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DRAFT AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA FOR

PERFLUOROOCTANE SULFONATE (PFOS)

April 2022

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	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 (or -alkyl) sulfonic acids or Perfluorokane 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 ratios
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
webICE	Web-based Interspecies Correlation Estimation
WQS	Water Quality Standards
ww	wet weight
WWTPs	Wastewater treatment plants

NOTICES

This draft document provides information to states and tribes authorized to establish water quality standards under the Clean Water Act, to protect aquatic life from toxic effects of perfluorooctane sulfonic acid (PFOS). Under the CWA, states and tribes are to 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 criteria to reflect site-specific conditions. While this document contains the Environmental Protection Agency's (EPA) draft scientific recommendations regarding ambient concentrations of PFOS that protect aquatic life, the draft PFOS Criteria Document does not substitute for the Clean Water Act or the EPA's regulations; nor is it a regulation itself. Thus, the document when final would not impose legally binding requirements on the EPA, states, tribes, or the regulated community, and might not apply to a particular situation based upon the circumstances. The EPA intends to finalize this document in the future. This draft 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 publish water quality criteria 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 is a draft ambient water quality criteria (AWQC) document for the protection of aquatic life based upon consideration of all available information relating to effects of perfluorooctane sulfonate (PFOS) on aquatic organisms.

The term Water Quality Criteria is used in two sections of the CWA, Section 304(a)(l) and Section 303(c)(2). The term has different meanings in each section. Under CWA section 304, the term represents a non-regulatory, scientific assessment of ecological and human health effects. Criteria presented in this draft document are such a scientific assessment of ecological effects. Under CWA section 303, when water quality criteria associated with specific surface water uses are adopted by a state or authorized tribe and approved by EPA as water quality standards, they become the CWA water quality standards applicable in ambient waters within that state or authorized tribe. Water quality criteria adopted in state/tribal water quality standards could have the same numerical values as recommended criteria developed under CWA section 304. However, in some situations, states/tribes might want to adjust water quality criteria developed under CWA section 304 to reflect local water chemistry or ecological conditions. Alternatively, states and authorized tribes may develop numeric criteria based on other scientifically defensible methods that are protective of designated uses. Guidelines to assist the states and authorized tribes in modifying the criteria presented in this draft document are contained in the Water Quality Standards Handbook (U.S. EPA 2014).

This document presents draft recommendations only. It does not establish or affect legal rights or obligations. It does not establish a binding requirement and cannot be finally determinative of the issues addressed. The EPA will make decisions in any particular situation by applying the CWA and the EPA regulations on the basis of specific facts presented and scientific information then available.

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Director
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EXECUTIVE SUMMARY

U.S. Environmental Protection Agency (EPA) developed the draft recommended perfluorooctane sulfonate (PFOS) aquatic life ambient water quality criteria in accordance with the provisions of section 304(a) of the Clean Water Act (CWA). This document provides EPA's basis for and derivation of the draft national PFOS ambient water quality criteria recommendations for freshwater environments to protect aquatic life. EPA has drafted the PFOS aquatic life criteria to be consistent with methods described in EPA's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" (U.S. EPA 1985).

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 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 draft criteria to protect aquatic life in freshwater from the acute and chronic toxic effects of PFOS.

The draft Aquatic Life Ambient Water Quality Criteria for PFOS 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 three of the eight MDRs for deriving chronic estuarine/marine criteria per the 1985 Guidelines. 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 ORD's peer-reviewed webICE tool (Raimondo et al. 2010). Both the freshwater criteria and estuarine/marine benchmarks are draft recommendations for states/authorized tribes to consider as protective values in their state/tribal water quality protection programs. However, the acute estuarine/marine benchmark is less certain than the freshwater criteria since it was based on both empirical and estimated acute toxicity data (Appendix L).

The draft freshwater acute water column-based criterion magnitude is 3.0 mg/L and the draft chronic water column-based criterion magnitude is 0.0084 mg/L. The draft chronic freshwater criteria also contains tissue-based criteria with magnitudes of 6.75 mg/kg wet weight (ww) for fish whole-body, 2.91 mg/kg ww for fish muscle tissue, and 0.937 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 be protective of the freshwater aquatic community.

Table Ex-1. Draft Recommended Perfluorooctane Sulfonate (PFOS) 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	3.0 mg/L	0.0084 mg/L	0.937 mg/kg ww	6.75 mg/kg ww	2.91 mg/kg ww
Duration	1-hour average	4-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 more than once in ten years on average		

[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 derived from the chronic water column concentration (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.

Table Ex-2. Draft Recommended 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	1 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) of the CWA states that aquatic life criteria serve as recommendations to states and tribes and define ambient water concentrations that will protect against unacceptable adverse ecological effects to aquatic life resulting from exposure to pollutants found in water. Once EPA publishes final CWA section 304(a) recommended water quality criteria, states and authorized tribes may consider these criteria and may adopt them or other scientifically defensible criteria into their water quality standards (WQS) to protect the designated uses of water bodies. States and authorized tribes may also modify these criteria to reflect site-specific conditions or use other scientifically defensible methods to develop criteria before adopting these into standards. States and authorized tribes are required to submit new and revised WQS to EPA for review and approval or disapproval. When approved by EPA, the state's/tribe's WQS become the applicable WQS for CWA purposes. Such purposes include derivation of water quality-based effluent limitations in permits issued under the CWA section 402 National Pollutant Discharge Elimination System (NPDES) permit program and identification of impaired waters and establishment of Total Maximum Daily Loads (TMDLs) under CWA section 303(d). For PFOS, EPA would recommend the adoption of all criteria, including the three chronic tissue criteria, to ensure the protection of aquatic life through all exposure pathways, including direct aqueous exposure and bioaccumulation. The draft estuarine/marine benchmarks are provided in Appendix L as additional protective values that states and tribes may consider in their water quality protection programs.

This assessment provides a critical review of all aquatic toxicity data identified in 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 draft criteria to protect aquatic life in freshwater from the acute and chronic toxic effects of PFOS.

EPA derived the draft recommended criteria using the best available data to reflect the latest scientific knowledge on the toxicological effects of PFOS to aquatic life. 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 draft PFOS freshwater criteria are expected to be protective of most aquatic organisms in the community (i.e., approximately 95 percent of tested aquatic organisms representing the aquatic community) and are derived to be protective of aquatic life designated uses established by states and tribes for freshwaters. The draft 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 draft criteria presented herein are EPA's best estimate of the maximum 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. And to date, no state or tribe has submitted a Water Quality Standard with criteria for PFOS to EPA for approval. However, two states (Michigan and Minnesota) have acute and chronic protective values that were developed to be numerical translations of CWA Section 303(c) narrative water quality

criteria. And other 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 (TCEQ 2021; Giesy et al. 2010) to 0.78 mg/L in Michigan (EGLE 2010). 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).

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). These publicly available values ranged from 0.00056 mg/L in California (San Francisco Bay RWQCB 2020; SERDP 2019; 99% species protection) to 0.14 mg/L in Michigan (EGLE 2010), 0.000023 mg/L in Europe (RIVM 2010), 0.00013 mg/L in Australia/New Zealand (CRC Care 2017; EPAV 2017) 95% species protection level), and 0.00680 mg/L in Canada (ECCC 2018) (Table 1-1).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). These publicly available values ranged from 0.00056 mg/L in California (San Francisco Bay RWQCB 2020; SERDP 2019; 99% species protection) to 0.14 mg/L in Michigan (EGLE 2010),

0.000023 mg/L in Europe (RIVM 2010), 0.00013 mg/L in Australia/New Zealand (CRC CARE 2017; EPAV 2016; HEPA 2020; 95% species protection level), and 0.00680 mg/L in Canada (ECCC 2018) (Table 1-1).

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 (TCEQ 2021;CRC Care 2017) 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 (CRC Care 2017; EPAV 2017); 95% species protection).

1.1.3 Previously Published Chronic Fish Tissue Criteria

Currently 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.

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.	TCEQ (2021); Giesy et al. (2010)
Minnesota	0.085	Final Acute Value (FAV) calculated as the acute curve-fitted and extrapolated 10-d EC50 for midge (*Chironomus tentans*) of 170 µg/L. And Maximum Criterion (MC) = 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.	(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	Final Acute Value (FAV) calculated from the lowest LC50 ÷ by a safety factor of 6.1 (following US EPA Great Lakes Initiative [GLI; U.S. EPA 1995]). And the Acute Maximum Value (AMV) was 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 (LC50) of 3.6 mg/L for a mysid (*Americamysis bahia*) ÷ by 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* (McDonald 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. (2017) 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)	HC1 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.	San Francisco Bay 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.	TCEQ (2021); Giesy et al. (2010)

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 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.	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. (2017) 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. (2017) and Batley et al. (2014) and consisting of nine of 16 species-specific values as reported in CRC CARE (2017). This is a chronic surface water benchmark and does not represent a CWA Section 303(c) approved water quality standard for PFOS.	TCEQ (2021); (CRCCare 2017)
California	0.0026 (99% species protection)	HC1 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.	San Francisco Bay 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 synthetic, organic compounds that consist of a carbon backbone and a functional group, such as sulfonate or carboxylic acid (C_nF_{2n+1}-R). EPA's Office of Pollution Prevention and Toxics (OPPT) defines a PFAS as: any chemical substance or mixture that structurally contains the unit R-(CF2)-C(F)(R′)R″. Both the CF2 and CF moieties are saturated carbons. And none of the R groups (R, R′ or R″) can be hydrogen (U.S.EPA 2021b). Specifically, PFOS consists of an eight-carbon backbone and a sulfonate functional group (formula is C_8F_{17} SO_3; CAS No. 45298-90-6 for anionic form; Buck et al. 2011). The carbon chain can be fully fluorinated (perfluorinated) or partially fluorinated (polyfluorinated), and therefore, these chemicals contain the perfluoroalkyl moiety (C_nF_{2n+1}-). The carbon-fluorine (C-F) bond is strong and stable due to the strong electronegativity and small atomic size of fluorine. The chemical and thermal stability offered by the perfluoroalkyl moiety, in addition to its hydrophobic and lipophobic characteristics, make PFAS water and oil repellent, thermally stabile, and have surfactant properties. Due to these properties, PFAS have been used in a wide range of industrial and consumer products since the 1940s and 1950s, including wetting agents, lubricants, corrosion inhibitors, firefighting foams, and stain-resistant treatments to leather, paper, and clothing. The PFAS subgroup of PFOS derivatives have been used in a broad range of consumer and industrial products since the 1950s, including surface treatments for soil and stain resistance of textiles, paper, metals, pesticides, and is used in applications such as in firefighting foams (Ahrens 2011; Ahrens and Bundschuh 2014; Buck et al. 2011; Lindstrom et al. 2011).

There are many families of PFAS and each contains many individual homologues and isomers (Buck et al. 2011). These PFAS families can be divided into two primary categories: nonpolymers and polymers. The nonpolymer PFAS include perfluoroalkyl and polyfluoroalkyl

substances. Polymer PFAS include fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers (Table 1-2).

Table 1-2. Two Primary Categories of PFAS.
Modified from Buck et al. (2011).

