development)	10.0-20.0	14.14	Atypical endpoint for an acute test	Huang et al. (2021)
Zebrafish (embryo, 4 hpf), *Danio rerio*	S, U	116.83 hr	PFOS Unreported	-	28.0	NOEC (mortality)	10.00->10.00	10.00[c]	Atypical duration	Lee et al. (2021)
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Potassium perfluoro-1-octanesulfonate >98%	-	28	NOEC (mortality)	-	0.2476[e]	Duration too long for an acute test; only one exposure concentration	Rericha et al. (2021)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo, <1 hpf), *Danio rerio*	R, U	96 hr	PFOS Unreported	-	28.5	LOEC (increase lauric C12:0 and myristic C14:0 fatty acids)	<8.002-8.002	8.002[e]	Atypical endpoint	Sant et al. (2021)
Zebrafish (dechorinated embryo, 1 dpf), *Danio rerio*	R, U	30 d	PFOS Unreported	-	28.5	NOEC (growth - length)	16->16	16[e]	Growth effects not the focus of study rather other non-apical endpoints	Sant et al. (2021)
Zebrafish (adult, 90 dpf), *Danio rerio*	R, U	10 d	PFOS-K >98%	7.21	28.0	LOEC (gene expression)	<0.5-0.5	0.5	Atypical endpoint	Zhu et al. (2021)
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, M	120 hr	PFOS-K Unreported	-	28	MATC (growth - length)	0.050-2.066	0.3214	Duration too long for an acute exposure, too short for a chronic test	Fey et al. (2022)
Zebrafish (embryo, ≤4 hpf), *Danio rerio*	R, U	5 d	PFOS 99.4%	-	27	NOEC (mortality and development)	-	>0.0024	Duration too long for an acute exposure, too short for a chronic test, test represents a greater than low value (followed decision rule; Section 2.10.3.2)	Haimbaugh et al. (2022)
Zebrafish (embryo, ≤4 hpf), *Danio rerio*	R, U	4-6 wk	PFOS 99.4%	-	27	NOEC (reproduction and growth)	-	>0.0024	Test represents a greater than low value (followed decision rule; Section 2.10.3.2)	Haimbaugh et al. (2022)
Zebrafish (embryo, 4 hpf), *Danio rerio*	S, U	116 hr	PFOS Unreported	-	-	NOEC (development, mortality)	-	10.00[e]	Duration too long for an acute exposure, too short for a chronic test, test represents a greater than low value (followed decision rule; Section 2.10.3.2)	Lee et al. (2022)
Zebrafish (embryo, 3 hpf), *Danio rerio*	S, U	117 hr	PFOS-NA >98%	-	-	LC50	-	16.47[e]	Duration too long for an acute exposure, too short for a chronic test	Lindqvist and Wincent (2022)
Zebrafish (embryo, 7 hpf), *Danio rerio*	R, M	30 d	PFOS-K >98%	6.86-7.39	24.9-25.3	EC10 (growth-weight)	0.004-0.140	0.098	Poor control survival (75%)	Krupa et al. (2022)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS Unreported	-	28	Benchmark Dose Value at 10% extra effect (morphology)	-	7.753[e]	Duration too long for an acute exposure, too short for a chronic test	Truong et al. (2022)
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOS-K Unreported	-	28	Benchmark Dose Value at 10% extra	-	5.930[e]	Duration too long for an acute exposure, too short for a chronic test	Truong et al. (2022)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
						effect (morphology)				
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, U	94 hr	PFOS 98.7%	7.2	28.5	NOEC (growth - length)	-	>0.5001[e]	Duration too short for an acute exposure. Greater than low value.	Wang et al. (2022)
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, U	82 hr	PFOS 98.7%	7.2	28.5	NOEC (hatching rate)	-	>0.5001[e]	Duration too short for an acute exposure. Greater than low value.	Wang et al. (2022)
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, M	118 hr	PFOS-K ≥98%	-	28.0	NOEC (mortality)	-	2.418	Duration too long for an acute test	Wu et al. (2022)
Zebrafish (embryo, 0.5 hpf), *Danio rerio*	S, U	119.5 hr	PFOS-K ≥98%	7.2	28	EC50 (abnormal development)	-	6	Duration too long for an acute exposure, too short for a chronic test, atypical endpoint	Gui et al. (2023)
Zebrafish (embryo, 5 hpf), *Danio rerio*	R, U	8 mo	PFOS Unreported	6.8-7.0	28	NOEC (mortality)	-	>0.5001[g]	Greater than low value	Hawkeye et al. (2023)
Zebrafish (embryo, 72 hpf), *Danio rerio*	R, U	48.83 hr	PFOS ≥98%	-	28.5	LOEC (swimming behavior)	-	0.1	Duration too short for an acute exposure, atypical endpoint	Kalyn et al. (2023)
Zebrafish (adult, 3 mo), *Danio rerio*	F, U	14 d	PFOS Unreported	7.0-7.4	28	LOEC (growth - weight)	<0.08-0.08	0.08	Duration too short for a chronic exposure	Lu et al. (2024)
Zebrafish (2 mo), *Danio rerio*	R, U	14 d	PFOS-K Unreported	7	28	LOEC (biochemistry and enzymatic changes)	-	0.03	Non-apical endpoints, duration too short for a chronic exposure	Liu et al. (2023a)
Zebrafish (embryo, 4 hpf), *Danio rerio*	R, U	92 hr	PFOS-K >98%	7.2-7.4	27	MATC (growth - length)	0.100-0.500	0.2236	Non-apical endpoints, duration too short for an acute exposure	Mahapatra et al. (2023)
Zebrafish (embryo, 4 hpf), *Danio rerio*	S, U	116 hr	PFOS-K >98%	-	28.5	LC50	-	24.77[g]	Duration too long for an acute exposure and too short for a chronic exposure	Min et al. (2023)
Zebrafish (embryo, 6 hpf), *Danio rerio*	R, U	90 hr	PFOS-K Unreported	-	28	AC50 (development)	-	1.965[g]	Duration too short for an acute exposure	Phelps et al. (2023)
Zebrafish (embryo, 6 hpf), *Danio rerio*	R, U	90 hr	PFOS-K Unreported	-	28	AC50 (growth length)	-	1.351[g]	Duration too short for an acute exposure	Phelps et al. (2023)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 6 hpf), *Danio rerio*	R, U	90 hr	PFOS-K Unreported	-	28	AC50 (morphology)	-	1.125[g]	Duration too short for an acute exposure	Phelps et al. (2023)
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, U	70 hr	PFOS-K >98%	-	26	LC50	-	2.104[g]	Duration too short for an acute exposure	Zhou et al. (2023)
Spottail shiner (female, mature, 8.9 cm, 6.7 g), *Notropis hudsonius*	Microcosm	14 d	PFOS 89%	-	-	NOEC (mortality)	-	3	Atypical exposure, not a true ELS test	Oakes et al. (2005)
Spottail shiner (female, mature, 8.9 cm, 6.7 g), *Notropis hudsonius*	Microcosm	14 d	PFOS 89%	-	-	LOEC (increase TBARS in liver/ovary and FAO activity in liver)	-	3	Atypical exposure, not a true ELS test	Oakes et al. (2005)
Fathead minnow (embryo, 48 hpf), *Pimephales promelas*	F, M	33 d	PFOS-K Unreported	6.6-7.3	22-26	EC10 (survival)	1.0-1.9	1.378	Another test within the same publication had 24.5% purity and this test purity was unknown, could be of low purity	3M Company (2000)
Fathead minnow (mature, 6.1 cm, 2.0 g), *Pimephales promelas*	Microcosm	28 d	PFOS 89%	9.2	16.6-22.8	LC10	-	3.5	Atypical exposure, not a true ELS test	Oakes et al. (2005)
Fathead minnow (embryo, <2 hpf), *Pimephales promelas*	R, M	14 d	PFOS-K ≥98%	8.4	27	MATC (mortality)	1.25-2.50	1.768	Duration, atypical exposure (5 d in toxicant + 9 d in clean water)	Krzykwa et al. (2021)
Topmouth gudgeon (juvenile female, 0.81 g, 4.03 cm), *Pseudorasbora parva*	R, U	96 hr	PFOS-K >99%	-	15	NOEC-LOEC (spontaneous swim behavior: swim distance)	0.5-2	-	Atypical endpoint and source of organisms	Xia et al. (2014)
Topmouth gudgeon (4.0 g, 4.0 cm), *Pseudorasbora parva*	S, M	96 hr	PFOS-K 99%	7	22	LC50	-	67.74	Source of organisms may be problematic	Yang et al. (2014)
Topmouth gudgeon (4.0 g, 4.0 cm), *Pseudorasbora parva*	R, M	30 d	PFOS-K 99%	7	22	EC10 (survival)	-	2.12	Not a true ELS test (started with older life stage), renewal chronic exposure, source of organisms may be problematic	Yang et al. (2014)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Creek chub (mature, 11.8 cm, 16.3 g), *Semotilus atromaculatus*	Microcosm	14 d	PFOS 89%	-	-	NOEC (mortality)	-	3	Atypical exposure, not a true ELS test	Oakes et al. (2005)
Creek chub (mature, 11.8 cm, 16.3 g), *Semotilus atromaculatus*	Microcosm	14 d	PFOS 89%	-	-	LOEC (increase TBARS in liver/ovary and FAO activity in liver)	-	3	Atypical exposure, not a true ELS test	Oakes et al. (2005)
Quinbo (juvenile, 2.77 g, 5.62 cm), *Spinibarbus sinensis*	R, U	30 d	PFOS-K >99%	6.8-7.5	18	MATC (% mobile, % highly mobile, swim distance, swim speed, freq. highly mobile, % social, resting metabolic rate)	0.32-0.80	0.506	Test was not replicated	Xia et al. (2015b)
Quinbo (juvenile, 2.77 g, 5.62 cm), *Spinibarbus sinensis*	R, U	30 d	PFOS-K >99%	6.8-7.5	18	MATC (decrease maximum linear acceleration)	0.32-0.80	0.506	Atypical endpoint	Xia et al. (2015c); Xia et al. (2015a)
Quinbo (juvenile, 2.77 g, 5.62 cm), *Spinibarbus sinensis*	R, U	30 d	PFOS-K >99%	6.8-7.5	28	MATC (decrease maximum linear acceleration)	0.32-0.80	0.506	Atypical endpoint	Xia et al. (2015a); Xia et al. (2015b)
White sucker (mature, 22.7 cm, 114.5 g), *Catostomus commersonii*	Microcosm	14 d	PFOS 89%	-	-	NOEC (mortality)	-	3	Atypical exposure, not a true ELS test	Oakes et al. (2005)
White sucker (mature, 22.7 cm, 114.5 g), *Catostomus commersonii*	Microcosm	14 d	PFOS 89%	-	-	LOEC (decrease LSI in females)	-	3	Atypical exposure, not a true ELS test	Oakes et al. (2005)
Bluegill (28.6 mm, 0.60 g), *Lepomis macrochirus*	S, U	96 hr	PFOS DEA salt Unreported	8.2-8.3	-	LC50	-	31	Only one replicate per treatment	3M Company (2000)
Nile tilapia, *Oreochromis niloticus*	Diet, U	144 hr	PFOS Unreported	-	22	MATC (weight and survival)	1.0-5.0 (mg/g bw)	2.236 (mg/g bw)	Duration too short for a chronic test, missing exposure details	Han et al. (2011)
Medaka (adult, male), *Oryzias latipes*	R, U	14 d	PFOS Unreported	-	25	NOEC (adult survival, GSI%, HSI%, condition factor)	1->1	1	Duration too long for an acute test and too short for a chronic test	Ji et al. (2008)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Medaka (adult, female), *Oryzias latipes*	R, U	14 d	PFOS Unreported	-	25	NOEC (adult survival, condition factor)	1->1	1	Duration too long for an acute test and too short for a chronic test	Ji et al. (2008)
Medaka (adult, female), *Oryzias latipes*	R, U	14 d	PFOS Unreported	-	25	LOEC (GSI%)	<0.01-0.01	0.01	Duration too long for an acute test and too short for a chronic test	Ji et al. (2008)
Medaka (adult, female), *Oryzias latipes*	R, U	14 d	PFOS Unreported	-	25	MATC (HSI%)	0.1-1	0.3162	Duration too long for an acute test and too short for a chronic test	Ji et al. (2008)
Medaka (F1 generation, <12 hr, embryo), *Oryzias latipes*	R, U	7-14 d (assumed)	PFOS Unreported	-	25	MATC (% hatchability, time to hatch)	0.1-1	0.3162	Duration too long for an acute test and too short for a chronic test	Ji et al. (2008)
Medaka (F1 generation, <12 hr, embryo), *Oryzias latipes*	R, U	~28 d post-hatch (assumed)	PFOS Unreported	-	25	MATC (swim up success)	0.1-1	0.3162	Duration too long for an acute test and too short for a chronic test	Ji et al. (2008)
Medaka (F1 generation, <12 hr, embryo), *Oryzias latipes*	R, U	100 d post-hatch	PFOS Unreported	-	25	EC10 (growth - length)	<0.01-0.01	0.0013	Duration too long for an acute test and too short for a chronic test	Ji et al. (2008)
Medaka (F1 generation, <12 hr, embryo), *Oryzias latipes*	R, U	28 d post-hatch	PFOS Unreported	-	25	LOEC (larval survival)	<0.01-0.01	0.01	Duration too long for an acute test and too short for a chronic test	Ji et al. (2008)
Medaka (adult, 16 week, 0.38g) *Oryzias latipes*	R, U	21 d	PFOS ≥98%	-	25	LOEC (fecundity)	<1.0-1.0	1	Only one exposure concentration	Kang et al. (2019)
African clawed frog (embryos), *Xenopus laevis*	R, M	96 hr	PFOS-K 86.9%	7.3	24	EC50 (teratogenesis)	-	12.1	Atypical acute endpoint	Palmer and Krueger (2001)
African clawed frog (embryos), *Xenopus laevis*	R, M	96 hr	PFOS-K 86.9%	7.27	24	EC50 (teratogenesis)	-	17.6	Atypical acute endpoint	Palmer and Krueger (2001)
African clawed frog (embryos), *Xenopus laevis*	R, M	96 hr	PFOS-K 86.9%	7.26	24	EC50 (teratogenesis)	-	16.8	Atypical acute endpoint	Palmer and Krueger (2001)
African clawed frog (embryos), *Xenopus laevis*	R, M	96 hr	PFOS-K 86.9%	7.3	24	NOEC (growth)	-	14.7	Atypical acute endpoint	Palmer and Krueger (2001)
African clawed frog (embryos), *Xenopus laevis*	R, M	96 hr	PFOS-K 86.9%	7.27	24	LOEC (growth)	-	7.97	Atypical acute endpoint	Palmer and Krueger (2001)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
African clawed frog (embryos), *Xenopus laevis*	R, M	96 hr	PFOS-K 86.9%	7.26	24	LOEC (growth)	-	8.26	Atypical acute endpoint	Palmer and Krueger (2001)
African clawed frog (tadpoles, NF stage 46/47), *Xenopus laevis*	R, U	67 d	PFOS >96%	-	22	NOEC (survival and forelimb emergence)	0.1->0.1	0.1	Control issues	Cheng et al. (2011)
African clawed frog (embryo, NF 10), *Xenopus laevis*	R, M	96 hr	PFOS >99%	-	24	LC50	-	>96	Non-definitive value	San-Segundo et al. (2016)
American toad (larva, Gosner stage 25), *Anaxyrus americanus*	R, M	26-45 d	PFOS ≥98%	-	20	NOEC (mortality, growth, development)	-	0.6162	Lack of dose response	Flynn et al. (2022)
Asiatic toad (tadpole, 1.8 cm, 0.048 g), *Bufo gargarizans*	R, M	96 hr	PFOS-K 99%	7	22	LC50	-	48.21	Source of organisms may be problematic	Yang et al. (2014)
Asiatic toad (tadpole, 1.8 cm, 0.048 g), *Bufo gargarizans*	R, M	30 d	PFOS-K 99%	7	22	EC10 (survival)	-	2.00	Renewal chronic exposure, not a true ELS test, source of organisms may be problematic	Yang et al. (2014)
Bullfrog (tadpole, Gosner stage 25), *Lithobates catesbeiana* (formerly, *Rana catesbeiana*)	R, M	65 d	PFOS-K >98%	-	23	LOEC (growth: snout-vent length)	-	0.002430	Potential mixture effects, missing exposure details (prior exposure)	Lech et al. (2022)
Northern leopard frog (Gosner stage 25), *Lithobates pipiens*	S, M	116 d	PFOS Unknown	7.41-8.54	13.1-29.8	NOEC (survival and growth)	0.0128->0.0128	0.0128	Outdoor mesocosm	Foguth et al. (2020)
Northern leopard frog (Gosner stage 26.5, 0.109 g), *Lithobates pipiens*	R, U	10 d	PFOS-K ≥98%	7.9	22	NOEC (development, growth, survival)	0.1->0.1	0.1	Duration too long for an acute test and too short for a chronic test	Brown et al. (2021)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Northern leopard frog (larva, Gosner stage 25), *Lithobates pipiens*	S, M	30 d	PFOS ≥96%	7.8	26.2	LOEC (developmental stage)	<0.00006-0.00006	0.00006	Duration too long for an acute test and too short for a chronic test	Flynn et al. (2021)
Leopard frog (larva, Gosner stage 25), *Lithobates pipiens*	R, M	30 d	PFOS ≥98%	-	20	MATC (developmental stage, growth-weight)	0.1219-1.437	0.4185	Lack of dose response; PFOS present in controls	Flynn et al. (2022)
Leopard frog (larva, Gosner stage 25), *Lithobates pipiens*	R, M	30 d	PFOS ≥98%	-	20	LOEC (scaled mass index)	-	0.00774	Lack of dose response; PFOS present in controls	Flynn et al. (2022)
Leopard frog (larva, Gosner stage 25), *Lithobates pipiens*	R, M	30 d	PFOS ≥98%	-	20	NOEC (mortality)	-	1.437	Lack of dose response; PFOS present in controls	Flynn et al. (2022)
Leopard frog (tadpole, Gosner stage 25), *Lithobates pipiens*	R, M	120 d	PFOS ≥98%	7.87 (7.40-8.25)	20.3 (19.4-21.0)	NOEC (mortality, growth)	-	>0.000934	Test represents a greater than low value (followed data rule)	Hoskins et al. (2022)
Black spotted frog, *Pelophylax nigromaculatus* (formerly, *Rana nigromaculata*)	R, M	21 d	PFOS >98%	6.5	20	LOEC (biochemistry changes and gene expression)	-	0.01	Non-apical endpoints	Lin et al. (2022a)
Black spotted frog (adult), *Pelophylax nigromaculatus*	R, M	21 d	PFOS-K >98%	6.5	20	LOEC (biochemistry changes and gene expression)	-	0.0009150	Non-apical endpoints	Lin et al. (2022b)
Black spotted frog, *Pelophylax nigromaculatus*	R, M	21 d	PFOS-K >98%	6.5	20	LOEC (gene expression)	-	0.00121	Non-apical endpoints	Liu et al. (2023b)
Black spotted frog (adult), *Pelophylax nigromaculatus*	R, M	21 d	PFOS ≥98%	6.5	20	LOEC (gene expression)	-	0.01114	Non-apical endpoint	Shi et al. (2023)
Tiger salamander (larva, 46 hr), *Ambystoma tigrinum*	R, M	30 d	PFOS ≥98%	-	20	NOEC (growth, survival)		0.6213	Lack of dose response	Flynn et al. (2022)

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

[b] Chemical concentrations made in a side-test representative of exposure and verified stability of concentrations of PFOS in the range of concentrations tested under similar conditions. Daily renewal of test solutions.

[c] Water concentrations were not measured, but PFOS concentrations were measured in the liver.

[d] 36 days corresponds to the first of ten generations, the one with the most consistent negative response. The value at 36 days is 1/10 of the duration of this year-long 10-generation study.

[e] Reported in moles converted to gram based on a molecular weight of 500.13 g/mol (PFOS); 538.22 g/mol (PFOS-K); 629.4 g/mol (PFOS-TEA); 522.111 g/mol (PFOS-Na).

Appendix H Other Estuarine/Marine PFOS Toxicity Studies

H.1 Summary Table of Acceptable Qualitative Estuarine/Marine PFOS Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Bacterium, *Vibrio fischeri*	S, M	15 min	PFOS-K 98%	-	18	-	EC50 (bioluminescence inhibition)	-	>500	Duration too short for a plant test, missing some exposure details, non-apical endpoint	Rosal et al. (2010)
Cyanobacterium, *Anabaena sp.*	S, M	24 hr	PFOS-K 98%	-	28	-	EC50 (bioluminescence inhibition)	-	143.27	Duration too short for a plant test, missing some exposure details, non-apical endpoint	Rosal et al. (2010)
Dinoflagellate, *Pyrocystis lunula*	S, M	24 hr	PFOS-K 98%	-	19	-	EC50 (bioluminescence inhibition)	-	4.9	Duration too short for a plant test	Hayman et al. (2021)
Dinoflagellate, *Symbiodiniaceae*	R, M	7 d	L-PFOS Unreported	-	25	-	NOEC (population abundance)	0.0001->0.0001	0.0001	Only one exposure concentration	Bednarz et al. (2022)
Dinoflagellate, *Symbiodiniaceae*	R, M	14 d	L-PFOS Unreported	-	25	-	LOEC (population abundance)	<0.0001-0.0001	0.0001	Only one exposure concentration	Bednarz et al. (2022)
Dinoflagellate, *Symbiodiniaceae*	R, M	7 d	L-PFOS Unreported	-	32	-	NOEC (population abundance)	0.0001->0.0001	0.0001	Only one exposure concentration	Bednarz et al. (2022)
Dinoflagellate, *Symbiodiniaceae*	R, M	14 d	L-PFOS Unreported	-	32	-	LOEC (population abundance)	<0.0001-0.0001	0.0001	Only one exposure concentration	Bednarz et al. (2022)
Dinoflagellate, *Symbiodiniaceae*	R, M	28 d	L-PFOS Unreported	-	32	-	NOEC (population abundance)	0.0001->0.0001	0.0001	Only one exposure concentration	Bednarz et al. (2022)
Golden brown alga, *Isochrysis galbana*	S, U	72 hr	PFOS 98%	-	20	-	EC50 (growth inhibition)	-	37.5	Duration too short for a plant test	Mhadhbi et al. (2012)
Alga, *Ceratoneis closterium*	S, U	72 hr	PFOS-K Unknown	-	-	33	NOEC (growth)	4.16->4.16	4.16	Sediment and other PFAS present in exposure	Simpson et al. (2021)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Diatom, *Skeletonema costatum*	S, M	96 hr	PFOS-K 86.9%	8.0-8.4	20	~30	EC50 (cell density)	-	>3.20	Only one exposure concentration	Desjardins et al. (2001a)
Sandworm (adult), *Perinereis wilsoni*	R, M	7 d	PFOS-K Unreported	8.1	17.1	36	NOEC (survival)	0.000028->0.000028	0.000028	Only one exposure concentration	Sakurai et al. (2017)
Sea urchin (adult), *Glyptocidaris crenularis*	R, U	21 d + 7 d observation	PFOS-K 98%	8.1	13	30	NOEC (mortality)	1.0->1.0	1.0	Not a true ELS test (started with adults); missing exposure details	Ding et al. (2015)
Sea urchin (adult), *Glyptocidaris crenularis*	R, U	21 d + 7 d observation	PFOS-K 98%	8.1	13	30	LOEC (SOD activity)	<0.01-0.01	0.01	Not a true ELS test (started with adults); missing exposure details; atypical endpoint	Ding et al. (2015)
Purple sea urchin (fertilized eggs), *Paracentrotus lividus*	S, U	48 hr	PFOS 98%	-	20	-	EC50 (growth inhibition)	-	20	Duration too short for an acute test	Mhadhbi et al. (2012)
Purple sea urchin (sperm), *Paracentrotus lividus*	S, U	65 min	PFOS Unreported	7.69	22	-	NOEC (reproduction - egg fertilization)	-	0.0005	Duration too short, greater than low value	Munari et al. (2022)
Sea urchin (embryo), *Psammechinus miliaris*	R, U[b] (tissue)	72 hr	PFOS-K ≥98%	8	19	31	EC50 (morphological abnormality)	-	>0.3999[c]	Interpolated endpoint; missing some exposure details	Anselmo et al. (2011)
Sea urchin (embryo), *Psammechinus miliaris*	R, U[b] (tissue)	16 d	PFOS-K ≥98%	8	19	31	NOEC (morphological abnormalities, hatch success, development)	0.3999->0.3999	0.3999[c]	Duration too short for chronic test and too long for acute test	Anselmo et al. (2011)
Sea urchin (larva), *Psammechinus miliaris*	S, U	85 min.	PFOS-K ≥98%	-	19	-	IC50 (cellular efflux pump inhibition)	-	1.399[c]	Duration too short for a chronic test and too long for an acute test, atypical endpoint	Anselmo et al. (2012)
Eastern oyster (33.8mm), *Crassostrea virginica*	S, M	96 hr	PFOS-K 90.49%	7.5-8.1	22	20-21	EC50 (shell deposition)	-	>3.0	Lack of replication; atypical endpoint	Drottar and Krueger (2000i)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Eastern oyster (adult, 70-100 mm), *Crassostrea virginica*	S, U	48 hr	PFOS ≥97%	7.5	24.9	20	LOEC (cellular lysosomal damage)	<3-3	3	Atypical endpoint	Aquilina-Beck et al. (2020)
Mediterranean mussel (6.4 cm), *Mytilus galloprovincials*	R, U	30 d	PFOS Analytical grade	8.1	17.5	34.5	LOEC (increase micronuclei nuclear aberrations in gills cells)	<2-2	2	Atypical endpoint; missing some exposure details	Nalbantlar and Arslan (2017)
Green mussel (adult), *Perna viridis*	R, M	7 d + 7 d observation	PFOS-K 98%	-	25	30	EC50 (integrative genotoxicity)	0.00095-0.0097	0.033	Duration too short for a chronic test and too long for an acute test, atypical endpoint	Liu et al. (2014a)
Green mussel (adult), *Perna viridis*	R, M	7 d	PFOS-K 98%	-	25	25	MATC (CAT and SOD activity)	0.106-0.968	0.3203	Duration too short for a chronic test and too long for an acute test, atypical endpoint	Liu et al. (2014b)
Green mussel (60-65 mm), *Perna viridis*	R, M	7 d	PFOS-K 98%	-	25	25	MATC (relative condition factor)	0.0096-0.106	0.0319	Duration too short for a chronic test and too long for an acute test, atypical endpoint	(Liu et al. 2014c)
Green mussel, *Perna viridis*	R, M	7 d + 7 d observation	PFOS-K 98%	8	25	30	MATC (hemocyte cell viability)	0.0096-0.106	0.0319	Duration too short for a chronic test and too long for an acute test, atypical endpoint	Liu and Gin (2018)
Green mussel, *Perna viridis*	R, U	7 d	PFOS >98%	-	25	3.2	MATC (biochemistry changes)	0.01-0.1	0.0316	Non-apical endpoint	Xu et al. (2022)
White sunset shell (15.0-20.3 mm), *Soletellina alba*	S, M	28 d	PFOS-K Unreported	8	19	30	NOEC (survival)	0.85->0.85	0.85	Other PFAS measured in the sediment and water	Simpson et al. (2021)
Bivalve (8.1-18.9 mm), *Tellina deltoidalis*	S, M	28 d	PFOS-K Unreported	8	19	30	MATC (growth - weight)	0.22-0.28	0.2482	Other PFAS measured in the sediment and water	Simpson et al. (2021)
Smooth cauliflower coral, *Stylophora pistillata*	R, U	7 d	L-PFOS Unreported	-	32	-	NOEC (lipid peroxidation)	0.0001->0.0001	0.0001	Atypical endpoint	Bednarz et al. (2022)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Smooth cauliflower coral, *Stylophora pistillata*	R, U	28 d	L-PFOS Unreported	-	32	-	LOEC (lipid peroxidation)	<0.0001-0.0001	0.0001	Atypical endpoint	Bednarz et al. (2022)
Mysid (juvenile), *Americamysis bahia*	S, M	96 hr	PFOS-K 90.49%	8.1-8.2	23.5-25.3	20	LC50	-	3.6	Percent recovery of test substance is low	Drottar and Krueger (2000a)
Copepod (adult), *Nitocra spinipes*	S, M	10 d	PFOS-K Unreported	8.1	21	30	NOEC (reproduction)	2.0->2.0	2.0	Other PFAS measured in the sediment and water	Simpson et al. (2021)
Copepod (adult), *Nitocra spinipes*	S, M	28 d	PFOS-K Unreported	8.1	21	30	NOEC (survival)	0.48->0.48	0.48	Other PFAS measured in the sediment and water	Simpson et al. (2021)
Copepod (adult, female), *Tigriopus japonicus*	R, U	10 d	PFOS Unreported	-	25	32	MATC (reproduction)	0.1-0.25	0.1581	Difficult to determine test methodology	Han et al. (2015)
Amphipod (adult), *Gammarus insensibilis*	S, U	48 hr	PFOS-K ≥98%	8	19	32.5	LC50	-	9.99	Duration too short for an acute test	Touaylia et al. (2019)
Amphipod (adult), *Melita plumulosa*	S, M	10 d	PFOS-K Unreported	-	21	30	EC10 (reproduction)	-	0.9	Other PFAS measured in the sediment and water	Simpson et al. (2021)
Smooth sentinel crab (6-15 mm carapace), *Macrophthalmus sp.*	S, M	28 d	PFOS-K Unreported	8	19	30	NOEC (survival)	0.85->0.85	0.85	Other PFAS measured in the sediment and water	Simpson et al. (2021)
Chinese mitten crab (11.89 g), *Eriocheir sinensis*	R, U	21 d	PFOS-K >98%	7.6-8.1	18-22	0.3	MATC (total hemocyte count)	0.01-0.1	0.03162	Duration too short for a chronic test and too long for an acute test	Zhang et al. (2015)
Mud crab (3cm), *Macrophthalmus japonicus*	R, U	96 hr	PFOS 98%	-	20	30	LC50	-	>0.03	Only three exposure concentrations, atypical source of organisms	Park et al. (2015)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Mud crab (3cm), *Macrophthalmus japonicus*	R, U	7 d	PFOS 98%	-	20	30	LOEC (mortality)	<0.001-0.001	0.001	Only three exposure concentrations, atypical source of organisms	Park et al. (2015)
Marine medaka (embryo, 2 dpf), *Oryzias melastigma*	R, U	8 d	PFOS 98%	-	28	30	MATC (sinus venosus–bulbus arteriosus distance)	4.0-16	8	Duration too short for a chronic test and too long for an acute test, only three exposure concentrations	Huang et al. (2011)
Marine medaka (embryo, 2 dpf), *Oryzias melastigma*	R, U	8 d	PFOS 98%	-	28	30	LOEC (decrease heart rate)	<1-1	1	Duration too short for a chronic test and too long for an acute test, only three exposure concentrations	Huang et al. (2011)
Marine medaka (embryo), *Oryzias melastigma*	R, M	8 d	PFOS 98%	-	28	30	NOEC (embryo mortality)	16->16	16	Duration too short for a chronic test and too long for an acute test	Fang et al. (2012)
Marine medaka (embryo), *Oryzias melastigma*	R, M	8 d	PFOS 98%	-	28	30	LOEC (malformation)	<1-1	1	Duration too short for a chronic test and too long for an acute test	Fang et al. (2012)
Marine medaka (embryo), *Oryzias melastigma*	R, U	≤21 d	PFOS 98%	-	28	30	MATC (increase hatching rate, decrease hatching time)	1.0-4	2.00	Duration too short for a chronic test, low control hatch success, only three exposure concentrations	Wu et al. (2012)
Marine medaka (embryo), *Oryzias melastigma*	R, U	≤21 d + 7 d observation	PFOS 98%	-	28	30	MATC (larval survival)	1.0-4	2.00	Duration too short for a chronic test, low control hatch success, only three exposure concentrations	Wu et al. (2012)
Atlantic Cod (juvenile), *Gadus morhua*	F, U[b] (tissue)	5 d (1 hr/day)	PFOS Technical grade	7.7	10	33.8	NOEC (survival, growth)	0.2->0.2	0.20	Duration too short for a chronic test and too long for an acute test, only two exposure concentrations. Pulsed exposure.	Preus-Olsen et al. (2014)
European seabass (juvenile), *Dicentrarchus labrax*	D, U	21 d	PFOS-K ≥98%	-	20	28	LOEC (histology, enzymatic and genetic changes)	-	4.83 µg/kg	Dietary exposure, non-apical endpoints	Espinosa-Ruiz et al. (2023)
Blackrock fish (5 mo. old), *Sebastes schlegelli*	R, U	6 d	PFOS 99%	8.0-8.2	8.0-12	10	NOEC (survival, growth)	1->1	1	Duration too short for a chronic test and too long	Jeon et al. (2010)

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
										for an acute test, only two exposure concentrations	
Blackrock fish (5 mo. old), *Sebastes schlegelli*	R, U	6 d	PFOS 99%	8.0-8.2	8.0-12	17.5	NOEC (survival, growth)	1->1	1	Duration too short for a chronic test and too long for an acute test, only two exposure concentrations	Jeon et al. (2010)
Blackrock fish (5 mo. old), *Sebastes schlegelli*	R, U	6 d	PFOS 99%	8.0-8.2	8.0-12	25	NOEC (survival, growth)	1->1	1	Duration too short for a chronic test and too long for an acute test, only two exposure concentrations	Jeon et al. (2010)
Blackrock fish (5 mo. old), *Sebastes schlegelli*	R, U	6 d	PFOS 99%	8.0-8.2	8.0-12	34	NOEC (survival, growth)	1->1	1	Duration too short for a chronic test and too long for an acute test, only two exposure concentrations	Jeon et al. (2010)
Turbot (embryo), *Scophthalmus maximus* (formerly, *Psetta maxima*)	R, U	6 d	PFOS 98%	-	18	-	LC50	-	0.11	Duration too short for a chronic test and too long for an acute test	Mhadhbi et al. (2012)

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

[b] Study did not measure water concentrations, but there are measured concentrations from analysis of the tissue of organisms.

[c] Reported in moles converted to gram based on a molecular weight of 500.13 g/mol (PFOS); 538.22 g/mol (PFOS-K); 629.4 g/mol (PFOS-TEA).

Appendix I Acute to Chronic Ratios

I.1 Acute to Chronic Ratios from Quantitatively Acceptable Toxicity Tests.

Species	Chemical / Purity	Acute Method[a]	Chronic Method[a]	Acute Test Duration	Chronic Test Duration	Chronic Effect	Acute Effect Conc. (mg/L)	Chronic Effect Conc. (mg/L)	ACR[c]	SMACR[c]	Reference
Fatmucket, *Lampsilis siliquoidea*	PFOS >98%	S, M	S, M	24 hour	36 day	MATC (metamorphosis success)	16.5	0.01768	**933.3**	**933.3**	Hazelton (2013); Hazelton et al. (2012)
Snail, *Physella heterostropha pomilia* (formerly, *Physa pomilia)*	PFOS-K ≥98%	S, M	R, M	96 hour	44 day	EC10 (clutch size)	161.8	8.527	**18.98**	**18.98**	Funkhouser (2014)
Rotifer, *Brachionus calyciflorus*	PFOS ≥98%	S, U[b]	R, U[b]	24 hour	Up to 158 hours	LOEC (reduced net reproductive rate)	61.8	0.25	**247.2**	**>247.2**	Zhang et al. (2013)
Cladoceran, *Daphnia carinata*	PFOS-K ≥98%	S, U	R, U	48 hour	21 day	MATC (days to first brood)	11.56	0.003162	3,656	3,656[b]	Logeshwaran et al. (2021)
Cladoceran, *Daphnia magna*	PFOS-K 90.49%	S, M	R, M	48 hour	21 day	EC10 (cumulative young)	58.51	11.19	**5.229**	-	Drottar and Krueger (2000c)
Cladoceran, *Daphnia magna*	PFOS-K 95%	S, U	R, U	48 hour	21 day	EC10 (survival)	67.2	16.35	**4.110**	-	Boudreau et al. (2003a)
Cladoceran, *Daphnia magna*	PFOS Unreported	S, U	R, U	48 hour	21 day	EC10 (# of young/brood)	35.46	1.051	**33.74**	-	Ji et al. (2008)
Cladoceran, *Daphnia magna*	PFOS-K >98%	S, U	R, U	48 hour	21 day	EC10 (total neonates/female)	63.84[d]	3.030	**21.07**	-	Li (2009); Li (2010)
Cladoceran, *Daphnia magna*	PFOS-K 99%	S, M	R, M	48 hour	21 day	EC10 (survival)	78.09	2.610	**29.92**	-	Yang et al. (2014)
Cladoceran, *Daphnia magna*	PFOS 98%	S, U	R, U	48 hour	21 day	EC10 (number of offspring/brood/female)	23.41	0.001818	12,877[b]	-	Lu et al. (2015)
Cladoceran, *Daphnia magna*	PFOS-K ≥98%	S, U	R, U	48 hour	21 day	EC10 (survival)	94.58	3.596	**26.30**	-	Liang et al. (2017)
Cladoceran, *Daphnia magna*	PFOS-K 98%	S, U	R, U	48 hour	21 day	EC10 (growth-length)	22.43	0.9093	**24.67**	**16.23**	Yang et al. (2019)

Species	Chemical / Purity	Acute Method[a]	Chronic Method[a]	Acute Test Duration	Chronic Test Duration	Chronic Effect	Acute Effect Conc. (mg/L)	Chronic Effect Conc. (mg/L)	ACR[c]	SMACR[c]	Reference
Cladoceran, *Moina macrocopa*	PFOS Unreported	S, U	R, U	48 hour	7 day	EC10 (# of young/starting adult)	17.20	0.1789	**96.14**	**96.14**	Ji et al. (2008)
Crayfish, *Procambarus fallax f. virginalis*	PFOS-K ≥98%	S, M	R, M	96 hour	28 day	LC20	59.87	0.167	**358.5**	**358.5**	Funkhouser (2014)
Mayfly, *Neocloeon triangulifer*	PFOS-K 98%	R, M	R, M	96 hour	23 day	EC10 (dry weight at day 14)	0.07617	0.000226	**337.0**	**337.0**	Soucek et al. (2023)
Zebrafish, *Danio rerio*	PFOS-K unknown/ PFOS 96%	R, U	R, U	96 hour	Life Cycle	EC10 (F1 offspring: % survival)	17	0.01650	**1,030**	**1,030**	Wang et al. (2011); Wang et al. (2013)
Fathead minnow, *Pimephales promelas*	PFOS-K 90.49%	S, M	F, M	96 hour	47 day	EC10 (survival)	9.020	0.4732	**19.06**	**19.06**	Drottar and Krueger (2000c)

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

[b] Value appears to be an outlier and is not used in SMACR calculation.