PFAS Non-polymers:	**Structural Elements:**	**Example PFAS Families:**
Perfluoroalkyl Substances	Compounds in which all carbon-hydrogen bonds, except those on the functional group, are replaced with carbon-fluorine bonds	Perfluoroalkyl acids, perfluoroalkane sulfonamides, perfluoroalkane sulfonyl fluorides
Polyfluoroalkyl Substances	Compounds in which all carbon-hydrogen bonds on at least one carbon (but not all) are replaced with carbon-fluorine bonds	Perfluoroalkane sulfonamido derivatives, semifluorinated *n*-alkanes and alkenes
PFAS Polymers:	**Structural Elements:**	**Example PFAS Families:**
Fluoropolymers	Carbon only polymer backbone with fluorines directly attached	Polyetrafluoroethylene, polyvinylidene fluoride
Perfluoropolyethers	Carbon and oxygen polymer backbone with fluorines directly attached	$F\text{-}(C_mF_{2m}O\text{-})_nCF_3$, where the $C_mF_{2m}O$ represents $-CF_2O$, $-CF_2CF_2O$, and/or $-CF(CF_3)CF_2O$ distributed randomly along polymer backbone
Side-chain fluorinated polymers	Non-fluorinated polymer backbone with fluorinated side chains with variable composition	Fluorinated acrylate and methacrylate polymers, fluorinated urethane polymers, and fluorinated oxetane polymers

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 family PFAAs 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).

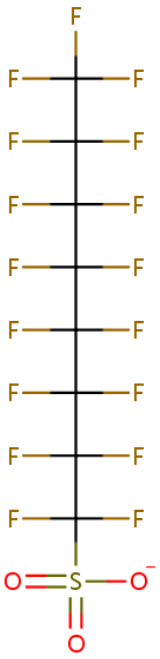

Figure 1-1. Chemical Structure of Linear Perfluorooctane Sulfonate (PFOS).
Source: United States EPA Chemistry Dashboard; https://comptox.epa.gov/dashboard

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} milligrams 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). Thus, 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 < 2.0×10^{-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 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 as 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.

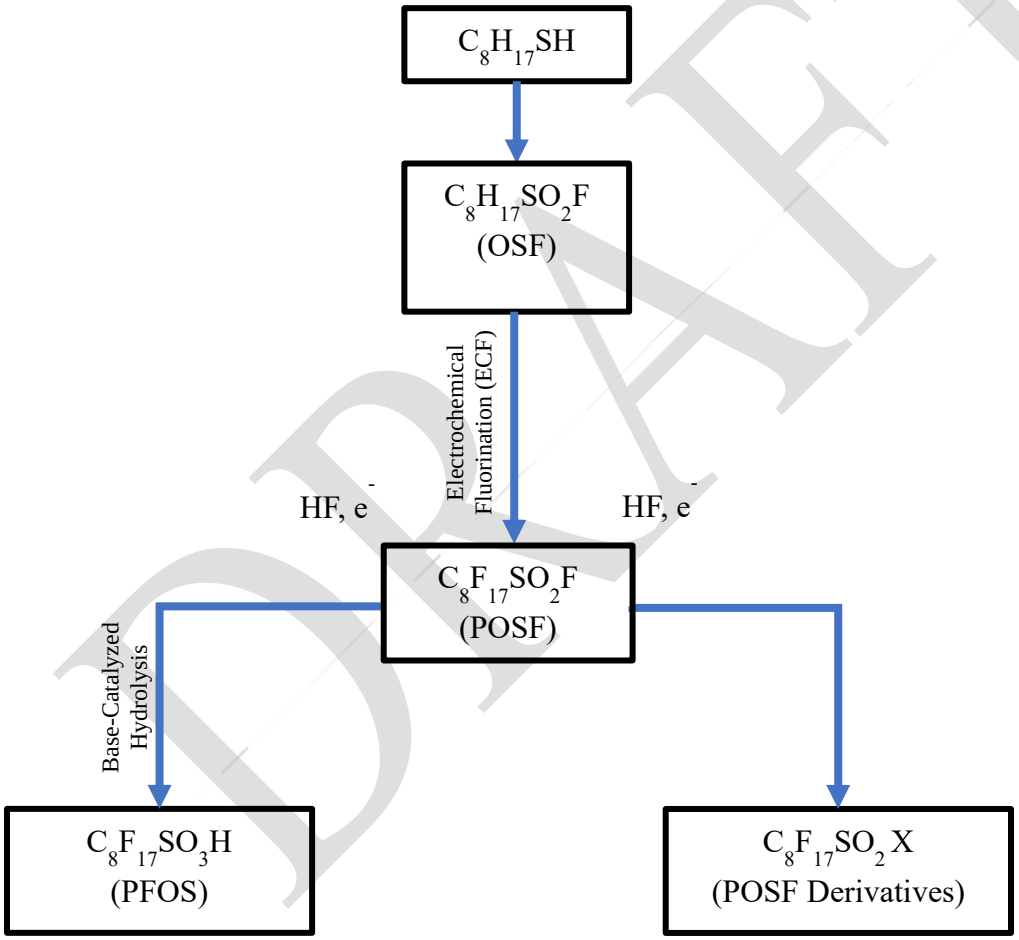

Figure 2-1. Synthesis of PFOS by electrochemical fluorination (ECF).
Modified from Buck et al. (2011).

The manufacture of PFOS started in 1949 with Minnesota Mining and Manufacturing (later name changed to the 3M Company) (3MCompany 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; 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 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. 2013b). 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 has 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, discharge of contaminated groundwater, 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 significant new use rules were put into place by EPA to restrict the production and use of PFOS and its precursors (Lindstrom et al. 2011). And generally, PFAS concentrations in the environment have been positively correlated with human population density. 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). 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). The metabolic transformation of PFAS precursors such as perfluoroalkyl sulfonamidoacetic acids (FASAAs) and the degradation of volatile PFAS such as perfluoroalkyl sulfonamidoethanols (FASEs), are known to degrade to

PFOS (Ahrens and Bundschuh 2014; Benskin et al. 2009; Boulanger et al. 2005; Buck et al. 2011; Lange 2000; Liu and Mejia Avendano 2013; Plumlee et al. 2008; Rhoads et al. 2008; Wang et al. 2017). However, 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; 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) will 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) 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. 2006; 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 2.4; 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. 2005; Hatfield 2001; Lange 2000; Plumlee et al. 2009; Rhoads et al. 2008). EtFOSE was a product of electrochemical fluorination and was a precursor compound for the synthesis of other products such as phosphate esters that were used to manufacture paper protectors (3MCompany 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. 2005; 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). Rhoades 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. 2005; 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).

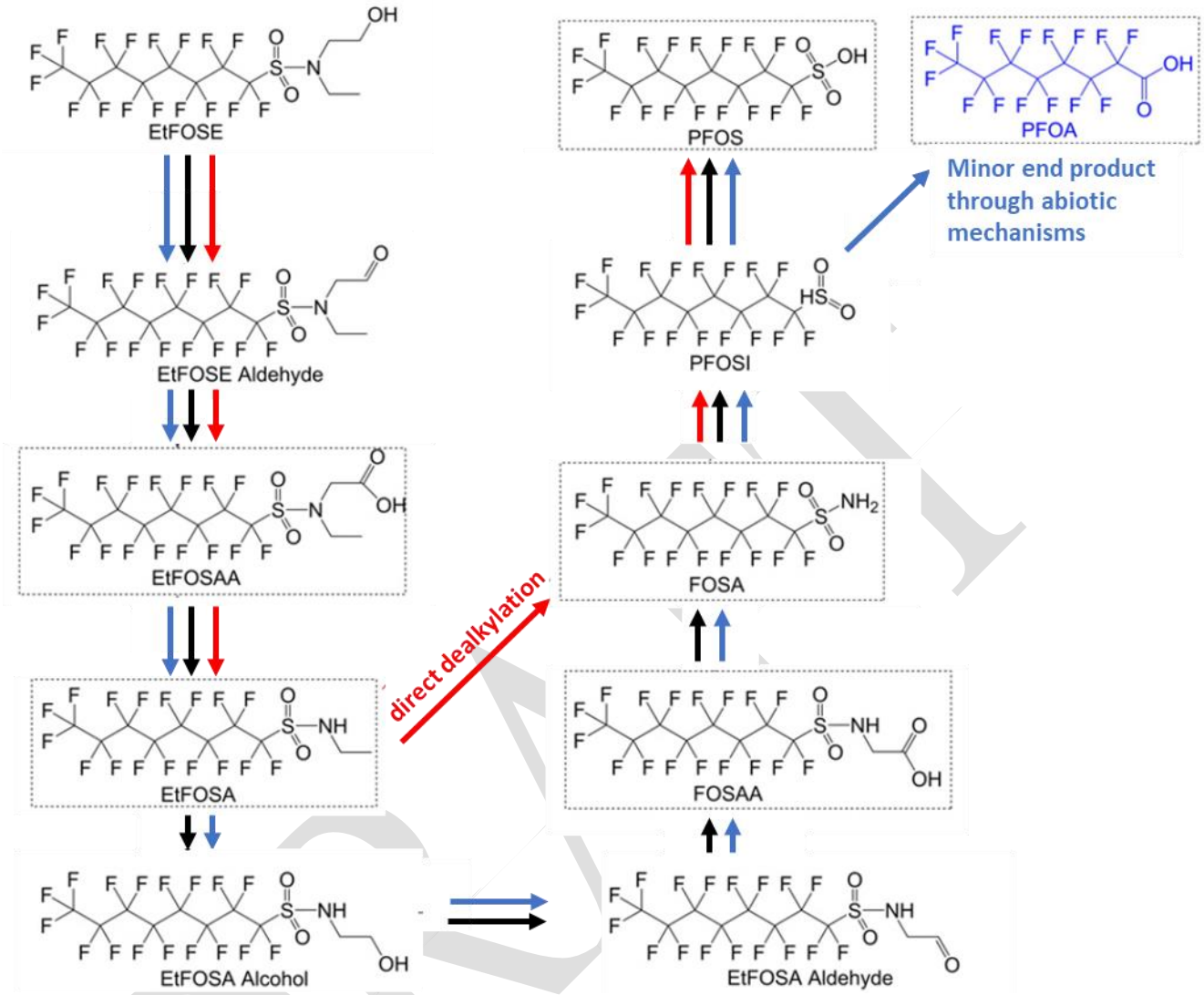

Figure 2-2. Aerobic Biodegradation of EtFOSE in Activated Sludge.
Black arrows show the Aerobic Biodegradation pathway as described by Liu and Mejia Avendano (2013). Blue pathway was observed by Lange (2000). Red pathway was observed by (Rhoads et al. 2008) Semi-stable compounds are shown inside boxes.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