[c] Values in bold are used in the SMACR and FACR calculations.

[d] Geometric mean of three LC50s.

I.2 Acute to Chronic Ratios from Qualitatively Acceptable Toxicity Tests.

Species	Acute / Chronic Chemical and Purity	Acute Method[a]	Chronic Method[a]	Acute Test Duration	Chronic Test Duration	Acute Effect	Chronic Effect	Acute Effect Conc. (mg/L)	Chronic Effect Conc. (mg/L)	ACR	References
Planaria, *Dugesia japonica*	PFOS-K >99%	R, U	R, U	96 hours	10 days	LC50	LOEC (regeneration: decreased appearance of auricles)	29.46	0.5	58.92	Yuan et al. (2014)
Snail, *Lymnaea stagnalis*	PFOS Unreported	S, M	R, M	96 hours	21 days	LC50	MATC (survival)	171.5	4.243	40.41	Olson (2017)
Midge, *Chironomus sp.*	PFOS-K (99%) / PFOS Unreported	S, M	S, M	96 hours	~36 days (1st of 10 generations)	LC50	LOEC (F1 developmental time, adult weight, exuvia length)	182.12	0.004	45,530	Marziali et al. (2019); Yang et al. (2014)
Midge, *Chironomus sp.*	PFOS-K (99%) / PFOS-K (95%)	S, M	S, M	96 hours	Life cycle (>50 days)	LC50	EC10 (total emergence)	182.12	0.05896	3,089	MacDonald et al. (2004); Yang et al. (2014)
Yellow fever mosquito, *Aedes aegypti*	PFOS Unreported	S, U	R, U	48 hours	~42 days	LC50	MATC (average time to emergence)	1.18	0.079	14.94	Olson (2017)
Rainbow trout, *Oncorhynchus mykiss*	PFOS-K (98%) / PFOS (89%)	R, M	S, U	96 hours	14 days	LC50	LOEC (decrease LSI)	2.5	1.0	2.500	Oakes et al. (2005); Sharpe et al. (2010)
Zebrafish, *Danio rerio*	PFOS ≥97%	S, U	R, U	96 hours	6 days	LC50	EC50 (uninflated swim bladder)	58.47	2.29	25.53	Hagenaars et al. (2014); Hagenaars et al. (2011b)
Zebrafish, *Danio rerio*	PFOS-K 98%	R, M	R, M	96 hours	21 days	LC50	LOEC (reduced fecundity)	22.2	0.5	44.40	Sharpe et al. (2010)
Zebrafish, *Danio rerio*	PFOS 98%	S, U	R, U	96 hours	70 days	LC50	MATC (increased malformation & decreased survival of F1 fish)	3.502	0.02236	156.6	Du et al. (2016a); Du et al. (2009)

Species	Acute / Chronic Chemical and Purity	Acute Method[a]	Chronic Method[a]	Acute Test Duration	Chronic Test Duration	Acute Effect	Chronic Effect	Acute Effect Conc. (mg/L)	Chronic Effect Conc. (mg/L)	ACR	References
African clawed frog, *Xenopus laevis*	PFOS-K (86.9%) / PFOS-K (86.9%)	R, M	R, M	96 hours	96 hours	LC50	LOEC (growth)	15.49	8.26	1.875	Palmer and Krueger (2001)

a S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

Appendix J Unused PFOS Toxicity Studies

J.1 Summary Table of Unused PFOS Toxicity Studies

Author	Citation	Reason Unused
Arukwe, A. and A.S. Mortensen	2011. Lipid peroxidation and oxidative stress responses of salmon fed a diet containing perfluorooctane sulfonic- or perfluorooctane carboxylic acids. Comp. Biochem. Physiol. Part C 154: 288-295.	Force-fed (oral gavage); only one exposure concentration
Arukwe, A., M.V. Cangialosi, R.J. Letcher, E. Rocha and A.S. Mortensen	2013. Changes in morphometry and association between whole-body fatty acids and steroid hormone profiles in relation to bioaccumulation patterns in salmon larvae exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids. Aquat. Toxicol. 130-131: 219-230.	Only one exposure concentration
Balbi, T., C. Ciacci, E. Grasselli, A. Smerilli, A. Voci and L. Canesi	2017. Utilization of *Mytilus* digestive gland cells for the in vitro screening of potential metabolic disruptors in aquatic invertebrates. Comp. Biochem. Physiol. Part C. 191: 26-35.	In vitro (excised cells)
Bilbao, E., D. Raingeard, O. Diaz de Cerio, M. Ortiz-Zarragoitia, P. Ruiz, U. Izagirre, A. Orbea, I. Marigómez, M.P. Cajaraville and I. Cancio	2010. Effects of exposure to Prestige-like heavy fuel oil and to perfluorooctane sulfonate on conventional biomarkers and target gene transcription in the thicklip grey mullet *Chelon labrosus*. Aquat. Toxicol. 98: 282-296.	Only one exposure concentration; the number of fish was not reported
Blanc, M., A. Karrman, P. Kukucka, N. Scherbak and S. Keiter	2017. Mixture-specific gene expression in zebrafish (*Danio rerio*) embryos exposed to perfluorooctane sulfonic acid (PFOS), perfluorohexanoic acid (PFHxA) and 3,3',4,4',5-pentachlorobiphenyl (PCB126). Sci. Total Environ. 590: 249-257.	Mixture (PFOS, PFHxA and PCB126)
Blanc, M., J. Ruegg, N. Scherbak and S.H. Keiter	2019. Environmental chemicals differentially affect epigenetic-related mechanisms in the zebrafish liver (zf-l) cell line and in zebrafish embryos. Aquat. Toxicol. 215:105272-9999.	Control absent from test
Chen, J., L. Zheng, L. Tian, N. Wang, L. Lei, Y. Wang, Q. Dong, C. Huang and D. Yang	2018. Chronic PFOS exposure disrupts thyroid structure and function in zebrafish. Bull. Environ. Contam. Toxicol. 101: 75-79.	Only one treatment concentration; severe lack of procedural details
Chen, K., N. Iwasaki, X. Qiu, H. Xu, Y. Takai, K. Tashiro, Y. Shimasaki and Y. Oshima	2020. Adipogenesis of perfluorooctanesulfonate (PFOS) on Japanese medaka (*Oryzias latipes*) embryo using ovo-nanoinjection-mRNA seq analysis. J. Fac. Agric. Kyushu Univ. 65(2): 295-303.	Injected toxicant in ova
Cheng, J., S. Lv, S. Nie, J. Liu, S. Tong, N. Kang, Y. Xiao, Q. Dong, C. Huang and D. Yang	2016. Chronic perfluorooctane sulfonate (PFOS) exposure induces hepatic steatosis in zebrafish. Aquat. Toxicol. 176: 45-52.	Only one exposure concentration; unmeasured chronic exposure
Consoer, D.M.	2017. A mechanistic investigation of perfluoroalkyl acid kinetics in rainbow trout (*Oncorhynchus mykiss*). A dissertation submitted to the faculty of the University of Minnesota.	Injected toxicant; only one exposure concentration
Cui, Y., W. Liu, W. Xie, W. Yu, C. Wang and H. Chen	2015. Investigation of the effects of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) on apoptosis and cell cycle in a zebrafish (*Danio rerio*) liver cell line. Int. J. Environ. Res. Public Health 12(12): 15673-15682.	Excised cells (liver cell line)
Dale, K., F. Yadetie, T. Horvli, X. Zhang, H.G. Froysa, O.A. Karlsen and A. Goksoyr	2022. Single PFAS and PFAS mixtures affect nuclear receptor- and oxidative stress-related pathways in precision-cut liver slices of Atlantic cod (*Gadus morhua*). Sci. Total Environ. 814: 1-12.	In vitro; no apical endpoints

Author	Citation	Reason Unused
Diaz de Cerio, O., E. Bilbao, M.P. Cajaraville and I. Cancio	2012. Regulation of xenobiotic transporter genes in liver and brain of juvenile thicklip grey mullets (*Chelon labrosus*) after exposure to Prestige-like fuel oil and to perfluorooctane sulfonate. Gene. 498: 50-58.	Only one exposure concentration
Dorts, J., P. Kestemont, P.A. Marchand, W. D'Hollander, M.L. Thezenas, M. Raes and F. Silvestre	2011. Ecotoxicoproteomics in gills of the sentinel fish species, *Cottus gobio*, exposed to perfluorooctane sulfonate (PFOS). Aquat. Toxicol. 103: 1-8.	Only two exposure concentrations, not North American species
Dragojevic, J., P. Maric, J. Loncar, M. Popovic, I. Mihaljevic, and T. Smital	2020. Environmental Contaminants Modulate Transport Activity of Zebrafish Organic Anion Transporters Oat1 and Oat3. Comp. Biochem. Physiol. C Toxicol. Pharmacol.231:8 p.	In vitro; no apical endpoints
Du, J., S. Wang, H. You and Z. Liu	2016b. Effects of ZnO nanoparticles on perfluorooctane sulfonate induced thyroid-disrupting on zebrafish larvae. J. Environ. Sci. 47: 153-164.	Only 72-75% control survival in 14-day test
Du, J., J. Tang, S. Xu, J. Ge, Y. Dong, H. Li and M. Jin	2018. Parental transfer of perfluorooctane sulfonate and ZnO nanoparticles chronic co-exposure and inhibition of growth in F1 offspring. Regul. Toxicol. Pharmacol. 98: 41-49.	Excessive control mortality in the F0 generation
Fang, C., Q. Huang, T. Ye, Y. Chen, L. Liu, M. Kang, Y. Lin, H. Shen and S. Dong	2013. Embryonic exposure to PFOS induces immunosuppression in the fish larvae of marine medaka. Ecotox. Environ. Saf. 92: 104-111.	Excessive control mortality (~60% control survival)
Fernández-Sanjuan, M., M. Faria, S. Lacorte and C. Barata	2013. Bioaccumulation and effects of perfluorinated compounds (PFCs) in zebra mussels (*Dreissena polymorpha*). Environ. Sci. Pollut. Res. 20:2661–2669.	Mixture
Garoche, C., A. Boulahtouf, M. Grimaldi, B. Chiavarina, L. Toporova, M.J. Den Broeder, J. Legler, W. Bourguet and P. Ba	2021. Interspecies differences in activation of peroxisome proliferator-activated receptor gamma by pharmaceutical and environmental chemicals. Environ. Sci. Technol. 55(24): 16489-16501.	In vitro
Gorrochategui, E., S. Lacorte, R. Tucker and F.L. Martin	2016. Perfluoroalkylated substance effects in *Xenopus laevis* A6 kidney epithelial cells determined by ATR-FTIR spectroscopy and chemometric analysis. Chem. Res. Toxicol. 29: 924-932.	The tests were performed on cell cultures obtained from an outside source. Whole organisms were not investigated.
Hagenaars A., I.J. Meyer, D. Herzke, B.G. Pardo, P. Martinez, M. Pabon, W. De Coen and D. Knapen	2011. The search for alternative aqueous film forming foams (AFFF) with a low environmental impact: Physiological and transcriptomic effects of two Forafac® fluorosurfactants in turbot. Aquat. Toxicol. 104: 168-176.	Only one exposure concentration; missing detail (focus is on other chemicals)
Hoff, P.T., W. Van Dongen, E.L. Esmans, R. Blust and W.M. De Coen	2003. Evaluation of the toxicological effects of perfluorooctane sulfonic acid in the common carp (*Cyprinus carpio*). Aquat. Toxicol. 62 (4): 349-359.	Exposure was from a single intra-peritoneal injection
Hoff, P.T., K. Van Campenhout, K. Van de Vijver, A. Covaci, L. Bervoets, L. Moens, G. Huyskens, G. Goemans, C. Belpaire, R. Blust and W. De Coen	2005. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. Environ. Pollut. 137: 324-333.	Field exposure, but concentrations were not measured so no BAFs could be calculated
Honda, M., A. Muta, T. Akasaka, Y. Inoue, Y. Shimasaki, K. Kanna, N. Okino and Y. Oshima	2014. Identification of perfluorooctane sulfonate binding protein in the plasma of tiger pufferfish *Takifugu rubripes*. Ecotox. Environ. Safety. 104: 409-413.	Injected toxicant; only one exposure concentration
Honda, M., A. Muta, A. Shimazaki, T. Akasaka, M. Yoshikuni, Y. Shimasaki and Y. Oshima	2018. High concentrations of perfluorooctane sulfonate in mucus of tiger puffer fish *Takifugu rubripes*: a laboratory exposure study. Environ. Sci. Pollut. Res. 25: 1551-1558.	Injected toxicant
Huang, T.S., P.A. Olsvik, A. Krovel, H.S. Tung and B.E. Torstensen	2009. Stress-induced expression of protein disulfide isomerase associated 3 (PDIA3) in Atlantic salmon (*Salmo salar* L.). Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 154(4): 435-442.	In vitro (cultured hepatocytes)

Author	Citation	Reason Unused
Huang, Q., S. Dong, C. Fang, X. Wu, T. Ye and Y. Lin	2012. Deep sequencing-based transcriptome profiling analysis of *Oryzias melastigma* exposed to PFOS. Aquat. Toxicol. 120-12: 54-58.	Only one or two exposure concentrations
Huang, Q., Y. Chen, Y. Chi, Y. Lin, H. Zhang, C. Fang and S. Dong	2015. Immunotoxic effects of perfluorooctane sulfonate and di(2-ethylhexyl) phthalate on the marine fish *Oryzias melastigma*. Fish Shell. Immunol. 44: 302-306.	Only two exposure concentrations
Huang, J., Q. Wang, S. Liu, H. Lai and W. Tu.	2022. Comparative chronic toxicities of PFOS and its novel alternatives on the immune system associated with intestinal microbiota dysbiosis in adult zebrafish. J. Hazard. Mater. 425: 11 p.	Only on exposure concentration; lack of apical endpoints
Jacobson, T., K. Holmstrom, G. Yang, A.T. Ford, U. Berger and B. Sundelin	2010. Perfluorooctane sulfonate accumulation and parasite infestation in a field population of the amphipod *Monoporeia affinis* after microcosm exposure. Aquat. Toxicol. 98(1): 99-106.	Dilution water not characterized, mixture
Jantzen, C.E.	2016. Toxicological Profiles of Perfluoroctanoic Acid (PFOA), Perfluorooctane Sulfonate (PFOS) and Perfluornonanoic Acid (PFNA) in Zebrafish (*Danio rerio*). Ph.D. Thesis, Rutgers, The State University of New Jersey, New Brunswick, NJ: 177 p.	Thesis publication; separate DERs were completed for individual components of the study
Jantzen, C.E., K.M. Annunziato and K.R. Cooper	2016. Behavioral, morphometric, and gene expression effects in adult zebrafish (Danio rerio) embryonically exposed to PFOA, PFOS, and PFNA. Aquat. Toxicol. 180:123–130.	Single concentration test where exposure to PFOS was of an acute (117 hours) duration but endpoints were measured at 6 months of age.
Keiter S., K. Burkhardt-Medicke, P. Wellner, B. Kais, H. Färber, D. Skutlarek, M. Engwall, T. Braunbeck, S.H. Keiter and T. Luckenbach	2016. Does perfluorooctane sulfonate (PFOS) act as chemosensitizer in zebrafish embryos? Sci. Total Environ. 548-549:317–324.	Mixture
Khan, E.A., X. Zhang, E.M. Hanna, F. Yadetie, I. Jonassen, A. Goksoyr and A. Arukwe	2021. application of quantitative transcriptomics in evaluating the ex vivo effects of per- and polyfluoroalkyl substances on Atlantic cod (Gadus morhua) ovarian physiology. Sci. Total Environ.755(1): 11 p.	In-vitro study
Kim, S., K. Ji, S. Lee, J. Lee, J. Kim, S. Kim, Y. Kho and K. Choi	2011. Perfluorooctane sulfonic acid exposure increases cadmium toxicity in early life stage of zebrafish, *Danio rerio*. Environ. Toxicol. Chem. 30(4): 870-877.	Only one exposure concentration; atypical duration (7 days)
Kovacevic, V., A.J. Simpson and M.J. Simpson	2018. Evaluation of Daphnia magna metabolic responses to organic contaminant exposure with and without dissolved organic matter using 1H nuclear magnetic resonance (NMR)-based metabolomics. Ecotoxicol. Environ. Saf.164:189-200.	Only one exposure concentration; test not focused on the toxicological effects of PFOS but on the effects of dissolved organic matter following exposure to PFOS and other contaminants
Kovacevic, V., A.J. Simpson and M.J. Simpson	2019. The concentration of dissolved organic matter impacts the metabolic response in *Daphnia magna* exposed to 17α-ethynylestradiol and perfluorooctane sulfonate. Ecotoxicol. Environ. Saf. 170: 468-478.	Only one treatment concentration (examined across a gradient of dissolved organic matter concentrations); endpoints measured were a suite of metabolic changes; atypical design for this test organism
Krovel, A.V., L. Softeland, B. Torstensen and P.A. Olsvik	2008. Transcriptional effects of PFOS in isolated hepatocytes from Atlantic salmon *Salmo salar* L. Comp. Biochem. Physiol., Part C. 148: 14-22.	In vitro

Author	Citation	Reason Unused
Lee, W. and Y. Kagami	2010. Effects of perfluorooctanoic acid and perfluorooctane sulfonate on gene expression profiles in medaka (*Oryzias latipes*). Abstracts. Toxicol. Let. 196S: S37-S351.	Abstract only, cannot judge against data quality objectives
Li, M.H.	2011. Changes of cholinesterase and carboxylesterase activities in male guppies, *Poecilia reticulata*, after exposure to ammonium perfluorooctanoate, but not to perfluorooctane sulfonate. Fresenius Environ. Bull. 20(8a): 2065-2070.	Each treatment group was run three times at separate times (not simultaneously) and the sample size for each treatment group was unclear; control mortality not reported
Li, Y., B. Men, Y. He, H. Xu, M. Liu and D. Wang	2017. Effect of single-wall carbon nanotubes on bioconcentration and toxicity of perfluorooctane sulfonate in zebrafish (*Danio rerio*). Sci. Total Environ. 607-608: 509-518.	Bioaccumulation (steady state no documented); only 4 days; static exposure
Li, R., T. Tang, W. Qiao and J. Huang	2020. Toxic effect of perfluorooctane sulfonate on plants in vertical-flow constructed wetlands. J. Environ. Sci. 92: 176-186.	PFOS added to a simulated wastewater (mixture) which was not properly characterized
Liu, C., Y. Dua and B. Zhoua	2007a. Evaluation of estrogenic activities and mechanism of action of perfluorinated chemicals determined by vitellogenin induction in primary cultured tilapia hepatocytes. Aquat. Toxicol. 85: 267-277.	In vitro (cultured hepatocytes)
Liu, C., K. Yu, X. Shi, J. Wang, P.K.S. Lam, R.S.S. Wu and B. Zhou	2007b. Induction of oxidative stress and apoptosis by PFOS and PFOA in primary cultured hepatocytes of freshwater tilapia (*Oreochromis niloticus*). Aquat. Toxicol. 82: 135-143.	Excised cells (cultured hepatocytes)
Martin, J.W., S.A. Mabury, K.R. Solomon and D.C.G. Muir	2003a. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 22: 196-204.	Bioaccumulation (steady state no documented); only 12 days
Martin, J.W., S.A. Mabury, K.R. Solomon and D.C.G. Muir	2003b. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 22(1): 189-195.	Mixture
Martin, J.W., S.A. Mabury, K.R. Solomon and D.C.G. Muir	2013. Progress toward understanding the bioaccumulation of perfluorinated alkyl acids. Environ. Toxicol. Chem. 32(11): 2421-2423.	Review paper
Mortensen, A.S., R.J. Letcher, M.V. Cangialosi, S. Chu and A. Arukwe	2011. Tissue bioaccumulation patterns, xenobioticbiotransformation and steroid hormone levels in Atlantic salmon (*Salmo salar*) fed a diet containing perfluoroactane sulfonic or perfluorooctane carboxylic acids. Chemosphere. 83: 1035-1044.	One dietary dosage level provided over a 6-day period; not intended as a toxicity test
Mylroie, J.E., M.S. Wilbanks, A.N. Kimble, K.T. To, C.S. Cox, S.J. Mcleod, K.A. Gust, D.W. Moore, E.J. Perkins and N. Garcia-Reyero	2021. Perfluorooctanesulfonic acid induced toxicity on zebrafish embryos in the presence or absence of the chorion. Environ. Toxicol. Chem. 40(3): 780-791.	Use of dilution medium (estradiol media) to prepare stock solutions inconsistent with EPA test guidelines
Oh, J.H., H.B. Moon and E.S. Choe	2013. Alterations in differentially expressed genes after repeated exposure to perfluorooctanoate and perfluorooctanesulfonate in liver of *Oryzias latipes*. Arch. Environ. Contam. Toxicol. 64(3): 475-483.	Only one exposure concentration, no concentration-response observed, not North American species
Otero-Sabio, C., M. Giacomello, C. Centelleghe, F. Caicci, M. Bonato, A. Venerando, J.M. Graic, S. Mazzariol, L. Finos	2022. Cell Cycle Alterations Due to Perfluoroalkyl Substances PFOS, PFOA, PFBS, PFBA and the New PFAS C6O4 on Bottlenose Dolphin (Tursiops truncatus) Skin Cell. Ecotoxicol. Environ. Saf.244:10 p	In vitro; no apical endpoints
Pablos, M.V., P. García-Hortigüela and C. Fernández	2015. Acute and chronic toxicity of emerging contaminants, alone or in combination, in *Chlorella vulgaris* and *Daphnia magna*. Environ. Sci. Pollut. Res. 22: 5417-5424.	Mixture

Author	Citation	Reason Unused
Popovic, M, R. Zaja, K. Fent and T. Smital	2014. Interaction of environmental contaminants with zebrafish organic anion transporting polypeptide, Oatp1d1 (Slco1d1). Toxicol. Appl. Pharmacol. 280(1): 149-158.	Excised cells
Prosser, R.S., K. Mahon, P.K. Sibley, D. Poirier and T. Watson-Leung	2016. Bioaccumulation of perfluorinated carboxylates and sulfonates and polychlorinated biphenyls in laboratory-cultured *Hexagenia* spp., *Lumbriculus variegatus* and *Pimephales promelas* from field-collected sediments. Sci. Total Environ. 543: 715-726.	Mixture (filed collected sediment, contained PFAS mixtures and PCBs)
Roland, K., P. Kestemont, L. Henuset, M.A. Pierrard, M. Raes, M. Dieu and F. Silvestre	2013. Proteomic responses of peripheral blood mononuclear cells in the European eel (*Anguilla anguilla*) after perfluorooctane sulfonate exposure. Aquat. Toxicol.128/129: 43-52.	In vitro (excised cells)
Shi, X., Y. Du, P.K.S. Lam, R.S.S. Wu and B. Zhou	2008. Developmental toxicity and alteration of gene expression in zebrafish embryos exposed to PFOS. Toxicol. Appl. Pharmacol. 230(1): 23-32.	Excessive control mortality
Shi, X., L.W.Y. Yeung, P.K.S. Lam, R.S.S. Wu and B. Zhou	2009b. Protein profiles in zebrafish (*Danio rerio*) embryos exposed to perfluorooctane sulfonate. Toxicol. Sci. 110(2): 334-340.	Only one exposure concentration; atypical duration (8 days)
Stanic, B., J. Petrovic, B. Basica, S. Kaisarevic, K. Schirmer and N. Andric	2021. Characterization of the ERK1/2 phosphorylation profile in human and fish liver cells upon exposure to chemicals of environmental concern. Environ. Toxicol. Pharmacol. 88: 9 p.	In vitro
Stevenson, C.N., L.A. MacManus-Spencer, T. Luckenbach, R.G. Luthy and D. Epel	2006. New perspectives on pefluorochemical ecotoxicology: inhibition and induction of an efflux transporter in marine mussel, *Mytilus californianus*. Environ. Sci. Technol. 40: 5580-5585.	Excised cells (mussel gill tissue)
Sun, X., Y. Xie, X. Zhang, J. Song, and Y. Wu	2023b. Estimation of Per- and Polyfluorinated Alkyl Substance Induction Equivalency Factors for Humpback Dolphins by Transactivation Potencies of Peroxisome Proliferator-Activated Receptors. Environ. Sci. Technol.57(9): 3713-3721.	In vitro
Thienpont, B., A. Tingaud-Sequeira, E. Prats, C. Barata, P.J. Babin and D. Raldua	2011. Zebrafish eleutheroembryos provide a suitable vertebrate model for screening chemicals that impair thyroid hormone synthesis. Environ. Sci. Technol. 45(17): 7525-7532.	Only one exposure concentration; atypical duration (3 days)
Qiu, X., N. Iwasaki, K. Chen, Y. Shimasaki and Y. Oshima	2019. Tributyltin and perfluorooctane sulfonate play a synergistic role in promoting excess fat accumulation in Japanese medaka (*Oryzias latipes*) via in ovo exposure. Chemosphere. 220: 687-695.	Injected toxicant into eggs, not North American species
Wagner, N.D., A.J. Simpson and M.J. Simpson	2016. Metabolomic responses to sublethal contaminant exposure in neonate and adult *Daphnia magna*. Environ. Toxicol. Chem. 36(4): 938-946.	Only one exposure concentration
Wagner, N.D., A.J. Simpson and M.J. Simpson	2018. Sublethal metabolic responses to contaminant mixture toxicity in *Daphnia magna*. Environ. Toxicol. Chem. 37(9): 2448-2457.	Only one exposure concentration
Wang, S., C. Zhuang, J. Du, C. Wu and H. You	2017. The presence of MWCNTs reduces developmental toxicity of PFOS in early life stage of zebrafish. Environ. Pollut. 222: 201-209.	The 96-hour LC50 reported in the publication is the same as the value in Du et al. 2016 (no details provided about this test)
Xia, X., X. Chen, X. Zhao, H. Chen and M. Shen	2012. Effects of carbon nanotubes, chars, and ash on bioaccumulation of perfluorochemicals by *Chironomus plumosus* larvae in sediment. Environ. Sci. Technol. 46: 12467-12475.	Mixture (PFCs mixed in sediment)

Author	Citation	Reason Unused
Xia, X., A.H. Rabearisoa, X. Jiang and Z. Dai	2013. Bioaccumulation of perfluoroalkyl substances by *Daphnia magna* in water with different types and concentrations of protein. Environ. Sci. Technol. 47: 10955-10963.	Bioaccumulation (steady state not documented); only 3 days; test was unmeasured
Xia, X., Z. Dai, A.H. Rabearisoa, P. Zhao and X. Jiang	2015a. Comparing humic substance and protein compound effects on the bioaccumulation of perfluoroalkyl substances by *Daphnia magna* in water. Chemosphere. 119: 978-986.	Bioaccumulation (steady state not documented); only 3 days; test was unmeasured
Xia, X., A.H. Rabaerisoa, Z. Dai, X. Jiang, P. Zhao and H. Wang	2015b. Inhibition effect of Na+ and Ca2+ on the bioaccumulation of perfluoroalkyl substances by Daphnia magna in the presence of protein. Environ. Toxicol. Chem. 34(2): 429-436.	Bioaccumulation (steady state not documented); only 3 days; test was unmeasured
Yang, Z., L. Fu, M. Cao, F. Li, J. Li, Z. Chen, A. Guo, H. Zhong, W. Li, Y. Liang, and Q. Luo	2023. PFAS-Induced Lipidomic Dysregulations and Their Associations with Developmental Toxicity in Zebrafish Embryos. Sci. Total Environ.861:9 p.	Injected toxicant
Zhang, L., Y.Y. Li, T. Chen, W. Xia, Y. Zhou, Y.J. Wan, Z.Q. Lv, G.Q. Li and S.Q. Xu	2011a. Abnormal development of motor neurons in perfluorooctane sulphonate exposed zebrafish embryos. Ecotoxicol. 20: 643-652.	Static, unmeasured exposure to single-concentration (1 mg/L) from 6 hours post-fertilization to 120 days post-fertilization
Zhang, L., Y.Y. Li, H.C. Zeng, J. Wei, Y.J. Wan, J. Chen and S.Q. Xu	2011b. MicroRNA expression changes during zebrafish development induced by perfluorooctane sulfonate. J. Appl. Toxicol. 31: 210-222.	Poor control survival (>80% at 24 hour and increasing)

Appendix K EPA Methodology for Fitting Concentration-Response Data and Calculating Effect Concentrations

Toxicity values, including LC_{50} and EC_{10} values, were independently-calculated from the data presented in the toxicity studies meeting the inclusion criteria described above (see Section 2.10) and when adequate concentrations-response data were published in the study or could be obtained from authors. When concentration-response data were not presented in toxicity studies, concentration-response data were requested from study authors to independently calculate toxicity values. In cases where study authors did not respond to the EPA's request for data or were unable to locate concentration-response data, the toxicity values were not independently-calculated by the EPA, and the reported toxicity values were retained for criteria deviation. The EPA also retained author-reported effect concentrations when data availability did not support effect concentration calculation by the EPA. This retention was done to be consistent with use of author-reported toxicity values in previous criteria documents and retain informative toxicity values (that would have otherwise not been used only on the basis of lacking the underlying C-R data). Where concentration-response data were available, they were analyzed using the statistical software program R (version 3.6.2) and the associated dose-response curve (drc) package.

In some cases, the author reported toxicity values were different than the corresponding effect concentrations calculated by the EPA. Overall, the magnitude of such discrepancies were limited and largely occurred for several potential reasons such as: (1) instances where authors were presumed to calculate effect concentrations using replicate level data, but the EPA only had access to treatment mean data; (2) the model selected to fit a particular set of C-R data, and; (3) the software used to fit a model to C-R data and calculate an effect concentration.

K.1 Fitting Concentration Response Data in R

Concentration-response data were obtained from quantitatively-acceptable toxicity studies when reported data were available. In many scenarios, toxicity studies report treatment-level mean concentrations and mean organismal responses; however, individual-replicate data may also be reported. When fitting C-R curves, replicate-level data were preferred over treatment-level data, if both types of data were available. Within R, the drc package can fit a variety of mathematical models to each set of C-R data.

K.1.1 Fitting Acute Mortality Data

K.1.1.1 Dichotomous Data

Dichotomous data are binary in nature (e.g., live/dead or 0/1) and are typical of survival experiments. They are usually represented as a proportion survived.

K.1.2 Fitting Chronic Growth, Reproduction, and Survival Data

K.1.2.1 Continuous Data

Continuous data take on any value along the real number line (e.g., biomass).

K.1.2.2 Count Data

Count data take on only integer values (e.g., number of eggs hatched).

K.1.2.3 Dichotomous Data

Dichotomous data are binary in nature (e.g., live/dead or 0/1) and are typical of survival experiments. They are usually represented as a proportion survived.

K.2 Determining Most Robust Model Fit for Each C-R curve

The R drc package was used to fit a variety of models to each individual C-R dataset. A single model was then selected from these candidate models to serve as the representative C-R model. The selected model represented the most statistically-robust model available. To determine the most-statistically-robust model for a C-R dataset, all individual model fits were assessed on a suite of statistical metrics.

K.2.1 Selecting Candidate Models

Initially, models were ranked according to the Akaike information criteria (AIC). The AIC provides a measure of the amount of information lost for a given model by balancing goodness of fit with model parsimony. The models with the lowest AIC, relative to other models based on the same data, tend to be optimal. In some instances, however, the model with the lowest AIC possessed a questionable characteristic that suggested said model was not the most appropriate. Rather than selecting a model based solely on the lowest AIC, the initial ranking step was only used to identify a subset of candidate models that were more closely examined before selecting a model fit for each C-R dataset.

K.2.2 Assessment of Candidate Models to Determine the Most Appropriate Model

Candidate models (i.e., models with low AIC scores relative to other models produced for a particular C-R dataset) were further evaluated based on additional statistical metrics to determine a single, statistically robust curve for each quantitatively-acceptable toxicity test. These additional statistical metrics were evaluated relative to the other candidate curve fits produced for each C-R dataset. Of these statistical metrics, residual standard errors, confidence intervals relative to effects concentration estimates, and confidence bands carried the most weight in determining the most appropriate model to be representative of an individual C-R dataset. These additional statistical metrics included:

K.2.2.1 Comparison of residual standard errors

As with AIC, smaller values were desirable. Residual standard errors were judged relative to other models.

K.2.2.2 Width of confidence intervals for EC estimates

Confidence intervals were assessed on standard error relative to estimate and confirming that the intervals were non-negative. Judged in absolute and relative to other models.

K.2.2.3 Width of confidence bands around the fitted model

A general visual inspection of the confidence bands for the fitted model. Wide bands in the area of interest were undesirable. Judged in absolute and relative to other models.

K.2.2.4 P-values of parameters estimates and goodness of fit tests

Hypothesis tests of parameter values to determine whether an estimate is significantly different from zero. Goodness of fit tests were used to judge the overall performance of the model fit. Typically, the level of significance was set at 0.05. There may have been occasional instances where the 0.05 criterion may not be met, but there was little recourse for choosing another model. Judged in absolute terms.

K.2.2.5 Residual plots

Residuals were examined for homoscedasticity and biasedness. Judged in absolute and relative to other models.

K.2.2.6 Overly influential observations

Observations were judged based on Cook's distance and leverage. When an observation was deemed overly influential, it was not reasonable to refit the model and exclude any overly influential observations given the limited data available with typical C-R curves. Judged in absolute terms.

K.3 Determining Curve Acceptability for use in Criteria Derivation

The final curve fits selected for each of the quantitatively-acceptable toxicity tests were further evaluated and classified to determine whether the curves were: 1) quantitatively acceptable for use, 2) qualitatively acceptable for use, or 3) unacceptable. To determine curve acceptability for use in deriving an effect concentration, each individual curve was considered based on the statistical metrics described above and assessed visually to compare how the calculated effect concentration aligned with the underlying raw C-R data. Instead of evaluating

curves fits relative to other curve fits for the same data (as was previously described to select the most-robust curve for each test), curve fit metrics were used to assign each curve a score:

- **Quantitatively Acceptable Model**. Model performed well on most/all statistical metrics and resultant effect concentrations were typically used in a quantitative manner.
- **Qualitatively Acceptable Model**. Model generally performed well on statistical metrics; however, the model presented some characteristic(s) that called estimates into question. Such models were considered with caution. These problems may have consisted of any number of issues such as a parameter with a high p-value, poor goodness of fit p-value, wide confidence bands for fit or estimate interval, or residuals that indicate model assumptions are not met. Broadly, effect concentrations from models that were deemed qualitatively acceptable were not used numerically in criteria derivation if quantitatively acceptable models for different endpoints or tests from the same publication were available. If quantitatively acceptable models for different endpoints or tests from the same publication were not available, effect concentrations from the qualitatively acceptable model were used numerically in criteria derivation on a case-by-case basis.
- **Unacceptable Model**. Model poorly fit the data. These models were not used for criteria derivation.

No single statistical metric can determine a given model's validity or appropriateness. Metrics should be considered as a whole. As such, there is a slightly subjective component to these evaluations. That said, this assessment scheme was developed to aid in evaluating models as to their quantitative or qualitative attributes in a transparent and relatively repeatable manner.

Appendix L Derivation of Acute Protective PFOS Benchmarks for Estuarine/Marine Waters through a New Approach Method (NAM): WebICE

The 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (U.S. EPA 1985) recommend that data for a minimum of eight families be available to fulfill taxonomic minimum data requirements (MDRs) to calculate criteria values, including to calculate estuarine/marine aquatic life criteria. Acute estuarine/marine test data are currently available for only five of the eight family MDRs (the dataset was missing another family in the Phylum Chordata, a family in a phylum other than Chordata, and any other family); thus, the EPA was not able to derive an acute estuarine/marine criterion element for PFOS based on the 1985 Guidelines MDR specifications (Section 3.2.1.2). However, the EPA was able to develop an acute PFOS protective benchmark for aquatic life using a New Approach Methods (NAMs) process, via the application of Interspecies Correlation Estimation (ICE) models (Raimondo et al. 2010). Although not a criterion based on 1985 Guidelines MDR specifications, because of gaps in available data for several of the taxonomic MDRs listed in the 1985 Guidelines for the derivation of aquatic life criteria, this benchmark represents an aquatic life value derived to be protective of aquatic communities. The ICE model predictions supplement the available test dataset to fulfill the missing MDRs and allow the derivation of an acute estuarine/marine benchmark for aquatic life using procedures consistent with those in the 1985 Guidelines. This is important as it provides an approach by which values that are protective of aquatic life communities can be developed, even when MDRs are not fulfilled by PFOS test data. This approach is consistent with both the 1985 Guidelines "good science" clause, the EPA's interest in providing useful information to states and Tribes regarding protective values for aquatic life, and the EPA's intention to reduce the use of animal testing via

application of NAMs (https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing).

L.1 Introduction to Web-ICE

ICE models, developed by the EPA's Office of Research and Development, are log-linear regressions of the acute toxicity (EC_{50}/LC_{50}) of two species across a range of chemicals, thus representing the relationship of inherent sensitivity between those species (Raimondo et al. 2010). Each model is derived from an extensive, standardized database of acute toxicity values by pairing each species with every other species for which acceptable toxicity data are available. Once developed, ICE models can be used to predict the sensitivity of an untested taxon (predicted taxa are represented by the y-axis) from the known, measured sensitivity of a surrogate species (represented by the x-axis) (Figure L-1).

ICE models have been developed for a broad range of different chemicals (e.g., metals and other inorganics, pesticides, solvents, and reactive chemicals) and across a wide range of toxicity values. There are approximately 3,400 significant ICE models for aquatic animal and plant species in the most recent version of web-ICE (v3.3, www3.epa.gov/webice, last updated June 2016; (Raimondo et al. 2015).

Models were validated using leave-one-out cross validation, which formed the basis for the analyses of uncertainty and prediction robustness. For this process, each datapoint within the model (representing the relative sensitivity of two species for a particular chemical) is systematically removed, one at a time. The model is then redeveloped with the remaining data (following each removal) and the removed value of the surrogate species is entered into the model. The estimated value for the predicted species is then compared to the measured value for that species (Raimondo et al. 2010; Willming et al. 2016).

ICE models have high prediction accuracy when values are derived from models with robust parameters (e.g., mean square error, R^2), that fall within a defined range of acceptability, and with close prediction confidence intervals that facilitate evaluating the fit of the underlying data (Brill et al. 2016; Raimondo et al. 2010; Willming et al. 2016). Results of these analyses provide the basis of the user guidance for selecting ICE predicted toxicity with high confidence (Box 1).

ICE models have undergone extensive peer review and their use has been supported for multiple applications, including direct toxicity estimation for endangered species (NRC 2013) (Willming et al. 2016) and development of Species Sensitivity Distributions (SSDs) (Awkerman et al. 2014; Bejarano et al. 2017; Dyer et al. 2006; Dyer et al. 2008; Raimondo et al. 2010; Raimondo and Barron 2020). The application of ICE-predicted values to develop protective aquatic life values by multiple independent, international groups confirms that values developed from ICE-generated SSDs provide a level of protection that is consistent with using measured laboratory data (Dyer et al. 2008; Feng et al. 2013; Fojut et al. 2012; Palumbo et al. 2012; Wang et al. 2020; Wu et al. 2015; Wu et al. 2016; Zhang et al. 2017). A recent external review of ICE models additionally supports their use in regulatory applications based on the reliability of underlying data, model transparency, statistical robustness, predictive reliability, proof of principle, applicability to probabilistic approaches, and reproducibility of model accuracy by numerous independent research teams (Bejarano and Wheeler 2020).

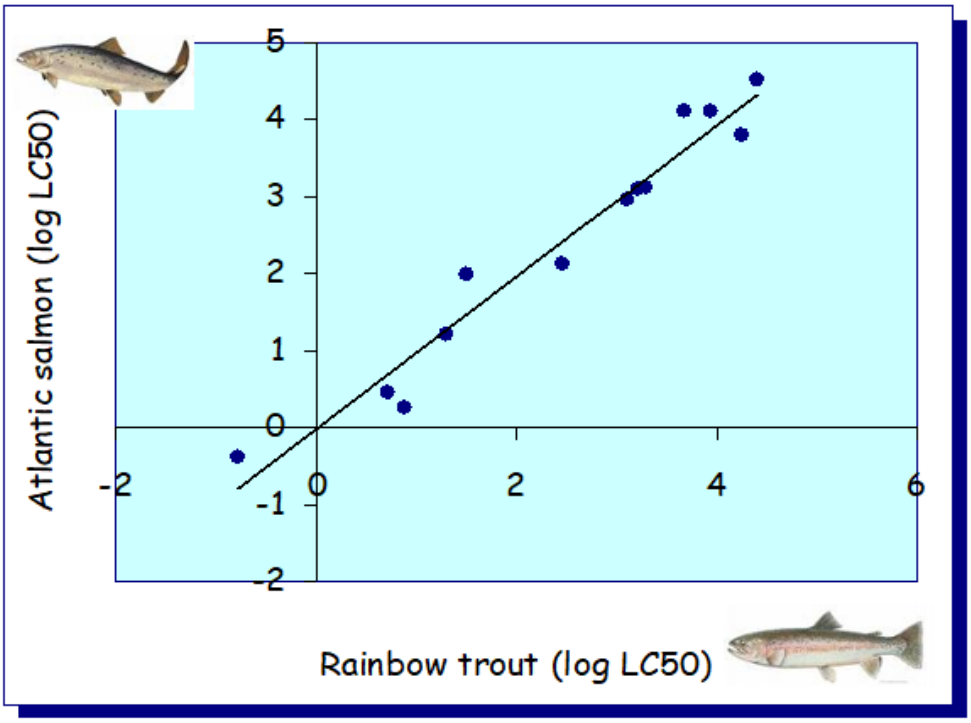


Figure L-1. Example ICE Model for Rainbow Trout (surrogate) and Atlantic Salmon (predicted).
Each model datapoint is a common chemical that was tested in both species to develop a log-linear regression.

Box 1. ICE Model User Guidance Recommended for Listed Species (Willming et al. 2016):

- Close taxonomic distance (within class)
- Low MSE (<~ 0.95)
- High R^2 (>~ 0.6)
- High slope (>~ 0.6)
- Prediction confidence intervals should be used to evaluate the prediction using professional judgement for the application (Raimondo et al. 2024).
- For models between vertebrates and invertebrates, using those with lower MSE or MOA-specific models (not available for PFAS) has been recommended for listed species predictions (Willming et al. 2016).

L.2 Application of Web-ICE with PFOS

ICE models are developed using a diversity of compounds (e.g., metals and other inorganics, pesticides, solvents, and reactive chemicals) across a wide range of toxicity values; however, PFAS are not included in web-ICE v3.3 due to the lack of available PFAS toxicity data when web-ICE v3.3 was created. PFAS acute values (typically reported as mg/L) can be greater than those used to develop an ICE model (ICE database toxicity range $1E^{-4}$ to $1E^{8}$ μg/L) such that the input PFAS value of the surrogate would be outside the model domain. In these cases, a user can either enter the value as μg/L and allow the model to extrapolate beyond its range or enter the toxicity as a "scaled" value (i.e., enter and estimate the value as mg/L). The principal assumptions of ICE models are: 1) they represent the relationship of inherent sensitivity between two species, which is conserved across chemicals, mechanisms of action, and ranges of toxicity; and 2) the nature of a contaminant that was tested on the surrogate reflects the nature of the contaminant in the predicted species (e.g., effect concentration (EC_{50}) or lethal concentration (LC_{50}), percentage of active ingredient, technical grade; Raimondo et al. (2010)). While neither of these assumptions are violated by either extrapolating beyond the range of the model or using scaled toxicity data, the uncertainty of using ICE models in either manner had not been thoroughly evaluated. Additionally, since PFAS were not included in the database used to develop web-ICE v3.3, the validation of ICE models to accurately and specifically predict to these compounds has not been previously explored. We address both these topics in the sections below.

L.2.1 Prediction Accuracy of Web-ICE for Scaled Toxicity and Values Beyond the Model Domain

The accuracy of using scaled toxicity data as input into ICE models was evaluated using an analysis with the existing ICE models (v3.3) and as described in detail in Raimondo et al.

(2024). Briefly, ICE models containing a minimum of 10 datapoints and spanning at least five orders of magnitude were separated into two subsets: 1) a lower subset that contained all paired chemical data corresponding to values below the 75th percentile of surrogate species values; and 2) an upper subset containing paired chemical data above the 75th percentile of surrogate values. The Raimondo et al. (2024) lower subset was used to develop "truncated" ICE models. The surrogate values in the upper subset were converted to mg/L and entered into the truncated ICE model. The predicted mg/L value was compared to the respective value of the measured predicted species. Prediction accuracy was determined as the fold difference (maximum of the predicted/measured and measured/predicted) between the predicted and the measured value, consistent with previously published evaluations of ICE models (Raimondo et al. 2010; Willming et al. 2016). Accuracy of using scaled toxicity as input into ICE models was compared to overall ICE prediction accuracy as previously reported and prediction accuracy of the respective upper subset data points that were entered into the models as µg/L (i.e., values beyond the model domain). A total of 3,104 datapoints from 398 models were evaluated. A match-paired comparison showed that the average fold differences of toxicity values predicted using scaled toxicity was not significantly different than the respective average fold differences of all cross-validated data points reported in Willming et al. (2016) (Wilcoxon paired rank sum test, V = 42741, p-value 0.11). Additionally, Raimondo et al. (2010) and Willming et al. (2016) showed a consistent and reproducible relationship between the taxonomic distance of the predicted and surrogate species, which was also reproduced using scaled values; the percentage of datapoints predicted using scaled toxicity was within 5-fold of the measured value for over 94% of all validated datapoints for species pairs within the same order, with a reduction in accuracy coinciding with decreasing taxonomic relatedness Raimondo et al. (2024). Comparison of scaled

values with those predicted from µg/L values beyond the model domain showed that predicted values varied by a factor of 10 for models with slopes ranging from 0.66 – 1.33. Toxicity values predicted from models with slopes within this range had a median fold difference of 2.4 using mg/L values and 2.8 using µg/L values (Wilcoxon paired rank sum test, V = 1334749, p-value 0.77). These results and a detailed review of ICE model assumptions are provided in Raimondo et al. (2024)[4].

L.2.2 Direct Comparison of Web-ICE and Measured Toxicity Values

Since limited PFOS toxicity test data are available for estuarine/marine species, the ability of ICE models to predict PFOS toxicity was evaluated using direct comparisons of freshwater species sensitivity as reported in the criteria document and predicted by web-ICE. In this comparison, the measured species mean acute values (SMAVs) for PFOS reported in Appendix A.1 and Appendix B.1 were used as values for surrogate species to predict all possible species that also had a measured PFOS SMAV reported. The available SMAVs for PFOS that could be used as ICE surrogate values along with the number of ICE models (i.e., potential predicted species) corresponding to each surrogate are shown in Table L-1.

[4] Use of scaled toxicity values and the use of surrogate toxicity values beyond the bounds of the ICE model that are input as µg/L are two approaches that both make extrapolations beyond the bounds of the underlying data. Actual predictions resulting from the two approaches from the same ICE model begin to deviate from one another the further the slope of the ICE model deviates from 1.0 (which is a primary reason why scaled toxicity data were only employed on ICE models with slopes ranging from 0.66 – 1.33). Overall, use of the scaled approach compared to direct extrapolation results a negligible change in the final estuarine/marine benchmark, primarily because the three of the four most sensitive estuarine/marine GMAVs were based on direct toxicity test results, and secondarily, because only a subset of ICE models required use of scaled toxicity data to account for predicting beyond the bounds of the underlying ICE model. For example, the final acute PFOS estuarine/marine benchmark was 0.55 mg/L (see section L.2.4). Had the values in Table L-4 been predicted using unscaled data that were input as µg/L only (and the model slope requirement of 0.66 -1.33 been retained), the final acute estuarine/marine benchmark would remain unchanged at 0.55 mg/L. Had the values in Table L-4 been predicted using unscaled data input as µg/L only (and the model slope requirement of 0.66 -1.33 was removed), the final acute estuarine/marine benchmark would increase slightly to 0.57 mg/L. While both approaches contain uncertainty, use of the scaled approach resulted in a more protective acute PFOS estuarine/marine benchmark (i.e., CMC = 0.55 mg/L) than an exploratory benchmark that used acute toxicity data estimated through direct extrapolation, with the model slope requirement of 0.66 -1.33 removed (i.e., exploratory CMC = 0.57 mg/L).

Table L-1. Surrogate Species Measured Values for PFOS and Corresponding Number of ICE Models for Each Surrogate.

For example, there are 53 species for which *Daphnia magna* can predict toxicity.

Broad Taxon	Species		PFOS SMAV (mg/L)	Number of ICE Models
	Common Name	Scientific		
Amphibian	Bullfrog	*Lithobates catesbeiana* [a]	133.3	9
Amphibian	African clawed frog	*Xenopus laevis*	15.99	2
Crustacean	Mysid	*Americamysis bahia*	4.914	28
Crustacean	Cladoceran	*Daphnia magna*	51.86	53
Fish	Zebrafish	*Danio rerio*	27.86	2 (juvenile models) 6 (embryo models)
Fish	Rainbow trout	*Oncorhynchus mykiss*	7.515	77
Fish	Fathead minnow	*Pimephales promelas*	6.95	74
Mollusc	Fatmucket	*Lampsilis siliquoidea*	16.5	29
Mollusc	Black sandshell	*Ligumia recta*	13.5	1

[a] *Lithobates catesbeianus* was used in web-ICE.

Table L-2 shows direct comparisons for PFOS measured and ICE-predicted values. The regressions for these comparisons are provided in the Appendix L.2.6. Comparisons are limited by the number of measured toxicity values and models available. To be included in this comparison, a measured value was needed for both species in an ICE model pair. For direct comparison of predicted and measured PFOS values, the measured SMAV of the surrogate species is entered into a model for which the measured SMAV for the intended predicted species is also known. The PFOS toxicity predicted by this model is then compared to the measured SMAV for the predicted species as listed in Appendix A.1, Appendix B.1 and Table L-1. This allows both species of an ICE model to serve as either the predicted or surrogate species. The exception to this was in cases involving zebrafish embryos, as web-ICE v3.3 only included models for which zebrafish embryos were used as surrogates. Accuracy of ICE predictions are presented as the "fold-difference" between the measured and the predicted species, such that fold

difference is the maximum of the ratio of the predicted LC_{50}/measured LC_{50} or measured LC_{50}/predicted LC_{50}. Analyses of ICE prediction accuracy have shown that ICE models over- and under-estimate toxicity values randomly, i.e., there is no systematic bias associated with the models (Table L-2) (Raimondo et al. 2010; Raimondo et al. 2024). For accuracy assessments, the fold difference provides a simplified metric to easily see how close predictions are to measured values at a glance. A 5-fold difference has been demonstrated to be the average interlaboratory variability of acute aquatic toxicity tests and represents a conservative amount of variance under standardized test conditions for a given life stage (Fairbrother 2008; Raimondo et al. 2010). This inter-test variation can increase significantly where experimental variables differ between tests; however, all ICE models are based on standardized life stages to minimize extraneous variability (Raimondo et al. 2010).

These comparisons are consistent with web-ICE user guidance (Raimondo et al. 2015), previously published reports on ICE model accuracy (Raimondo et al. 2010; Willming et al. 2016), and the above presented uncertainty analysis of using scaled toxicity as model input. ICE models predict with acceptable accuracy for PFOS when invertebrates were used to predict to invertebrate species and vertebrates were used to predict to vertebrate species in these comparisons. Models validated across a wide range of species, chemicals, and toxicity values show an acceptable level of prediction accuracy (>90% values predicted within 5-fold of measured value) when adhering to the model guidance listed in Box 1 (Raimondo et al. 2010; Willming et al. 2016).

The results summarized in Sections L.2.1 and L.2.2 demonstrate that the relationship of inherent sensitivity represented by ICE models is preserved across taxa, chemicals, and range of toxicity values when using robust ICE models. While the current analysis uses freshwater species

to predict to estuarine/marine species, previous model validation and uncertainty analyses did not indicate the habitat of the species to be an influential source of ICE model uncertainty (Raimondo et al. 2010; Willming et al. 2016).

Table L-2. Comparison of ICE-predicted and measured values of PFOS for species using both scaled values (entered as mg/L) and values potentially beyond the model domain (entered as µg/L).

Measured SMAVs are for the predicted species as listed in Appendix A.1, Appendix B.1 and Table L-1. Footnotes indicate where predictions or models do not meet one or more of the user guidance criteria.

Predicted Species	Surrogate Species	Toxicity Values Potentially Beyond Model Domain				Scaled Toxicity Values			
		Measured SMAV (µg/L)	web-ICE Predicted (µg/L)	95% Confidence Intervals (ug/L)	Fold Difference	Measured SMAV (mg/L)	web-ICE Predicted (mg/L)	Confidence Interval (mg/L)	Fold Difference
Bullfrog (*Lithobates catesbeianus*)	Daphnid (*Daphnia magna*)	133,300	59755.54	12281.24 - 290746.24	2.23	133.3	63.35	7.50 - 534.65	2.1[a]
	Fathead minnow (*Pimephales promelas*)		8356.68	3748.61 - 18629.28	15.95		13.26	4.13 - 42.57	10.05
	Rainbow trout (*Oncorhynchus mykiss*)		15140.53	8139.36 - 28163.81	8.8		33.9	13.63 - 84.26	3.93
African clawed frog (*Xenopus laevis*)	Fathead minnow (*Pimephales promelas*)	15,990	7034.49	800.65 - 61804.35	2.27[a]	15.99	18.93	0.306 - 1170.65	1.18[ab]
Mysid (*Americamysis bahia*)	Daphnid (*Daphnia magna*)	4,914	9221.68	5220.28 - 16290.18	1.88	4.914	28.55	19.59 - 41.60	5.81
	Fathead minnow (*Pimephales promelas*)		359.91	135.34 - 957.15	13.65[c]		0.481	0.104 - 2.21	10.22[c]
	Rainbow trout (*Oncorhynchus mykiss*)		1172.37	702.88 - 1955.47	4.19[c]		2.01	1.08 - 3.75	2.44[c]
Daphnid (*Daphnia magna*)	Bullfrog (*Lithobates catesbeianus*)	51,860	81946.04	17394.84 - 386042.67	1.58	51.86	199.47	32.95 - 1207.24	3.85
	Fathead minnow (*Pimephales promelas*)		1697.85	1149.29 - 2508.22	30.54[c]		3.29	1.36 - 7.96	15.76[c]
	Fatmucket (*Lampsilis siliquoidea*)		23122.84	7634.81 - 70030.01	2.24		7.73	1.46 - 40.85	6.71[b]
	Mysid (*Americamysis bahia*)		6096.75	3829.31 - 9706.79	8.51		21.29	13.73 - 33.02	2.44
	Rainbow trout (*Oncorhynchus mykiss*)		2775.45	2007.74 - 3836.72	18.69[c]		8.83	5.26 - 14.80	5.87[c]
	Zebrafish embryo (*Danio rerio*)		4515.51	1042.06 - 19566.76	11.48[c]		2.85	0.171 - 47.42	18.2[abc]
Rainbow trout (*Oncorhynchus mykiss*)	Bullfrog (*Lithobates catesbeianus*)	7,515	82395.25	38247.48 - 177501.32	10.96	7.515	39.73	16.29 - 96.91	5.29
	Daphnid (*Daphnia magna*)		22196.99	15080.46 - 32671.85	2.95[c]		245.99	182.01 - 332.46	32.73[cd]
	Fathead minnow (*Pimephales promelas*)		2771.13	2136.90 - 3593.60	2.71		3.43	2.01 - 5.85	2.19
	Fatmucket (*Lampsilis siliquoidea*)		48028.61	3264.96 - 706515.68	6.39[ac]		13.14	1.03 - 167.64	1.75[abc]

Predicted Species	Surrogate Species	Toxicity Values Potentially Beyond Model Domain				Scaled Toxicity Values			
		Measured SMAV (µg/L)	web-ICE Predicted (µg/L)	95% Confidence Intervals (ug/L)	Fold Difference	Measured SMAV (mg/L)	web-ICE Predicted (mg/L)	Confidence Interval (mg/L)	Fold Difference
	Mysid (*Americamysis bahia*)		6169.68	3855.10 - 9873.91	1.22		68.63	45.85 - 102.73	9.13[d]
	Zebrafish embryo (*Danio rerio*)		11721	4212.88 - 32610.00	1.56		3.46	0.618 - 19.40	2.17[b]
Fathead minnow (*Pimephales promelas*)	African clawed frog (*Xenopus laevis*)	6,950	16080.14	1020.67 - 253332.73	2.31[a]	6.95	7.89	0.071 - 868.40	1.14[ab]
	Bullfrog (*Lithobates catesbeianus*)		121541.51	44334.20 - 333204.08	17.49		91.08	33.84 - 245.08	13.11
	Daphnid (*Daphnia magna*)		46651.96	30060.61 - 72400.58	6.71[c]		712.85	474.15 - 1071.71	102.57[cd]
	Fatmucket (*Lampsilis siliquoidea*)		116669.9	19477.15 - 698863.06	16.79[c]		595.88	48.52 - 7317.09	85.74[abc]
	Mysid (*Americamysis bahia*)		13672.93	5348.62 - 34952.76	1.97[c]		254.88	118.25 - 549.38	36.67[cd]
	Rainbow trout (*Oncorhynchus mykiss*)		14424.97	11028.30 - 18867.80	2.08		36.58	23.89 - 56.02	5.26
	Zebrafish embryo (*Danio rerio*)		31446.57	17390.46 - 56863.77	4.52		56.87	17.21 - 187.92	8.18[b]
Fatmucket (*Lampsilis siliquoidea*)	Black sandshell (*Ligumia recta*)	16,500	11412.52	2418.09 - 53863.02	1.45	16.5	8.15	0.319 - 208.15	2.02[ab]
	Daphnid (*Daphnia magna*)		23821.82	9341.74 - 60746.62	1.44		138.32	48.08 - 397.96	8.38
	Fathead minnow (*Pimephales promelas*)		717.52	149.82 - 3436.35	23[c]		3.21	0.065 - 158.39	5.14[abc]
	Rainbow trout (*Oncorhynchus mykiss*)		1585.37	485.38 - 5178.24	10.41[c]		44.11	9.18 - 211.95	2.67[cd]
Black sandshell (*Ligumia recta*)	Fatmucket (*Lampsilis siliquoidea*)	13,500	19191.22	4438.79 - 82973.68	1.42	13.5	26.59	1.49 - 472.22	1.97[ab]

[a] Confidence interval >1.5 order magnitude
[b] Input data outside model range
[c] Guidance for model mean square error, R^2, and/or slope not met.
[d] Does not meet slope criteria for using scaled toxicity (0.66-1.33).

L.2.3 Prediction of Estuarine/Marine Species Sensitivity to PFOS

A value of PFOS sensitivity was predicted with web-ICE v3.3 for all possible species using all available surrogate species (Table L-1). Predicted values were obtained by entering all available surrogate species into the web-ICE SSD generator, which predicts to all possible species from all available surrogates simultaneously and exports results into an excel spreadsheet. Web-ICE results were generated using both mg/L and µg/L values to evaluate the full set of possible predictions using both units of measure against the model domain, confidence intervals, and model parameters. First, all available models were evaluated based on the parameter (MSE, R^2, slope) guidance in Box 1, which are the same for an ICE species pair regardless of input value (Table L-3). Models that did not meet the parameter criteria in Box 1 were rejected in this first pass. In the next step, values that were predicted using µg/L were evaluated against the model domain and selected for the next tier of evaluation when the surrogate value was within the range of data used to develop the model. If the surrogate value reported as µg/L was beyond the model domain, the mg/L value was evaluated if it was within the model domain and if the model slope was between 0.66-1.33 (Raimondo et al. 2024). Cases in which both units were outside the model domain were not included quantitatively, but the value with the narrowest confidence intervals was included for qualitative considerations. Values (using either µg/L or mg/L input value) were excluded quantitatively from the SMAVs but retained for qualitative consideration if an evaluation of confidence intervals, model parameters, and the model domain indicated the relationship between surrogate and predicted species was not informed by robust underlying data. At this stage, specific predictions should be based on holistic evaluation of all available information provided by the model, confidence interval, and data used to develop the model. Decisions to exclude a prediction from the SMAV are clarified

in footnotes. Because the sensitivity of a single species can be predicted by multiple surrogates, we calculated the SMAV where multiple robust models were available for a predicted species. Each predicted species was then assigned to the appropriate saltwater MDRs as defined in the 1985 *Guidelines.*

Saltwater MDRs:

a. Family in the phylum Chordata
b. Family in the phylum Chordata
c. Either the Mysidae or Penaeidae family
d. Family in a phylum other than Arthropoda or Chordata
e. Family in a phylum other than Chordata
f. Family in a phylum other than Chordata
g. Family in a phylum other than Chordata
h. Any other family

The acute sensitivity of estuarine/marine species to PFOS is presented in Table L-4. A total of 36 models representing 19 estuarine/marine species were available in web-ICE to predict the toxicity of PFOS to saltwater species (Table L-3). Of these, 12 models were initially rejected based on model parameters not meeting the guidance in Box 1, reducing the number of predicted species to 17 represented by 24 models. Further evaluation of ICE predictions resulted in 12 SMAVs. The range of sensitivity for the predicted taxa is consistent with the range of sensitivity of freshwater species for this compound.

Table L-3. All ICE Models Available in web-ICE v3.3 for Saltwater Predicted Species Based on Surrogates with Measured PFOS.

Model parameters are used to evaluate prediction robustness. Cross-validation success is the percentage of all model data that were predicted within 5-fold of the measured value through leave-one-out cross-validation (Willming et al. 2016). Taxonomic distance describes the relationship between surrogate and predicted species (e.g., 1 = shared genus, 2 = shared family, 3 = shared order, 4 = shared class, 5 = shared phylum, 6 = shared kingdom).

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate Model Minimum Value (µg/L)	Surrogate Model Maximum Value (µg/L)	Cross-Validation Success (%)	Taxonomic Distance	Use in Criteria
Acartia tonsa	*Daphnia magna*	0.59	1.31	2	0.91	0.0443	0.17	2.24	38514.70	50	5	Rejected
Allorchestes compressa	*Daphnia magna*	0.83	1.59	3	0.8	0.039	0.12	5.00	184.54	100	5	Accepted
Allorchestes compressa	*Pimephales promelas*	0.84	0.15	3	0.96	0.0028	0.02	163.05	26895.72	100	6	Accepted
Americamysis bahia	*Daphnia magna*	0.83	0.02	160	0.68	<0.001	0.93	0.07	840000.00	64	5	Accepted
Americamysis bahia	*Oncorhynchus mykiss*	0.92	-0.5	150	0.6	<0.001	1.08	0.06	1100000.00	57	6	Rejected
Americamysis bahia	*Pimephales promelas*	0.95	-1.12	46	0.55	<0.001	1.75	2.27	70200000.00	35	6	Rejected
Chelon labrosus	*Lampsilis siliquoidea*	1.27	1.5	1	0.99	0.0403	0	19.01	281.00	NA	6	Accepted qualitatively
Chelon macrolepis	*Pimephales promelas*	1.51	-1.04	2	0.97	0.0114	0.05	26.00	2533.38	100	4	Accepted qualitatively
Crassostrea virginica	*Americamysis bahia*	0.44	1.76	114	0.34	<0.001	0.88	0.003	117648.20	55	6	Rejected
Crassostrea virginica	*Daphnia magna*	0.44	1.54	116	0.28	<0.001	1.08	0.08	137171.43	58	6	Rejected
Crassostrea virginica	*Lampsilis siliquoidea*	0.82	-0.28	3	0.95	0.0041	0.06	30.00	22000.00	100	4	Accepted
Crassostrea virginica	*Oncorhynchus mykiss*	0.59	0.97	120	0.5	<0.001	0.68	0.02	570000.00	68	6	Rejected
Crassostrea virginica	*Pimephales promelas*	0.75	0.44	24	0.61	<0.001	0.68	1.24	206300.75	69	6	Accepted
Cyprinodon bovinus	*Oncorhynchus mykiss*	0.72	0.8	2	0.91	0.0427	0.08	4.93	1637.92	100	4	Accepted qualitatively
Cyprinodon bovinus	*Pimephales promelas*	0.67	0.65	2	0.99	0.0043	0	10.49	7847.42	100	4	Accepted
Cyprinodon variegatus	*Americamysis bahia*	0.57	1.88	88	0.56	<0.001	0.67	0.003	182000.00	64	6	Rejected
Cyprinodon variegatus	*Daphnia magna*	0.53	1.79	84	0.49	<0.001	0.72	0.08	304000.00	64	6	Rejected
Cyprinodon variegatus	*Lampsilis siliquoidea*	0.72	0.76	1	0.99	0.0392	0	30.00	22000.00	NA	6	Accepted qualitatively
Cyprinodon variegatus	*Oncorhynchus mykiss*	0.75	0.9	87	0.65	<0.001	0.56	0.82	12700000.00	75	4	Accepted
Cyprinodon variegatus	*Pimephales promelas*	0.69	0.98	24	0.74	<0.001	0.43	2.27	16500000.00	77	4	Accepted
Farfantepenaeus duorarum	*Americamysis bahia*	1.03	0.06	6	0.81	0.0022	0.55	0.01	720.00	50	4	Accepted
Farfantepenaeus duorarum	*Daphnia magna*	1.08	0.14	16	0.76	<0.001	1.32	0.04	65686.02	44	5	Rejected
Farfantepenaeus duorarum	*Oncorhynchus mykiss*	1.2	-1.36	15	0.72	<0.001	1.54	0.57	221000.00	47	6	Rejected
Fenneropenaeus merguiensis	*Daphnia magna*	0.82	1.43	4	0.66	0.0473	0.4	5.00	1251.41	67	5	Accepted
Gasterosteus aculeatus	*Oncorhynchus mykiss*	1.05	0.29	4	0.9	0.0038	0.18	0.61	890.00	83	4	Accepted
Hydroides elegans	*Daphnia magna*	0.49	1.59	2	0.96	0.0182	0.01	5.00	1251.41	100	6	Rejected
Hydroides elegans	*Oncorhynchus mykiss*	0.2	2.3	1	0.99	0.0179	0	1.84	13390.93	NA	6	Rejected
Litopenaeus stylirostris	*Americamysis bahia*	1.04	0.01	5	0.6	0.0401	0.29	0.58	24.09	57	4	Accepted
Menidia menidia	*Oncorhynchus mykiss*	1.28	-1.4	3	0.94	0.005	0.23	11.24	91000.00	60	4	Accepted qualitatively
Menidia peninsulae	*Americamysis bahia*	0.63	0.91	3	0.88	0.0162	0.32	0.01	1160.00	80	6	Accepted qualitatively
Menidia peninsulae	*Oncorhynchus mykiss*	1.01	-0.36	2	0.91	0.0421	0.35	0.82	1600.00	50	4	Accepted qualitatively

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate Model Minimum Value (µg/L)	Surrogate Model Maximum Value (µg/L)	Cross-Validation Success (%)	Taxonomic Distance	Use in Criteria
Metamysidopsis insularis	*Daphnia magna*	0.86	0.93	3	0.94	0.0057	0.18	6.97	317472.74	80	5	Accepted
Metamysidopsis insularis	*Lampsilis siliquoidea*	1.03	0.62	2	0.99	0.0027	0.02	19.01	87705.88	75	6	Accepted
Mugil cephalus	*Oncorhynchus mykiss*	1.44	-0.37	3	0.89	0.0144	0.12	0.82	29.18	100	4	Accepted qualitatively
Tigriopus japonicus	*Pimephales promelas*	0.81	1.12	5	0.76	0.0103	0.11	195.14	27000.00	86	6	Accepted
Tisbe battagliai	*Daphnia magna*	0.86	1.25	2	0.94	0.0289	0.08	0.61	184.54	100	5	Accepted

NA = Not Available.

Table L-4. ICE-Estimated Species Sensitivity to PFOS.

Values in bold and underlined are used for SMAV.

Common Name	Scientific	Surrogate	Input Unit	Estimated Toxicity (mg/L)	95% Confidence Intervals (mg/L)	SMAV
Calanoid copepod	*Acartia tonsa*	*Daphnia magna*	µg/L	13.12[abc]	(0.66 - 259.64)	NA
Amphipod	*Allorchestes compressa*	*Daphnia magna*	mg/L	**1072.28**	(323.49 - 3554.23)	50.94
		Pimephales promelas	µg/L	**2.42**	(1.29 - 4.54)	
Mysid	*Americamysis bahia*	*Daphnia magna*	µg/L	**9.22**	(5.22 - 16.29)	9.22
		Oncorhynchus mykiss	µg/L	1.17[c]	(0.7 - 1.96)	
		Pimephales promelas	µg/L	0.36[c]	(0.14 - 0.96)	
Thicklip mullet	*Chelon labrosus*	*Lampsilis siliquoidea*	mg/L	1144.93[ab]	(126.12 - 10393.7)	NA
Bigscale mullet	*Chelon macrolepis*	*Pimephales promelas*	µg/L	61.79[ab]	(4.94 - 772.16)	NA
Eastern oyster	*Crassostrea virginica*	*Americamysis bahia*	µg/L	2.52[c]	(1.45 - 4.37)	1.89
		Daphnia magna	µg/L	4.31[c]	(2.02 - 9.2)	
		Lampsilis siliquoidea	µg/L	**1.56**	(0.44 - 5.55)	
		Oncorhynchus mykiss	µg/L	2.01[c]	(1.3 - 3.1)	
		Pimephales promelas	µg/L	**2.28**	(0.78 - 6.67)	
Leon springs pupfish	*Cyprinodon bovinus*	*Oncorhynchus mykiss*	mg/L	27.57[a]	(3.2 - 236.94)	1.82
		Pimephales promelas	µg/L	**1.82**	(0.78 - 4.24)	
Sheepshead minnow	*Cyprinodon variegatus*	*Americamysis bahia*	µg/L	9.87[c]	(5.58 - 17.46)	5.77
		Daphnia magna	µg/L	20.32[c]	(9.75 - 42.39)	
		Lampsilis siliquoidea	µg/L	6.76[a]	(0.56 - 81.92)	
		Oncorhynchus mykiss	µg/L	**7.08**	(4.53 - 11.06)	
		Pimephales promelas	µg/L	**4.7**	(2.32 - 9.52)	
Pink shrimp	*Farfantepenaeus duorarum*	*Americamysis bahia*	mg/L	**6.02**	(1.34 - 26.97)	6.02
		Daphnia magna	µg/L	173.22[ac]	(14.83 - 2023.16)	
		Oncorhynchus mykiss	µg/L	2.12[c]	(0.38 - 11.71)	
Banana prawn	*Fenneropenaeus merguiensis*	*Daphnia magna*	mg/L	**722.81**	(131.83 - 3963)	722.81
Threespine stickleback	*Gasterosteus aculeatus*	*Oncorhynchus mykiss*	mg/L	**16.46**	(5.22 - 51.84)	16.46
Polychaete	*Hydroides elegans*	*Daphnia magna*	µg/L	8.45[bc]	(1.31 - 54.56)	NA
		Oncorhynchus mykiss	µg/L	1.28[c]	(0.89 - 1.83)	
Blue shrimp	*Litopenaeus stylirostris*	*Americamysis bahia*	mg/L	**5.41**	(1.59 - 18.41)	5.41
Atlantic silverside	*Menidia menidia*	*Oncorhynchus mykiss*	µg/L	3.97[a]	(0.52 - 30.32)	NA
Tidewater silverside	*Menidia peninsulae*	*Americamysis bahia*	mg/L	22.65[d]	(3.47 - 147.72)	NA

Common Name	Scientific	Surrogate	Input Unit	Estimated Toxicity (mg/L)	95% Confidence Intervals (mg/L)	SMAV
		Oncorhynchus mykiss	mg/L	3.35[a]	(0.1 - 118.6)	
Mysid	*Metamysidopsis insularis*	*Daphnia magna*	mg/L	**258.03**	(48.24 - 1380.1)	156.17
		Lampsilis siliquoidea	µg/L	**94.52**	(27.87 - 320.53)	
Striped mullet	*Mugil cephalus*	*Oncorhynchus mykiss*	mg/L	7.66[d]	(2.17 - 27.01)	NA
Harpacticoid copepod	*Tigriopus japonicus*	*Pimephales promelas*	µg/L	**18.04**	(7.2 - 45.24)	18.04
Harpacticoid copepod	*Tisbe battagliai*	*Daphnia magna*	mg/L	**550.44**	(107.35 - 2822.37)	550.44

NA = Not Available

[a] Both confidence intervals >1.5 order magnitude

[b] Input data outside model range

[c] Guidance for model mean square error, R^2, and/or slope not met

[d] Does not meet slope criteria for using scaled toxicity (0.66-1.33)

L.2.4 Derivation of Acute Water Quality Benchmark for Estuarine/Marine Water

The web-ICE predicted acute dataset for PFOS contains 15 genera, representing the eight MDR groups that would be necessary for developing an estuarine/marine criterion. The EPA fulfilled these eight MDRs by integrating the acceptable quantitative study data (discussed in Section 3.1.1.2) with data derived using web-ICE to support calculating a protective benchmark. In scenarios where both empirical LC_{50} values and estimated LC_{50} values were available for the same species, only the empirical data were used to derive the species mean acute value. The ranked GMAVs for these combined data along with the MDR met by each GMAV is summarized in Table L-5. From this dataset, an acute benchmark was calculated using procedures consistent with the 1985 Guidelines and with those used for the derivation of freshwater criteria values for PFOS. GMAVs for the four most sensitive genera were within a factor of 1.7 of each other (Table L-6). The estuarine/marine FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFOS is 1.096 mg/L (Table L-6). The FAV is lower than all of the GMAVs for both the tested species and for values derived using web-ICE. The FAV was then divided by two to obtain a concentration yielding a minimal effects acute benchmark. The FAV/2, which is the estuarine/marine acute water column benchmark magnitude, is 0.55 mg/L PFOS (rounded to two significant figures) and is expected to be protective of 95% of estuarine/marine genera potentially exposed to PFOS under short-term conditions of one-hour of duration, if the one-hour average magnitude is not exceeded more than once in three years (Figure L-2). This acute benchmark for estuarine/marine aquatic life is greater than the recommended acute freshwater criterion (0.071 mg/L), suggesting that estuarine/marine species may be less acutely sensitive to PFOS and emphasizing the importance of having a separate benchmark value for the protection of estuarine/marine aquatic life.

Table L-5. Ranked Estuarine/Marine Genus Mean Acute Values.

Values in bold were derived from empirical toxicity tests with the species.