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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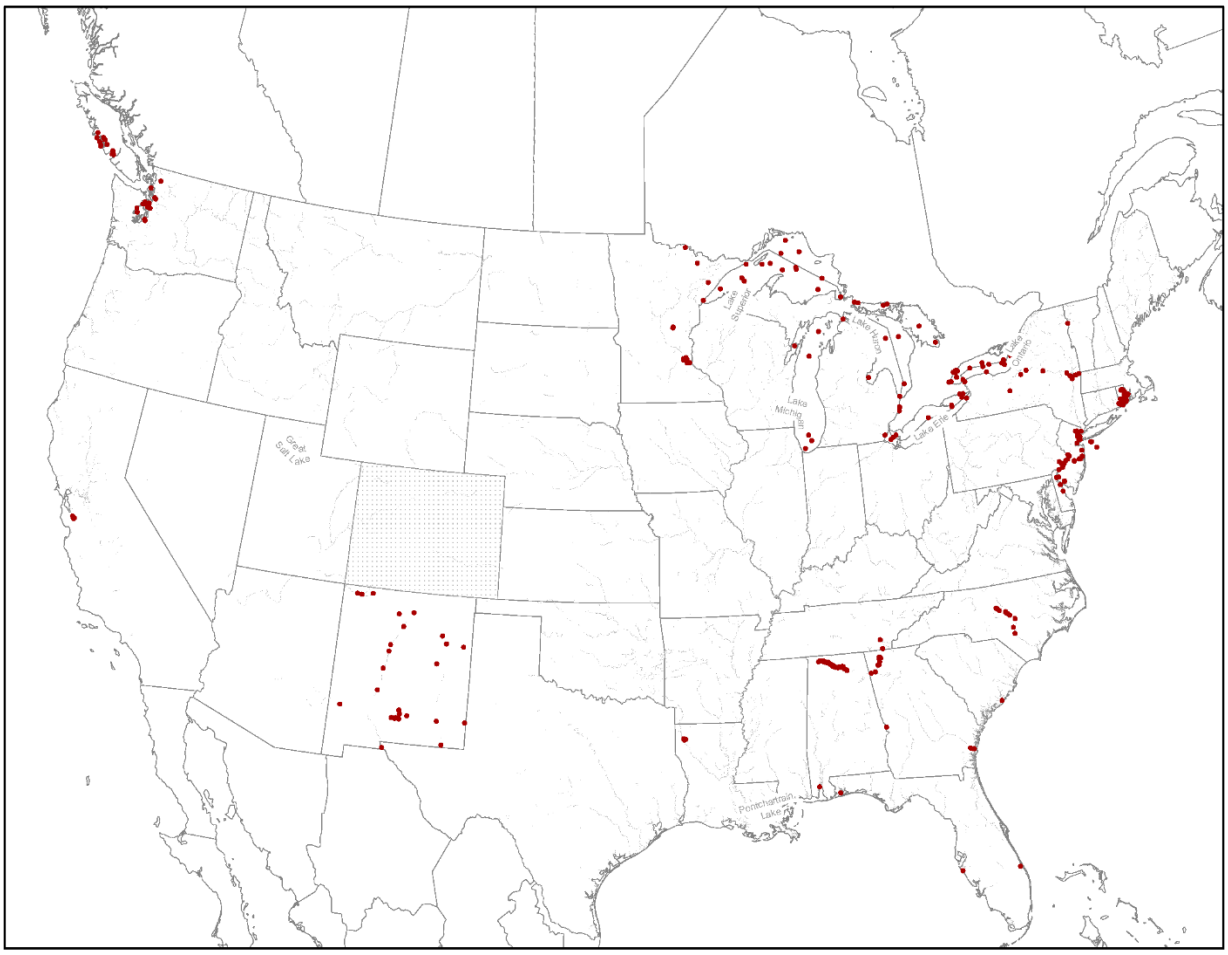

Figure 2-3. Map Indicating Sampling Locations for Perfluorooctane Sulfonate (PFOS) Measured in Surface Waters across the United States (U.S.).

Based on data reported in the current, publicly available literature. Sampling locations for the Colorado data were not available and these data are represented by the dash marks to indicate measured PFOS surface water concentrations are available. Detailed information on sampling locations, including references, coordinates, and sampling site identification numbers and names, 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 and are generally detected between picogram and nanogram per liter 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 (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).

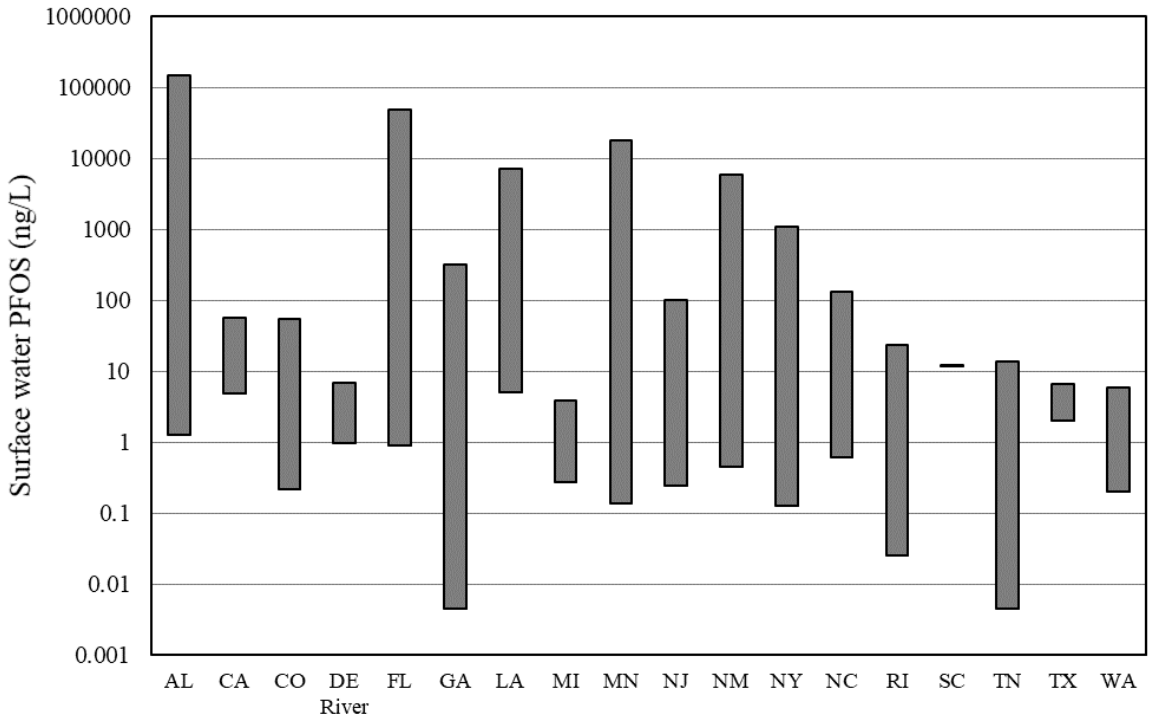

Figure 2-4. Distribution of the Minimum and Maximum Concentrations (ng/L) of Perfluorooctane Sulfonate Measured in Surface Waters for Each State or Waterbody (excluding the Great Lakes) with Reported Data in the Publicly Available Literature.
The distribution is arranged alphabetically by state and waterbody. 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, 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 waters across the U.S. remains very limited. More recent sampling efforts indicate that PFOS

[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)

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, is needed to better understand PFOS occurrence in aquatic ecosystems across the U.S. (Jarvis et al. 2021). See Appendix N for further discussion of PFOA 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 2.3.3; Haukas et al. 2007; Houde et al.

2006). 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 (Martin et al. 2003a; Martin et al. 2003b). 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, 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 2.3 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. 2014; 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. 2006; 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 2.4; 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. 2006). 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. 2006).

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) 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 studies of PFOS concentrations included fish muscle tissue data from 164 urban river sites (5^{th} order or higher) across the conterminous U.S. selected following a probabilistic design, as 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 (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 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 at unimpacted sites to 2,000 ng/g 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 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. (2006) 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 in pinfish to 92 ng/g in spot. In Sarasota Bay, PFOS concentrations averaged 0.2 ng/g in zooplankton, and ranged from 3.1 ng/g in pigfish to 8.8 ng/g 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, indicating the importance of sediments as a route of exposure into the base of the food web (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. 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. 2013). 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 the diets of 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 2.2.1, 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

Appendices A through 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). 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; 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 Section 3.1.1.3.4).

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 is 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; Ahrens 2011; Ahrens and Bundschuh 2014; Giesy and Kannan 2002; Houde et al. 2006; Keiter et al. 2012; Wang et al. 2017). Although 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. Findings of the studies 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 aegyptii*; 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. For example, in a single *in vivo* exposure of zebrafish (*D. rerio*) embryos, synergism, additivity and antagonism were all reported for different

combinations/ratios of PFOS and PFOA and endpoints (Ding et al. 2013), illustrating the complexity and uncertainty associated with mixture studies. Importantly, neither the concentration addition model nor the independent-action model could predict the combined effects when strong interactive effects existed. More recently, 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 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. 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), a mosquito (*Aedes aegyptii*; Olson 2017), a bioluminescent cyanobacterium (*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. For example, in a single *in vivo* exposure of zebrafish (*D. rerio*) embryos, synergism, additivity and antagonism were all reported for different combinations/ratios of PFOS and PFOA and endpoints (Ding et al. 2013), illustrating the complexity and uncertainty associated with mixture studies. Importantly, neither the concentration addition model nor the independent-action model could predict the combined effects when strong interactive effects existed. More recently, 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 (2021, unpublished) 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 and 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.

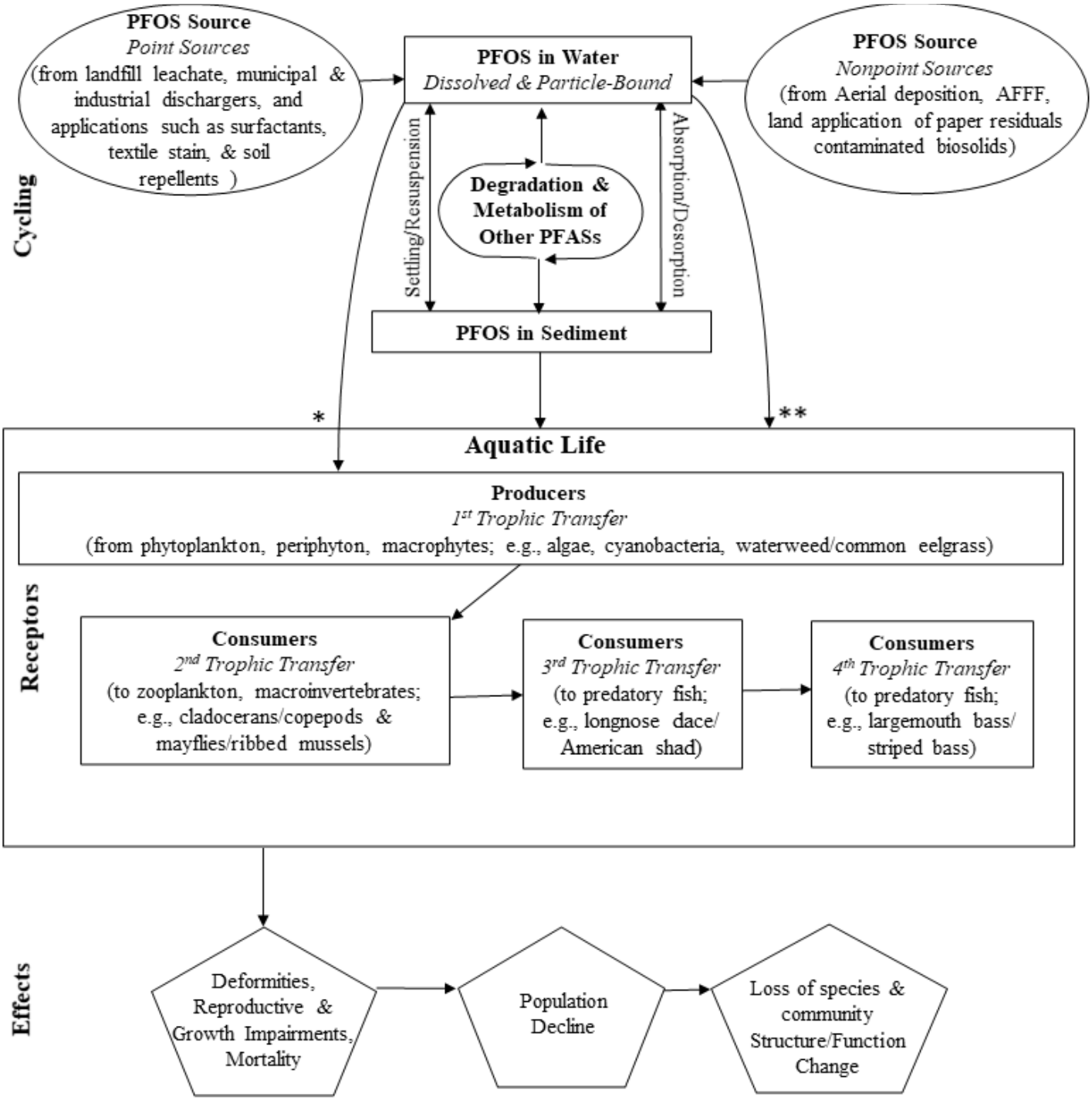

Figure 2-5. Conceptual Model Diagram of Sources, Compartmental Partitioning, and Trophic Transfer Pathways of Perfluorooctane Sulfonate (PFOS) in the Aquatic Environment and its Bioaccumulation and Effects in Aquatic Life.