MDR Group	Name	Species (lifestage)	SMAV	GMAV	Rank	Percentile
D	**Mediterranean mussel**	***Mytilus galloprovincialis***	**1.1**	**1.1**	1	0.06
F	**Purple sea urchin**	***Strongylocentrotus purpuratus***	**1.7**	**1.7**	2	0.13
E	**Sea urchin**	***Paracentrotus lividus***	**1.795**	**1.795**	3	0.19
D	Eastern oyster	*Crassostrea virginica*	1.89	1.89	4	0.25
C	**Mysid**	***Americamysis bahia***	**4.914**	**4.914**	5	0.31
A	Leon springs pupfish	*Cyprinodon bovinus*	1.82	**5.225**	6	0.38
	Sheepshead minnow	***Cyprinodon variegatus***	**>15**			
F	Blue shrimp	*Litopenaeus stylirostris*	5.41	5.41	7	0.44
F	Pink shrimp	*Farfantepenaeus duorarum*	6.02	6.02	8	0.50
C	**Mysid**	***Siriella armata***	**6.9**	**6.9**	9	0.56
B	Threespine stickleback	*Gasterosteus aculeatus*	16.46	16.46	10	0.63
G	Harpacticoid copepod	*Tigriopus japonicus*	18.04	18.04	11	0.69
E	Amphipod	*Allorchestes compressa*	50.94	50.94	12	0.75
C	Mysid	*Metamysidopsis insularis*	156.2	156.2	13	0.81
H	Harpacticoid copepod	*Tisbe battagliai*	550.4	550.4	14	0.88
F	Banana prawn	*Fenneropenaeus merguiensis*	722.8	722.8	15	0.94

MDR Groups

a. Family in the phylum Chordata
b. Family in the phylum Chordata
c. Either the Mysidae or Panaeidae family
d. Family in a phylum other than Arthropoda or Chordata
e. Family in a phylum other than Chordata
f. Family in a phylum other than Chordata
g. Family in a phylum other than Chordata
h. Any other family

Table L-6. Estuarine/Marine Final Acute Value and Protective Aquatic Acute Benchmark.
Bold values represent genera for which empirical toxicity data were available.

Calculated Estuarine/Marine FAV based on 4 lowest values; n=15						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	***Mytilus***	1.1	0.10	0.01	0.063	0.250
2	***Strongylocentrotus***	1.7	0.53	0.28	0.125	0.354
3	***Paracentrotus***	1.795	0.59	0.34	0.188	0.433
4	*Crassostrea*	1.89	0.64	0.41	0.250	0.500
		Σ (Sum):	**1.85**	**1.04**	**0.63**	**1.54**

S^2 =	5.32	S = slope
L =	-0.424	L = X-axis intercept
A =	0.092	A = lnFAV
FAV =	1.096	P = cumulative probability
PVAL=	**0.55 mg/L PFOS** (rounded to two significant figures)	

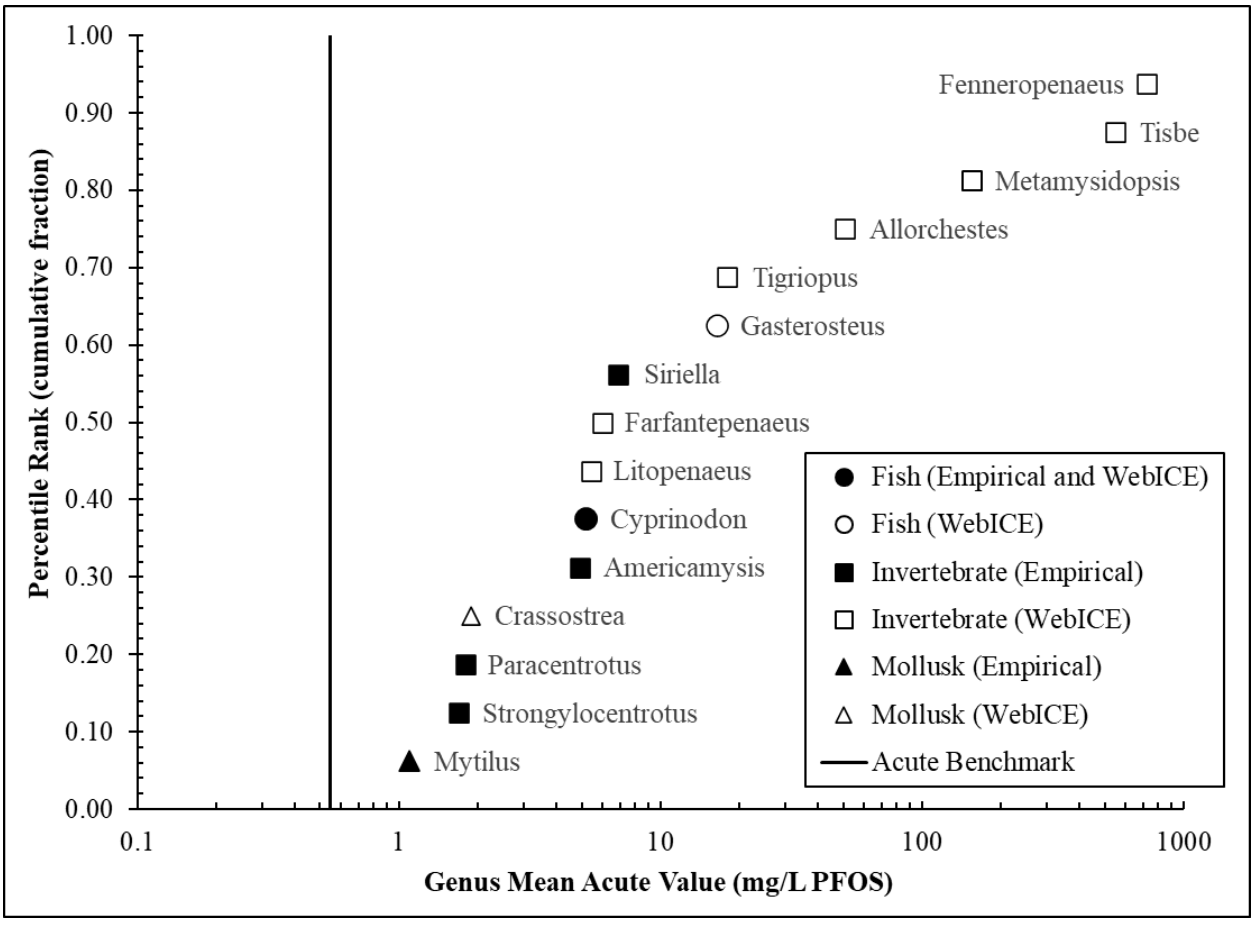


Figure L-2. Ranked Estuarine/Marine Acute PFOS GMAVs used for the Aquatic Life Acute Benchmark Calculation.

L.2.5 Estuarine Marine/Benchmark Uncertainty

Epistemic uncertainty of individual ICE estimates used for SMAV calculation was quantified through the calculation of corresponding 95% confidence intervals for each ICE estimate. Of the individual models and resultant ICE-estimated LC_{50} values estimates from the available and quantitatively acceptable models (see bolded and underlined values in Table L-4; n =16), the range of individual 95% CIs (i.e., 95% CI range = upper 95% CI – lower 95% CI) as a percent of the corresponding LC_{50} estimate (i.e., = [95% CI range/LC50 estimate]*100) ranged from 92.23% to 530.04%. The ICE model with the lowest 95% CI range relative to the LC_{50} estimate (i.e., 92.23%) employed *Oncorhynchus mykiss* as the predictor species and *Cyprinodon variegatus* as the predicted species. The ICE model with the largest 95% CI range relative to the LC_{50} estimate (i.e., 530.04%) employed *Daphnia magna* as the predictor species and *Fenneropenaeus merguiensis* as the predicted species. Fifteen of the 16 ICE-predicted values in Table L-4 that were used for SMAV calculation had 95% CI ranges that were greater than the corresponding LC_{50} estimate (i.e., 95% CI range was >100% of the LC_{50} estimate). The relatively wide ranging 95% CIs demonstrate the underlying uncertainty in the PFOS estuarine/marine benchmark.

Six of the 15 GMAVs used to derive the acute PFOS estuarine/marine benchmark were based on empirical toxicity tests. The six GMAVs based on empirical data were not evenly distributed across the GSD, with all empirical data falling below the 60^{th} percentile of sensitivity (Table L-2). Also, three of the four most sensitive GMAVs in the GSD (Figure L-2) were based on empirical data and five of the six most sensitive GMAVs were based empirical acute values, meaning final estuarine/benchmark magnitude was primarily based on relatively certain empirical toxicity tests and the inherent uncertainty in the ICE models had little influence on the final acute estuarine/marine benchmark magnitude.

The estuarine/marine benchmark appears adequately protective based on the available high quality empirical data (Appendix B.1). The acute PFOS estuarine/marine benchmark (i.e., 0.55 mg/L) is two times lower than the lowest GMAV (i.e., 1.1 mg/L), which was based on empirical data for *Mytilus*. The EPA further evaluated the appropriateness of the estuarine/marine benchmark by comparing it to empirical, but qualitatively acceptable, data for estuarine/marine species. The EPA specifically focused on qualitatively-acceptable estuarine/marine tests reported in Table H.1 that: (1) tested an animal species; (2) exposed test organisms to PFOS for a continuous exposure duration that was reasonably similar to standard acute exposures (e.g., 48 hours to seven days); (3) reported acute apical effects; and (4) reported effect concentrations that were lower than the acute estuarine/marine benchmark final acute value (i.e., 1.096 mg/L). The EPA identified three individual tests in Table H.1 as meeting the previous criteria:

1. Park et al. (2015) conducted a seven-day test with the mud crab, *Macrophthalmus japonicus*. Exposures lasted seven days, but survival was also recorded at 96 hours. The authors did not calculate an LC_{50}, but at 96 hours there was 36% mortality in the highest test concentration (i.e., 0.03 mg/L). Therefore, the 96-hour LC_{50} was >0.03 mg/L. The test was not used quantitatively because an LC_{50} could not be calculated based on the three exposure concentrations used. Overall, 36% mortality after 96 hours in the 0.03 mg/L treatment suggests this species may be sensitive to acute PFOS exposures relative to the acute estuarine/marine benchmark. However, the source of the organisms (fish market) could be problematic as there is no mention of potential previous exposure or measures of PFOS in test organisms at any point during the experiment.

2. Mhadhbi et al. (2012) conducted a 6-day test with the turbot, *Schophthalmus maximus*. Endpoints included dead embryos, malformation, hatch success at 48 hours and larval survival (missing heartbeat and a non-detached tail) at six days. The 6-day LC_{50} of 0.11 mg/L PFOS was not acceptable for acute benchmark derivation because of the relatively long exposure duration. Nevertheless, the 6-day LC_{50} is nearly an order of magnitude lower than the acute estuarine/marine benchmark final acute value (i.e., 1.096 mg/L) and five times lower than the acute estuarine/marine benchmark, suggesting *S. maximus* is sensitive to acute PFOS exposures at concentrations below the acute estuarine/marine benchmark.
3. Jeon et al. (2010) performed a 6-day test on blackrock fish, *Sebastes schlegeli*. There were no significant differences in total length, weight and survival (no mortality observed in any of the exposures) over the 6 - day exposure. The NOEC (survival and growth) was 1 mg/L at each test salinity (10, 17.5, 25 and 34 ppt), which is less than the acute estuarine/marine benchmark final acute value (i.e., 1.096 mg/L). The lack of effects observed at 1.0 mg/L preclude this test from providing meaningful information about the protectiveness of the acute estuarine/marine benchmark.

Results from Mhadhbi et al. (2012), which was determined to only be acceptable for qualitative use, suggests *S. maximus* is sensitive to acute PFOS exposures at concentrations below the acute estuarine/marine benchmark, but at an exposure duration that was 50% longer than the standard 96- hour exposure duration from quantitatively acceptable tests. Additionally, results of quantitatively acceptable empirical toxicity studies with estuarine/marine organisms do not provide any evidence that the aquatic estuarine/marine community will experience unacceptable acute effects at the acute estuarine/marine PFOS benchmark.

L.2.6 ICE Regressions Supporting the Acute Estuarine/Marine Benchmark

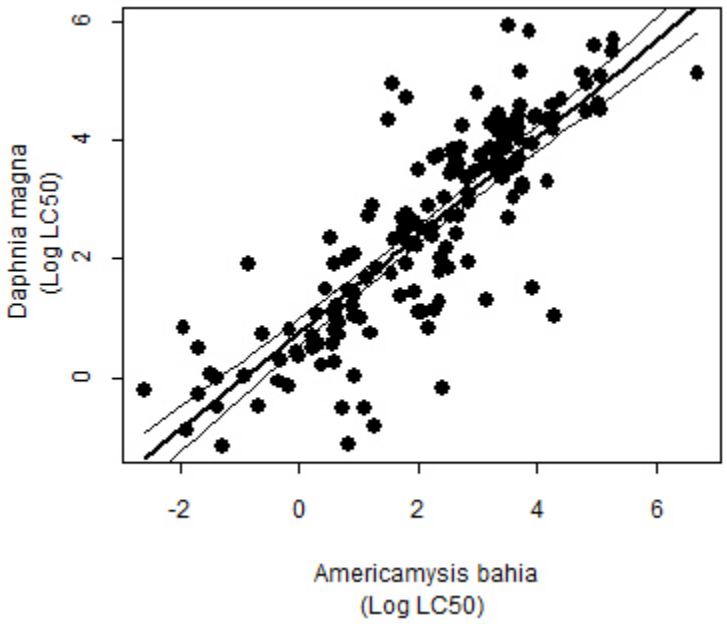


Figure L-3. *Americamysis bahia* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

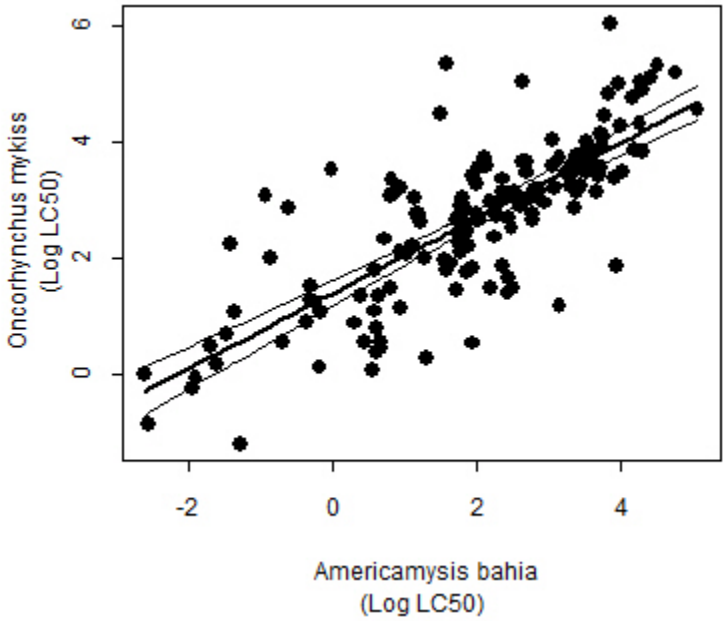


Figure L-4. *Americamysis bahia* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

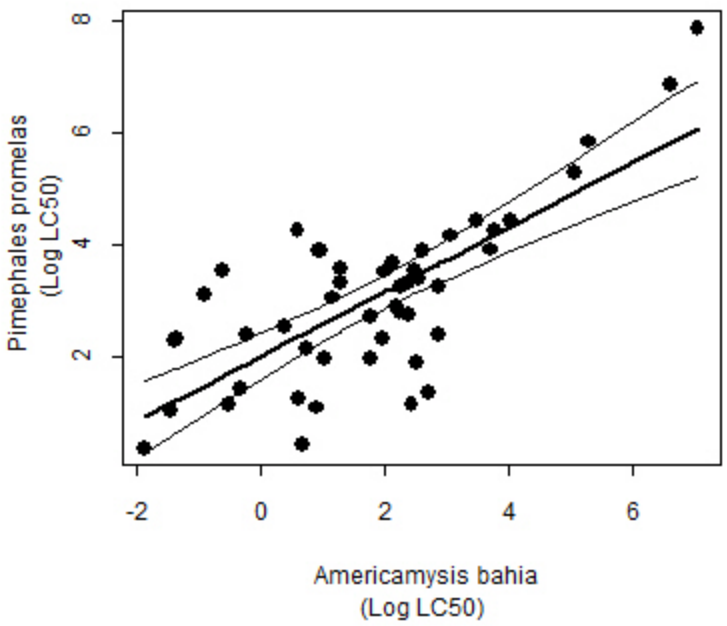


Figure L-5. *Americamysis bahia* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

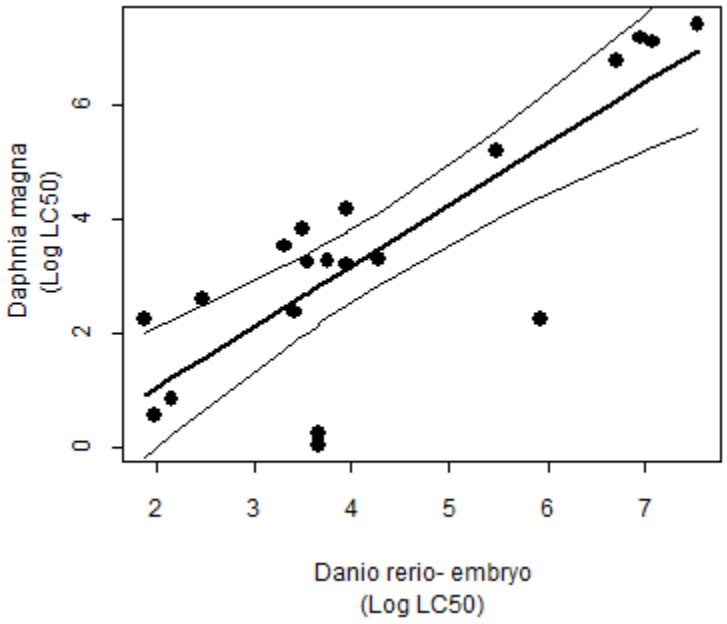


Figure L-6. *Danio rerio* -embryo (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

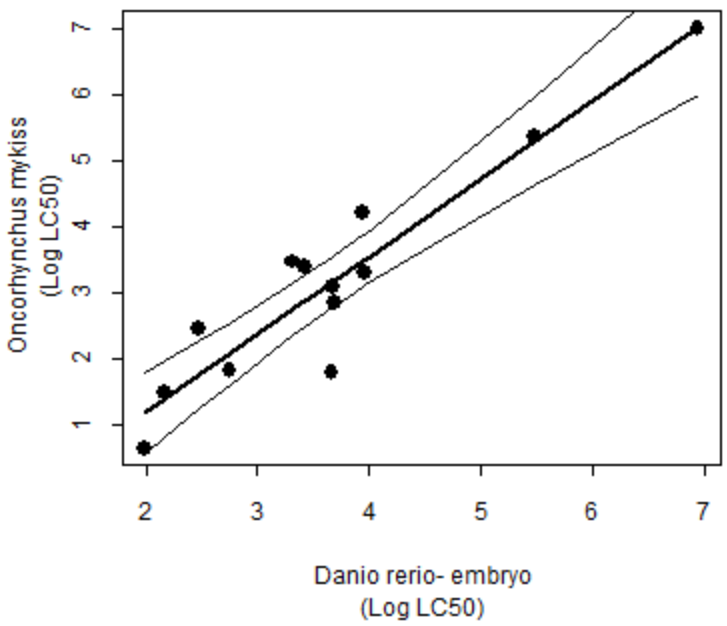


Figure L-7. *Danio rerio* - embryo (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

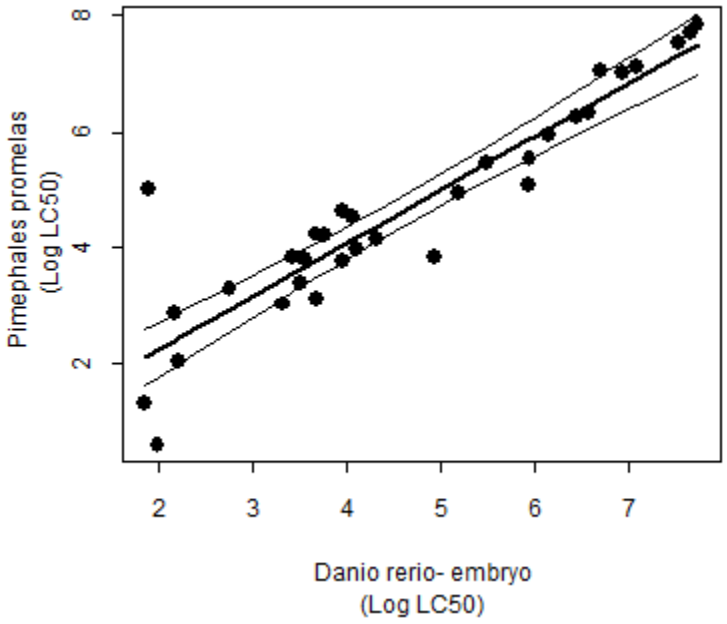


Figure L-8. *Danio rerio* - embryo (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

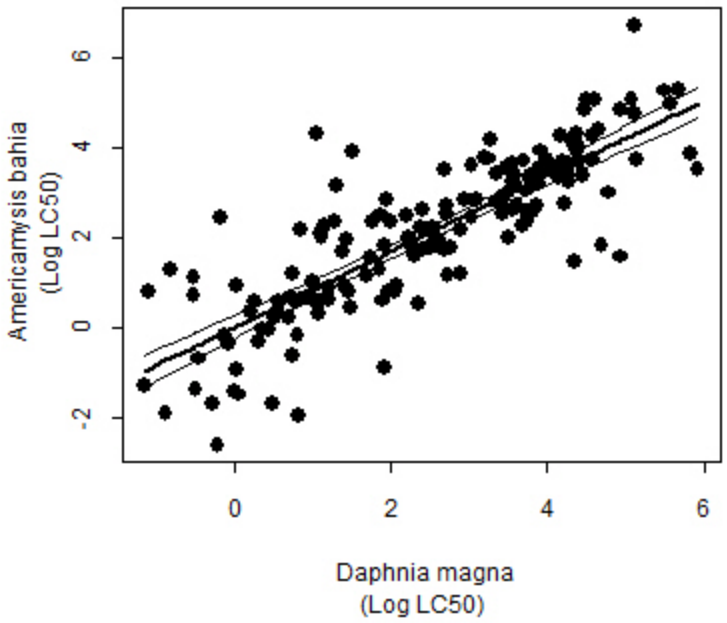


Figure L-9. *Daphnia magna* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

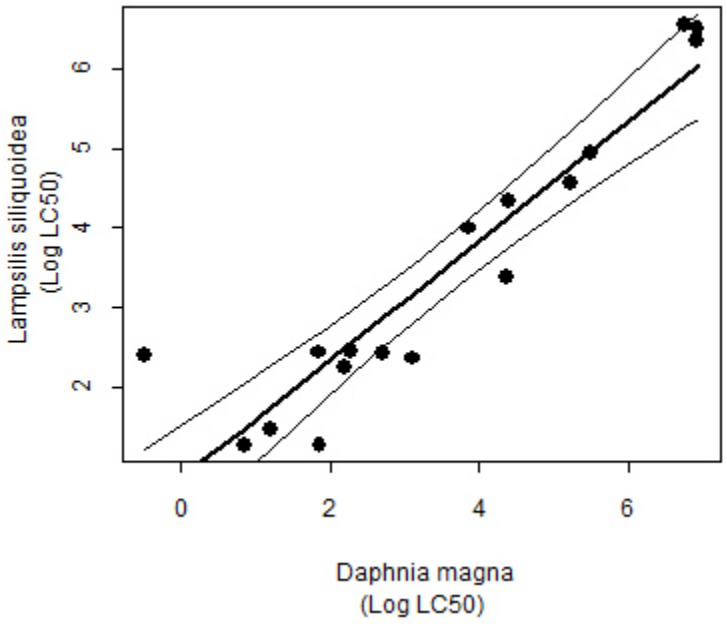


Figure L-10. *Daphnia magna* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

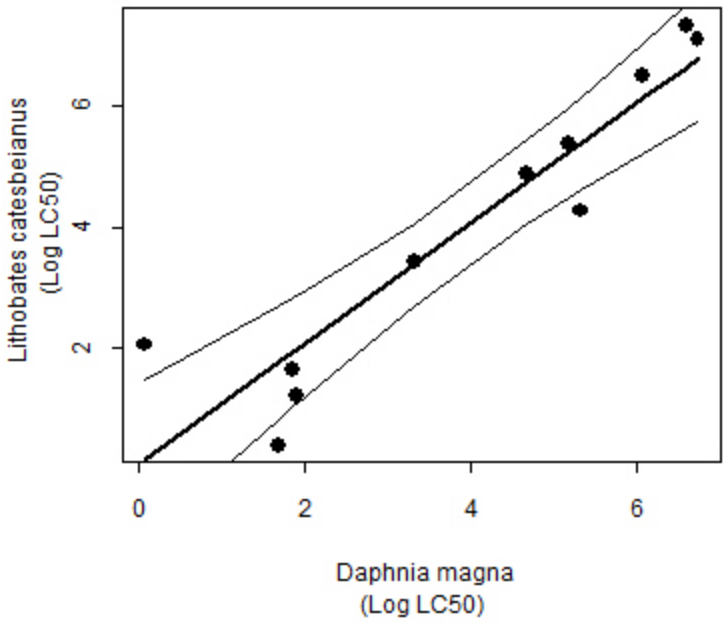


Figure L-11. *Daphnia magna* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

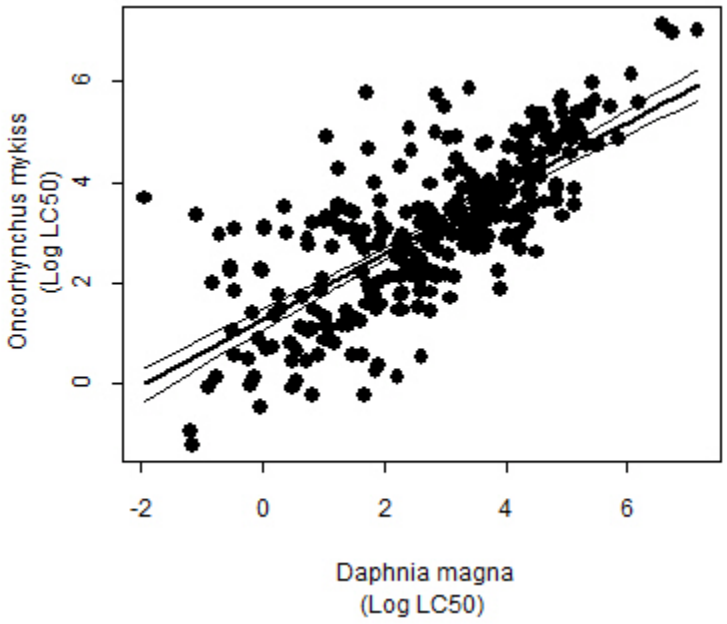


Figure L-12. *Daphnia magna* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

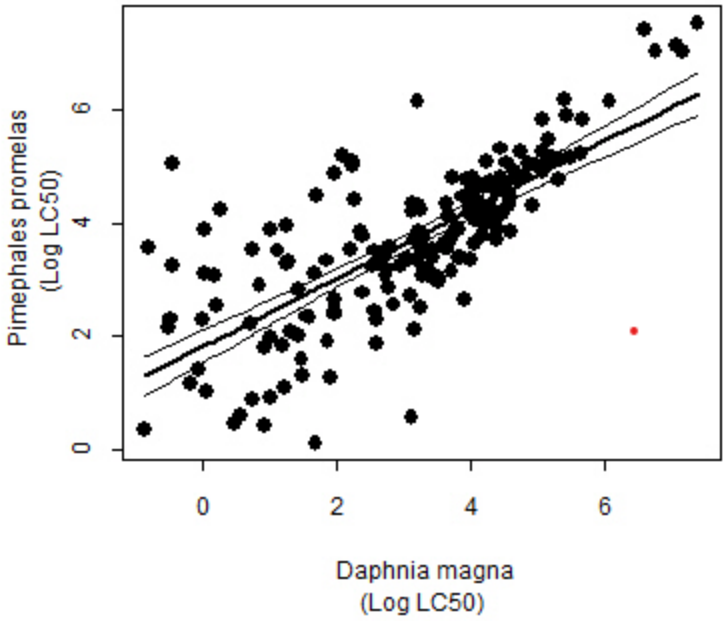


Figure L-13. *Daphnia magna* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

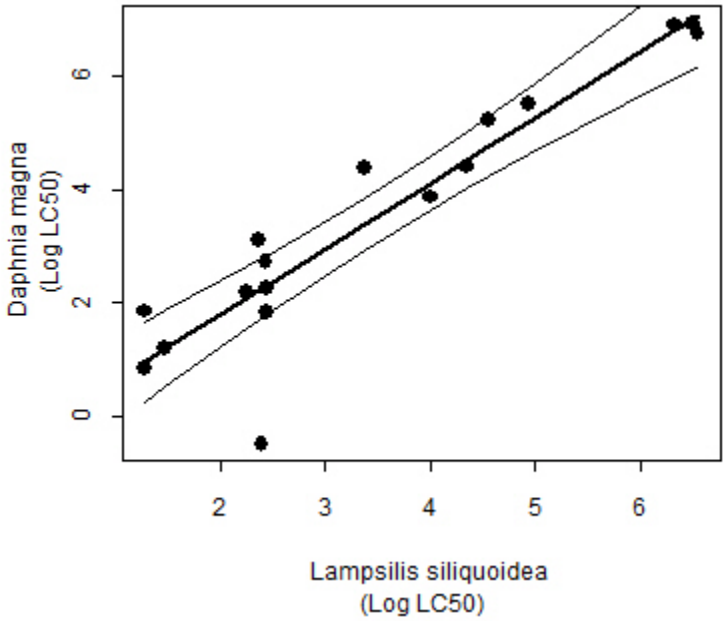


Figure L-14. *Lampsilis siliquoidea* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

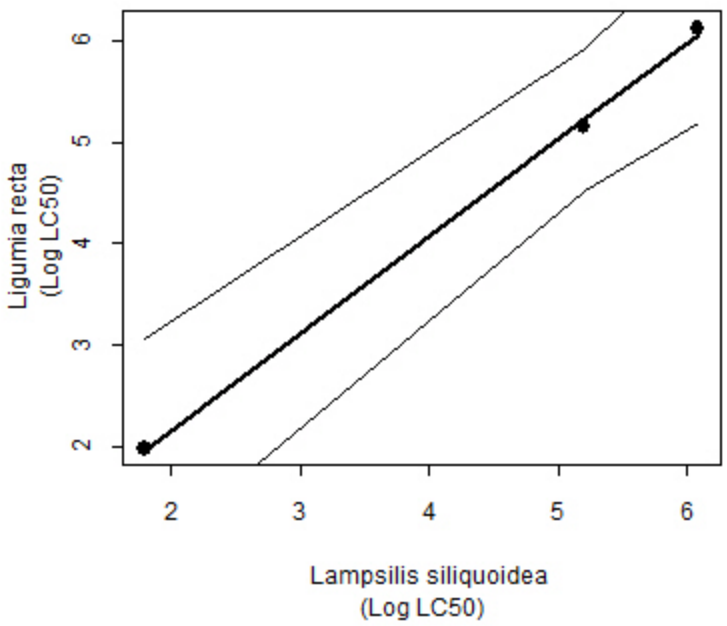


Figure L-15. *Lampsilis siliquoidea* (X-axis) and *Ligumia recta* (Y-axis) regression model used for ICE predicted values.

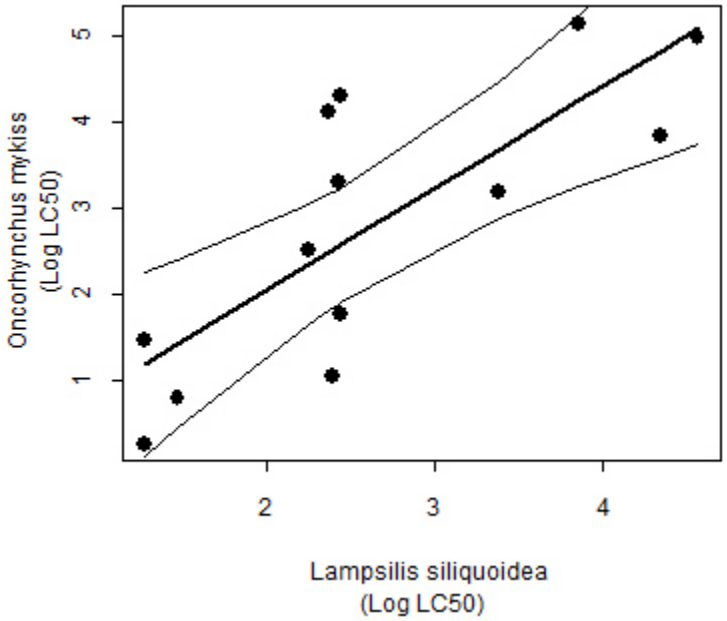


Figure L-16. *Lampsilis siliquoidea* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

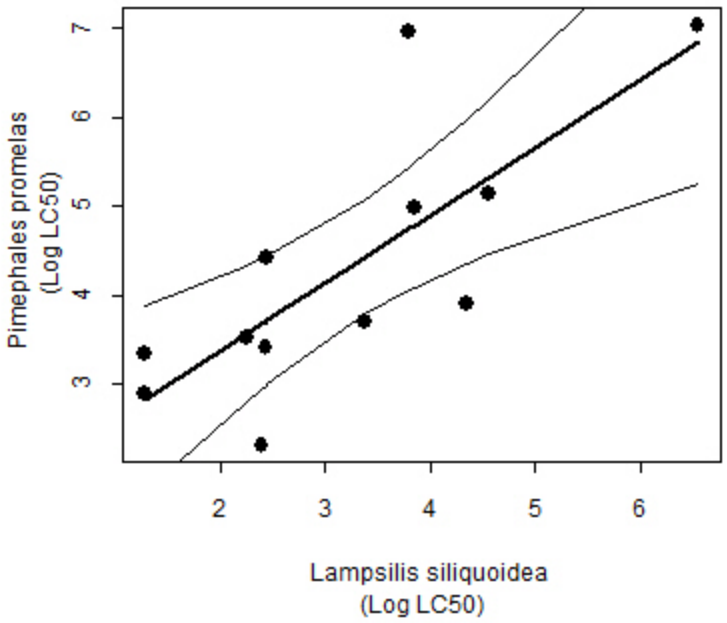


Figure L-17. *Lampsilis siliquoidea* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

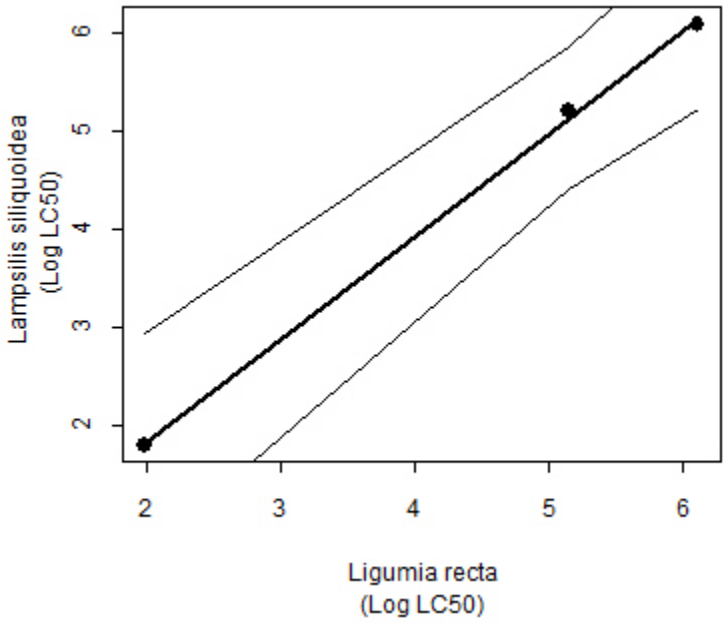


Figure L-18. *Ligumia recta* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

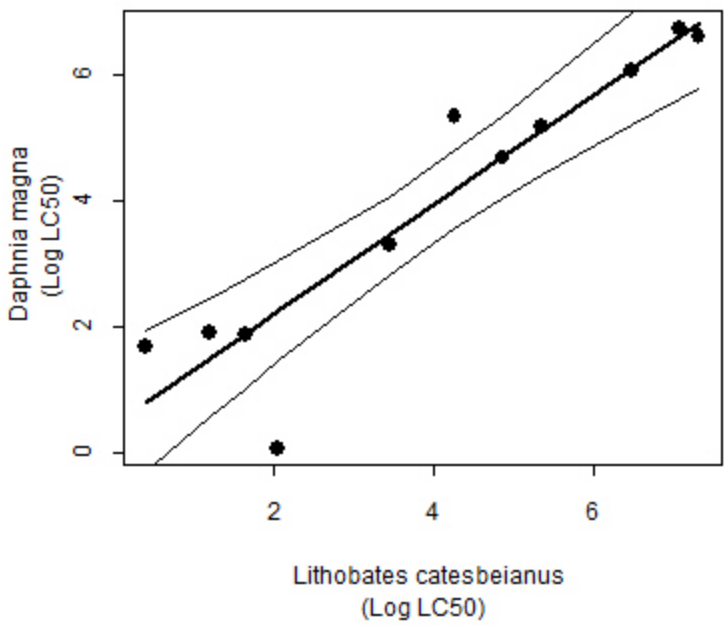


Figure L-19. *Lithobates catesbeianus* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

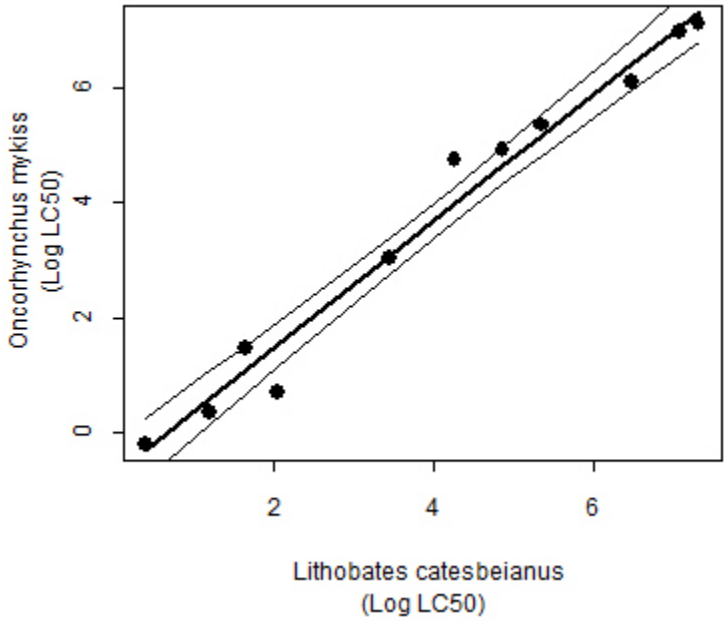


Figure L-20. *Lithobates catesbeianus* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

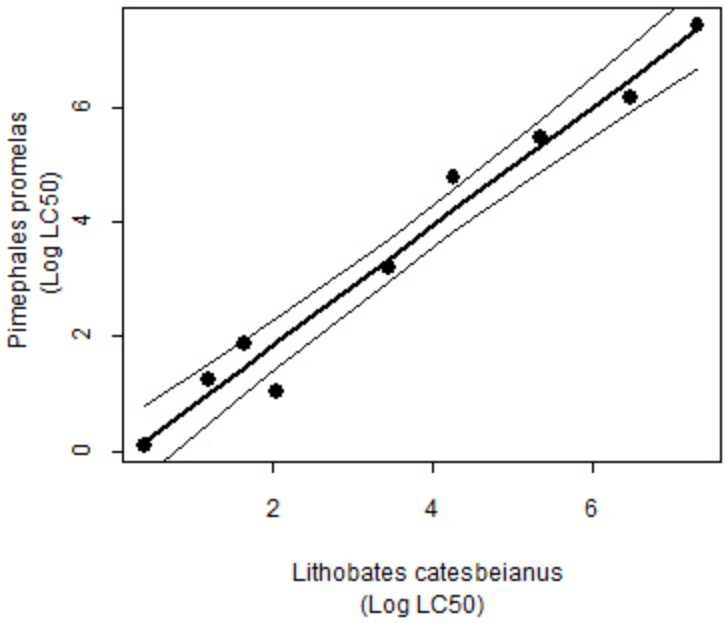


Figure L-21. *Lithobates catesbeianus* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

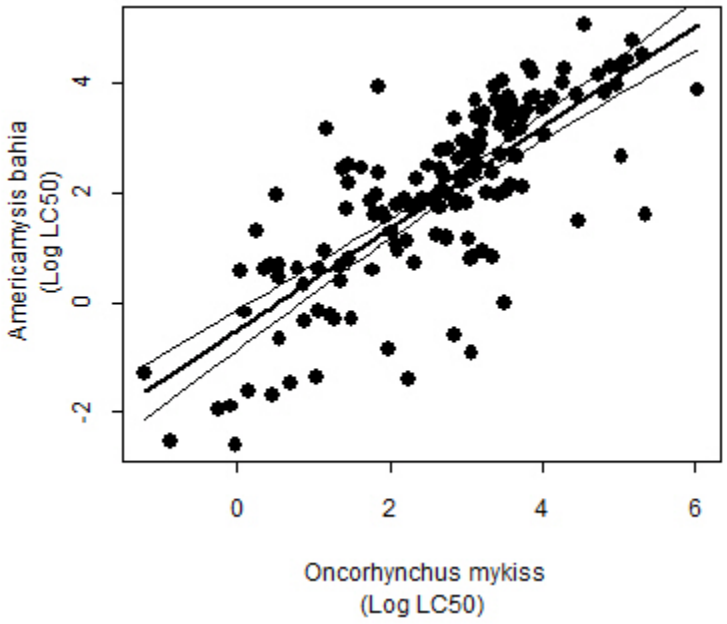


Figure L-22. *Oncorhynchus mykiss* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

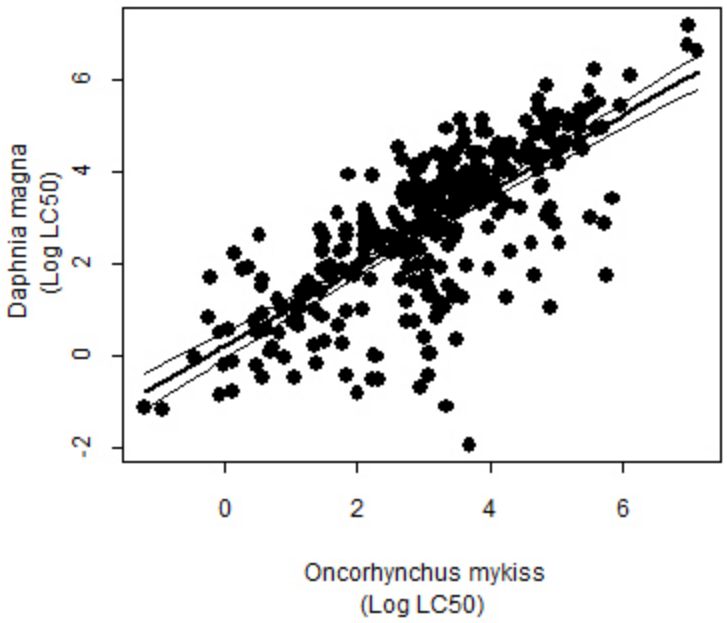


Figure L-23. *Oncorhynchus mykiss* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

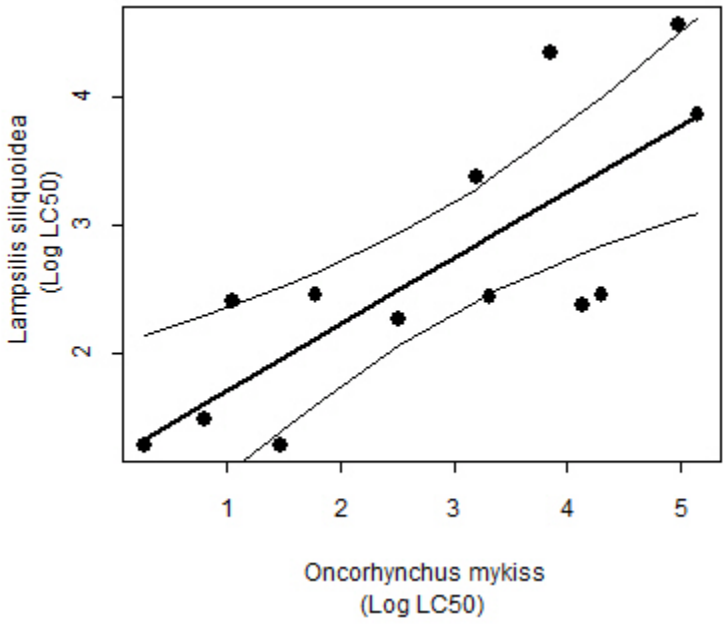


Figure L-24. *Oncorhynchus mykiss* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

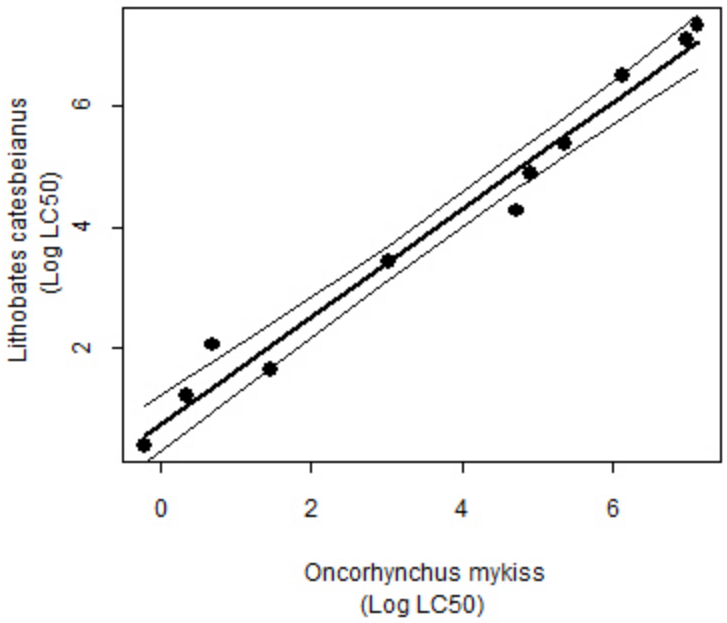


Figure L-25. *Oncorhynchus mykiss* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

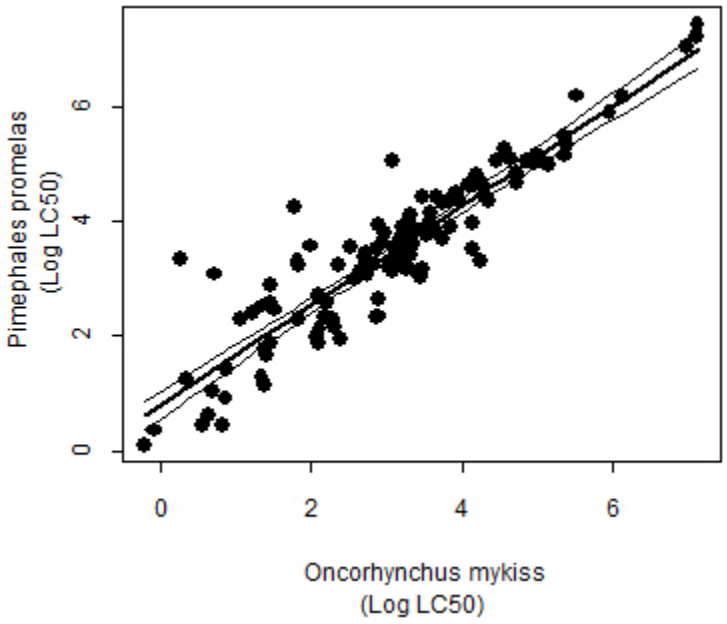


Figure L-26. *Oncorhynchus mykiss* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

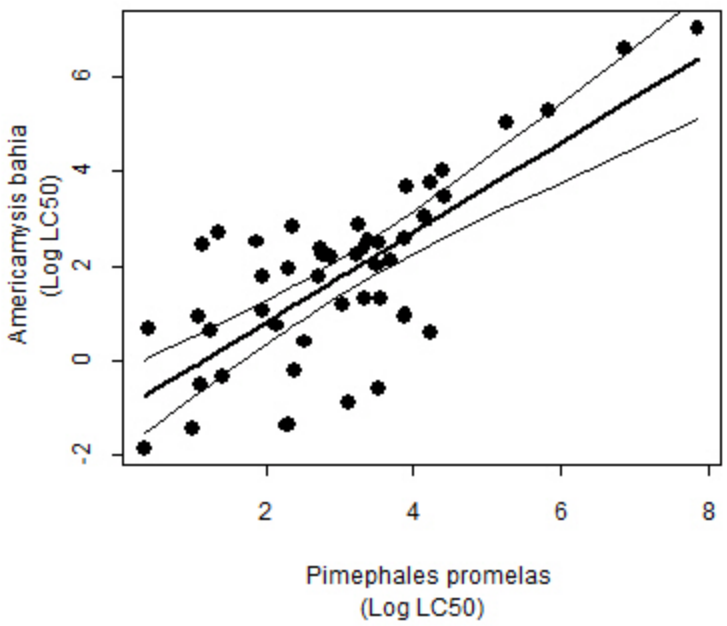


Figure L-27. *Pimephales promelas* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

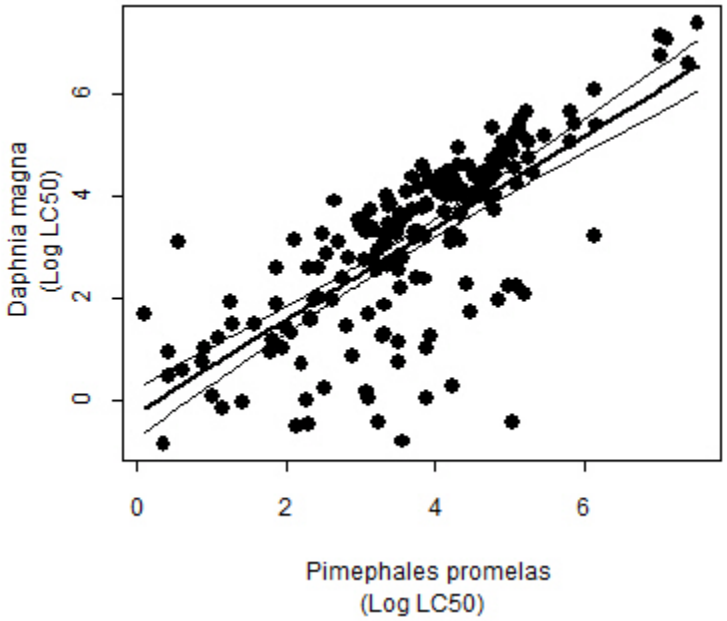


Figure L-28. *Pimephales promelas* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

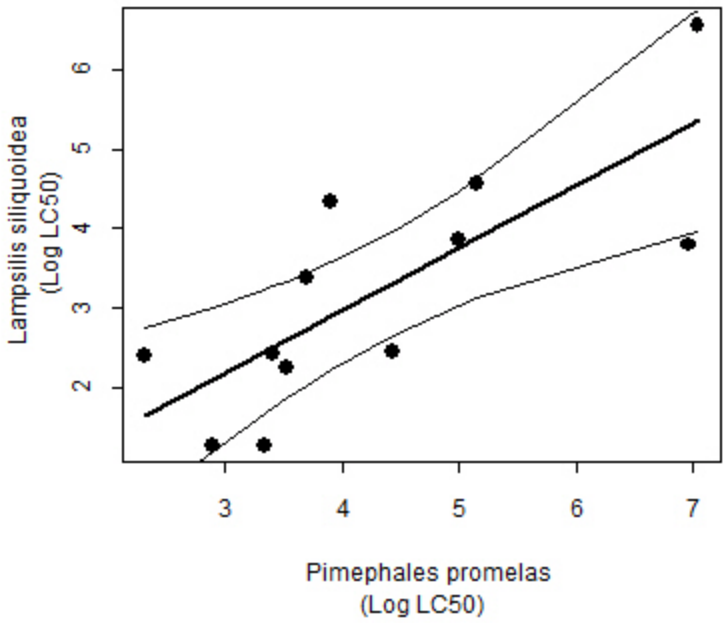


Figure L-29. *Pimephales promelas* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

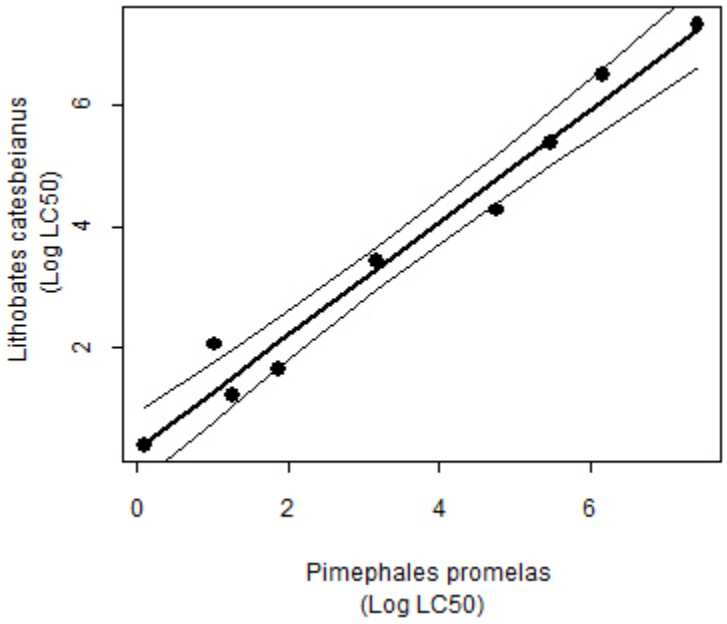


Figure L-30. *Pimephales promelas* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

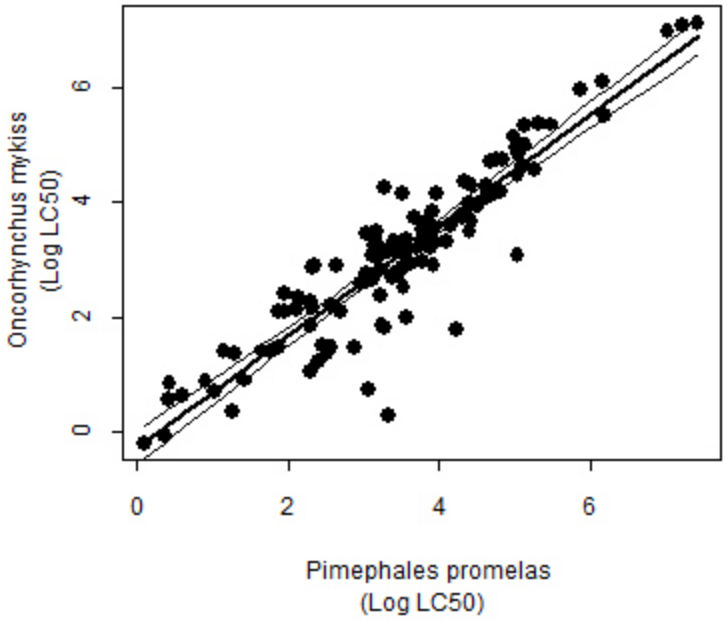


Figure L-31. *Pimephales promelas* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

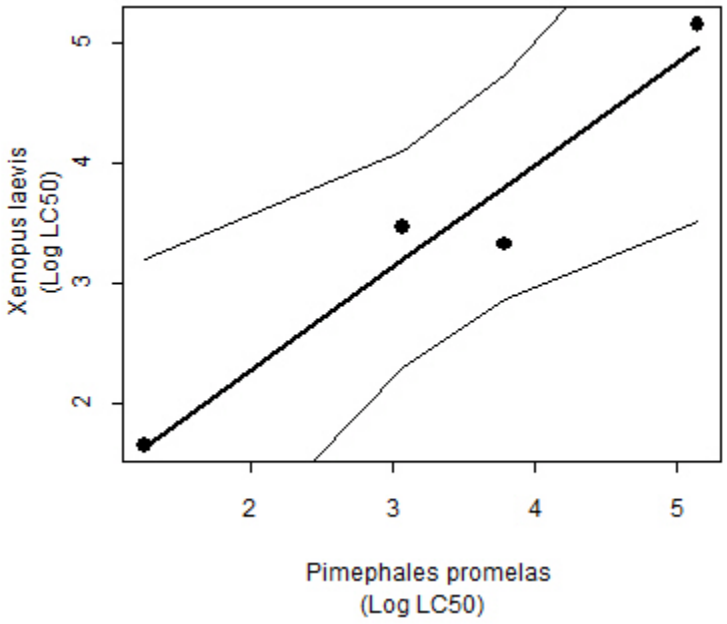


Figure L-32. *Pimephales promelas* (X-axis) and *Xenopus laevis* (Y-axis) regression model used for ICE predicted values.

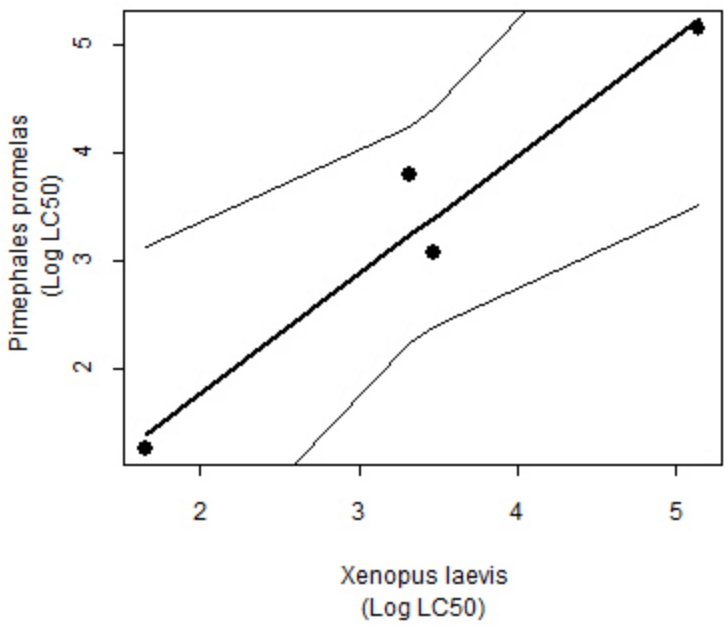


Figure L-33. *Xenopus laevis* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

Appendix M Environmental Fate of PFOS in the Aquatic Environment

Natural degradation of PFOS has not been observed. As described above in Section 2.2 above, under environmental conditions, PFOS does not photolyze, hydrolyze, or biodegrade and is thermally stable. For these reasons, PFOS is considered to be highly persistent in the environment (Beach et al. 2006; OECD 2002).

M.1 Photolysis

PFOS does not appear to photolyze (OECD 2002). No experimental evidence of direct or indirect photolysis was available (Hatfield 2001). The indirect photolytic half-life of PFOS using an iron oxide photo-initiator matrix model was estimated to be ≥ 3.7 years at 25°C. This half-life was based on the analytical method of detection (Giesy et al. 2010).

M.2 Hydrolysis

No hydrolytic loss of PFOS was observed in a 49 day study under experimental conditions of 50°C and pH conditions of 1.5, 5, 7, 9 or 11 (Hatfield 2001). Instead, the half-life of PFOS was estimated to be ≥41 years at 25°C. However, this estimate was influenced by the analytical limit of quantification and that no loss of PFOS was actually detected (Giesy et al. 2010).

M.3 Biodegradation

Several studies have demonstrated that PFOS does not biodegrade under aerobic or anaerobic conditions (Gledhill and Markley 2000c; Gledhill and Markley 2000b; Gledhill and Markley 2000a; Key et al. 1998; Laboratory 2002; Lange 2001; Remde and Debus 1996; Saez et al. 2008). Results from a study conducted by Kurume Laboratory in 2002 showed no biodegradation of PFOS after 28 days as measured by net oxygen demand, loss of total organic carbon, and loss of parent material. Key et al. (1998) demonstrated that even under sulfur-

limiting conditions, PFOS did not degrade. Similarly, Saez et al. (2008) observed no PFOS degradation under aerobic or anaerobic conditions in municipal sewage sludge. In contrast, Schröder (2003) reported that PFOS was anaerobically degraded; however, the reported results are uncertain as the results could likely be attributed to sorption and there was a lack of increased fluoride concentrations reported (Frömel and Knepper 2010).

The persistence of PFOS has been attributed to the strong C-F bond. Additionally, there have been limited indications that naturally occurring, defluorinating enzymes exist that can break a C-F bond, which is likely due to the rarity of fluorinated molecules in nature (Frömel and Knepper 2010). To date, no laboratory data exist that demonstrates the PFOS undergoes significant biodegradation in environmental conditions (Beach et al. 2006; Giesy et al. 2010; OECD 2002).

M.4 Thermal Stability

Based on carbon-sulfur (C-S) bond energy, which is weaker than the carbon-carbon (C-C) or the C-F bond energies, PFOS is considered to have relatively low thermal stability. Thus, PFOS would more easily breakdown under incineration conditions and would be nearly completely destroyed when incinerated (Beach et al. 2006; Giesy et al. 2010).

M.5 Adsorption/Desorption

In general, PFOS may adsorb to sediments (with a K_d greater than 1 mL/g; (Giesy et al. 2010)). However, this sorption to sediment is limited since PFOS has a K_{oc} of 2.57, indicating that PFOS is relatively mobile in water and the physicochemical characteristics of the sediment ultimately influence the sorption of PFOS (Ahrens et al. 2011b; Beach et al. 2006; Giesy et al. 2010; Higgins and Luthy 2006). Sediment characteristics have a strong influence on the partitioning of PFOS (You et al. 2010). Specifically, organic content was found to have a

significant influence on the partitioning of PFOS. Density of the sediment was also found to be an important factor influencing partitioning (Ahrens et al. 2011b). PFOS has a high affinity to bind to organic carbon with log K_{oc} values ranging between 2.57 and 3.8 cm^3/g ((Higgins and Luthy 2006) and (Ahrens et al. 2010); respectively). A sorption mechanism could be a salting-out and calcium-bridging effect, as PFOS sorption to sediment increased with increased salinity, pH, and calcium (You et al. 2010). Thus, the sorption of PFOS is a complicated process that is partially dependent on other factors such as metal anion concentrations, pH, temperature, and salinity; however, the strong relationship between PFOS concentrations and organic carbon in soil, sediment, and sludge indicates that these other factors have a minor influence on PFOS sorption (Ahrens et al. 2011b; Chen et al. 2012; Higgins and Luthy 2006; You et al. 2010).

Appendix N Occurrence of PFOS in Abiotic Media

N.1 Summary of Measured Perfluorooctane Sulfonate Concentrations in Surface Waters Across the United States.

Modified from: Jarvis et al. (2021).

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	Lake Erie	3.77	3	2.8 - 5.5	Sinclair et al. (2006)
		31.3	32.5	21.5 – 38.5	Boulanger et al. (2004)
		2.84	2.63	2.49 - 3.41	De Silva et al. (2011)
		4.5	4.2	4.0 - 5.3	Furdui et al. (2008)
	Lake Huron	2.25	1.96	0.239 - 5.46	De Silva et al. (2011)
		1.73	1.5	1.2 – 2.7	Furdui et al. (2007)
	Lake Michigan	2.03	2.03	0.93 – 3.13	Simcik and Dorweiler (2005)
		2.00	1.96	1.73 – 2.36	De Silva et al. (2011)
	Lake Ontario	not provided	4.9	2.9 – 30	Sinclair et al. (2006)
		55.4	59.8	16.5 – 85.5	Boulanger et al. (2004)
		5.96	5.63	2.60 – 9.48	De Silva et al. (2011)
		8.69	6.6	3.6 – 37.6	Furdui et al. (2008)
		2.20	not provided	not provided	Houde et al. (2008)
	Lake Superior	0.255	0.236	0.095 – 0.395	De Silva et al. (2011)
		0.233	0.3	0.1 – 0.3	Furdui et al. (2008)
		0.246	0.124	0.074 – 0.996	Scott et al. (2010)
Alabama	Waterbody near Decatur	58,016	41,027	9 – 150,000	OECD (2002)

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	Waterbody in Decatur	2.5< x <25	2.5< x <25	2.5< x <25	3M Company (2001)
	Pond in Decatur	111	111	111	
	Waterbody in Mobile	30.3	35.5	< 25 – 41.5	3M Company (2001)
	Pond in Mobile	32.5	32.5	32.5	
	Tennessee River (upstream of Baker's Creek)	30.85	29.80	16.0 – 52.6	Hansen et al. (2002)
	Tennessee River (downstream of Baker's Creek)	103.9	107.0	30.3 – 144	Hansen et al. (2002)
California	Upper Silver Creek	not provided	not provided	27 – 56	Plumlee et al. (2008)
	Coyote Creek	not provided	not provided	4.8 – 25	
Colorado	Animas River	<0.48	<0.48	<0.48	CDPHE (2020)
	Arkansas River	1.96	0.62	0.23 - 5.00	
	Arvada Blunn Reservoir	0.77	0.77	0.77	
	Barker Reservoir	<0.49	<0.49	<0.49	
	Bessemer Ditch	14.0	14.0	14.0	
	Big Thompson River	3.90	3.90	3.90	
	Blue River	1.20	1.20	1.20	
	Boulder Feeder Canal	<0.45	<0.45	<0.45	
	Boyd Lake	1.00	1.00	1.00	
	Cache la Poudre River	5.61	5.61	<0.45 - 11.0	
	Clear Creek	7.95	7.95	7.20 - 8.70	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	Colorado River	0.67	0.66	0.65 - 0.69	
	Coon Creek	<0.48	<0.48	<0.48	
	Eagle River	0.68	0.68	0.68	
	East Plum Creek	<0.43	<0.43	<0.43	
	Erie Lake	3.70	3.70	3.70	
	Fairmount Reservoir	<2.50	<2.50	<2.50	
	Fountain Creek	16.9	20.0	3.50 -24.0	
	Fraser River	1.00	1.00	1.00	
	Gore Creek	0.98	0.98	0.98	
	Gunnison River	0.71	0.71	0.71	
	Horsetooth Reservoir	0.51	0.51	0.51	
	Jackson Creek	<0.44	<0.44	<0.44	
	Jerry Creek	<0.485	<0.485	<0.48 – <0.49	
	Kannah Creek Flowline	<0.49	<0.49	<0.49	
	Lakewood Reservoir	<0.45	<0.45	<0.45	
	Little Fountain Creek	<0.46	<0.46	<0.46	
	Maple Grove Reservoir	10.0	10.0	10.0	
	Marstron Reservoir	0.48	0.48	0.48	
	McBroom Ditch	4.90	4.90	4.90	
	Mclellen Reservoir	1.30	1.30	1.30	
	Mesa Creek	<0.49	<0.49	<0.49	
	Michigan River	<0.46	<0.46	<0.46	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	Molina Power Plant Tail	<0.50	<0.50	<0.50	
	North Fork Gunnison River	<0.47	<0.47	<0.47	
	Purdy Mesa Flowline	<0.49	<0.49	<0.49	
	Purgatoire River	0.47	0.47	0.47	
	Ralston Reservoir	<0.46	<0.46	<0.46	
	Rio Grande	<0.47	<0.47	<0.47	
	Roaring Fork River	<0.50	<0.50	<0.50	
	San Juan River	<0.44	<0.44	<0.44	
	Sand Creek	30.3	30.3	6.50 - 54.0	
	Severy Creek	<0.47	<0.47	<0.47	
	Somerville Flowline	<0.48	<0.48	<0.48	
	South Boulder Creek	0.50	0.50	0.50	
	South Platte River	10.5	11.5	3.80 - 16.0	
	St. Vrain River	3.90	3.90	3.90	
	Strontia Springs	<0.51	<0.51	<0.51	
	Taylor River	<0.45	<0.45	<0.45	
	Uncompahgre River (delta)	0.54	0.54	0.54	
	Welton Reservoir	2.60	2.60	2.60	
	White River	<0.46	<0.46	<0.46	
	Yampa River	<0.47	<0.47	<0.47	
Delaware, New Jersey, Pennsylvania	Delaware River	3.98	3.5	0.97 - 6.92	Pan et al. (2018)

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
Florida	Waterbody in Pensacola	16.29	2.5< x <25	<25 – 29	3M Company (2001)
	Pond in Pensacola	2.5< x <25	2.5< x <25	2.5< x <25	
	Waterbody in Port St. Lucie	50.83	2.5< x <25	<2.5 – 137.5	
	Small pond in Port St. Lucie[3]	9,784	1,945	1,830 – 48,200	
	Sarasota Bay	0.90	not provided	not provided	Houde et al. (2006a)
Georgia	Waterbody in Columbus	59.9	55	44.6 – 80	3M Company (2001)
	Pond in Columbus	<2.5	<2.5	<2.5	
	Conasauga River	162.1	192	<1.5 - 321	Konwick et al. (2008)
	Altamaha River	2.63	2.6	2.6 – 2.7	
	Streams and ponds in Dalton	70.36	70.73	10.5-119.5	
	Oostanaula River	150.3	151	148 - 152	Lasier et al. (2011)
Louisiana	Waterbodies (locations of concern) near Barksdale A.F.B.	776.7	195.0	<10 – 7,070	Cochran (2015); Lanza et al. (2017)
	Reference waterbodies near Barksdale A.F.B.	<10	<10	<10	
Michigan	Raisin River	3.5	3.5	3.5	Kannan et al. (2005)
	St Clair River	2.6	2	1.9 – 3.9	
	Siskiwit Lake	0.283	0.283	0.277 – 0.289	Scott et al. (2010)
Minnesota	Upper Mississippi River	528.9	<2	<2 – 18,200	Newsted et al. (2017)

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	Lake of the Isles	2.47	2.47	2.47	Simcik and Dorweiler (2005)
	Lake Calhoun	50.4	50.4	50.4	
	Lake Harriet	22.1	22.1	22.1	
	Minnesota River	9.21	9.21	9.21	
	Lake Tettegouche	0.23	0.23	0.23	
	Lake Nipisiquit	<0.27	<0.27	<0.27	
	Lake Loiten	<0.27	<0.27	<0.27	
	Little Trout Lake	1.2	1.2	1.2	
New Jersey	Echo Lake Reservoir	<2	<2	<2	NJDEP (2019)
	Passaic River	13.1	13.1	13.0 – 13.2	
	Raritan River	6.9	6.9	6.9	
	Metedeconk River	1.65	1.65	<2 – 2.8	
	Pine Lake	102	102	102	
	Horicon Lake	10	10	10	
	Little Pine Lake	100	100	100	
	Mirror Lake	72.9	72.9	72.9	
	Woodbury Creek	6.4	6.4	6.4	
	Fenwick Creek	3.1	3.1	3.1	
	Cohansey River	<2	<2	<2	
	Harbortown Road	1.93	1.93	1.93	Zhang et al. (2016)
	Passaic River	4.59	4.07	0.244 – 9.99	
New Mexico	Alamogordo Domestic Water Sys.	<	<1	<1	NMED (2020)
	Animas River	0.799	0.625	< 0.89 - 1.5	
	Canadian River	0.848	0.9	< 0.89 - 1.2	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	Cloud Country Estates WUA	<0.93	<0.93	<0.93	
	Gila River	<0.93	<0.93	<0.93	
	Holloman AFB Golf Course Pond 1	1,220	1,220	1,220	
	Holloman AFB Golf Course Pond 2	878	878	878	
	Holloman AFB Lagoon G	310	310	310	
	Holloman AFB Outfall	951	951	951	
	Holloman AFB Sewage Lagoon	2,200	2,200	2,200	
	Karr Canyon Estates	<0.93	< 0.93	< 0.93	
	La Luz MDWCA	<1.3	<1.3	<1.3	
	Lake Holloman	4,033	4,500	1,700 - 5,900	
	Mountain Orchard MDWCA	< 0.93	< 0.93	< 0.93	
	Pecos River	1.223	1.50	<0.94 - 1.70	
	Rio Chama	<0.98	<0.98	<0.96 - <1	
	Rio Grande	1.052	0.474	<0.465 - 2.90	
	Rio Puerco	4.35	4.35	3.10 - 5.60	
	San Juan River	<1.15	<1.15	<1.06 – <1.24	
	Tularosa Water System	0.723	0.723	<0.89 - 1.0	
New York	Washington Park Lake	1.67	1.77	<0.25 – 2.88	Kim and Kannan (2007)
	Rensselaer Lake	7.11	6.58	5.85 – 9.3	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	Iroquois Lake	not provided	not provided	not provided	
	Unnamed lake 1 outside Albany, NY	not provided	not provided	not provided	
	Unnamed lake 2 outside Albany, NY	not provided	not provided	not provided	
	Niagara River	5.17	5.5	3.3 – 6.7	Sinclair et al. (2006)
	Finger Lakes	not provided	1.6	1.3 – 2.6	
	Lake Onondaga	681	756	198 – 1,090	
	Lake Oneida	3.5	3.5	3.5	
	Erie Canal	8.37	6.4	5.7 - 13	
	Hudson River	not provided	1.7	1.5 – 3.4	
	Lake Champlain	not provided	2.7	0.8 – 7.7	
	Lower NY Harbor	0.755	0.755	0.755	Zhang et al. (2016)
	Staten Island	1.66	1.66	1.66	
	Hudson River	1.81	1.81	0.79 – 2.84	
North Carolina	Cape Fear River	31.2	28.9	<1 - 132	Nakayama et al. (2007)
Rhode Island	Narragansett Bay	2.2	2.2	2.2	Benskin et al. (2012)
	Allen Cove Inflow	1.20	1.20	1.20	Zhang et al. (2016)
	Bristol Harbor	0.508	0.46	0.437 – 0.626	
	Brook at Mill Cove	9.80	9.80	9.80	
	Buckeye Brook	4.13	4.13	4.13	
	Chickasheen Brook	< 0.05	< 0.05	< 0.05	
	EG Town Dock	0.735	0.735	0.735	
	Fall River	0.238	0.238	0.238	
	Green Falls River	0.291	0.291	0.29 – 0.292	
	Hunt River	1.48	1.48	1.48	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	Mill Brook	3.94	3.94	3.94	
	Narrow River	0.298	0.264	0.176 – 0.488	
	Pawcatuck River	0.561	0.561	0.509 – 0.612	
	Pawtuxet River	2.19	2.19	2.19	
	Queens River	0.334	0.334	0.334	
	Sand Hill Brook	1.82	1.82	1.82	
	Secret Lake – Oak Hill Brook	<0.05	<0.05	<0.05	
	Slack's Tributary	0.777	0.777	0.777	
	South Ferry Road Pier	0.161	0.161	0.161	
	Southern Creek	3.74	3.74	3.74	
	Woonasquatucket River	14.6	14.6	5.87 – 23.2	
South Carolina	Charleston Harbor	12.0	not provided	not provided	Houde et al. (2006a)
Tennessee	Waterbody near Cleveland	2.5 < x <25	2.5 < x <25	<2.5 - <25	3M Company (2001)
	Conasauga River	<0.009[4]	<0.009[4]	<0.009[4]	Lasier et al. (2011)
Texas	Rio Grande	4.17	4.1	2.0 - 6.5	NMED (2020)
Washington	Puget Sound	2.3	1.45	0.2 – 5.9	Dinglasan-Panlilio et al. (2014)
	Clayoquot Sound	0.32	0.3	0.25 – 0.4	
	Barkley Sound	0.7	0.7	0.7	
Multiple States (10 Air Force Bases across the continental U.S.)	Surface waters impacted by aqueous film forming foam use	not provided	2,170	8,970,000 (maximum)	Anderson et al. (2016)

Less than (<) values based on study specific LOD and LOQ values that the study authors reported, LOD = limit of detection and LOQ = limit of quantitation

[1] Name of Waterbody Sampled for PFOS. Name or description of waterbody above is consistent with that provided in cited reference.
[2]Calculation of arithmetic mean and median includes lower of ½ LOD or ½ LOQ, depending on information provided. See full occurrence table in Appendix N for waterbody-specific details.
[3] Study authors conducted additional sampling of this waterbody but were unable to detect the initial high PFOS concentrations in any of the additional samples.
[4] Reported as ng/g by the study authors.