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; 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, 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. Available data for aquatic plants and algae were reviewed to determine if they were more sensitive to PFOS than aquatic animals (see Appendices A, C and E for freshwater species).

2.10.2 Measure of PFOS Exposure Concentrations

This PFOS aquatic life ambient water quality criteria document provides a critical review of all data identified in 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)

Based on EPA's data review, PFOS toxicity studies typically used the linear PFOS isomer for dosing with fewer studies using the branched isomer. Studies that conducted PFOS-only exposures were considered for possible inclusion. For most 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 this 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 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 4.4).

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 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 the Office of Water. 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 2016c), 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 R 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 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 4.3). These supporting analyses provided an additional line-of-evidence that further suggested it is appropriate to consider nonresident 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. Therefore, EPA examined whether nominal and measured concentrations for PFOS are typically in close agreement with each other. Among the PFOS studies that were used quantitatively (Sections 3.1.1.1 and 3.1.1.3 and Appendices A.1 and C.1) and qualitatively (Section 4.4 and Appendix G) in the freshwater water column-based criteria, 65 freshwater studies had measured concentrations, yielding 477 pairs of measured and nominal concentrations (excluding controls, where PFOS was rarely detected). Furthermore, there were 20 estuarine/marine studies with measured concentrations, yielding 171 pairs of measured and nominal concentrations. The data were grouped by classifications including water type (salt/fresh) and experimental conditions (acute/chronic; solvent/no solvent; fed/unfed, etc.). Data

displayed a high degree of linear correlation, and the measured and nominal concentrations were in close agreement. Details of this meta-analysis can be found below in Appendix O.

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 media (e.g., 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. 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 if 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, U.S. EPA 2016c) when available. For example, 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 and to relate 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 (or immobilize) 50 percent of the test organisms. EC_{50} is the concentration of a chemical that is estimated to produce a specific effect 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). 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 2016a), 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 MSt.J. 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 2. NOEC and LOEC 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 EPA. Occasionally, individual replicate-level data or treatment-level data needed to be obtained 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 concentration-response 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 study summary below and were utilized to derive this criteria for aquatic life, where available (for acute criterion in Table 3-9 and chronic criterion in Table 3-10).

2.11 Analysis Plan

2.11.1 Derivation of Water Column Criteria

During CWA section 304(a) criteria development, 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 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, with the exception that the acute MDR for an insect was not fulfilled with quantitatively acceptable data (Appendix A). Therefore, qualitatively acceptable acute insect data (Appendix G) were evaluated relative to the sensitivities of other species/MDRs. EPA will continue to seek and evaluate acute PFOS insect data to further evaluate the sensitivity of aquatic insects. Acute and chronic MDRs for PFOS estuarine/marine criteria derivation were not met. Consequently, EPA used the available toxicity data and a New Approach Method (NAM) 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 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). 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 2016a). 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 4.7). 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, EPA examined 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 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 from 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 2016a), a BAF is an expression of all exposure routes, i.e., dietary, water, maternal transfer, and contact with 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 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

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

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 Burkhard (2021).

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 P). 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) – Draft Manuscript.

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	Collocated 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 EPA, Office of Water 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 September 2021.

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

Quantitative data for acute PFOS toxicity were available for 26 freshwater species, representing 18 genera and 15 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 16 freshwater species, representing 14 genera and 13 families in four phyla, and three estuarine/marine species, representing three genera and three families in two 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	4	2	3
Benthic Crustacean	2	2	1	1
Insect	0[a]	0[a]	2	2
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	**18**	**26**	**14**	**16**
MDR	**Saltwater[b]**			
	GMAV	**SMAV**	**GMCV**	**SMCV**
Family in the phylum Chordata	1	1	0	0
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	0	0
Any other family	0	0	0	0
Total	**6**	**6**	**3**	**3**

[a] One MDR, for aquatic insects, was not fulfilled with acute quantitative data. However, EPA considered acute qualitative data for insects, as discussed below, and concluded that there were sufficient data to conclude that not having met the MDR would not substantively affect the resulting FAV to develop an acute freshwater criterion.

[b] 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, EPA cannot derive an estuarine/marine acute criterion, based on the 1985 Guidelines. However, EPA has developed draft estuarine/marine benchmarks through use of surrogate data to fill in missing MDRs using EPA's Web-based inter-species correlation estimation 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 summarized in Appendices E, F, G and H while the remaining, unused studies are summarized in Appendix J.

Acute and chronic values were presented as reported by the study authors for each individual study. 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 EPA's independently-calculated values (where available) were included for each study throughout the document (in the study summaries and appendices as applicable), and the specific value utilized to derive the criteria were identified along with a justification. 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

Acceptable data on the acute effects of PFOS in freshwater were available for a total of 26 species representing 18 genera and 15 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), 13 freshwater invertebrate species (four 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 (5 most sensitive genera) and Table 3-3 (all genera) and plotted in Figure 3-1. The aquatic insect MDR was not fulfilled with quantitatively acceptable acute data; however, qualitatively acceptable acute insect data (as discussed below) were evaluated and these data demonstrate the variability in the sensitivity of aquatic insects. Therefore, EPA will continue to seek additional acute PFOS insect data to further understand the sensitivity of this taxon. Additionally, EPA evaluated the effect of the current qualitative studies on the Final Acute Value (FAV) in Section 4.2.1. Thus, the current development of an acute freshwater criterion was based on seven of the eight MDRs.

Table 3-2. The Five Most Sensitive Genera Used in Calculating the Acute Freshwater Criterion (Sensitivity Rank 1-5).

Ranked below from most to least sensitive. Note: five genera are shown with the intent to discuss sensitive genera to the point where the four most sensitive North American resident species are represented. The fourth most sensitive genus, *Neocaridina*, is based on toxicity data from a non-North American resident.

Rank	Genus	Species	GMAV (mg/L)[1]	Comment
1	*Pimephales*	Fathead minnow, *P. promelas*	6.950	North American resident species
2	*Oncorhynchus*	Rainbow trout, *O. mykiss*	7.515	North American resident species
3	*Ligumia*	Black sandshell, *L. recta*	13.5	North American resident species
4	*Neocaridina*	Japanese swamp shrimp, *N. denticulata*	15.61	Not a resident species in North America[2]
5	*Xenopus*	African clawed frog, *X. laevis*	15.99	North American resident species[3]

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

[2] Species not resident to North America were included since the relative sensitivities between native and non-native species were similar. Therefore, for the PFOS criteria derivation, it was determined that species not native to North American can serve as surrogates for other sensitive resident North American organisms.

[3] Not native to North America; however, is considered a resident to North America in the 1985 Guidelines (U.S.EPA 1985).

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

Drottar and Krueger (2000d) 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 a LC_{50} of 9.5 mg/L PFOS. EPA's independently-calculated 96-hour LC_{50} was 9.020 mg/L and was used quantitatively to derive the draft acute water column criterion for freshwater.

(3MCompany 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% 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 is deemed acceptable for quantitative use in the derivation of the acute freshwater criterion for PFOS.

The geometric mean of the two acute toxicity values provided 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 most sensitive GMAV in EPA's acute dataset used to derive the freshwater aquatic life criterion.

3.1.1.1.2 Second 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. 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 calculate the SMAV and GMAV for derivation of the draft acute water column criterion.

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 calculate the SMAV and GMAV for derivation of the draft acute water column criterion.

The geometric mean of the two toxicity values provided 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*, which was used to derive the freshwater aquatic life criterion. This 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).

3.1.1.1.3 Third 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 3rd and 6th most sensitive genus values (respectively) in the PFOS freshwater acute toxicity database (The *L. siliquoidea* results are reported in Appendix A). Acute toxicity was observed under static conditions over a 24-hour period (< 24-hour old glochidia) or a 96-hour period (4 to 6-week-old juveniles). The tests followed the ASTM (2006) test method. The 24-hour EC_{50} reported by the study authors for glochidia of *L. recta* was 13.5 mg/L. The 96-hour LC_{50} reported by the study authors for juvenile *L. recta* was 141.7 mg/L. Both acute values are

acceptable for quantitative use but because the juvenile life stage was less sensitive, only the glochidia LC_{50} was used to calculate the SMAV. The independently calculated toxicity values could not be calculated at this time given the lack of data presented in the paper. No other quantitative toxicity values were available for this species or genus. Therefore, the author-reported 24-hour EC_{50} of 13.5 mg/L for the glochidia life stage of *L. recta* served directly as the SMAV and GMAV which are utilized to derive the freshwater aquatic life acute criterion.

3.1.1.1.4 Fourth Most Sensitive Freshwater Genus for Acute Toxicity: Neocaridina (shrimp)

Li (2009) conducted three independent repeats of a 96-hour static test on PFOS-K (potassium salt, >98% purity) with the freshwater shrimp species, *Neocaridina denticulata* (a non-North American species). The author-reported 96-hour LC_{50} was 10 mg/L based on the average of three repeat tests. The independently-calculated LC_{50} values for the three independent experimental repeats were 12.91, 28.55, 10.32 mg/L, respectively. These independently-calculated LC_{50} values were used to calculate the geometric mean of 15.61 mg/L. No other quantitative toxicity values were available for this species or genus; therefore, the geometric mean of the three independently-calculated 96-hour LC_{50}s of 15.61 mg/L served directly as the SMAV and GMAV utilized in the acute water column criterion.