N.2 PFOS occurrence and concentrations in the Great Lakes region

The Great Lakes are among the most widely studied waterbodies in the U.S. for PFOS occurrence. However, occurrence data are still relatively limited for this system. Comparisons across the Great Lake system indicate PFOS concentrations are higher in Lakes Erie and Ontario, ranging between 2.8 and 38.5 ng/L and 2.6 and 85.5 ng/L, respectively (Figure 2-3) (Boulanger et al. 2004; De Silva et al. 2011; Furdui et al. 2008; Sinclair et al. 2006), compared to the more northern Great Lakes. These northern Great Lakes (i.e., Lakes Huron, Michigan, and Superior) have a maximum observed concentration of 5.46 ng/L, which was observed in Lake Huron (Remucal 2019). However, current measured PFOS concentrations were not from sampling sites around urbanized areas (such as Chicago and Detroit) and may not be representative of the potential sources of PFOS related to these areas. The measured concentrations of PFOS in the surface waters of Lakes Huron and Michigan range between 0.24 and 5.46 ng/L (De Silva et al. 2011; Furdui et al. 2008) and 0.93 and 3.13 ng/L (De Silva et al. 2011; Simcik and Dorweiler 2005), respectively. In contrast, measured PFOS concentrations observed in Lake Superior were considerably lower and range between 0.074 and 0.996 ng/L (De Silva et al. 2011; Furdui et al. 2008; Scott et al. 2010). The higher PFOS concentrations in Lakes Erie and Ontario are likely due to higher levels of industrial activities and urbanization around these lakes (Boulanger et al. 2004; Remucal 2019) and could also be associated with the sampling locations. A mass balance constructed for Lake Ontario by Boulanger et al. (2004) indicated wastewater effluent was the

major source of PFOS to the lake. In contrast, inputs from Canadian tributaries and atmospheric deposition of PFOS, and other PFAS that may be transformed into PFOS, were the major contributing sources of PFOS to Lake Superior. Inputs from Canadian tributaries and atmospheric deposition were estimated to contribute 57 and 32% of PFOS inputs into Lake Superior, respectively (Scott et al. 2010).

N.3 PFOS occurrence and concentrations in the southeastern U.S.

Measured PFOS concentrations in southeastern U.S. surface waters were similar to those measured in Lakes Erie and Ontario, with some of the highest observed concentrations occurring in waterbodies near areas with PFOS manufacturing. In 2001, the 3M Company conducted a multi-city study measuring PFOS concentrations across waterbodies with known manufacturing and/or industrial uses of PFOS (3M Company 2001). In the 3M Company's 2001 report, PFOS concentrations from sites with known PFOS discharges were compared to PFOS concentrations measured in waterbodies with no known sources of any PFAS (3M Company 2001). In this comparison study, cities with known PFOS exposure were Mobile and Decatur, Alabama, Columbus, Georgia, and Pensacola, Florida. Measured PFOS concentrations ranged from not detected (reported detection limit of 2.5 ng/L; 3M Company 2001) to 80 ng/L in the cities with known PFOS discharges. These PFOS concentrations were compared to those measured in control cities. These control cities were Cleveland, Tennessee and Port St. Lucie, Florida and PFOS concentrations ranged from not detected to 137.5 ng/L (3M Company 2001). The PFOS concentrations measured in Cleveland, Tennessee were below the limit of quantification (25 ng/L) and were lower than the PFOS concentrations observed in the cities with known PFOS exposure, as was expected in the report for the control cities. However, PFOS concentrations around Port St. Lucie, Florida, the other control city, were unexplainably similar to, and at times

higher than, the waterbodies with known PFOS discharges. The sources of PFOS near Port St. Lucie, Florida remain unknown; however, observed PFOS concentration suggest the presence of a potential manufacturing/industrial source or the use of AFFF in this area (3M Company 2001).

Water samples were collected from ponds near all of the sampling sites except those in Cleveland, Tennessee. PFOS concentrations in these additional pond sites were similar to those measured in Mobile, Alabama (ranging between 32 and 33 ng/L), lower than those observed in Columbus, Georgia (as PFOS was not detected with a detection limit of 2.5 ng/L), and higher than those measured in Decatur, Alabama (ranging between 108 and 111 ng/L) and in Port St. Lucie, Florida (ranging between 1,830 and 48,200 ng/L). Samples collected from the pond site near Port St. Lucie, Florida had some of the highest measured PFOS concentrations in publicly available literature with the maximum concentration of 48,200 ng/L. In the report, the 3M Company conducted additional sampling at the pond site in Port St. Lucie, Florida and determined that the measured PFOS concentrations at this site were more variable than the initial measurements indicated and were lower than the previous measurements, ranging between below detection (i.e., <2.5 ng/L) and 2,340 ng/L. Aside from the samples collected in Port St. Lucie, Florida, this report demonstrated that measured PFOS concentrations in surface waters tend to be higher in areas with PFOS manufacturing and/or industrial use (3M Company 2001).

In separate studies, PFOS and PFOA concentrations were measured in surface waters by Hansen et al. (2002) near Decatur, Alabama, and Konwick et al. (2008) in Georgia. Hansen et al. (2001) studied a stretch of the Tennessee River near Decatur, Alabama, and Konwick et al. (2008) focused on the Conasauga River in Georgia, both areas with known PFOS discharge and use. In Hansen et al. (2002), discharge from a fluorochemical manufacturing facility entered the Tennessee River towards the middle of the study area. In contrast, Konwick et al. (2008)

compared the PFOS concentrations measured in the Conasauga River with those from sites with no known exposure along the Altamaha River. In both studies, mean PFOS concentrations were higher in the study areas with PFOS sources. Specifically, Hansen et al. (2002) observed mean PFOS concentrations upstream of the fluorochemical manufacturing facility were 30.85 ng/L (ranging between 16.0 and 52.6 ng/L) and were 103.9 ng/L (ranging between 30.3 and 144 ng/L) downstream of the fluorochemical manufacturing facility. Similarly, Konwick et al. (2008) observed higher measured PFOS concentrations in the Conasauga River, which ranged from below the limit of detection (i.e., 1.5 ng/L) to 321 ng/L, compared to those in the Altamaha River, ranging between 2.6 and 2.7 ng/L. Consistent with the report from the 3M Company summarized above, effluents from manufacturing facilities, WWTP, and carpet mill effluents were determined to be the source of increased PFOS concentrations in both the Tennessee and Conasauga Rivers (Hansen et al. 2002; Konwick et al. 2008; respectively). These PFOS concentrations are relatively consistent with those measured in Alabama and Georgia as reported by the 3M Company (3M Company 2001).

Nakayama et al. (2007) and Cochran (2015) measured PFAS, including PFOS, in the Cape Fear Drainage Basin in North Carolina and waterbodies on Barksdale Air Force Base in Bossier City, Louisiana, respectively. PFOA and PFOS were found to be the dominant PFAS detected in both studies. Nakayama et al. (2007) detected PFOS in 97.5% of all samples above the limit of quantification of 1 ng/L. PFOS concentrations in the Cape Fear Drainage Basin ranged between <1 (the lower limit of quantification) and 132 ng/L with a mean concentration of 31.2 ng/L. As in other studies summarized above, lower PFAS concentrations, including PFOS, were found in the upland tributaries and concentrations were highest in the middle reaches of the Cape Fear Drainage Basin, nearer expected sources. Wastewater treatment plant effluents were

identified as the source of PFAS to the study area. AFFF usage at the Department of Defense base in Fayetteville, North Carolina and the land application of contaminated biosolids likely contributed as well (Nakayama et al. 2007). Cochran (2015) detected PFOS in 79% of all water samples collected and concentrations ranged between below the limit of quantification (i.e., 10 ng/L) and 7,070 ng/L, with an average concentration of 776.7 ng/L. PFOS concentrations collected in Barksdale Air Force Base varied based on proximity to fire training areas. Cochran (2015) attributed the evaluated PFOS concentrations to runoff and ground infiltration of AFFF formerly used on the base during firefighting and/or training.

N.4 PFOS occurrence and concentrations in the midwestern U.S.

Similar PFOS concentrations were reported in the publicly available literature for waterbodies in urban areas across the midwestern U.S., with lower PFOS concentrations reported in remote areas in the same states (Newsted et al. 2017; Simcik and Dorweiler 2005). In Minnesota, Simcik and Dorweiler (2005) observed PFOS concentrations ranged between 2.4 and 50.4 ng/L in urban areas near Minneapolis and between less than the limit of quantification (i.e., 0.27 ng/L) and 1.2 ng/L in remote areas in northern Minnesota. Additionally, Newsted et al. (2007) reported an average PFOS concentration of 528.9 ng/L (ranging between below limit of quantification and 18,200 ng/L; limit of quantification not provided) in surface waters collected from the Upper Mississippi River near the Minneapolis/St. Paul, Minnesota metropolitan area. The source of PFOS at these urban sites was attributed to manufacturing (3M plant), runoff, and wastewater discharge (Newsted et al. 2017; Simcik and Dorweiler 2005).

N.5 PFOS occurrence and concentrations in the northeastern U.S.

Several studies measured PFOS concentrations in surface waters in the northeastern U.S. that are comparable to those reported in Minnesota (NJDEP 2019; Sinclair et al. 2006). Sinclair

et al. (2006) measured PFOS in various waterbodies across New York state and observed a median concentration of 756 ng/L in surface waters collected from the Superfund site at Lake Onondaga (ranging between 198 and 1,090 ng/L; Table N.1) and attributed these elevated concentrations to several industries located along Lake Onondaga. All other observed concentrations of PFOS in New York, including sites along the Niagara River, the Finger Lakes, Lakes Oneida and Champlain, the Erie Canal, and the Hudson River, had lower median PFOS concentrations ranging between 0.8 and 13 (Table N.1)(Sinclair et al. 2006).

The New Jersey Department of Environmental Protection (NJDEP) measured PFOS in surface water samples collected from 14 different sites across New Jersey. PFOS concentrations ranged from below the detection limit of 2.0 ng/L and 102 ng/L (NJDEP 2019). Individual samples collected along Pine, Little Pine, and Mirror Lakes had measured PFOS concentrations of 102, 100, and 72.9 ng/L, respectively. All other observed concentrations of PFOS in New Jersey freshwaters were below 15 ng/L. NJDEP attributed the elevated concentrations of PFOS observed at Pine, Little Pine, and Mirror Lakes to the use of AFFF in training and/or fire-fighting on the Department of Defense (DoD) Joint Base McGuire-Lix-Lakehurst (NJDEP 2019).

N.6 PFOS occurrence and concentrations in the western U.S.

PFOS concentrations in surface waters of western U.S. states are consistent with the lower-end concentrations (less than 100 ng/L) measured in eastern states; however, the monitoring data for PFOS was limited in the western U.S. Plumlee et al. (2008) measured PFOS concentrations in Coyote Creek and a tributary of Upper Silver Creek in San Jose, California and found concentrations to be similar to those measured in eastern states. Concentrations of PFOS in Coyote Creek ranged from 4.8 to 25 ng/L and concentrations in Upper Silver Creek ranged from 27 to 56 ng/L. The source of PFOS to these aquatic systems was unknown, however,

Plumlee et al. (2008) stated that a combination of atmospheric deposition of volatile precursors and surface runoff were likely sources of PFOS to both Coyote and Upper Silver Creeks.

Lastly, Dinglasan-Panlilio et al. (2014) measured PFOS concentrations in surface waters along the Puget Sound in Washington, as well as Clayoquot and Barkley Sounds in British Columbia, Canada. PFOS concentrations measured by Dinglasan-Panlilio et al. (2014) were lower than those observed from sites in eastern states (such as those summarized above for Alabama, Florida, and North Carolina with known manufacturing and/or industrial use of PFOS). Concentrations ranged from 0.2 to 5.9 ng/L in Puget Sound and 0.25 to 0.7 ng/L in Clayoquot and Barkley Sound, British Columbia. These concentrations are consistent with those reported in the publicly available literature for remote areas, such as in Minnesota (Simcik and Dorweiler 2005) and in New York (Sinclair et al. 2006), as summarized above. The study authors indicated specific regional sources and atmospheric deposition were likely PFOS sources to these remote areas (Dinglasan-Panlilio et al. 2014).

N.7 Comparison of PFOS occurrence in the U.S. to global surface waters

Similar to surface waters in the U.S., generally PFOS and PFOA were the most commonly detected PFAS in surface waters around the world (Ahrens 2011). On a global scale, PFOS concentrations in surface waters generally range between picogram/liter and nanogram/liter with some concentrations in the milligram/liter range. However, PFOS occurrence data were limited for surface waters in Africa and South America. Based on the currently available data, PFOS concentrations in the U.S. were relatively similar to those reported in studies with sampling sites in other countries. Global surface water PFOS concentrations reported in the public literature ranged between not detected and 2,100,000 ng/L

(Jarvis et al. 2021). These global surface water concentrations are summarized in Jarvis et al. (2021) to provide a comparison with those observed in the U.S.

Overall, the currently available data on PFOS occurrence in ambient surface waters show the widespread distribution and variability of PFOS concentrations in surface waters around the world and that surrounding land use has a large influence on PFOS concentrations in surface waters. In general, urbanized areas with high population densities tended to have elevated PFOS concentrations in surface waters (Jarvis et al. 2021). Like in the U.S., PFOS concentrations in surface waters around the world vary widely and current information on the environmental distribution of PFOS in surface waters around the world is relatively limited.

N.8 PFOS Occurrence and Detection in Aquatic Sediments

PFOS has been detected in sediments of aquatic environments across various countries (Lau et al. 2007). Typically, in the U.S., soil and sediment measurements of PFOS occur in the µg/kg dry weight (dw) range with measured concentrations in the public literature ranging from not detected (with a detection limit of 0.08 µg/kg dw) to 31.38 µg/kg dw (3M Company 2001; Cochran 2015). Anderson et al. (2016), measured concentrations of PFAS in sediment across ten U.S. Air Force bases where there is a known history of use of AFFF use and found that PFOS concentrations were detected in 94% of samples. The median concentration of PFOS across all sample sites was 31.0 µg/kg, with a maximum concentration of 190,000 µg/kg (Anderson et al. 2016). Arias et al. (2015) measured PFOS in sediment from an evaporation pond used to collect the wastewater arising from fire-fighting exercises at an Australian military air base. Despite the discontinued use of PFOS/PFOA-based foams six years earlier, the PFOS sediment concentration was 38,000,000 µg/kg, a million times higher than the average global values for sediments (0.28 - 3.8 µg/kg PFOS) reported by the authors (Arias et al. 2015).

These observed concentrations were similar to other sediment concentrations in areas with known perfluorinated chemical discharges and manufacturing. Lasier et al. (2011) measured PFOS in sediment from the Coosa River, Georgia watershed, upstream, and downstream of a land-application site of municipal/industrial wastewater with sediment concentrations ranging from less than the method detection limit (MDL) to 1.73 µg/kg dw upstream of the land-application and 1.66 - 20.18 µg/kg dw PFOS downstream. Giesy and Kannan (2001), as presented in OECD (2002), measured PFOS in sediments collected from locations upstream and downstream of the 3M facility in Decatur, Alabama. The two closest sites downstream of the 3M facility had significantly greater concentrations (1,299 and 5,930 µg/kg ww) than the two upstream sites (~0.18 and 0.98 µg/kg ww; OECD 2002).

Other sediment concentrations across the U.S. were much lower: <4 µg/kg across sites in Puget Sound, Washington, San Francisco and Monterey Bay California, the Niagara River in New York, and Lake Michigan. These concentrations appeared to be similar to other sediment concentrations across the globe (Table N-1).

Table N-1. Global Sediment Concentration of PFOS.

Location	PFOS concentration	Reference
Tokyo Bay, Japan	0.29-0.36 µg/kg dw	Ahrens et al. (2010)
Ariake Sea, Japan	0.11 µg/kg ww	Nakata et al. (2006)
Toronto, Canada	<0.1-2.2 µg/kg ww	Vedagiri et al. (2018)
Lake Ontario, Canada	10 µg/kg dw	ECCC (2018)
Lake Ontario, Niagara Basin	27-47 µg/kg	Meyers et al. (2012)
Lake Ontario, Mississauga Basin	4.4-19 µg/kg	Meyers et al. (2012)
Lake Ontario, Rochester Basin	8.1-49 µg/kg	Meyers et al. (2012)
Resolute Lake, Canada	24-85 µg/kg ww	Butt et al. (2010)
Gufunes Bay, Iceland	< 50 µg/kg ww	Butt et al. (2010); Kallenborn (2004)
Faroe Islands	< 50 - 0.11 µg/kg	Butt et al. (2010); Kallenborn (2004)
Urban reservoir, Singapore	2.8-3.6 µg/kg dw	Nguyen et al. (2016)

N.9 PFOS Occurrence and Detection in Air and Rain

Air concentrations of PFOS in the atmosphere varied widely across the globe. In an urban area in Albany, NY, perfluorinated acids were measured in air samples in both the gas and particulate phase in May and July of 2006 (Kim and Kannan 2007). PFOS in the gas phase had a mean concentration of 1.70 pg/m^3 (range: 0.94-3.0) and the particulate phase had a mean concentration of 0.64 pg/m^3 (range: 0.35-1.16) (Kim and Kannan 2007). Kim and Kannan (2007) also reported mean PFOS concentrations of 0.36 ng/L and 0.62 ng/L in rain and snow, respectively.

Above Lake Ontario, concentrations of PFOS in the particulate phase measured in air samples over the lake were higher than those observed by Kim and Kannan (2007) near Albany, NY. The mean concentration of PFOS at Lake Ontario was 6.4 ± 3.3 pg/m^3 (Boulanger et al. 2005a), with a range of concentrations from not detected to 8.1 pg/m^3 (Martin et al. 2010). In an urban area in Minneapolis, Minnesota, PFOS was measured in both the particulate and gas phase. PFOS in the particulate phase ranged from 2.1 - 7.9 pg/m^3 and the gas phase ranged from 1.8 - 5.0 pg/m^3 in across the five samples (MPCA/STS 2007).

In Canada, PFOS air concentrations measured in 2009 showed widespread distribution with remote sites having similar concentrations as urban sites (ECCC 2018). Using passive samplers, PFOS concentrations were detected in Toronto, Ontario (8 pg/m^3), an agricultural site in Saskatchewan (5 pg/m^3), Whistler, British Columbia (4 pg/m^3), and Alert, N Nunavut (2 pg/m^3) (ECCC 2013).

Other reported concentrations of PFOS in air samples included Sydney, Florida (3.4 pg/m^3), Tudor Hill, Bermuda (6.1 pg/m^3), Malin Head, Ireland (3.3 pg/m^3), and Hilo, Hawaii (6.6 pg/m^3) are similar to the concentrations reported in Canada (ECCC 2018) and Japan (Sasaki et al. 2003). The annual geometric mean concentration of PFOS in air samples collected monthly

from 2001-2002 in the town of Oyamazaki and Fukuchiyama City were 5.3 and 0.6 pg/m^3, respectively (Sasaki et al. 2003).

Across Europe, PFOS air concentrations were reported to be variable. In the particulate phase PFOS concentrations ranged from < 1.8 - 46 pg/m^3. Most locations had low (~1-2 pg/m^3) to less than the reported Minimum Detection Limit (MDL) and included Hazelrigg, United Kingdom, Kjeller Norway, and Mace Head, Ireland (Barber et al. 2007). The highest concentrations were reported in Manchester, United Kingdom. Similarly, high concentrations were reported for another urban area, 150 pg/m^3 for Paris, France (ECCC 2018).

Even in the Arctic, PFOS, its precursors, and degradation products, have been detected in air samples in Resolute Bay, Nunavut, Canada, during the summer of 2004 (Stock et al. 2007). PFOS in the filter samples were 1-2 orders of magnitude greater than other compounds, with a mean concentration of 5.9 pg/m^3 (Butt et al. 2010). These concentrations are greater than PFOS concentrations measured in the particle phase of air samples measured in Zeppelinstasjonen, Svalbard, Norway (Butt et al. 2010). PFOS was measured in September and December, 2006 and August and December, 2007, with mean concentrations of 0.11 pg/m^3 (range: 0.03 - 0.50 pg/m^3) and 0.18 pg/m^3 (range: 0.02 - 0.97 pg/m^3), respectively (NILU 2007).

N.10 PFOS Occurrence and Detection in Groundwater

Similar to surface water, PFOS and PFOA are the dominant PFAS detected in groundwater. Generally, PFOS concentrations tend to occur in the ng/L range, with some elevated detections in the µg/L range (Ahrens 2011; Xiao 2017). Concentrations of PFOS were detected in groundwater samples across Minnesota in 2006 and 2007, approximately five years after the 3M Corporation phased out PFOS production in Minnesota in 2002 (MPCA/STS 2007). Data collected from shallow aquifers across Minnesota in both urban and agricultural areas were

likely affected by a variety of different contamination sources (i.e., industrial and municipal stormwater, pesticides, land application of contaminated biosolids and atmospheric deposition) and indicated that perfluorinated chemicals are present in areas beyond the disposal sites and aquifers associated with these disposal sites (MPCA/STS 2007). Groundwater samples of PFOS ranged from < 0.00222 - 0.037 µg/L across urban areas, with most of the perfluorinated compound detections in the Twin Cities metro area (MPCA/STS 2007). Concentrations in rural areas of Minnesota were all less than the analytical method reporting limit (0.025 µg/L).

Detections of PFOS in groundwater have been associated with the use of AFFF and fire-training locations (Ahrens 2011; Xiao 2017). The use of AFFF to suppress fires resulted in the release of various PFAS into the environment as AFFF contains high levels of PFAS (Ahrens 2011; Moody and Field 2000). The use of AFFF in particular has been identified as an important source of groundwater contamination with PFAS (Moody and Field 2000). This contamination is often persistent, lasting for many years after the release (Moody and Field 2000; Xiao 2017). The transformation of PFOS precursor compounds (see Section 2.3) by soil micro-organisms may be a contributing source of PFOS in groundwater (Xiao 2017).

Groundwater samples from wells in the area of a known plume were measured in 1998 and 1999. Samples were taken at the Wurtsmith Air Force Base in northeastern Michigan, a base where fire-training exercises were conducted from the 1950's until the base was decommissioned in 1993. PFOS concentrations ranged from 4.0 to 110 µg/L depending on the proximity to the training pad, demonstrating that PFOS is still present in measurable quantities for at least five or more years after the use of AFFF (Moody et al. 2003). These values are consistent with ten other U.S. Air Force bases where there is a known historic use of AFFF to extinguish hydrocarbon-based fires but were not active fire-training areas. Anderson et al. (2016) measured groundwater

samples between March and September 2014 at the ten locations with PFOS concentrations detected in 96% of samples. The median groundwater concentration of PFOS across all sites was 2.17 µg/L, with a maximum concentration of 8,970 µg/L (Anderson et al. 2016). Other reported groundwater concentrations at other U.S. military installations summarized by Cousins et al. (2016) include: Tyndall Air Force Base (147 - 2,300 µg/L; Schultz et al. 2004), Fallon Naval Air station (< LOD - 380 µg/L; Schultz et al. 2004) and Ellsworth Air Base (5 - 75 µg/L; McGuire et al. 2014). Similar concentrations are reported at other airports and bases globally, including at a fire training area in Cologne, Germany (0.02 - 8.35 µg/L; Weiß et al. 2012); air force base F18 in Sweden (< 0.001 - 42.2 µg/L; Filipovic et al. 2015) and the Jersey airport in the United Kingdom (10 - 98 µg/L; Rumsby et al. 2009).

N.11 PFOS Occurrence and Detection in Ice

Very little information was provided about PFOS concentrations in ice. Saez et al. (2008) found PFOS in a Russian Artic ice core sampled in 2007. The PFOS concentration reported was 0.0053 ng/L.

Appendix O Bioaccumulation Factors (BAFs) Used to Calculate PFOS Tissue Values

O.1 Summary Table of PFOS BAFs used to calculate tissue criteria and supplemental fish tissue values

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
common carp	*Carassius auratus*	Blood	11167	**11167**	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
mandarin	*Siniperca scherzeri*	Blood	73612	73612	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
lefteye flounder	*Paralichthys olivaceus*	Blood	5625	**5625**	medium	Ariake Bay	Taniyasu et al. (2003)
crucian carp	*Carassius carassius*	Blood	80168	**80168**	high	Beijing Airport, China	Wang et al. (2016)
crucian carp	*Carassius carassius*	Blood	22484	**22484**	high	Gaobeidian Lake, China	Shi et al. (2020)
carp	*Cyprinus carpio*	Blood	84211	84211	medium	Lake Biwa	Taniyasu et al. (2003)
bluegill	*Lepomis macrochirus*	Blood	11053	**11053**	medium	Lake Biwa	Taniyasu et al. (2003)
largemouth bass	*Micropterus salmoides*	Blood	169737	169737	medium	Lake Biwa	Taniyasu et al. (2003)
European perch	*Perca fluviatilis*	Blood	58000	**58000**	medium	Lake Halmsjön, near Stockholm, Sweden	Wang et al. (2016)
black seabream	*Acanthopagrus schlegeli*	Blood	14138	**14138**	medium	Osaka Bay	Taniyasu et al. (2003)
white croaker	*Argyrosomus argentatus*	Blood	19540	19540	medium	Osaka Bay	Taniyasu et al. (2003)
Japanese scad	*Trachurus japonicus*	Blood	14138	**14138**	medium	Osaka Bay	Taniyasu et al. (2003)
crucian carp	*Carassius carassius*	Blood	9638	**9638**	high	Tangxum Lake, China	Shi et al. (2015)
conger eel	*Conger myriaster*	Blood	3500	**3500**	medium	Tokyo Bay	Taniyasu et al. (2003)
rockfish	*Sebastes inermis*	Blood	9423	9423	medium	Tokyo Bay	Taniyasu et al. (2003)
Japanese stingfish	*Sebastiscus marmoratus*	Blood	5154	5154	medium	Tokyo Bay	Taniyasu et al. (2003)
crucian carp	*Carassius carassius*	Blood	19999	19999	high	Xiaoqing River, China	Shi et al. (2015)
common carp	*Cyprinus carpio*	Blood	7244	**7244**	high	Xiaoqing River, China	Pan et al. (2017)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
crucian carp	*Carassius carassius*	Blood	21275	**21275**	high	Yubei River, China	Shi et al. (2020)
crucian carp	*Carassius carassius*	Gonad	25645	**25645**	high	Beijing Airport, China	Wang et al. (2016)
crucian carp	*Carassius carassius*	Gonad	8012	**8012**	high	Gaobeidian Lake, China	Shi et al. (2020)
European perch	*Perca fluviatilis*	Gonad	16000	**16000**	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. (2015)
European chub	*Leuciscus cephalus*	Gonad	10000	**10000**	high	Orge River, near Paris, France	Labadie and Chevreuil (2011)
chub	*Leuciscus cephalus*	Gonad	2222	**2222**	medium	Roter Main, Upper Franconia, Germany	Becker et al. (2010)
crucian carp	*Carassius carassius*	Gonad	5888	**5888**	high	Tangxum Lake, China	Shi et al. (2015)
crucian carp	*Carassius carassius*	Gonad	11482	**11482**	high	Xiaoqing River, China	Shi et al. (2015)
crucian carp	*Carassius carassius*	Gonad	7990	**7990**	high	Yubei River, China	Shi et al. (2020)
common carp	*Carassius auratus*	Liver	4572	**4572**	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
mandarin	*Siniperca scherzeri*	Liver	24718	24718	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
Mozambique tilapia	*Oreochromis mossambicus*	Liver	436.8	436.8	medium	Matikulu, N2 Bridge	Fauconier et al. (2020)
cape stumpnose	*Rhabdosargus holubi*	Liver	111.2	**111.2**	medium	Matikulu, N2 Bridge	Fauconier et al. (2020)
lefteye flounder	*Paralichthys olivaceus*	Liver	23958	**23958**	medium	Ariake Bay	Taniyasu et al. (2003)
crucian carp	*Carassius carassius*	Liver	83753	**83753**	high	Beijing Airport, China	Wang et al. (2016)
crucian carp	*Carassius carassius*	Liver	11180	**11180**	high	Gaobeidian Lake, China	Shi et al. (2020)
tilapia	tilapia	Liver	5108	**4176**	medium	Key River, Taiwan	Lin et al. (2014)
tilapia	tilapia	Liver	4181		medium	Key River, Taiwan	Lin et al. (2014)
tilapia	tilapia	Liver	3409		medium	Key River, Taiwan	Lin et al. (2014)
carp	*Cyprinus carpio*	Liver	1053	**1053**	medium	Lake Biwa	Taniyasu et al. (2003)
bluegill	*Lepomis macrochirus*	Liver	74211	74211	medium	Lake Biwa	Taniyasu et al. (2003)
largemouth bass	*Micropterus salmoides*	Liver	61579	61579	medium	Lake Biwa	Taniyasu et al. (2003)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
European perch	*Perca fluviatilis*	Liver	39000	**39000**	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. (2015)
European chub	*Leuciscus cephalus*	Liver	19953	**19953**	high	Orge River, near Paris, France	Labadie and Chevreuil (2011)
white croaker	*Argyrosomus argentatus*	Liver	1609	1609	medium	Osaka Bay	Taniyasu et al. (2003)
common seabass	*Lateolabrax japonicus*	Liver	459.8	**459.8**	medium	Osaka Bay	Taniyasu et al. (2003)
Japanese scad	*Trachurus japonicus*	Liver	1034	1034	medium	Osaka Bay	Taniyasu et al. (2003)
crucian carp	*Carassius auratus*	Liver	19953	19953	high	Pearl River Delta, China	Pan et al. (2014)
mud_carp	*Cirrhinus molitorella*	Liver	25119	25119	medium	Pearl River Delta, China	Pan et al. (2014)
leather_catfish	*Clarias fuscus*	Liver	5012	5012	high	Pearl River Delta, China	Pan et al. (2014)
grass carp	*Ctenopharyngodon idellus*	Liver	39811	39811	high	Pearl River Delta, China	Pan et al. (2014)
common_carp	*Cyprinus carpio*	Liver	25119	25119	high	Pearl River Delta, China	Pan et al. (2014)
chub	*Hypophthalmichthys molitrix*	Liver	7943	7943	high	Pearl River Delta, China	Pan et al. (2014)
snakehead	*Ophicephalus argus*	Liver	15849	15849	high	Pearl River Delta, China	Pan et al. (2014)
bream	*Parabramis pekinensis*	Liver	3162	**3162**[c]	high	Pearl River Delta, China	Pan et al. (2014)
tilapia	*Tilapia aurea*	Liver	3162	**3162**[c]	high	Pearl River Delta, China	Pan et al. (2014)
chub	*Leuciscus cephalus*	Liver	4556	**4556**	medium	Roter Main, Upper Franconia, Germany	Becker et al. (2010)
silver perch	*Bidyanus bidyanus*	Liver	26000	**26000**	high	Shoalhaven region, Australia	Terechovs et al. (2019)
common shiner	*Notropis cornutus*	Liver	12589	**12589**	high	Spring/Etobicoke Creek, Toronto, Canada	Awad et al. (2011)
sea mullet	*Mugil cephalus*	Liver	5000	**5000**	medium	Sydney Harbour, Australia	Thompson et al. (2011)
crucian carp	*Carassius carassius*	Liver	4426	**4426**	high	Tangxum Lake, China	Shi et al. (2015)
common seabass	*Lateolabrax japonicus*	Liver	3269	3269	medium	Tokyo Bay	Taniyasu et al. (2003)
flatfish	*Pleuronectidae*	Liver	6846	6846	medium	Tokyo Bay	Taniyasu et al. (2003)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
rockfish	*Sebastes inermis*	Liver	2462	**2462**	medium	Tokyo Bay	Taniyasu et al. (2003)
Japanese stingfish	*Sebastiscus marmoratus*	Liver	4423	4423	medium	Tokyo Bay	Taniyasu et al. (2003)
crucian carp	*Carassius carassius*	Liver	9226	9226	high	Xiaoqing River, China	Shi et al. (2015)
common carp	*Cyprinus carpio*	Liver	4467	**4467**	high	Xiaoqing River, China	Pan et al. (2017)
crucian carp	*Carassius carassius*	Liver	10735	**10735**	high	Yubei River, China	Shi et al. (2020)
Mozambique tilapia	*Oreochromis mossambicus*	Muscle	17.44	17.44	medium	Matikulu, N2 Bridge	Fauconier et al. (2020)
cape stumpnose	*Rhabdosargus holubi*	Muscle	8.718	**8.718**	medium	Matikulu, N2 Bridge	Fauconier et al. (2020)
crucian carp	*Carassius carassius*	Muscle	50234	**50234**	high	Beijing Airport, China	Wang et al. (2016)
juvenile char (muscle)	*Salvelinus alpinus*	Muscle	10800	**20785**	high	Char Lake, Canadian High Arctic	Lescord et al. (2015)
adult char (muscle)	*Salvelinus alpinus*	Muscle	40000		high	Char Lake, Canadian High Arctic	Lescord et al. (2015)
crucian carp	*Carassius carassius*	Muscle	1130	**1130**	high	Gaobeidian Lake, China	Shi et al. (2020)
meagre	*Argyrosomus regius*	Muscle	2496	2496	high	Gironde estuary, SW France	Munoz et al. (2017)
common seabass	*Dicentrarchus labrax*	Muscle	3257	3257	high	Gironde estuary, SW France	Munoz et al. (2017)
spotted seabass	*Dicentrarchus punctatus*	Muscle	2535	3844	high	Gironde estuary, SW France	Munoz et al. (2017)
spotted seabass	*Dicentrarchus punctatus*	Muscle	5830		high	Gironde estuary, SW France	Munoz et al. (2017)
anchovy	*Engraulis encrasicolus*	Muscle	1761	1761	high	Gironde estuary, SW France	Munoz et al. (2017)
mullet	*Liza ramada*	Muscle	1226	1226	high	Gironde estuary, SW France	Munoz et al. (2017)
sprat	*Sprattus sprattus*	Muscle	808.7	**808.7**	medium	Gironde estuary, SW France	Munoz et al. (2017)
tilapia	tilapia	Muscle	245.0	**256.4**	medium	Key River, Taiwan	Lin et al. (2014)
tilapia	tilapia	Muscle	323.0		medium	Key River, Taiwan	Lin et al. (2014)
tilapia	tilapia	Muscle	213.0		medium	Key River, Taiwan	Lin et al. (2014)
European perch	*Perca fluviatilis*	Muscle	3400	**3400**	high	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. (2015)
brown bullhead	*Ameiurus nebulosus*	Muscle	794.3	794.3	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
common carp	*Cyprinus carpio*	Muscle	7943	7943	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
northern pike	*Esox lucius*	Muscle	1000	1000	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
channel catfish	*Ictalurus punctatus*	Muscle	3162	3162	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
pumpkinseed	*Lepomis gibbosus*	Muscle	631.0	**631.0**	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
smallmouth bass	*Micropterus dolomieu*	Muscle	6310	6310	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
largemouth bass	*Micropterus salmoides*	Muscle	5012	5012	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
yellow perch	*Perca flavescens*	Muscle	794.3	794.3	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
white crappie	*Pomoxis annularis*	Muscle	1000	1000	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
black crappie	*Pomoxis nigromaculatus*	Muscle	1585	1585	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
juvenile char (muscle)	*Salvelinus alpinus*	Muscle	1878	**1048**	high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)
adult char (muscle)	*Salvelinus alpinus*	Muscle	585.4		high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)
eel	*Anguilla anguilla*	Muscle	3236	**3236**	high	Netherlands	Kwadijk et al. (2010)
European chub	*Leuciscus cephalus*	Muscle	2512	**2512**	high	Orge River, near Paris, France	Labadie and Chevreuil (2011)
crucian carp	*Carassius auratus*	Muscle	1585	1585	high	Pearl River Delta, China	Pan et al. (2014)
mud_carp	*Cirrhinus molitorella*	Muscle	2512	2512	medium	Pearl River Delta, China	Pan et al. (2014)
leather_catfish	*Clarias fuscus*	Muscle	251.2	**251.2**[c]	high	Pearl River Delta, China	Pan et al. (2014)
grass carp	*Ctenopharyngodon idellus*	Muscle	2512	2512	high	Pearl River Delta, China	Pan et al. (2014)
common_carp	*Cyprinus carpio*	Muscle	1585	1585	high	Pearl River Delta, China	Pan et al. (2014)
chub	*Hypophthalmichthys molitrix*	Muscle	631.0	631.0	high	Pearl River Delta, China	Pan et al. (2014)
snakehead	*Ophicephalus argus*	Muscle	398.1	398.1	high	Pearl River Delta, China	Pan et al. (2014)
bream	*Parabramis pekinensis*	Muscle	398.1	398.1	high	Pearl River Delta, China	Pan et al. (2014)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
tilapia	*Tilapia aurea*	Muscle	251.2	**251.2**[c]	high	Pearl River Delta, China	Pan et al. (2014)
juvenile char (muscle)	*Salvelinus alpinus*	Muscle	1038	**2162**	high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
adult char (muscle)	*Salvelinus alpinus*	Muscle	4500		high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
goby	*Gobio gobio*	Muscle	2963	2963	medium	Roter Main, Upper Franconia, Germany	Becker et al. (2010)
chub	*Leuciscus cephalus*	Muscle	481.5	**481.5**	medium	Roter Main, Upper Franconia, Germany	Becker et al. (2010)
eel	*Anguilla anguilla*	Muscle	1148	**518.8**	medium	Schiphol Amsterdam Airport	Kwadijk et al. (2014)
eel	*Anguilla anguilla*	Muscle	234.4		medium	Schiphol Amsterdam Airport	Kwadijk et al. (2014)
silver perch	*Bidyanus bidyanus*	Muscle	6000	**6000**	high	Shoalhaven region, Australia	Terechovs et al. (2019)
sea mullet	*Mugil cephalus*	Muscle	157.1	**157.1**	medium	Sydney Harbour, Australia	Thompson et al. (2011)
crucian	*Carassius cuvieri*	Muscle	15599	15599	high	Taihu Lake, China	Fang et al. (2014)
lake saury	*Coilia mystus*	Muscle	9190	9190	high	Taihu Lake, China	Fang et al. (2014)
gobies	*Ctenogobius giurinus*	Muscle	6144	6144	high	Taihu Lake, China	Fang et al. (2014)
Mongolian culter	*Culter mongolicus*	Muscle	15088	15088	high	Taihu Lake, China	Fang et al. (2014)
common carp	*Cyprinus carpio*	Muscle	7623	7623	high	Taihu Lake, China	Fang et al. (2014)
minnow	*Hemiculter leucisculus*	Muscle	6092	6092	high	Taihu Lake, China	Fang et al. (2014)
silver carp	*Hypophthalmichthys molitrix*	Muscle	1761	**1761**	high	Taihu Lake, China	Fang et al. (2014)
mudfish (Oriental weatherfish)	*Misgurnus anguillicaudatus*	Muscle	10810	10810	high	Taihu Lake, China	Fang et al. (2014)
white bait	*Reganisalanx brachyrostralis*	Muscle	2835	2835	high	Taihu Lake, China	Fang et al. (2014)
Chinese bitterling	*Rhodeus sinensis Gunther*	Muscle	6444	6444	high	Taihu Lake, China	Fang et al. (2014)
Crucian carp	*Carassius carassius*	Muscle	741.3	**741.3**	high	Tangxum Lake, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	Muscle	1567	1567	high	Xiaoqing River, China	Shi et al. (2015)
common carp	*Cyprinus carpio*	Muscle	537.0	**537.0**	high	Xiaoqing River, China	Pan et al. (2014)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
crucian carp	*Carassius carassius*	Muscle	1167	**1167**	high	Yubei River, China	Shi et al. (2020)
grass goby	*Zosterisessor ophiocephalus*	WB	863.7	**863.7**	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
ommon carp	*Cyprinus carpio*	WB	11749	**11749**	high	Baiyangdian Lake, China	Zhou et al. (2012)
herring	*Clupea harengus membras*	WB	20893	**20893**	medium	Baltic Sea	Gebbink et al. (2016)
spat	*Sprattus sprattus*	WB	22387	22387	medium	Baltic Sea	Gebbink et al. (2016)
grass carp	*Ctenopharyngodon idellus*	WB	7960	**7960**	medium	Bantou Reservoir - Xiamen Sea, China	Dai and Zheng (2019)
crucian carp	*Carassius carassius*	WB	43954	**43954**	high	Beijing Airport, China	Wang et al. (2016)
juvenile char (whole body)	*Salvelinus alpinus*	WB	30000	**30000**	high	Char Lake, Canadian High Arctic	Lescord et al. (2015)
grass goby	*Zosterisessor ophiocephalus*	WB	332.0	**332.0**	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
goby	*Pomatoschistus*	WB	2400	**2400**	medium	Gironde estuary, SW France	Munoz et al. (2017)
chameleon goby	*Tridentiger trigonocephalus*	WB	8086	**8086**	medium	Gulf Park - Xiamen Sea, China	Dai and Zheng (2019)
Chinese icefish	*Neosalanx tangkahkeii taihuensis*	WB	2267	**2267**	medium	Lake Chaohu, China	Pan et al. (2019)
lake trout	*Salvelinus namaycush*	WB	84598	**46098**	high	Lake Erie	De Silva et al. (2011)
lake trout	*Salvelinus namaycush*	WB	25119		medium	Lake Erie	Furdui et al. (2007)
walleye	*Sander vitreus*	WB	47659	47659	high	Lake Erie	De Silva et al. (2011)
European perch	*Perca fluviatilis*	WB	6400	**6400**	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. (2015)
lake trout	*Salvelinus namaycush*	WB	21142	**18305**	high	Lake Huron	De Silva et al. (2011)
lake trout	*Salvelinus namaycush*	WB	15849		medium	Lake Huron	Furdui et al. (2007)
lake trout	*Salvelinus namaycush*	WB	6310	**6310**	medium	Lake Michigan	Furdui et al. (2007)
alewife	*Alosa pseudoharengus*	WB	24000	24000	medium	Lake Ontario	Houde et al. (2008)
slimy sculpin	*Cottus cognatus*	WB	234000	234000	medium	Lake Ontario	Houde et al. (2008)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
rainbow smelt	*Osmerus mordax*	WB	45000	45000	medium	Lake Ontario	Houde et al. (2008)
lake trout	*Salvelinus namaycush*	WB	17293	**16715**	high	Lake Ontario	De Silva et al. (2011)
lake trout	*Salvelinus namaycush*	WB	7943		medium	Lake Ontario	Furdui et al. (2007)
lake trout	*Salvelinus namaycush*	WB	34000		medium	Lake Ontario	Houde et al. (2008)
lake trout	*Salvelinus namaycush*	WB	14453	**16982**	high	Lake Superior	De Silva et al. (2011)
lake trout	*Salvelinus namaycush*	WB	19953		medium	Lake Superior	Furdui et al. (2007)
flag-tailed glass perchlet	*Ambassis miops*	WB	774.6	774.6	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
small snakehead	*Channa asiatica*	WB	1283	1283	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
ladyfish	*Elops saurus*	WB	550.9	550.9	high	Mai Po Marshes, Hong Kong	Loi et al. (2011)
grey mullet	*Mugil cephalus*	WB	821.6	821.6	high	Mai Po Marshes, Hong Kong	Loi et al. (2011)
Mozambique tilapia	*Oreochromis mossambicus*	WB	422.5	**422.5**	high	Mai Po Marshes, Hong Kong	Loi et al. (2011)
juvenile char (whole body)	*Salvelinus alpinus*	WB	4415	**4415**	high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)
grass goby	*Zosterisessor ophiocephalus*	WB	565.0	**565.0**	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
yellowfin goby	*Acanthogobius flavimanus*	WB	576.9	576.9	medium	Omuta River mouth and estuary, Japan	Kobayashi et al (2018)
sea bass	*Lateolabrax sp.*	WB	384.6	**384.6**	medium	Omuta River mouth and estuary, Japan	Kobayashi et al (2018)
grey mullet	*Mugil cephalus*	WB	1038	1038	medium	Omuta River mouth and estuary, Japan	Kobayashi et al (2018)
juvenile char (whole body)	*Salvelinus alpinus*	WB	8615	**8615**	high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
perch	*Esox lucius*	WB	2344	**3846**	medium	Schiphol Amsterdam Airport	Kwadijk et al. (2014)
perch	*Esox lucius*	WB	6310		medium	Schiphol Amsterdam Airport	Kwadijk et al. (2014)
medaka	*Oryzias latipes*	WB	5500	**5500**	high	Seven locations across Japan	Iwabuchi et al. (2015)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
juvenile char (whole body)	*Salvelinus alpinus*	WB	8889	**8889**	high	Small Lake, Canadian High Arctic	Lescord et al. (2015)
common shiner	*Notropis cornutus*	WB	1995	**1995**	high	Spring/Etobicoke Creek, Toronto, Canada	Awad et al. (2011)
crucian carp	*Carassius carassius*	WB	1963	**1963**	high	Tangxum Lake, China	Shi et al. (2015)
bleak	*Alburnus alburnus*	WB	251.2	251.2	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
common carp	*Cyprinus carpio*	WB	1000	1000	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
mullet	*Liza sp.*	WB	4.786	**4.786**	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
roach	*Rutilus rutilus*	WB	199.5	199.5	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
rudd	*Scardinius erythrophtalmus*	WB	79.43	79.43	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
European catfish	*Silurus glanis*	WB	100.0	100.0	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
ebro chub	*Squalius laietanus*	WB	100.0	100.0	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
crucian carp	*Carassius carassius*	WB	2818	**2818**	high	Xiaoqing River, China	Shi et al. (2015)
mesozooplankton	Mesozooplankton	Invert	3450	3450	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
microzooplankton	Microzooplankton	Invert	3017	**3017**	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
chironomids	Diptera	Invert	550000	550000	high	9-Mile Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	100000	**100000**	high	9-Mile Lake, Canadian High Arctic	Lescord et al. (2015)
snail	Gastropoda	Invert	15.26	**15.26**	medium	aMatikulu N2 Bridge	Fauconier et al. (2020)
zooplankton	zooplankton	Invert	295.1	**295.1**	medium	Baltic Sea	Gebbink et al. (2016)
chironomids	Diptera	Invert	280000	280000	high	Char Lake, Canadian High Arctic	Lescord et al. (2015)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
zooplankton	zooplankton	Invert	2400	**2400**	high	Char Lake, Canadian High Arctic	Lescord et al. (2015)
ghost crab	*Ocypode stimpsoni*	Invert	3270	**3270**	medium	Fenglin - Xiamen Sea, China	Dai and Zheng (2019)
copepods	Copepoda	Invert	3.400	**68.50**	high	Gironde Estuary, SW France	Munoz et al. (2019)
copepods	Copepoda	Invert	1380		medium	Gironde Estuary, SW France	Munoz et al. (2017)
brown shrimp	*Crangon crangon*	Invert	3.900	166.5	high	Gironde Estuary, SW France	Munoz et al. (2019)
brown shrimp	*Crangon crangon*	Invert	7110		medium	Gironde Estuary, SW France	Munoz et al. (2017)
oyster	*Crassostrea gigas*	Invert	122.0	122.0	high	Gironde Estuary, SW France	Munoz et al. (2017)
gammarids	*Gammarus sp.*	Invert	2380	2380	medium	Gironde Estuary, SW France	Munoz et al. (2017)
mysids	Mysidacea	Invert	3.900	117.8	high	Gironde Estuary, SW France	Munoz et al. (2017)
mysids	Mysidacea	Invert	3560		medium	Gironde Estuary, SW France	Munoz et al. (2017)
white shrimp	*Palaemon longirostris*	Invert	3.400	97.62	high	Gironde Estuary, SW France	Munoz et al. (2019)
white shrimp	*Palaemon longirostris*	Invert	2803		medium	Gironde Estuary, SW France	Munoz et al. (2017)
Pacific oyster	*Crassostrea gigas*	Invert	6430	**6430**	medium	Gulf Park - Xiamen Sea, China	Dai and Zheng (2019)
snails	*Bithynia tentaculata*	Invert	128.4	128.4	high	Hogsmill River, Chertsey Bourne River, Blackwater River	Wilkinson et al. (2018)
amphipod	*Gammarus pulex*	Invert	118.0	**118.0**	high	Hogsmill River, Chertsey Bourne River, Blackwater River	Wilkinson et al. (2018)
Manila clam	*Ruditapes philippinarum*	Invert	3991	**3991**	high	Jiaozhou Bay, China	Cui et al. (2019)
orange-striped hermit crab	*Clibanarius infraspinatus*	Invert	3879	**3879**	medium	Jimei Bridge - Xiamen Sea, China	Dai and Zheng (2019)
Pacific oyster	*Crassostrea gigas*	Invert	4180	4180	medium	Jimei Bridge - Xiamen Sea, China	Dai and Zheng (2019)
ghost crab	*Ocypode stimpsoni*	Invert	4240	4240	medium	Jimei Bridge - Xiamen Sea, China	Dai and Zheng (2019)
diporeia	*Diporeia hoyi*	Invert	32000	32000	medium	Lake Ontario	Houde et al. (2008)
mysis	*Mysis relicta*	Invert	3000	3000	medium	Lake Ontario	Houde et al. (2008)
zooplankton	zooplankton	Invert	650.0	**650.0**	high	Lake Ontario	Houde et al. (2008)
worms	Capitellidae	Invert	913.93	913.93	high	Mai Po Marshes, Hong Kong	Loi et al. (2011)
gastropoda	Gastropoda	Invert	92.33	92.33	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
sand prawn	*Metapenaeus ensis*	Invert	286.4	286.4	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
worms	Nereidae	Invert	78.25	**78.25**	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
black tiger prawn	*Penaeus monodon*	Invert	220.7	220.7	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
worms	*Sabellidae*	Invert	364.6	364.6	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
zooplankton	zooplankton	Invert	266.0	266.0	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
chironomids	Diptera	Invert	7000	7000	high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	1195	**1195**	high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)
chironomids	Diptera	Invert	243333	243333	high	North Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	36667	**36667**	high	North Lake, Canadian High Arctic	Lescord et al. (2015)
Snail	*Cerithidea rhizophorarum*	Invert	2.692	**2.692**	medium	Omuta River mouth and estuary, Japan	Kobayashi et al. (2018)
crab	*Carcinus aestuarii*	Invert	1623	1623	medium	Orbetell lagoon, AC Site, Italy	Renzi et al. (2013)
bivalve	*Mytilus galloprovincialis*	Invert	5029	5029	medium	Orbetell lagoon, AC Site, Italy	Renzi et al. (2013)
prawn	*Palaemon serratus*	Invert	451.4	**451.4**	medium	Orbetell lagoon, AC Site, Italy	Renzi et al. (2013)
bivalve	*Ruditapes decussatus*	Invert	1150	1150	medium	Orbetell lagoon, AC Site, Italy	Renzi et al. (2013)
crab	*Carcinus aestuarii*	Invert	551.5	551.5	medium	Orbetell lagoon, FC Site, Italy	Renzi et al. (2013)
bivalve	*Mytilus galloprovincialis*	Invert	1137	1137	medium	Orbetell lagoon, FC Site, Italy	Renzi et al. (2013)
prawn	*Palaemon serratus*	Invert	187.5	**187.5**	medium	Orbetell lagoon, FC Site, Italy	Renzi et al. (2013)
bivalve	*Ruditapes decussatus*	Invert	392.2	392.2	medium	Orbetell lagoon, FC Site, Italy	Renzi et al. (2013)
bivalve	*Ruditapes decussatus*	Invert	1059	**1059**	medium	Orbetell lagoon, M Site, Italy	Renzi et al. (2013)
crab	*Carcinus aestuarii*	Invert	1140	1140	medium	Orbetell lagoon, NC Site, Italy	Renzi et al. (2013)
bivalve	*Mytilus galloprovincialis*	Invert	2728	2728	medium	Orbetell lagoon, NC Site, Italy	Renzi et al. (2013)
prawn	*Palaemon serratus*	Invert	302.6	**302.6**	medium	Orbetell lagoon, NC Site, Italy	Renzi et al. (2013)
bivalve	*Ruditapes decussatus*	Invert	577.2	577.2	medium	Orbetell lagoon, NC Site, Italy	Renzi et al. (2013)
chironomids	Diptera	Invert	17115	17115	high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	2308	**2308**	high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
waterlouse, water boatmen,	Isopoda, Hemiptera, amphipoda, nematoda	Invert	942.0	**942.0**	medium	site A Stockholm Arlanda Airport	Koch et al. (2019)

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
amphipods, roundworm							
mayflies, caddisflies, dragonflies, water boatmen, waterlouse, fresh water amphipods	Ephemeroptera, Trichoptera, Odonata, Hemiptera, Isopoda, Amphipoda	Invert	534.0	**534.0**	medium	site K the Kvarntorp area	Koch et al. (2019)
fresh water amphipods	Amphipoda	Invert	905.0	**905.0**	medium	site R Ronneby Airport	Koch et al. (2019)
chironomids	Diptera	Invert	58889	58889	high	Small Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	2444	**2444**	high	Small Lake, Canadian High Arctic	Lescord et al. (2015)
rock oyster	*Saccostrea commercialis*	Invert	85.71	**85.71**	medium	Sydney Harbour, Australia	Thompson et al. (2011)
freshwater mussel	Unionidae	Invert	572.2	572.2	high	Taihu Lake, China	Fang et al. (2014)
pearl mussel	Unionidae	Invert	1011	1011	high	Taihu Lake, China	Fang et al. (2014)
zooplankton	zooplankton	Invert	380.3	**380.3**	high	Taihu Lake, China	Fang et al. (2014)
amphipod	*Gammarus sp., Hyalella sp.*	Invert	6015	**6015**	high	Welland River, Hamilton, Ontario, Canada	De Solla et al. (2012)

a – WB (Fish whole body); Invert (Invertebrate whole body)
b – Lowest species level BAF (highlighted in bold) at each site represents the site-level BAF
c – One site level BAF represented by two species tied for lowest species level BAF

O.2 Summary of PFOS BAFs used to calculate tissue criteria and supplemental fish tissue values

Field measured BAFs used to calculate fish and invertebrate PFOS tissue criteria (fish muscle, fish whole body, and invertebrate whole body) and supplemental fish tissue values (blood, reproductive tissue, liver) are shown in Appendix O.1. Summary statistics for the BAFs from this table used to derive tissue criteria and additional tissue values (i.e., lowest species-level BAF from each site) are reported in Table 3-11 and Table P-2, respectively. Rankings for individual BAFs were determined by Burkhard (2021), who devised a ranking system based on five characteristics: 1) number of water samples; 2) number of tissue samples; 3) spatial coordination of water and tissue samples; 4) temporal coordination of water and tissue samples; and 5) general experimental design. For the first four characteristics, a score of one to three was assigned, based on number of samples or how closely the water and tissue measurements were paired. For the experimental design characteristic, a default value of zero was assigned; unless the measured tissues were composites of mixed species, in which case it was assigned a three (Burkhard 2021). These sub-scores were then summed and assigned a rank based on the final score. Studies with high quality rankings had scores of four or five, studies with medium quality rankings had scores of five or six, and studies with low quality rankings had scores of seven or higher (Burkhard 2021). Parameters for the scores assigned to the five characteristics are listed in Table 2-2, and additional details can be found in Burkhard (2021). Only BAFs from studies with high or medium quality rankings were included for the final BAF geometric mean calculations used to derive tissue criteria (Table 3-12) and supplemental tissue values (Table P-3).

O.3 PFOS BAFs References

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Appendix P Translation of Chronic Water Column Criterion into Other Fish Tissue Types (liver, blood, reproductive tissues)

The PFOS aquatic life criteria (summarized in Section 3.3) include chronic tissue criteria for fish whole body, fish muscle, and invertebrate whole-body. Additional values for fish liver, fish blood, and fish reproductive tissues were also calculated by transforming the chronic water column criterion (i.e., 0.00025 mg/L) into representative tissue concentrations using tissue-specific bioaccumulation factors (BAFs). Fish BAFs for liver, blood, and reproductive tissues were identified following the same approaches used to identify fish whole body, muscle, and inverterbrate whole body BAFs, which are described in detail in Section 2.11.3.1. Briefly, BAFs were determined from field measurements and calculated using the equation:

$$BAF = \frac{C_{biota}}{C_{water}} \qquad (Eq.\ P\text{-}1)$$

Where:
C_{biota} = PFOS concentration in organismal tissue(s)
C_{water} = PFOS concentration in water

For further details on BAFs compilation and ranking, see Section 2.11.3.1 and Burkhard (2021). BAFs based on reproductive tissues identified by Burkhard (2021) were further screened to evaluate characteristics that influence reproductive tissue BAFs. These characteristics included timing of sample collection and organism sex, age, length and weight. However, since the data were limited, the influence of these characteristics could not be fully evaluated to determine their potential influence on PFOS BAFs for reproductive tissues. Therefore, characteristics of timing of sample collection and organism age, length or weight were currently not considered to be influential given available data. Reproductive tissue BAFs were additionally screened to ensure only BAFs based on adult females were considered, because female

reproductive tissues are most relevant to potential maternal transfer to offspring. This subset of reproductive-based BAFs and corresponding species and sampling locations are described in Table P-1.

Table P-1. Characteristics of adult fish sampled for the calculation of PFOS reproductive tissue BAFs.

All sampled fish were adults, and all reproductive tissues identified as gonad. Weights, lengths, and BAFs are averages.

Author	Species	Collection Date	n	Sex	Age (yr.)	Weight (g-ww)	Length (cm)	BAF (L/kg)
Ahrens et al. (2015)	European perch (*Perca fluviatilis*)	10/12/2012	3	F	7, 8, 9	N.R.	N.R.	16,000
Becker et al. (2010)	European chub (*Leuciscus cephalus*)	8/28/2007	6	N.R.	4	178.5	25.5	2,222
Labadie and Chevreuil (2011)	European chub (*Leuciscus cephalus*)	April 2010	5	3 M 2 F	N.R.	228.0 (M) 258.2 (F)	28.5 (M) 27.8 (F)	10,000
Shi et al. (2015, 2018)	Crucian carp (*Carassius carassius*)	July 2014[1]	30	24 F 6 M	N.R.	79.4 (F) 60.5 (M)	15.0 (F) 13.7 (M)	11,482
Shi et al. (2015, 2018)	Crucian carp (*Carassius carassius*)	July 2014[2]	13	9 F 4 M	N.R.	352.3 (F) 320.7 (M)	24.6 (F) 24.8 (M)	5,888
Shi et al. (2020)	Crucian carp (*Carassius carassius*)	N.R.	30[3]	N.R.	N.R.	N.R.	N.R.	7,990
Shi et al. (2020)	Crucian carp (*Carassius carassius*)	N.R.	20[3]	N.R.	N.R.	N.R.	N.R.	8,012
Wang et al. (2016)	Crucian carp (*Carassius carassius*)	April 2014	8	N.R.	N.R.	(16.8 - 65.1)[5]	(10.0 - 14.7)[5]	25,645

N.R.= Not Reported
[1]Xiaoqing River, China
[2]Tangxun Lake, China
[3]Yubei River, China
[4]Gaobeidian Lake, China
[5]Range

The distributions of fish liver, fish blood, and fish reproductive BAFs identified in the literature used to calculate tissue-specific BAFs were determined in the same manner as invertebrate, fish muscle, and fish whole body BAFs (Section 3.2.3.1). Briefly, distributions of BAFs used to derive additional tissue values were based on the lowest species-level BAF

reported at a site. When more than one BAF was available for the same species at the same site, the species-level BAF was calculated as the geometric mean of all BAFs for that species at that site. Summary statistics for the PFOA BAFs used in the derivation of the additional tissue-based values are presented below (Table P-2) and individual BAFs are provided in Appendix O.

Table P-2. Summary Statistics for PFOS BAFs in Additional Fish Tissues[1].

Category	n	Geometric Mean BAF (L/kg-wet weight)	Median BAF (L/kg-wet weight)	20th Centile BAF (L/kg-wet weight)	Minimum (L/kg-wet weight)	Maximum (L/kg-wet weight)
Liver	19	5,688	4,572	2,462	111	83,753
Blood	11	14,355	11,167	6,273	3,500	80,168
Reproductive Tissue	8	8,903	9,006	5,155	2,222	25,645

1- Based on the lowest species-level BAF measured at a site (i.e., when two or more BAFs were available for the same species at the same site, the species-level geometric mean BAF was calculated, and the lowest species-level BAF was used).

The chronic freshwater column criterion (see Section 3.2.1.3) was then translated into tissue values using the 20th centile BAFs from the distributions of BAFs summarized in Table P-2 using the following equation:

$$Tissue\ Value = Chronic\ Water\ Column\ Criterion \times 20th\ \ Centile\ BAF \qquad (Eq.\ Q\text{-}2)$$

The resulting tissue values that correspond to the 20th centile tissue-specific BAF used in equation Q-2 are reported in Table P-3. The values reported in Table P-3 represent tissue-based concentrations that offer a level of protection that is equal to the magnitude components of the chronic water column criterion as well as the fish whole body, fish muscle, and invertebrate whole-body tissue-based criteria; however, the tissue-based values reported in Table P-3 are only presented for comparative purposes and are not recommended criteria.

Table P-3. PFOS Concentrations for Additional Fish Tissue.[1, 2]

Category	PFOS Concentration (mg/kg ww)
Liver	0.616
Blood	1.57
Reproductive Tissue	1.29

[1] These PFOS concentrations are provided as supplemental information and are not intended to replace the PFOS fish tissue criteria provided in Table .

[2] Tissue criteria derived from the chronic water column concentration (CCC) with the use of bioaccumulation factors and are expressed as wet weight (ww) concentrations.

Appendix Q Example Data Evaluation Records (DERs)

The PFOS toxicity literature evaluated and used to derive the PFOS aquatic life criteria was identified using the ECOTOXicology database (ECOTOX; https://cfpub.epa.gov/ecotox/) as meeting data quality standards. ECOTOX is a source of high-quality toxicity data for aquatic life, terrestrial plants, and wildlife. The database was created and is maintained by the EPA, Office of Research and Development, Center for Computational Toxicology and Exposure. The ECOTOX search generally begins with a comprehensive chemical-specific literature search of the open literature conducted according to ECOTOX Standard Operating Procedures (SOPs). The search terms are often comprised of chemical terms, synonyms, degradates and verified Chemical Abstracts Service (CAS) numbers. After developing the literature search strategy, ECOTOX curators conduct a series of searches, identify potentially applicable studies based on title and abstract, acquire potentially applicable studies, and then apply the applicability criteria for inclusion in ECOTOX. Applicability criteria for inclusion into ECOTOX generally include:

1. The toxic effects are related to single chemical exposure (unless the study is being considered as part of a mixture effects assessment);
2. There is a biological effect on live, whole organisms or *in vitro* preparation including gene chips or omics data on adverse outcome pathways potentially of interest;
3. Chemical test concentrations are reported;
4. There is an explicit duration of exposure;
5. Toxicology information that is relevant to OW is reported for the chemical of concern;
6. The paper is published in the English language;
7. The paper is available as a full article (not an abstract);
8. The paper is publicly available;
9. The paper is the primary source of the data;
10. A calculated endpoint is reported or can be calculated using reported or available information;
11. Treatment(s) are compared to an acceptable control;
12. The location of the study (*e.g.*, laboratory vs. field) is reported; and
13. The tested species is reported (with recognized nomenclature).

Following inclusion in the ECOTOX database, toxicity studies are subsequently evaluated by the Office of Water. All studies were evaluated for data quality generally as described by U.S. EPA (1985) in the 1985 Guidelines and in the EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S. EPA 2016b), and the EPA OW's internal data quality SOP, which is consistent with OCSPP's data quality review approach (U.S. EPA 2018). These toxicity data were further screened to ensure that the observed effects could be primarily attributed to PFOS exposure. Office of Water completed a DER for each species by chemical combination from the PFOS studies identified by ECOTOX. Example DERs are presented here to convey the meticulous level of evaluation, review, and documentation each PFOS study identified by ECOTOX was subject to. Appendix Q.1 shows an example fish DER and Appendix Q.2 shows an example aquatic invertebrate DER.

Q.1 Example Fish DER

Part A: Overview

I. Test Information

Chemical name:

CAS name: CAS Number:
Purity: Storage conditions:
Solubility in Water (units):

____ **Controlled Experiment** (*manipulated*) ____ **Field Study/Observation** (*not manipulated*) (*Place X by One*)

Primary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)

Secondary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)
(*At least one reviewer should be from EPA for sensitive taxa*)

Citation: *Indicate: author(s), year, study title, journal, volume, and pages*.
(e.g., Slonim, A.R. 1973. Acute toxicity of beryllium sulfate to the common guppy. J. Wat. Pollut. Contr. Fed. 45(10): 2110-2122)

Companion Papers: *Identify any companion papers associated with this paper using the citation format above.*

Were other DERs completed for Companion Papers? ____ **Yes** ____ **No** (*If yes, list file names of DERs below*)

Study Classification for Aquatic Life Criteria Development: *Place X by One Based on Highest Use*

_____ Acceptable for Quantitative Use
_____ Acceptable for Qualitative Use
_____ Not Acceptable for Use/Unused

General Notes: *Provide any necessary details regarding the study's use classification for all pertinent endpoints, including non-apical endpoints within the study (e.g., note all study classifications for each endpoint if the use varies)*

Major Deficiencies (note any stated exclusions): *Check all that apply. Checking any of these items make the study "**Not Acceptable for Use**"*

_____ Mixture (for controlled experiments only)
_____ No Controls (for controlled experiments only)
_____ Excessive Control Mortality (> 10% for acute and > 20% for chronic)
_____ Dilution water not adequately characterized
_____ Bioaccumulation: steady state not reached
_____ Dermal or Injection Exposure Pathway
_____ Review paper or previously published without modification

_____ Other: *(if any, list here*)

POTENTIAL CHEMICAL MIXTURES: *Describe any potential chemicals mixtures as characterized by study authors (including any confirmation of chemical mixtures).*

DESCRIPTION OF DILUTION WATER: *Describe concerns with characterization of and/or major deficiencies with dilution water.*

General Notes:

Minor Deficiencies: *List and describe any minor deficiencies or other concerns with test. These items may make the study "**Acceptable for Qualitative Use**"* **(exceptions may apply as noted)**

***For Field Studies/Observations**: A field study/observation may be considered "**Acceptable for Quantitative Use**" if it consisted of a range of exposure concentrations and the observed effects are justifiably contributed to a single chemical exposure*

_____ Mixture (observed effects not justifiably contributed to single chemical exposure)
_____ Uncharacterized Reference Sites/Conditions

POTENTIAL CHEMICAL MIXTURES PRESENT AT SITE: *Describe any potential chemicals mixtures present at the site as characterized by study authors (including any confirmation of chemicals present at study site).*

EXPOSURE VARIABILITY ACROSS STUDY SITE(S): *Describe any exposure variability across study site(s) as characterized by study authors (i.e., description of study design with reference and contaminated sites).*

General Notes:

Reviewer's Comments: *Provide additional comments that do not appear under other sections of the DER*.

ABSTRACT: *Copy and paste abstract from publication.*

SUMMARY*: Fill out and modify as needed.*

Acute:

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration (mg/L)	Classification
											Quantitative / Qualitative / Unused

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

Chronic:

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Chronic Limits	Reported Chronic Value (mg/L or µg/g)	Verified Chronic Value (mg/L or µg/g)	Chronic Value Endpoint	Classification
												Quantitative / Qualitative / Unused

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

II. Results *Provide results as reported in the publication (including supplemental materials). Include screen shots of tables and/or figures reporting results from the article <u>following</u> tabulated data table in each associated results section for <u>all studies</u>. Complete tabulated data tables for all studies for studies marked "**Acceptable for Quantitative Use"** and **"Acceptable for Qualitative Use**".*

Water Quality Parameters: *If only general summary data of water quality parameters is provided by study authors (i.e., no specific details of water quality parameters on a treatment level is provided), summarize any information regarding water quality parameters under General Notes below and indicate data not provided in* Table A.II.1.

General Notes: *For aquatic life criteria development, measured water quality parameters in the treatments nearest the toxicity test endpoint(s), e.g., LC_{50}, EC_{20}, etc., are most relevant.*

Table A.II.1. Measured Water Quality Parameters in Test Solutions.

Dissolved oxygen, temperature, pH and [other parameters (hardness, salinity, DOC)] in test solutions during the *[X]*-day exposure of *[test organism]* to *[concentration of treatment(s)]* of *[test substance]* under *[static renewal/flow-through]* conditions.

Parameter	Treatment	Mean	Range
Dissolved Oxygen (% saturation or mg/L)	*[1]*		
	[2]		
	j		
	j		
Temperature (℃)	*[1]*		
	[2]		
	j		
	j		
pH	*[1]*		
	[2]		
	j		
	j		
Other (e.g., hardness, salinity, DOC)	*[1]*		
	[2]		
	j		
	j		

Chemical Concentrations: *Summarize the concentration verification data from test solutions/media. Expand table to include measured concentration data for each media type (i.e., water, diet, muscle, liver, blood, etc.).*

General Notes: *Provide any necessary detail regarding the measured concentrations, including any identified cause for substantial differences between nominal and measured concentrations, if samples were collected on separate days (and if so provide details), and any potential cross contamination.*

Table A.II.2. Measured (and Nominal) Chemical Concentrations in Test Solutions/Media.

[Analytical Method] verification of test and control concentrations during an [X]-day exposure of [test organism] to [test substance] under [static renewal/flow-through] conditions.

Treatment	Nominal Concentration (units)	[Mean] Measured Concentration (units)	Number of Samples	Non-Detect[a]	Number of Samples Below Non-Detect	[Standard Deviation or Standard Error]	Range
Control							
[1]							
[2]							
[3]							
[4]							
[5]							
[6]							
j							

[a]Non-Detect: 0 = measured and detected; 1= measured and not detected; if not measured or reported enter as such

Mortality: *Briefly summarize mortality results (if any).*

General Notes: *Comment on concentrations response relationship and slope of response if provided. Compare mortality in treatments with control group and/or the reference chemical.*

Table A.II.3. Mean Percent [Mortality or Survival].

Mean percent mortality [or number of immobilized, survival] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment	[Mean % Mortality]	[Standard Deviation or Standard Error]
Control		
[1]		
[2]		
[3]		
[4]		
[5]		
[6]		
[LCx]		
NOEC		
LOEC		

[a] Use superscript to identify the values reported to be significantly different from control.

Growth: *Briefly summarize growth results (if any).*

General Notes: *Comment on concentrations response relationship and slope of response if provided. Compare growth endpoints in treatments with control group and/or the reference chemical.*

Table A.II.4. Mean [Growth].

Mean growth [length and/or weight] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment	Mean Growth [Length/Weight] (units)	[Standard Deviation or Standard Error]	Mean Percent Change in [Length/ Biomass]	[Standard Deviation or Standard Error]
Control				
[1]				
[2]				
[3]				
[4]				
[5]				
[6]				
j				
[ECx]				
NOEC				
LOEC				

[a] Use superscript to identify the values reported to be significantly different from control.

Reproductive: *Briefly summarize reproduction endpoint results (if any). For multi-generational studies, copy and paste* Table A.II.5 *below for each generation with reproductive effects data.*

General Notes: *Comment on concentrations response relationship and slope of response if provided. Compare reproductive endpoints in treatments with control group and/or the reference chemical.*

Table A.II.5. Mean [Reproductive] Effect.

Mean [reproductive] effects for [generation] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment (units)	[Mean Number of Spawns]	[Standard Deviation or Standard Error]	[Mean Number of Eggs]	[Standard Deviation or Standard Error]	[Mean Percent Hatch]	[Standard Deviation or Standard Error]	[Mean Hatch Percent Survival Post]	[Standard Deviation or Standard Error]
Control								
[1]								
[2]								
[3]								
[4]								
[5]								
[6]								
j								
[ECx]								
NOEC								
LOEC								

[a] Use superscript to identify the values reported to be significantly different from control.

Sublethal Toxicity Endpoints: *Include other sublethal effect(s), including behavioral abnormalities or other signs of toxicity, if any. Copy* Table A.II.6 *as needed to provide details for each sublethal effect observed.*

General Notes: *Briefly summarize observed sublethal effects otherwise not captured in the results table(s) below.*

Table A.II.6. Mean [Sublethal] Effect.

*Mean [*Sublethal effect*, (e.g., behavioral abnormalities, etc.)]* in *[test organism]* during [test duration (*acute/chronic*)] exposure to *[test substance]* under *[static/renewal/flow-through]* conditions.