3.1.1.1.5 Fifth Most Sensitive Freshwater Genus for Acute Toxicity: Xenopus (frog)

Palmer and Krueger (2001) conducted three independent, renewal assays with *Xenopus laevis.* The author-reported 96-hour LC_{50} values were 13.8, 17.6 and 15.3 mg/L PFOS, the teratogenesis EC_{50}s were 12.1, 17.6 and 16.8 mg/L PFOS, and the minimum concentrations to inhibit growth values (effectively LOECs) were > 14.7, 7.97 and 8.26 mg/L for the same three tests, respectively. LC_{50} values for teratogenesis and mortality were similar, suggesting there was no apparent difference in endpoint sensitivity. Mortality was used to derive the *X. laevis* SMAV

since it is the more established endpoint for deriving acute criteria (U.S. EPA 1985). The independently-calculated 96-hour mortality-based LC_{50} values were 15.53, 18.04, and 14.60 mg/L, which were taken together as a geometric mean to calculate the *X. laevis* SMAV of 15.99 mg/L. No additional quantitative, acute toxicity data were available for other members of this genus. Therefore, the *X. laevis* SMAV of 15.99 mg/L served directly as the *Xenopus* GMAV.

3.1.1.1.6 Missing Insect MDR

The PFOS acute dataset based on direct aqueous exposures contains 18 genera (Table 3-3) representing seven of the eight MDRs. The missing MDR is a representative from an insect family. As the derivation of a PFOS acute freshwater criterion is important for the protection of aquatic life exposed to PFOS, EPA considered qualitative data (see Appendix G) to determine if the relative sensitivity of aquatic insects could be ascertained. There were qualitative data from two acute studies focused on aquatic insects. First, Yang et al. (2014) conducted a 96-hour renewal, acute test with measured concentrations on the midge, *Chironomus plumosus*. Second, Olson (2017) conducted a 40-day static test with unmeasured concentrations on the yellow fever mosquito (*Aedes aegypti*) and reported a 48-hour LC_{50} value. Yang et al. (2014) was classified as acceptable for qualitative use because the test organisms were considered to be from a problematic source since the test organisms were obtained from the Beijing City Big Forest Flower Market and no further quantification of previous exposure to contaminants or husbandry was provided (Yang et al. 2014). The reported PFOS LC_{50} was 182 mg/L (see Appendix Section G.2.1.5), indicating that insects, as represented by the midge in this test, is one of the least sensitive taxonomic groups to acute exposures of PFOS (Figure 3-1). However, as previously mentioned the source of the test organisms was problematic and it is difficult to ascertain the role

any potential previous exposure or husbandry issues might have played in the results of this toxicity study and the relative sensitivity of chironomids to acute exposures of PFOS.

In contrast, qualitative data from Olson (2017) on the yellow fever mosquito, an invasive species to the U.S., indicated that this species is relatively sensitive to acute exposures of PFOS. A LC_{50} of 1.18 mg/L was reported following 48 hours of exposure in this 40-day, static test with unmeasured test concentrations (Olson 2017; see Appendix G.2.1.5). This study was not acceptable for quantitative use primarily because the test duration was too short for the species and secondarily because the test organism is an invasive, pest species. However, the reported PFOS 48-hour LC_{50} was 1.18 mg/L, which indicated that this species of insect may be one of the most sensitive species to acute exposures of PFOS (Figure 3-1).

These two qualitatively acceptable studies demonstrated the variability in the sensitivity and indicated contrasting sensitivity of aquatic insects to acute exposures of PFOS. Therefore, EPA will continue to seek additional acute PFOS insect data to further understand the sensitivity of this taxon. Additionally, EPA evaluated the effect of the current qualitative studies on the Final Acute Value (FAV) in Section 4.2.1. As such additional insect toxicity data for PFOS are needed to further examine the relative sensitivity of insects to PFOS exposures. And the current development of an acute freshwater criterion was based on seven of the eight MDRs.

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	6.950	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	6.950
2	7.515	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	7.515
3	13.50	G	*Ligumia*	Black sandshell, *Ligumia recta*	13.50
4	15.61	E	*Neocaridina*	Japanese swamp shrimp, *Neocaridina denticulata*	15.61
5	15.99	C	*Xenopus*	African clawed frog, *Xenopus laevis*	15.99
6	16.50	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	16.50
7	17.20	D	*Moina*	Cladoceran, *Moina macrocopa*	17.20
8	19.88	C	*Hyla*	Gray treefrog, *Hyla versicolor*	19.88
9	22.48	G	*Dugesia*	Planaria, *Dugesia japonica*	22.48
10	24.44	B	*Danio*	Zebrafish, *Danio rerio*	24.44
11	42.30	D	*Daphnia*	Cladoceran, *Daphnia carinata*	11.56
				Cladoceran, *Daphnia magna*	48.87
				Cladoceran, *Daphnia pulicaria*	134.0
12	47.40	C	*Ambystoma*	Jefferson salamander, *Ambystoma jeffersonianum*	51.71
				Small-mouthed salamander, *Ambystoma texanum*	30.00
				Eastern tiger salamander, *Ambystoma tigrinum*	68.63
13	56.49	C	*Anaxyrus*	American toad, *Anaxyrus americanus*	56.49
14	59.87	E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	59.87
15	61.80	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	61.80
16	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)
17	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
18	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.

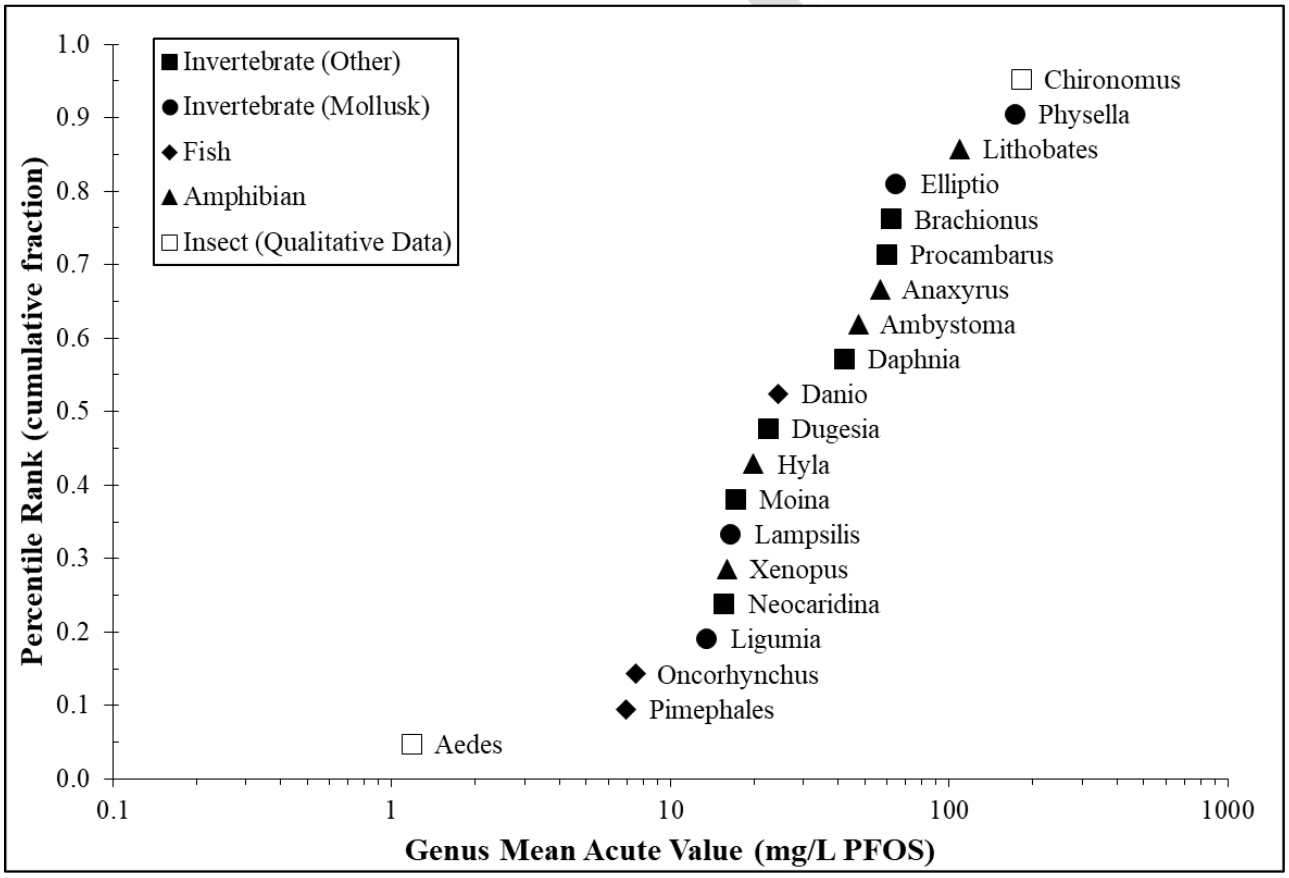

Figure 3-1. Freshwater Acute PFOS GMAVs Fulfilling the Acute MDRs.
Qualitative data for insect species taken into consideration to understand the aquatic insect MDR are denoted by the open white boxes. The GMAVs for these qualitative data were not used to derive the freshwater acute criterion for PFOS.

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 only six genera and five families. The data available for saltwater species fulfilled only five of the eight MDRs. In the interest of providing recommendations to states/authorized tribes on protective values, EPA developed an estuarine/marine acute benchmark using the available empirical data supplemented with toxicity values generated through the use of new approach methods, 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)	Comments
1	*Mytilus*	Mediterranean mussel, *M. galloprovincialis*	1.1	Not a resident species in North America, but other species in this genus are resident, commercially, or ecologically important species
2	*Strongylocentrotus*	Purple sea urchin, *S. purpuratus*	1.7	North American resident species
3	*Paracentrotus*	Sea urchin, *P. lividus*	1.795	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.
4	*Americamysis*	Mysid, *A. bahia*	4.914	North American resident species

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*. 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 controls for a percentage of normal D-shell stage larvae, > 75% (ASTM 2004a). 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 (PFOS potassium salt, CAS # 2795-39-3, 98% purity) on *Mytilus galloprovincialis*. Authors noted that tests followed (U.S.EPA 1995) and (ASTM 2004b) 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, is 1.1 mg/L. 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 that latter (1.1 mg/L) serves as the basis for the SMAV and GMAV 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 USEPA (1995) and ASTM (2004b) 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, is 1.7 mg/L. 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 taken, given 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 USEPA (1995, 2002) and ASTM (2004b) 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 urchins, *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.

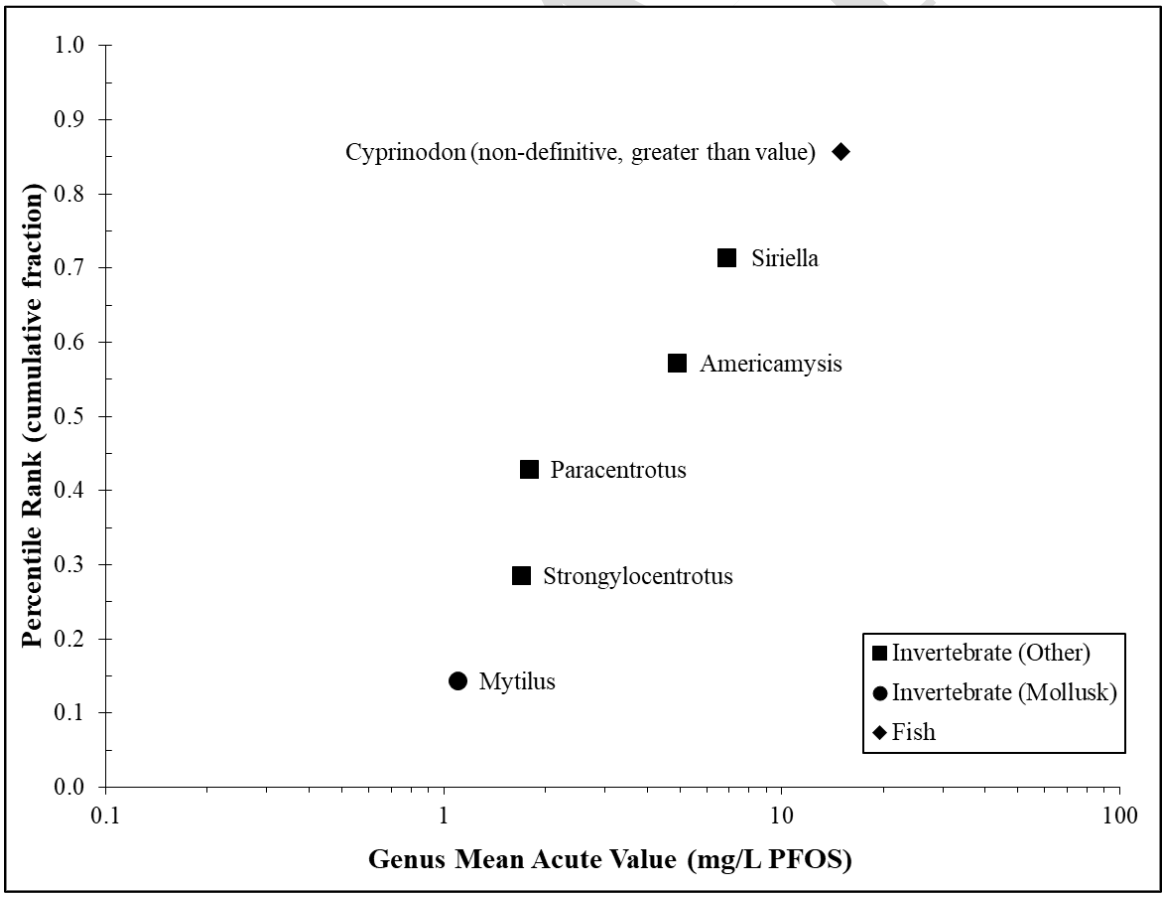

Figure 3-2. Acceptable Estuarine/Marine GMAVs.

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 16 freshwater species, representing 14 genera and 13 families in four phyla. These data included four freshwater fish species, representing four genera and three families. The data available for freshwater fish were relatively diverse and fulfilled three of the eight MDRs. Chronic data were also available for nine freshwater invertebrate species, representing eight genera and eight families. The data available for freshwater invertebrate were also relatively diverse and fulfilled five of the eight MDRs. The chronic dataset also includes data for three amphibian species, representing two genera in two families. Ranked GMCVs for PFOS in freshwater based on chronic toxicity were identified 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]	Comments
1	*Chironomus*	Midge, *Chironomus dilutus*	0.009676	North American resident species
2	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	0.01768	North American resident species
3	*Enallagma*	Blue damselfly, *Enallagma cyathigerum*	0.03162	North American resident species
4	*Danio*	Zebrafish, *Danio rerio*	0.03217	Uncertain as resident species of North America.[2] *D. rerio* is a common ecotoxicity test species that serves as a surrogate for untested fish species residing in North America.

[1]Values used in additional analyses supporting the criterion calculation to examine the effects of less certain toxicity studies and non-resident species on chronic freshwater criterion. See Section 4.1 below for more details.
[2] Not native to North America; however, is considered a resident to North America in the 1985 Guidelines.

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

(MacDonald et al. 2004) conducted chronic larval and 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). **(MacDonald et al. 2004)** conducted chronic larval and 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. 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 PFOS draft criteria. 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 acceptable for quantitative use in the derivation of the freshwater chronic water column criterion for PFOS.

McCarthy et al. (2021) also conducted two chronic toxicity tests with PFOS (98% purity) on the midge, *Chironomus dilutus*, a 10-day and a 20-day exposure, following standard protocols (ASTM 2005; 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 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 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. EPA was unable to independently calculate EC_{10}s for survival and mean weight. However, 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 acceptable for quantitative use in the derivation of 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.2.1) 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). Although over an order of magnitude different, both the EC_{10} of 0.05896 mg/L for 10-day growth and EC_{10} of 0.0015879 mg/L for 16-day mean biomass were used quantitatively to derive the chronic aquatic life criterion with a SMCV and GMCV equal to the geometric mean of the two values or 0.009676 mg/L. As mentioned in the Bots et al. (2010) summary and in Section 4.1.1, 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) and McCarthy et al. (2021). 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.

3.1.1.3.2 Second 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. The *in marsupia* exposure was followed by a 24-hour free glochidia exposure consisting of 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.2.2 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.2.2). 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 *Lampsilis siliquoidea.* Additionally, as there were only two PFOS treatment groups and the gap in these exposure concentrations is large (about 15-fold), 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 ratio of $EC_{10}/EC_{35.4}$ levels from that study, which was: $EC_{10}/EC_{35.4}$ = 0.0033/0.0186 = 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. This MATC is currently used quantitatively to derive the draft chronic water column criterion, and 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 PFOS dataset. No other quantitative, chronic toxicity values were available for this species or genus; therefore, the MATC of 0.01768 mg/L served directly as the SMCV and GMCV used for deriving the chronic aquatic life criterion for PFOS.

3.1.1.3.3 Third 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, 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 4.2.2 below).

3.1.1.3.4 Fourth Most Sensitive Freshwater Genus for Chronic Toxicity: Danio (zebrafish)

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. Following the receipt of treatment level data from the study authors, EPA independently-calculated an EC_{10} value of 0.01650 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 4.4.2.1.4 and Appendix G). Thus, this study and the EC_{10}

value for F1 survival was used quantitatively in the derivation of the aquatic life chronic criterion.

Guo et al. (2019) evaluated the chronic effects of PFOS of unknown form and purity to AB strain zebrafish (*Danio rerio*) males in a 21-day static-renewal, unmeasured study. Growth as weight was the most sensitive endpoint at 21 days, with an author-reported NOEC and LOEC of 0.02 and 0.04 mg/L PFOS, respectively. Although fish weight was the most sensitive endpoint identified by the study authors, EPA was only able to independently calculate and EC_{10} based on mean body length. Therefore, EPA's independently-calculated EC_{10} for the test based on mean body length (in cm) at 21 days is 0.06274 mg/L PFOS and was used quantitatively to derive the draft chronic water column criterion for freshwater.

Given the wide (10x) spacing of the treatment concentrations in the full life-cycle test by Wang et al. (2011) which creates some uncertainty with regards to deriving a more definitive chronic point estimate for the species, EPA is using the two independently-calculated EC_{10} values from both studies above (0.01650 and 0.06274 mg/L) to calculate the SMCV and GMCV for *D. rerio*. The geometric mean of the two EC_{10} values is 0.03217 mg/L, which was used to derive the freshwater chronic aquatic life criterion.

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	0.009676	F	*Chironomus*	Midge, *Chironomus dilutus*	0.009676
2	0.01768	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	0.01768
3	0.03162	F	*Enallagma*	Blue damselfly, *Enallagma cyathigerum*	0.03162
4	0.03217	B	*Danio*	Zebrafish, *Danio rerio*	0.03217

Rank[a]	GMCV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMCV[b] (mg/L PFOS)
5	0.06329	D	*Daphnia*	Cladoceran, *Daphnia carinata*	0.003162
				Cladoceran, *Daphnia magna*	1.267
6	> 0.1	A	*Salmo*	Atlantic salmon, *Salmo salar*	> 0.1
7	0.1555	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	0.1555
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	0.8872	C	*Xenopus*	African clawed frog, *Xenopus laevis*	> 1
				Clawed frog, *Xenopus tropicalis*	0.7871
13	1.316	C	*Lithobates*	Northern leopard frog, *Lithobates pipiens*	1.316
14	8.831	G	*Physella*	Snail, *Physella heterostropha pomilia*	8.831

[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.

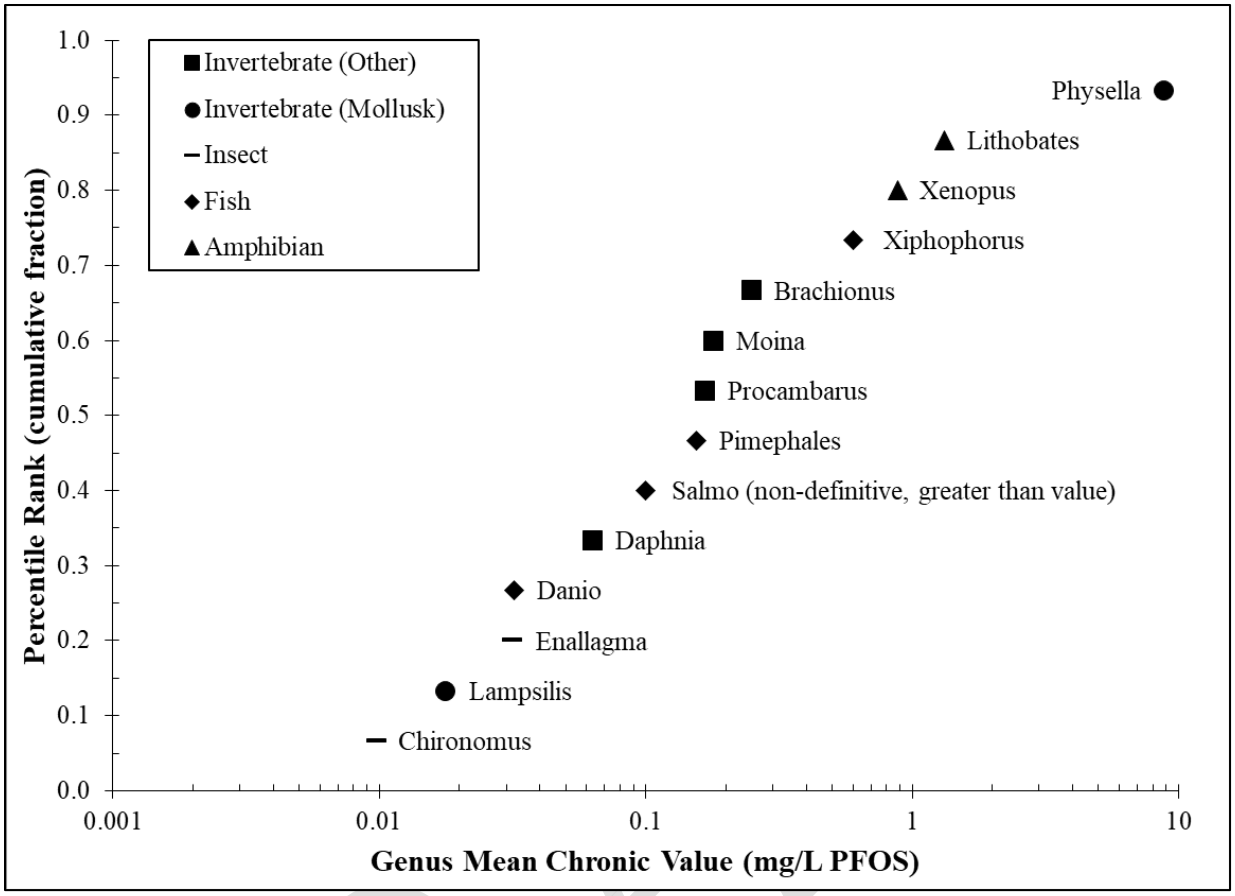

Figure 3-3. Ranked Freshwater Chronic PFOS Used Quantitatively to Derive the Criterion.