Treatment	[Mean Sublethal Response] (units)	[Standard Deviation or Standard Error]
Control		
[1]		
[2]		
[3]		
[4]		
[5]		
[6]		
j		
[ECx]		
NOEC		
LOEC		

[a] Use superscript to identify the values reported to be significantly different from control

Reported Statistics: *Copy and paste statistical section from publication.*

Part B: Detailed Review
I. Materials and Methods

Protocol/Guidance Followed: *Indicate if provided by authors.*

Deviations from Protocol: *If authors report any deviations from the protocol noted above indicate here.*

Study Design and Methods: *Copy and paste methods section from publication.*

TEST ORGANISM: *Provide information under Details and any relevant or related information or clarifications in Remarks.*

Parameter	Details	Remarks
Species:	Common Name: Scientific Name:	North American species? ____ Surrogate for North American Taxon? ____ *(Place X if applicable)*

Strain/Source:
• Wild caught from unpolluted areas [1]
  o Quarantine for at least 14 days or until they are disease free, before acclimation [1]
• Must originate from same source and population [1]
• Should not be used:
  o If appeared stressed, such as discoloration or unusual behavior [1]
  o If more than 5% die during the 48 hours before test initiation [1]
  o If they were used in previous test treatments or controls [2]
• No treatments of diseases may be administered:
  o Within 16 hour of field collection [1]
  o Within 10 days or testing or during testing [1]		

Age at Study Initiation:
Acute:
• Juvenile stages preferred [1]
Chronic:
• Life-cycle test:
  o Embryos or newly hatched young < 48 hours old [2]
• Partial life-cycle test:
  o Immature juveniles at least 2 months prior to active gonad development [2]
• Early life-stage test:
  o Shortly after fertilization [2]		

Was body weight or length recorded at test initiation?	____ Yes ____ No	
Was body weight or length recorded at regular intervals?	____ Yes ____ No *If yes, describe regular intervals:*	

STUDY PARAMETERS: *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for <u>both Controlled Experiments and Field Studies/Observations.</u>*

	Parameter	Details	Remarks
For Both Controlled Experiments and Field Observations	**Number of Replicates per Treatment Group:** • At least 2 replicates/treatment recommended for acute tests [1] • At least 2 replicates/treatment recommended for chronic tests [3]	Control(s): Treatment(s):	
	Number of Organisms per Replicate/ Treatment Group: • At least 10 organisms/treatment recommended [3] • At least 7 organisms/treatment acceptable [4]	Control(s): Treatment(s):	
	Exposure Pathway: *(i.e., water, sediment, gavage, or diet).* *Note: all other pathways (e.g., dermal, single dose via gavage, and injection) are unacceptable.*		
	Exposure Duration: **Acute** • Should be 96 hours [2] **Chronic** • Life-cycle tests: o Ensure that all life stages and life processes are exposed [2] o Begin with embryos (or newly hatched young), continue through maturation and reproduction, and should end not less than 24 days (90 days for salmonids) after the hatching of the next generation [2] • Partial life-cycle tests: o Allowed with species that require >1 year to reach sexual maturity, so that all major life stages can be exposed to the test material in <15 months [2] o Begin with immature juveniles at least 2 months prior to active gonad development, continue through maturation and reproduction, and end not less than 24 days (90 days for salmonids) after the hatching of the next generation [2] • Early life-cycle tests: o 28 to 32 day (60 day post hatch for salmonids) exposures from shortly after fertilization through embryonic, larval, and early juvenile development [2]	____ Acute ____ Partial Life Cycle ____ Early Life Stage ____ Full Life Cycle ____ Other *(please remark):*	
	Test Concentrations (remember units): *Recommended test concentrations include at least three concentrations other than the control; four or more will provide a better statistical analysis* [3]	Nominal: Measured: Media measured in:	
	Observation Intervals: • Should be an appropriate number of observations over the study to ensure water quality is being properly maintained [4]		

CONTROLLED EXPERIMENT STUDY PARAMETERS: *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for Controlled Experiments only.*

For Controlled Experiments Only

Parameter	Details	Remarks

Acclimation/Holding:
• Should be placed in a tank along with the water in which they were transported
  o Water should be changed gradually to 100% dilution water (usually 2 or more days) [1]
  o For wild-caught animals, test water temperature should be within 5°C of collection water temperature [1]
  o Temperature change rate should not exceed 3°C within 72 hours [1]
• To avoid unnecessary stress and promote good health:
  o Organisms should not be crowded [1]
  o Water temperature variation should be limited [1]
  o Dissolved oxygen:
  ▪ Maintain between 60 - 100% saturation [1]
  ▪ Continuous gentle aeration if needed [1]
  o Unionized ammonia concentration in holding and acclimation waters should be < 35 µg/L [1]
	Duration:
	Feeding:
	Water type:
	Temperature (°C):
	Dissolved Oxygen (mg/L):
	Health (*any mortality observed?*):
		Identify number of individuals excluded from testing and/or analysis (if any):

Acclimation followed published guidance? *Describe, if any*	____ Yes ____ No *If yes, indicate which guidance:*	

Test Vessel:
• Test chambers should be loosely covered [1]
• Test chamber material:
  o Should minimize sorption of test chemical from water [1]
  o Should not contain substances that can be leached or dissolved in solution and are free of substances that could react with exposure chemical [1]
  o Glass, No. 316 stainless steel, nylon screen and perfluorocarbon (e.g. Teflon) are acceptable [1]
  o Rubber, copper, brass, galvanized metal, epoxy glues, lead and flexible tubing should not come into contact with test solution, dil. water, or stock [1]
• Size/volume should maintain acceptable biomass loading rates (see Biomass Loading Rate below) [1]
	Material:
	Size:
	Fill Volume:
		Briefly describe the test vessel:

Test Solution Delivery System/Method:
• Flow-through preferred for some highly volatile, hydrolysable or degradable materials [2]
  o Concentrations should be measured often enough using acceptable analytical methods [2]
• Chronic exposures:
  o Flow-through, measured tests required [2]
	Test Concentrations Measured
	____ Yes ____ No
	
	Test Solution Delivery System:
	____ Static
	____ Renewal
	Indicate Interval:
	
	____ Flow-through
	Indicate Type of Diluter:	

Source of Dilution Water:
• Freshwater hardness range should be < 5 mg/L or < 10% of the average (whichever is greater) [1]
• Saltwater salinity range should be < 2 g/kg or < 20% of the average (whichever is greater) [1]
• Dilution water must be characterized (natural surface water, well water, etc.) [3]
  o Distilled/deionized water without the addition of appropriate salts should not be used [2]
• Dilution water in which total organic carbon or particulate matter >5 mg/L should not be used [2]
  o Unless data show that organic carbon or particulate matter do not affect toxicity [2]		

Dilution Series (*e.g., 0.5x, 0.6x, etc.*):		

	Parameter	Details	Remarks
For Controlled Experiments Only	**Dilution Water Parameters:** *Measured at the beginning of the experiment or averaged over the duration of the experiment (details of water quality parameters measured in test solutions should be included under the results section)*	Dissolved Oxygen (mg/L): pH: Temperature (°C): Hardness (mg/L as $CaCO_3$): Salinity (ppt): Total Organic Carbon (mg/L): Dissolved Organic Carbon (mg/L):	
	Aeration: • Acceptable to maintain dissolved oxygen at 60 - 100% saturation at all times [1] • Avoid aeration when testing highly oxidizable, reducible and volatile materials [1] • Turbulence should be minimized to prevent stress on test organisms and/or re-suspend fecal matter [1] • Aeration should be the same in all test chambers at all times [1]	____ Yes ____ No	
	Describe Preparation of Test Concentrations (e.g., water exposure, diet):		
	Test Chemical Solubility in Water: *List units and conditions (e.g., 0.01% at 20ºC)*		
	Were concentrations in water or diet verified by chemical analysis? *Measured test concentrations should be reported in* Table A.II.2 *above.*	__Yes __No *Indicate media:*	
	Were test concentrations verified by chemical analysis in tissue? *Measured test concentrations can be verified in test organism tissue (e.g., blood, liver, muscle) alone if a dose-response relationship is observed.* *Measured test concentrations should be reported in* Table A.II.2 *above.*	__Yes __No *Indicate tissue type:*	*If test concentrations were verified in test organism tissue, was a dose-response relationship observed?*
	Were stability and homogeneity of test material in water/diet determined?	__Yes __No	
	Was test material regurgitated/avoided?	__Yes __No	
	Solvent/Vehicle Type (Water or Dietary): • When used, a carrier solvent should be kept to a minimum concentration [1] • Should not affect either survival or growth of test organisms [1] • Should be reagent grade or better [1] • Should not exceed 0.5 ml/L (static) or 0.1 ml/L (flow through) unless it was shown that higher concentrations do not affect toxicity [3]		
	Negative Control:	____ Yes ____ No	
	Reference Toxicant Testing:	____ Yes ____ No	*If Yes, identify substance:*
	Other Control: *If any (e.g. solvent control)*		

	Biomass Loading Rate:
	• Loading should be limited so as not to affect test results. Loading will vary depending on temperature, type of test (static vs. flow-through), species, food/feeding regime, chamber size, test solution volume, etc. [1]
	• This maximum number would have to be determined for the species, test duration, temperature, flow rate, test solution volume, chamber size, food, feeding regime, etc.
	• Loading should be sufficiently low to ensure:
	  ○ Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [1,5]
	  ○ Unionized ammonia does not exceed 35 µg/L [1]
	  ○ Uptake by test organisms does not lower test material concentration by > 20% [1]
	  ○ Growth of organisms is not reduced by crowding
	• Generally, at the end of the test, the loading (grams of organisms; wet weight; blotted dry) in each test chamber should not exceed the following:
	  ○ Static tests: > 0.8 g/L (lower temperatures); > 0.5 g/L (higher temperatures) [1]
	  ○ Flow through tests: > 1 g/L/day or > 10 g/L at any time (lower temperatures); > 0.5 g/L/day or > 5 g/L at any time (higher temperatures) [1]
	• Lower temperatures are defined as the lower of 17˚C or the optimal test temperature for that species [1]		

	Parameter	Details	Remarks

For Controlled Experiments Only	**Feeding:**
	• Unacceptable for acute tests [2]
	  o Exceptions:
	  ▪ Data indicate that the food did not affect the toxicity of the test material [2]
	  ▪ Test organisms will be severely stressed if they are unfed for 96 hours [2]
	  ▪ Test material is very soluble and does not sorb or complex readily (e.g., ammonia) [2]
		____ Yes ____ No	

	Lighting:
	• Depends on the type of test (acute or chronic) and endpoint (e.g., reproduction) of interest.
	  o Embryos should be incubated under dim incandescent lighting (≤ 20 fc) or total darkness during early life-stage toxicity testing
	  o Embryos must not be subjected to prolonged exposure to direct sunlight, fluorescent lighting, or high intensity incandescent lighting
	• Generally, ambient laboratory levels (50-100 fc) or natural lighting should be acceptable, as well as a diurnal cycle consisting of 50% daylight or other natural seasonal diurnal cycle.
	• Artificial light cycles should have a 15 – 30-minute transition period to avoid stress due to rapid increases in light intensity [1]		

Study Design/Methods Classification: *(Place X by One Based on Overall Study Design/Methods Classification)*
Provide details of Major or Minor Deficiencies/Concerns with Study Design in Associated Sections of Part A: Overview
This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A.

_____ Study Design Acceptable for Quantitative Use
_____ Study Design Acceptable for Qualitative Use
_____ Study Design Not Acceptable for Use

Additional Notes: *Provide additional considerations for the classification of study use based on the study design.*

OBSERVATIONS: *Provide information under Details and any relevant information in Remarks. This information should be consistent with the Results Section in Part A.*

Parameter	Details	Remarks

Parameters measured including sublethal effects/toxicity symptoms:
Common Apical Parameters Include:
Acute
• EC_{50} based on percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus percentage of organisms killed [2]
  ○ If not available, the 96-hr LC_{50} should be used [2]
Chronic
• Life-cycle/Partial Life-cycle test:
  ○ Survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability [2]
• Early life-cycle test:
  ○ Survival and growth [2]
	List parameters:	

Was control survival acceptable? **Acute** • > 90% control survival at test termination [2] **Chronic** • > 80% control survival at test termination [2]	____ Yes ____ No Control survival (%):	
Were individuals excluded from the analysis?	____ Yes ____ No *If yes, describe justification provided:*	
Was water quality in test chambers acceptable? • If appropriate, describe any water quality issues (e.g., dissolved oxygen level below 60% of saturation)	____ Yes ____ No	
Availability of concentration-response data: • Were treatment level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • Were replicate level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • If treatment and/or replicate level concentration-response data were included, how was data presented? *(check all that apply)* • Were concentration-response data estimated from graphs study publication or supplemental materials? • Should additional concentration-response data be requested from study authors? *If concentration-response data are available, complete **Verification of Statistical Results (Part C)** for sensitive species.*	____ Yes ____ No ____ Yes ____ No ____ Tables ____ Graphs ____ Supplemental Files ____ Yes ____ No *If yes, indicate software used:* ____ Yes ____ No Requested by: Request date: Date additional data received:	

Part C: Statistical Verification of Results

I. Statistical Verification Information: *Report the statistical methods (e.g., EPA TRAP, BMDS, R, other) used to verify the reported study or test results for the five (5) most sensitive genera and sensitive apical endpoints (including for tests where such estimates were not provided). If values for the LC_{50}, LT_{50} and NOEC are greater than the highest test concentration, use the ">" symbol.*

Primary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)

Secondary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)
(*At least one reviewer should be from EPA for sensitive taxa*)

Endpoint(s) Verified:

Additional Calculated Endpoint(s):

Statistical Method (e.g., TRAP, BMDS, R, other):

II. Toxicity Values: *Include confidence intervals if applicable*

NOEC:
LOEC:
MATC:

EC_5:
EC_{10}:
EC_{20}:
EC_{50} or LC_{50}

Dose-Response Curve Classification: *(Place X by One)*
This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A

_____ Dose-Response Curve Acceptable for Quantitative Use
_____ Dose-Response Curve Acceptable for Qualitative Use
_____ Dose-Response Curve Not Acceptable for Use

Summary of Statistical Verification: *Provide summary of methods used in statistical verification.*

Additional Notes:

Attachments:

1. *Provide attachments to ensure all data used in Part C are captured, whether from study results reported in the publication and/or from additional data requested from study authors*
    - *Data from study results of the publication should be reported in Results section of Part A*
    - *Additional data provided upon request from study authors should be reported in Table C.II.1 below and original correspondence with study authors should be included as attachments*
2. *Model assessment output (including all model figures, tables, and fit metrics)*
3. *Statistical code used for curve fitting*

III. Attachments: *Include all attachments listed above after the table below.*

Additional Data Used in Response-Curve: *Provide all data used to fit dose-response curve not captured in Results section of DER above in Part A. Add rows as needed. First row in italicized text is an example.*

Table C.II.1 Additional Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[a] N = number of individuals per treatment; k = number of replicates per treatment level; n = number of individuals per replicate

Part D: References to Test Guidance

1. ASTM Standard E 739, 1980. 2002. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, West Conshohocken, PA.
2. Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses. PB85-227049. National Technical Information Service, Springfield, VA.
3. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.
4. OECD 203. 1992. Test No. 203: Fish, Acute Toxicity Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264069961-en.
5. American Public Health Association (APHA). 2012. Standard methods for the examination of water and wastewater. Part 8000 - Toxicity. APHA. Washington, DC.

Q.2 Example Aquatic Invertebrate DER

Part A: Overview

I. Test Information

Chemical name:

CAS name: CAS Number:
Purity: Storage conditions:
Solubility in Water (units):

____ **Controlled Experiment** (*manipulated*) ____ **Field Study/Observation** (*not manipulated*) (*Place X by One*)

Primary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)

Secondary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)
(*At least one reviewer should be from EPA for sensitive taxa*)

Citation: *Indicate: author(s), year, study title, journal, volume, and pages.*
(e.g., Keller, A.E and S.G. Zam. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis*. Environ. Toxicol. Chem. 10(4): 539-546.)

Companion Papers: *Identify any companion papers associated with this paper using the citation format above.*

Were other DERs completed for Companion Papers? ____ **Yes** ____ **No** (*If yes, list file names of DERs below*)

Study Classification for Aquatic Life Criteria Development:

_____ Acceptable for Quantitative Use
_____ Acceptable for Qualitative Use
_____ Not Acceptable for Use/Unused

General Notes: *Provide any necessary details regarding the study's use classification for all pertinent endpoints, including non-apical endpoints within the study (e.g., note all study classifications for each endpoint if the use varies)*

Major Deficiencies (note any stated exclusions): *Check all that apply. Checking any of these items make the study "**Not Acceptable for Use**"*

_____ Mixture (for controlled experiments only)
_____ No Controls (for controlled experiments only)
_____ Excessive Control Mortality (> 10% for acute and > 20% for chronic)
_____ Dilution water not adequately characterized
_____ Bioaccumulation: steady state not reached
_____ Dermal or Injection Exposure Pathway
_____ Review paper or previously published without modification

_____ Other: *(if any, list here)*

POTENTIAL CHEMICAL MIXTURES: *Describe any potential chemicals mixtures as characterized by study authors (including any confirmation of chemical mixtures).*

DESCRIPTION OF DILUTION WATER: *Describe concerns with characterization of and/or major deficiencies with dilution water.*

General Notes:

Minor Deficiencies: *List and describe any minor deficiencies or other concerns with test. These items may make the study "**Acceptable for Qualitative Use**"* **(exceptions may apply as noted)**

***For Field Studies/Observations**: A field study/observation may be considered "**Acceptable for Quantitative Use**" if it consisted of a range of exposure concentrations and the observed effects are justifiably contributed to a single chemical exposure*

_____ Mixture (observed effects not justifiably contributed to single chemical exposure)
_____ Uncharacterized Reference Sites/Conditions

POTENTIAL CHEMICAL MIXTURES PRESENT AT SITE: *Describe any potential chemicals mixtures present at the site as characterized by study authors (including any confirmation of chemicals present at study site).*

EXPOSURE VARIABILITY ACROSS STUDY SITE(S): *Describe any exposure variability across study site(s) as characterized by study authors (i.e., description of study design with reference and contaminated sites).*

General Notes:

Reviewer's Comments: *Provide additional comments that do not appear under other sections of the template*.

ABSTRACT: *Copy and paste abstract from publication.*

SUMMARY*: Fill out and modify as needed.*

Acute:

Species (lifestage)	Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration (mg/L)	Classification
											Quantitative / Qualitative / Unused

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

Chronic:

Species (lifestage)	Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Chronic Limits	Reported Chronic Value (mg/L or µg/g)	Verified Chronic Value (mg/L or µg/g)	Chronic Value Endpoint	Classification
												Quantitative / Qualitative / Unused

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

II. Results *Provide results as reported in the publication (including supplemental materials). Include screen shots of tables and/or figures reporting results from the article <u>following</u> tabulated data table in each associated results section for <u>all studies</u>. Complete tabulated data tables for all studies for studies marked "**Acceptable for Quantitative Use"** and **"Acceptable for Qualitative Use**".*

Water Quality Parameters: *If only general summary data of water quality parameters is provided by study authors (i.e., no specific details of water quality parameters on a treatment level is provided), summarize any information regarding water quality parameters under General Notes below and include data not provided in* Table A.II.1.

General Notes: *For aquatic life criteria development, measured water quality parameters in the treatments nearest the toxicity test endpoint(s), e.g., LC_{50}, EC_{20}, etc., are most relevant.*

Table A.II.1. Measured Water Quality Parameters in Test Solutions.

Dissolved oxygen, temperature, pH and [other parameters (hardness, salinity, DOC)] in test solutions during the *[X]*-day exposure of *[test organism]* to *[concentration of treatment(s)]* of *[test substance]* under *[static renewal/flow-through]* conditions.

Parameter	Treatment	Mean	Range
Dissolved oxygen (% saturation or mg/L)	*[1]*		
	[2]		
	j		
	j		
Temperature (℃)	*[1]*		
	[2]		
	j		
	j		
pH	*[1]*		
	[2]		
	j		
	j		
Other (e.g., hardness, salinity, DOC)	*[1]*		
	[2]		
	j		
	j		

Chemical Concentrations: *Summarize the concentration verification data from test solutions/media. Expand table to include each measured concentration data for each media type (i.e., muscle, liver, blood, etc.).*

General Notes: *Provide any necessary detail regarding the measured concentrations, including any identified cause for substantial differences between nominal and measured concentrations, if samples were collected on separate days (and if so provide details), and any potential cross contamination.*

Table A.II.2. Measured (and Nominal) Chemical Concentrations in Test Solutions/Media.

[Analytical Method] verification of test and control concentrations during an [X]-day exposure of [test organism] to [test substance] under [static renewal/flow-through] conditions.

Treatment	Nominal Concentration (units)	[Mean] Measured Concentration (units)	Number of Samples	Non-Detect[a]	Number of Samples Below Non-Detect	[Standard Deviation or Standard Error]	Range
Control							
[1]							
[2]							
[3]							
[4]							
[5]							
[6]							
j							

[a]Non-Detect: 0 = measured and detected; 1=measured and not detected; if not measured or reported enter as such

Mortality: *Briefly summarize mortality results (if any).*

General Notes: *Comment on concentrations response relations and slope of response if provided. Compare mortality with control treatment and/or the reference chemical.*

Table A.II.3. Mean Percent [Mortality or Survival].

Mean percent mortality [or number of immobilized] or survival of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment	[Mean % Mortality]	[Standard Deviation or Standard Error]
Control		
[1]		
[2]		
[3]		
[4]		
[5]		
[6]		
[LC_x]		
NOEC		
LOEC		

[a] Use superscript to identify the values reported to be significantly different from control.

Growth: *Briefly summarize growth results (if any).*

General Notes: *Comment on concentrations response relations and slope of response if provided. Compare growth endpoints with control treatment and/or the reference chemical.*

Table A.II.4. Mean [Growth].

Mean growth [length and/or weight] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment	Mean Growth [Length/Weight] (units)	[Standard Deviation or Standard Error]	Mean Percent Change in [Length/ Biomass]	[Standard Deviation or Standard Error]
Control				
[1]				
[2]				
[3]				
[4]				
[5]				
[6]				
j				
[EC_x]				
NOEC				
LOEC				

[a] Use superscript to identify the values reported to be significantly different from control.

Reproductive: *Briefly summarize reproduction endpoint results (if any). For multi-generational studies, copy and paste* Table A.II.5 *below for each generation with reproductive effects data.*

General Notes: *Comment on concentrations response relations and slope of response if provided. Compare reproduction endpoints with control treatment and/or the reference chemical.*

Table A.II.5. Mean [Reproductive] Effect.

Mean [reproductive] effects for [generation] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment (units)	[Mean Number of Spawns]	[Standard Deviation or Standard Error]	[Mean Number of Eggs]	[Standard Deviation or Standard Error]	[Mean Number of Offspring]	[Standard Deviation or Standard Error]
Control						
[1]						
[2]						
[3]						
[4]						
[5]						
[6]						
j						
[ECx]						
NOEC						
LOEC						

[a] Use superscript to identify the values reported to be significantly different from control.

Sublethal Toxicity Endpoints: *Include other sublethal effect(s), including behavioral abnormalities or other signs of toxicity, if any. Copy Table A.II.6 as needed to provide details for each sublethal effect observed.*

General Notes: *Briefly summarize observed sublethal effects otherwise not captured in the results table(s) below.*

Table A.II.6. Mean [Sublethal] Effect.

*Mean [*Sublethal effect, *(e.g., behavioral abnormalities, etc.)]* in *[test organism]* during [test duration (*acute/chronic*)] exposure to *[test substance]* under *[static/renewal/flow-through]* conditions.

Treatment	[Mean Sublethal Response] (units)	[Standard Deviation or Standard Error]
Control		
[1]		
[2]		
[3]		
[4]		
[5]		
[6]		
j		
[ECx]		
NOEC		
LOEC		

[a] Use superscript to identify the values reported to be significantly different from control

Reported Statistics: *Copy and paste statistical section from publication.*

Part B: Detailed Review

I. Materials and Methods

PROTOCOL/GUIDANCE FOLLOWED: *Indicate if provided by authors.*

DEVIATIONS FROM PROTOCOL: *If authors report any deviations from the protocol noted above indicate here.*

Study Design and Methods: *Copy and paste methods section from publication.*

TEST ORGANISM: *Provide information under Details and any relevant or related information or clarifications in Remarks.*

Parameter	Details	Remarks
Species:	Common Name: Scientific Name:	North American species? ____ Surrogate for North American Taxon? ____ *(Place X if applicable)*

Strain/Source:
• Wild caught from unpolluted areas [1]
  o Quarantine for at least 7 days or until they are disease free, before acclimation [1]
• Must originate from same source and population [1]
• Should not be used:
  o If appeared stressed, such as discoloration or unusual behavior [1]
  o If more than 5% die during the 48 hours before test initiation [1]
  o If they were used in previous test treatments or controls [2]
• No treatments of diseases may be administered:
  o Within 16 hours of field collection [1]
  o Within 10 days of testing or during testing [1]		

Age at Study Initiation:
Acute:
• Larval stages preferred [1]
• Mayflies and Stoneflies
  o Early instar [1]
• Daphnids/cladocerans:
  o < 24-hr old [1]
• Midges:
  o 2nd or 3rd instar larva [1]
• *Hyalella azteca* (chronic exposure)
  o Generally, 7 - 8 days old [3]
• Freshwater mussels (chronic exposure)
  o Generally, 2 month old juveniles [4]
• Mysids (chronic exposure)
  o < 24-hr old [1]		

Was body weight or length recorded at test initiation and/or at regular intervals?	____ Yes ____ No	
Was body weight or length recorded at regular intervals?	____ Yes ____ No *If yes, describe regular intervals:*	

STUDY PARAMETERS: *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for both Controlled Experiments and Field Studies/Observations.*

For Both Controlled Experiments and Field Observations

Parameter	Details	Remarks
Number of Replicates per Treatment Group: • At least 2 replicates/treatment recommended for acute tests [1] • At least 2 replicates/treatment recommended for chronic tests [5]	Control(s):	
	Treatment(s):	
Number of Organisms per Replicate/ Treatment Group: • At least 10 organisms/treatment recommended.	Control(s):	
	Treatment(s):	
Exposure Pathway: *(i.e., water, sediment, or diet). Note: all other pathways (e.g., dermal, injection) are unacceptable.*		
Exposure Duration: **Acute** • Cladocerans and midges should be 48 hours [2] o Longer durations acceptable if test species not fed and had acceptable controls [2] • Freshwater mussel glochidia should be a maximum of 24 hours [4] o Shorter durations (6, 12, 18 hours) acceptable so long as 90% survival of control animals achieved (see below) [4] • Embryo/larva (bivalve mollusks, sea urchins, lobsters, crabs, shrimp and abalones) should be 96 hours, but at least 48 hours [2] • Other invertebrate species should be 96 hours **Chronic** • Daphnids/cladocerans should be 21 days (3-brood test) [2] o Exception 7 days acceptable for *Ceriodaphnia dubia* [2] • Freshwater juvenile mussels should be at least 28 days [4] • *Hyalella azteca* should be at least 42 days o Beginning with 7 - 8 day old animals [3] • Mysids should continue until 7 days past the median time of first brood release in the controls [4]	____ Acute ____ Chronic ____ Other *(please remark):*	
Test Concentrations (remember units): *Recommended test concentrations include at least three concentrations other than the control; four or more will provide a better statistical analysis.*	Nominal:	
	Measured:	
	Media measured in:	
Observation Intervals: • Should be an appropriate number of observations over the study to ensure water quality is being properly maintained [1]		

CONTROLLED EXPERIMENT STUDY PARAMETERS: *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for Controlled Experiments only.*

For Controlled Experiments Only

Parameter	Details	Remarks

Acclimation/Holding:
• Should be placed in a tank along with the water in which they were transported [1]
  o Water should be changed gradually to 100% dilution water (usually 2 or more days) [1]
  o For wild-caught animals, test water temperature should be within 5°C of collection water temperature [1]
  o Temperature change rate should not exceed 3°C within 72 hours [1]
• To avoid unnecessary stress and promote good health:
  o Organisms should not be crowded [1]
  o Water temperature variation should be limited
  o Dissolved oxygen:
  ▪ Maintain between 60 - 100% saturation [1]
  ▪ Continuous gentle aeration if needed [1]
  o Unionized ammonia concentration in holding and acclimation waters should be < 35 µg/L [1]
	Duration:
	Feeding:
	Water:
	Temperature (°C):
	Dissolved Oxygen (mg/L):
	Health (*any mortality observed?*):
		Identify number of individuals excluded from testing and/or analysis (if any):

Acclimation followed published guidance? *Describe, if any*	____ Yes ____ No *If yes, indicate which guidance:*	

Test Vessel:
• Test chambers should be loosely covered [1]
• Test chamber material:
  o Should minimize sorption of test chemical from water [1]
  o Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical [1]
  o Glass, No. 316 stainless steel, nylon screen and perfluorocarbon (e.g. Teflon) are acceptable [1]
  o Rubber, copper, brass, galvanized metal, epoxy glues, lead and flexible tubing should not come into contact with test solution, dilution water or stock [1]
• Size/volume should maintain acceptable biomass loading rates (see below) [1]
• Substrate:
  o Required for some species (e.g., *Hyalella azteca*) [3]
  o Common types: stainless steel screen, nylon screen, quartz sand, cotton gauze and maple leaves [3]
  o More inert substances preferred over plant material, since plants may break down during testing and promote bacterial growth [3]
  o Consideration should be given between substrate and toxicant [3]
  ▪ Hydrophobic organic compounds in particular can bind strongly to Nitex® screen, reducing exposure concentrations, especially for studies using static or intermittent renewal exposure methods [3]
	Material:
	Size:
	Fill Volume:
	Substrate Used *(if applicable)*:
		Briefly describe the test vessel here

	Parameter	Details	Remarks

For Controlled Experiments Only	**Test Solution Delivery System/Method:**
	• Flow-through preferred for some highly volatile, hydrolyzable or degradable materials [2]
	  o Concentrations should be measured often enough using acceptable analytical methods [2]
	• Chronic exposures:
	  o Flow-through, measured tests required [2]
	  o Exception: renewal is acceptable for daphnids [2]
		Test Concentrations Measured
		____ Yes ____ No
		
		Test Solution Delivery System:
		____ Static
		____ Renewal
		Indicate Interval:
		
		____ Flow-through
		Indicate Type of Diluter:	

	Source of Dilution Water:
	• Freshwater hardness range should be < 5 mg/L or < 10% of the average (whichever is greater) [1]
	• Saltwater salinity range should be < 2 g/kg or < 20% of the average (whichever is greater) [1]
	• Dilution water must be characterized (natural surface water, well water, etc.) [2]
	  o Distilled/deionized water without the addition of appropriate salts should not be used [2]
	• Dilution water in which total organic carbon or particulate matter exceed 5 mg/L should not be used
	  o Unless data show that organic carbon or particulate matter do not affect toxicity [2]
	• Dilution water for tests with *Hyalella azteca*
	  o Reconstituted waters should have at least 0.02 mg bromide/L; natural ground or surface water presumed to have sufficient bromide [3]
	  o Recommended that control/dilution waters have chloride concentrations at or above 15 mg/L [3]		

	Dilution Series (*e.g., 0.5x, 0.6x, etc.*):		
	Dilution Water Parameters: *Measured at the beginning of the experiment or averaged over the duration of the experiment (details of water quality parameters measured in test solutions should be included under the results section)*	Dissolved Oxygen (mg/L): pH: Temperature (°C): Hardness (mg/L as $CaCO_3$): Salinity (ppt): Total Organic Carbon (mg/L): Dissolved Organic Carbon (mg/L):	
	Aeration: • Acceptable to maintain dissolved oxygen at 60 - 100% saturation at all times [1] • Avoid aeration when testing highly oxidizable, reducible and volatile materials • Turbulence should be minimized to prevent stress on test organisms and/or re-suspend fecal matter [1] • Aeration should be the same in all test chambers at all times [1]	____ Yes ____ No	
	Describe Preparation of Test Concentrations (e.g., water exposure, diet):		

	Parameter	Details	Remarks
For Controlled Experiments Only	**Test Chemical Solubility in Water:** • List units and conditions (e.g., 0.01% at 20ºC)		
	Were concentrations in water or diet verified by chemical analysis? *Measured test concentrations should be reported in* Table A.II.2 *above.*	____ Yes ____ No *Indicate media:*	
	Were test concentrations verified by chemical analysis in tissue? *Measured test concentrations can be verified in test organism tissue (e.g., blood, liver, muscle) alone if a dose-response relationship is observed.* *Measured test concentrations should be reported in* Table A.II.2 *above.*	____ Yes ____ No *Indicate tissue type:*	*If test concentrations were verified in test organism tissue, was a dose-response relationship observed?*
	Were stability and homogeneity of test material in water/diet determined?	____ Yes ____ No	
	Was test material regurgitated/avoided?	____ Yes ____ No	
	Solvent/Vehicle Type: • When used, a carrier solvent should be kept to a minimum concentration [1] • Should not affect either survival or growth of test organisms [1] • Should be reagent grade or better [1] • Should not exceed 0.5 ml/L (static), or 0.1 ml/L (flow through) unless it was shown that higher concentrations do not affect toxicity [5]		
	Negative Control:	____ Yes ____ No	
	Reference Toxicant Testing:	____ Yes ____ No *If yes, identify substance:*	
	Other Control: *If any (e.g. solvent control)*		
	Biomass Loading Rate: • Loading should be limited so as not to affect test results. Loading will vary depending on temperature, type of test (static vs. flow-through), species, food/feeding regime, chamber size, test solution volume, etc. [1] • This maximum number would have to be determined for the species, test duration, temperature, flow rate, test solution volume, chamber size, food, feeding regime, etc. • Loading should be sufficiently low to ensure: o Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [1,6] o Unionized ammonia does not exceed 35 µg/L [1] o Uptake by test organisms does not lower test material concentration by > 20% [1] o Growth of organisms is not reduced by crowding • Generally, at the end of the test, the loading (grams of organisms; wet weight; blotted dry) in each test chamber should not exceed the following: o Static tests: > 0.8 g/L (lower temperatures); > 0.5 g/L (higher temperatures) [1] o Flow through tests: > 1 g/L/day or > 10 g/L at any time (lower temperatures); > 0.5 g/L/day or > 5 g/L at any time (higher temperatures) [1] o Lower temperatures are defined as the lower of 17˚C or the optimal test temperature for that species. [1]		

For Controlled Experiments Only	**Feeding:**
	• Unacceptable for acute tests [2]
	  o Exceptions:
	  ▪ Data indicate that the food did not affect the toxicity of the test material [2]
	  ▪ Test organisms will be severely stressed if they are unfed for 96 hours [2]
	  ▪ Test material is very soluble and does not sorb or complex readily (e.g., ammonia) [2]
		____ Yes ____ No	

	Lighting: • No specific requirements for lighting • Generally, ambient laboratory levels (50 - 100 fc) or natural lighting should be acceptable, as well as a diurnal cycle consisting of 50% daylight or other natural seasonal diurnal cycle • Artificial light cycles should have a 15 - 30 minute transition period to avoid stress due to rapid increases in light intensity [1] • Depends on the type of test (acute or chronic) and endpoint (e.g., reproduction) of interest.		

Study Design/Methods Classification: *(Place X by One Based on Overall Study Design/Methods Classification)*
Provide details of Major or Minor Deficiencies/Concerns with Study Design in Associated Sections of Part A: Overview
This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A.

_____ Study Design Acceptable for Quantitative Use
_____ Study Design Acceptable for Qualitative Use
_____ Study Design Not Acceptable for Use

Additional Notes: *Provide additional considerations for the classification of study use based on the study design.*

OBSERVATIONS: *Provide information under Details and any relevant information in Remarks. This information should be consistent with the Results Section in Part A.*

Parameter	Details	Remarks
Parameters measured including sublethal effects/toxicity symptoms: **Common Apical Parameters Include:** **Acute** • Daphnids/cladocerans: o EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed [2] • Embryo/larva (bivalve molluscs, sea urchins, lobsters, crabs, shrimp, and abalones): o EC_{50} based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed [2] ▪ If not available, the lower of the 96 hour EC_{50} based on the percentage of organisms with incompletely developed shells and the 96-hr LC_{50} should be used [2] • Freshwater mussel (glochidia and juveniles): o Glochidia: EC_{50} based on 100 x number closed glochidia after adding NaCl solution - number closed glochidia before adding NaCl solution) / Total number open and closed glochidia after adding NaCl solution [4] o Juvenile: EC_{50} based on percentage exhibiting foot movement within a 5-min observation period [4] • All other species and older life stages: o EC_{50} based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed [2] ▪ If not available, the 96 hour LC_{50} should be used [2] **Chronic** • Daphnid: o Survival and young per female [2] • Mysids: o Survival, growth and young per female [2]	*List parameters:*	
Was control survival acceptable? **Acute** • > 90% control survival at test termination [2] o Glochidia 90% after 24 hours, or, the next longest duration less than 24 hours that had at least 90% survival [4] **Chronic** • > 80% control survival at test termination [2] o 80% in 42 day test with *Hyalella azteca*, slightly lower in tests substantially longer than 42 days [3]	____ Yes ____ No Control survival (%):	

Parameter	Details	Remarks
Were individuals excluded from the analysis?	____ Yes ____ No *If yes, describe justification provided:*	
Was water quality in test chambers acceptable? • If appropriate, describe any water quality issues (e.g., dissolved oxygen level below 60% of saturation)	____ Yes ____ No	
Availability of concentration-response data: • Were treatment level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • Were replicate level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • • If treatment and/or replicate level concentration-response data were included, how was data presented? *(check all that apply)* • Were concentration-response data estimated from graphs study publication or supplemental materials? Should additional concentration-response data be requested from study authors? *If concentration-response data are available, complete **Verification of Statistical Results (Part C)** for sensitive species*.	____ Yes ____ No ____ Yes ____ No ____ Tables ____ Graphs ____ Supplemental Files ____ Yes ____ No *If yes, indicate software used:* ____ Yes ____ No Requested by: Request date: Date additional data received:	

Part C: Statistical Verification of Results

I. Statistical Verification Information: *Report the statistical methods (e.g., EPA TRAP, BMDS, R, other) used to verify the reported study or test results for the five (5) most sensitive genera and sensitive apical endpoints (including for tests where such estimates were not provided). If values for the LC_{50}, LT_{50} and NOEC are greater than the highest test concentration, use the ">" symbol.*

Primary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)

Secondary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)
(*At least one reviewer should be from EPA for sensitive taxa*)

Endpoint(s) Verified:

Additional Calculated Endpoint(s):

Statistical Method (e.g., TRAP, BMDS, R, other):

II. Toxicity Values: *Include confidence intervals if applicable*

NOEC:
LOEC:
MATC:

EC_5:
EC_{10}:
EC_{20}:
EC_{50} or LC_{50}

Dose-Response Curve Classification: *(Place X by One)*
This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A

_____ Dose-Response Curve Acceptable for Quantitative Use
_____ Dose-Response Curve Acceptable for Qualitative Use
_____ Dose-Response Curve Not Acceptable for Use

Summary of Statistical Verification: *Provide summary of methods used in statistical verification.*

Additional Notes:

Attachments:

1. *Provide attachments to ensure all data used in Part C is captured, whether from study results reported in the publication and/or from additional data requested from study authors*
    - *Data from study results of the publication should be reported in Results section of Part A*
    - *Additional data provided upon request from study authors should be reported in Table C.II.1 below and original correspondence with study authors should be included as attachments*
2. *Model assessment output (including all model figures, tables, and fit metrics)*
3. *Statistical code used for curve fitting*

III. Attachments: *Include all attachments listed above after the table below.*

Additional Data Used in Response-Curve: *Provide all data used to fit dose-response curve not captured in Results section of DER above in Part A, rows as needed. First row in italicized text is an example.*

Table C.II.1 Additional Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[a] N = number of individuals per treatment; k = number of replicates per treatment level; n = number of individuals per replicate

Part D: References to Test Guidance

6. ASTM Standard E 739, 1980. 2002. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, West Conshohocken, PA.
7. Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses. PB85-227049. National Technical Information Service, Springfield, VA.
8. Mount, D.R. and J.R. Hockett. 2015. Issue summary regarding test conditions and methods for water only toxicity testing with *Hyalella azteca*. Memorandum to Kathryn Gallagher, U.S. EPA Office of Water. U.S. EPA Office of Research and Development. MED. Duluth, MN. 9 pp.
9. Bringolf, R.B., M.C. Barnhart, and W.G. Cope. 2013. Determining the appropriate duration of toxicity tests with glochidia of native freshwater mussels. Submitted to Edward Hammer. U.S. EPA. Chicago, IL, May 8, 2013. 39 pp.
10. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.
11. American Public Health Association (APHA). 2012. Standard methods for the examination of water and wastewater. Part 8000 - Toxicity. APHA. Washington, DC.