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 three saltwater invertebrate species, representing three genera and three families. The data available for saltwater fish fulfilled only three of the eight MDRs.

Table 3-8. The Three 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	*Americamysis*	Mysid, *Americamysis bahia*	0.3708	North American resident species
3	*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. 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 a marine/estuarine aquatic life dataset.

3.1.1.4.2 Second 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 *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.

3.1.1.4.3 Third 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. 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). 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 toxicity dataset.

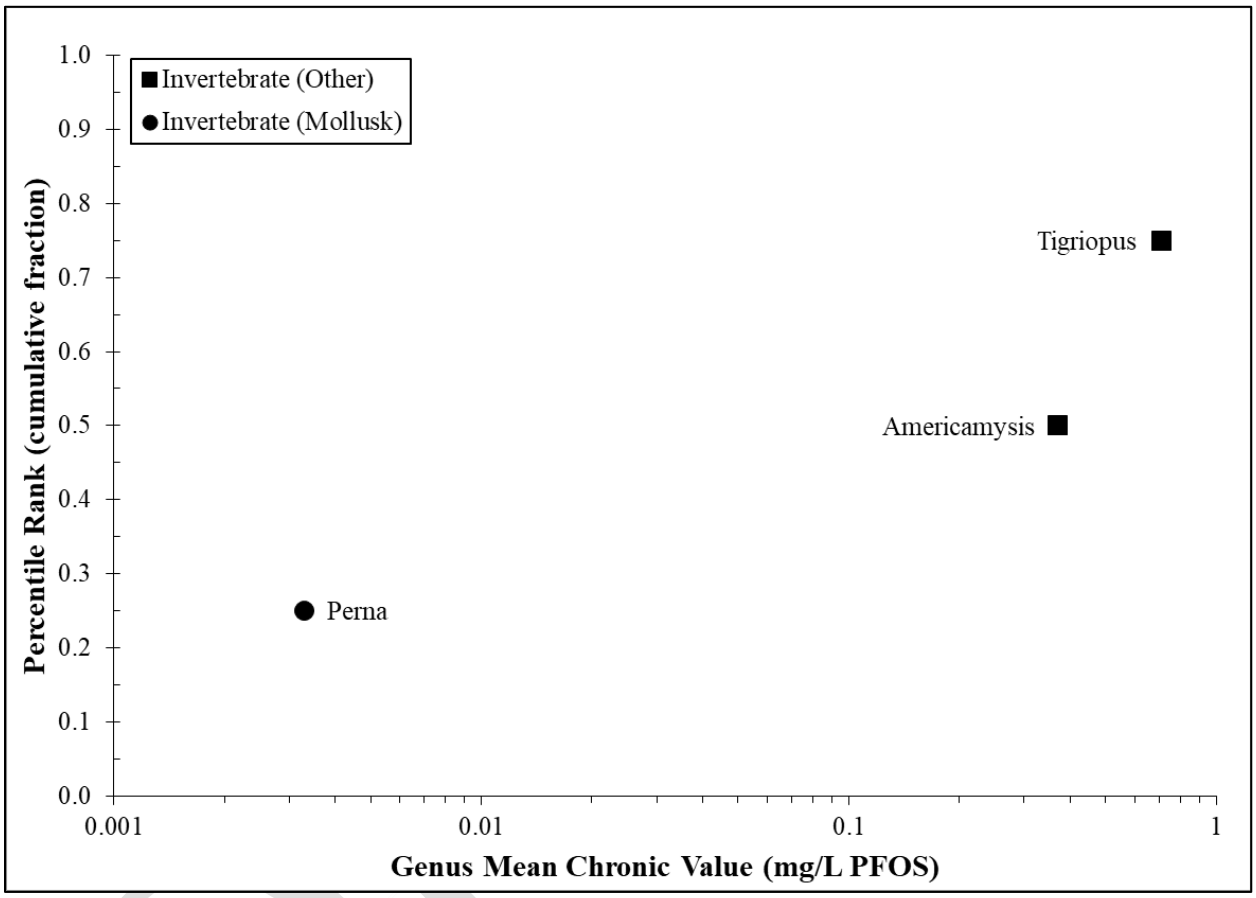

Figure 3-4. Acceptable Estuarine/Marine GMCVs.

3.2 Derivation of the PFOS Aquatic Life Criteria

3.2.1 Derivation of Water Criteria for Direct Aqueous Exposure

3.2.1.1 Derivation of Acute Water Criterion for Freshwater

The PFOS acute dataset for freshwater based on direct aqueous exposures contained 18 genera (Table 3-3) representing seven of the eight MDRs. The missing MDR was a representative from an insect family. Details on the two qualitative aquatic insect toxicity tests were given above (Section 3.1.1.1.6). These two qualitatively acceptable studies demonstrated

the variability in the sensitivity of aquatic insects. Therefore, EPA will continue to seek additional acute PFOS insect data to further understand the sensitivity of this taxon. Additionally, EPA evaluated the effect of the current qualitative studies on the Final Acute Value (FAV) in Section 4.2.1. Thus, the current development of an acute freshwater criterion was based on seven of the eight MDRs.

GMAVs for 18 freshwater genera are provided in Table 3-3, and the four most sensitive genera were within a factor of 2.2 of each other. The freshwater FAV, the 5th percentile of the genus sensitivity distribution, for PFOS was 6.011 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 for the tested species. 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 3.0 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 = 18						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Pimephales*	6.950	1.94	3.76	0.053	0.229
2	*Oncorhynchus*	7.515	2.02	4.07	0.105	0.324
3	*Ligumia*	13.5	2.60	6.77	0.158	0.397
4	*Neocaridina*	15.61	2.75	7.55	0.211	0.459
		Σ (Sum):	**9.31**	**22.15**	**0.53**	**1.41**

S^2 = 17.093 **S = slope**
L = 0.869 **L = X-axis intercept**
A = 1.794 **A = lnFAV**
FAV = 6.011 **P = cumulative probability**
CMC = 3.0 mg/L PFOS (rounded to two significant figures)

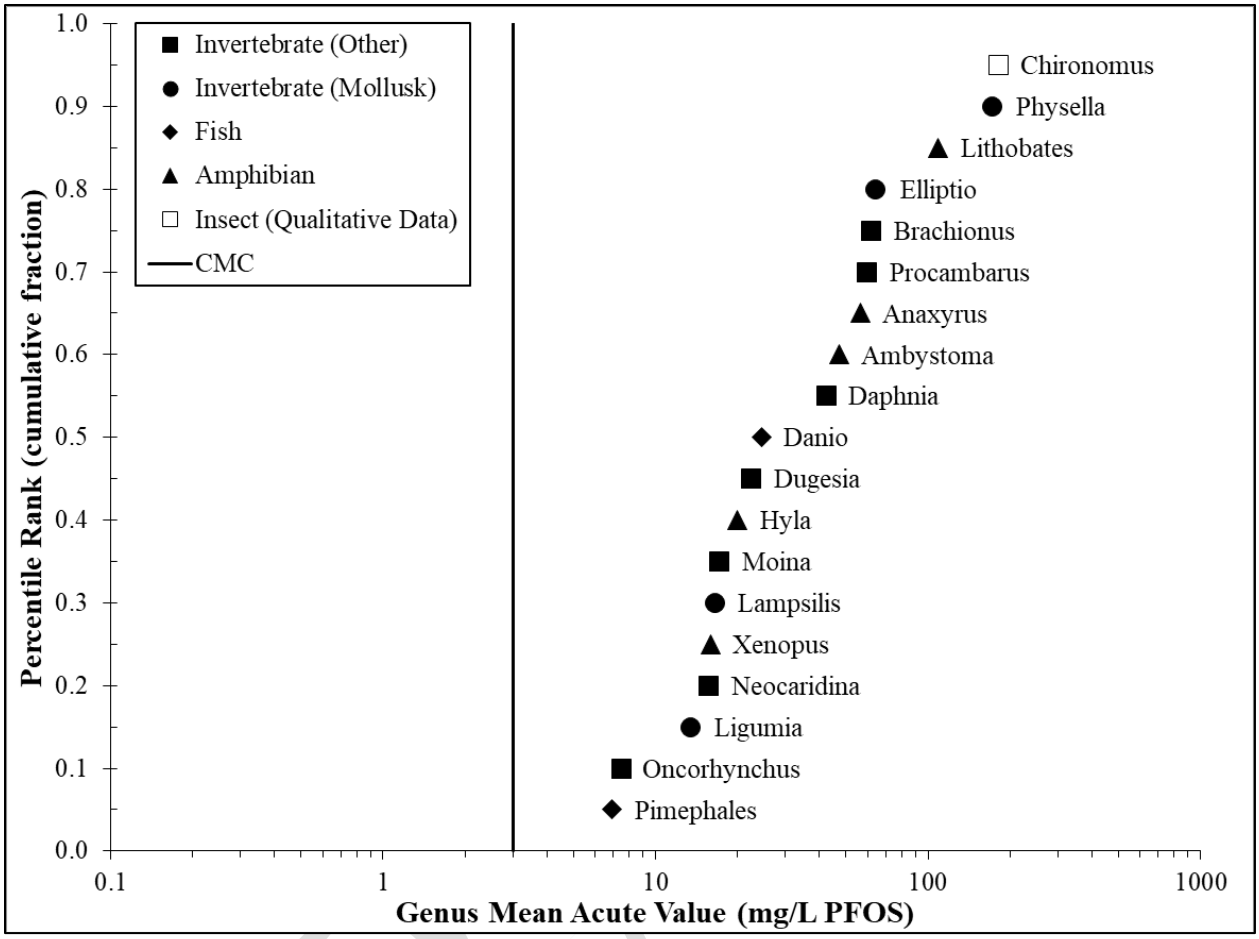

Figure 3-5. Ranked Freshwater Acute PFOS Used Quantitatively to Derive the Criterion. Qualitative data for an insect species was taken into consideration to understand the relative sensitivity of aquatic insects and is denoted by the hollow black box. The GMAV for this qualitative data was not used to derive the freshwater acute criterion for PFOS.

3.2.1.2 Derivation of Acute Water 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, EPA developed an estuarine/marine acute benchmark using the available empirical data supplemented with toxicity values generated through the use of New Approach Methods, specifically through the use of the

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

3.2.1.3 Derivation of Chronic Water 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 14 freshwater genera (Table 3-7). The four most sensitive genera were within a factor of 3.3 of each other. The freshwater FCV for PFOS was 0.008398 mg/L, calculated using the procedures described in the 1985 Guidelines (U.S.EPA 1985). The FCV is the 5^{th} 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. 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 0.0084 mg/L PFOS (rounded to two significant figures), or 8.4 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 = 14						
Rank	**Genus**	**GMCV (mg/L)**	**ln(GMCV)**	**ln(GMCV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Chironomus*	0.009676	-4.64	21.51	0.067	0.258
2	*Lampsilis*	0.01768	-4.04	16.28	0.133	0.365
3	*Enallagma*	0.03162	-3.45	11.93	0.200	0.447
4	*Danio*	0.03217	-3.44	11.81	0.267	0.516
		Σ (Sum):	**-15.56**	**61.54**	**0.67**	**1.59**

S^2 = 26.35 **S = slope**
L = -5.927 **L = X-axis intercept**
A = -4.780 **A = lnFCV**
FCV = 0.008398 **P = cumulative probability**
CCC = 0.0084 mg/L PFOS (rounded to two significant figures)

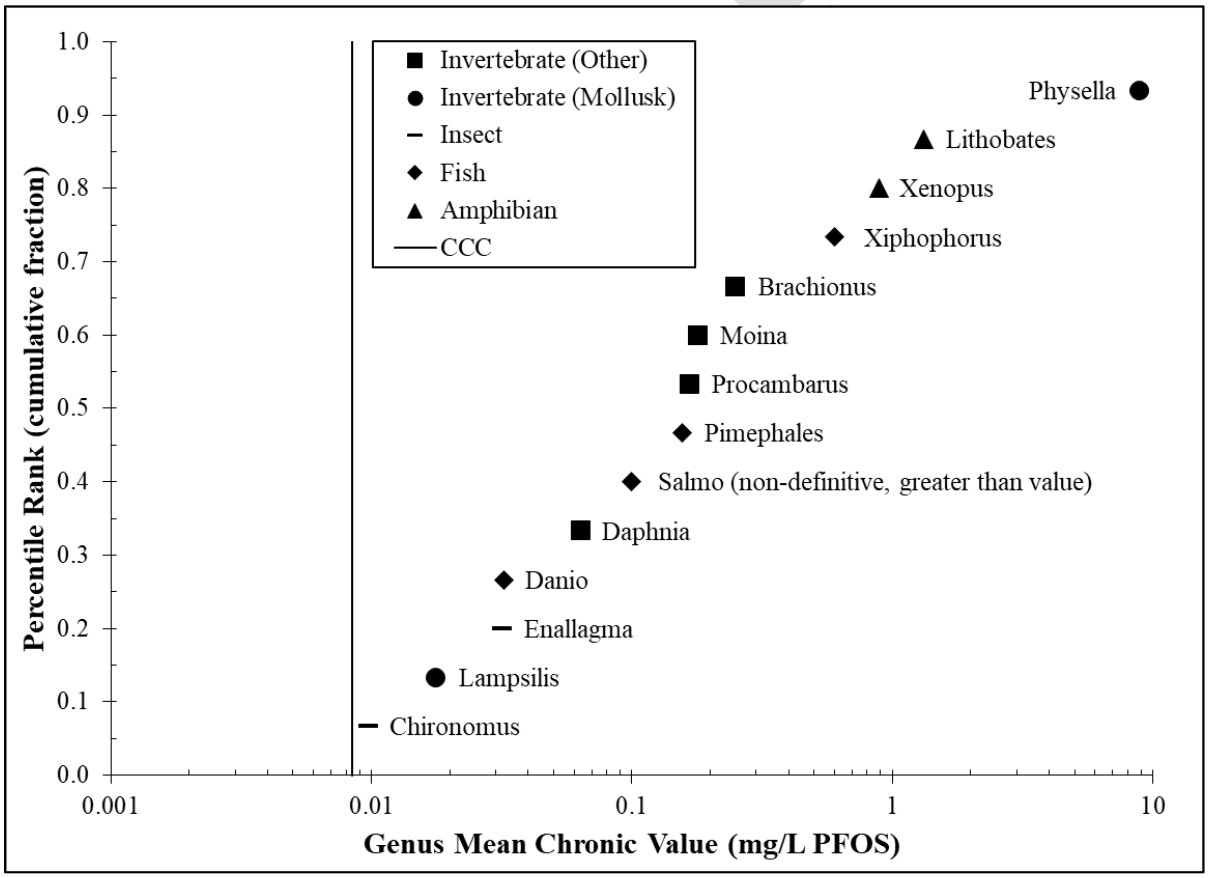

Figure 3-6. Ranked Freshwater Chronic PFOS Used Quantitatively to Derive the Criterion.

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 *Chironomus dilutus* and the *Chironomus* GMCV (most sensitive genus) are based on EPA’s independently-calculated EC_{10} of 0.05896 mg/L for a 10-day larval growth test by MacDonald et al. (2004) and EC_{10} of 0.001588 mg/L for a 16-day larval mean biomass test by McCarthy et al. (2021). 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. 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 *Lampsilis siliquoidea* and the *Lampsilis* GMCV (second most sensitive genus) are 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, w_i = 0.78) and 83% of the metamorphosis success (AIC = 21955, w_i = 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 1-hour 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* and the *Enallagma* GMCV (third 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 10 times 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.

PFOS effects observed for chronically sensitive species indicate that a 4-day chronic duration is appropriate. For example, the EC_{10} for *Danio rerio* (fourth most sensitive genus) is based on survival of the F1 offspring at eight days post fertilization (dpf), suggesting chronic PFOS effects may occur in relatively brief periods of time following fertilization. Pronounced effects occurring 8 dpf, with relatively lesser effects later in the course of the 5-month study suggest the potential presence of a uniquely sensitive life stage occurring from 0 – 8 dpf.

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 days ELS) and a chronic 4-day duration component of the water column criterion was considered protective for these species/genera. Therefore, 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 Criterion for Estuarine/Marine Water

The estuarine/marine chronic dataset for PFOS contained GMCVs for three genera. GMCVs for three 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 five MDR groups are not available (two

families in the phylum Chordata, a family in a phylum other than Arthropoda or Chordata, a family in a phylum other than 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.

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).

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 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 from 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 P.

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 Q.

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 0.0084 mg/L, resulting in an invertebrate whole-body tissue criterion of 0.937 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 0.0084 mg/L, resulting in a fish whole-body tissue criterion of 6.75 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 0.0084 mg/L, resulting in a fish muscle tissue criterion of 2.91 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 more than once in ten years. The duration component of the tissue-based criteria is expressed as an instantaneous duration because the tissue-based criteria are protective long-term conditions and represent an integrated measure of bioaccumulated PFOS concentrations over time.

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

3.2.3.3.1 Duration: Chronic Criterion 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 Criterion Tissue-Based Criteria

Ecological recovery times following chemical disturbances 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 a chemical and potential for residual effects. Given the large variation in possible biological and physical variables influencing ecological recovery, EPA focused on the known chemical attributes of PFOS to inform a recommended ten-year exceedance frequency for the chronic tissue-based criteria.

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 et al. 2016). 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 is 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 a lack of 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 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) 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 dischargers that led to severe reproductive failure and deformities in fish, was still measurable (fish deformities) in 1992 (seven years later) and in 1996 (ten years later). Lemly (1997, pg. 280) 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 vs. persistence may also provide a mechanism influencing ecological recovery rates. The persistence of PFOS has been attributed to the strong C-F bond, with no known biodegradation or abiotic degradation processes for PFOS. Somewhat similarly, as elements, 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).

The bioaccumulative nature and persistence of PFOS in aquatic systems, including sediments (Ahrens 2011), in combination with the documented recovery times of pollutants with similar chemical attributes (Gergs et al. 2016; Lemly 1997), suggested 10 years was a protective exceedance frequency for the tissue-based criteria for PFOS. The tissue-based criteria are protective if they are not exceeded more than once in ten years to allow sufficient time for PFOS concentrations built up in tissues and source reservoirs in the freshwater system to diminish while simultaneously providing freshwater organisms adequate time to recover following elevated PFOS exposures in tissues.

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 that fed test organisms. 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 in tissues. The tissue criteria will provide

information to states, tribes, and stakeholders on potential effects to aquatic organisms based on aquatic tissue monitoring data. Quantitatively acceptable data on the effects of dietary exposures to aquatic species were relatively limited, thus EPA elected to develop protective values for aquatic organism tissues 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.

3.3 Summary of the PFOS Aquatic Life Criteria

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 freshwaters. The freshwater acute water column-based criterion magnitude is 3.0 mg/L, and the chronic water column-based criterion magnitude is 0.0084 mg/L (Table 3-12). The chronic freshwater tissue-based criteria magnitudes are 6.75 mg/kg wet weight (ww) for fish whole-body, 2.91 mg/kg ww for fish muscle tissue and 0.937 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 above 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. Draft Recommended Perfluorooctane Sulfonate (PFOS) 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	3.0 mg/L	0.0084 mg/L	0.937 mg/kg ww	6.75 mg/kg ww	2.91 mg/kg ww
Duration	1 hour average	4 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 more than once in ten years on average		

[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 derived from the chronic water column concentration (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.

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 criteria that contributed to the weight-of-evidence for the derivation. This section includes: (1) comparison of quantitative insect data used to derive freshwater criteria (Section 4.1); (2) 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 4.2); (3) assesses the influence of including non-North American resident species in criteria derivation (i.e., species not resident to North America removed from dataset; Section 4.3); (4) provides summaries of the toxicity studies with apical endpoints (e.g., effects on survival, growth, or reproduction) that were not used directly to derive the criteria, but were used qualitatively to support the PFOS criteria (Section 4.4); (5) evaluation of the acute insect MDR through the use of inter-species correlation estimates (Section 4.5); (6) discussion of acute to chronic ratios (Section 4.6); (7) comparison of empirical tissue concentrations to translated tissue criteria (Section 4.7); and (8) discussion of the effects of PFOS on aquatic plants (Section 4.8). EPA is proposing the national recommended PFOS aquatic life criteria 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 Comparison of Quantitative Data used to Derive Freshwater Criteria

4.1.1 Aquatic Insects

While comparing the effects of PFOS across studies presented several challenges, especially with differences in test species, methodologies, exposure durations, and observed endpoints, in general there appeared to be several similarities and few differences between the three aquatic insect toxicity studies used quantitatively to derive the PFOS chronic freshwater

criterion. In all three studies (Bots et al. 2010; MacDonald et al. 2004; McCarthy et al. 2021) effects of chronic exposures to PFOS on survival and/or emergence were observed in damselfly (*Enallagma cyathigerum*) and midge (*Chironomus dilutus*), respectively. Additionally, all three studies measured effects of PFOS on growth. However, since the studies focused on very different life stages for this endpoint (on growth in emerged adult damselfly from Bots et al. (2010) and on growth in larval midge from MacDonald et al. (2004) and McCarthy et al. (2021), the toxicity data for growth could not be compared.

During the early phases and exposure durations of the experiments, it appeared that the effects of PFOS are not similar between the two species and that midge is more sensitive than the damselfly. This is particularly true for McCarthy et al. (2021) where the author-reported 16-day EC_{10} was 0.00136 mg/L. However, after this initial phase, the effects of PFOS on damselfly and midge became more similar. In the later phases of the tests, the independently-calculated EC_{10} of 0.0171 mg/L and the author-reported 20-day MATC of 0.0454 mg/L for chironomid survival in MacDonald et al. (2004) were similar to the author-reported 150-day MATC of 0.03162 mg/L for damselfly survival. The test organisms at these phases of each respective test likely occurred in a similar life stage (later development and about to undergo metamorphosis). Therefore, they were more comparable than any of the other survival toxicity values from these studies (i.e., the 10-day values for damselfly and the 10-day values for midge), which were focused on the effects of PFOS on much less comparable instars, especially given that odonates have a much longer development and life span compared to midges.

These results indicated that PFOS exposures to aquatic insects in later life stages are likely similar. These apparent similarities in the chronic effects of PFOS to aquatic insects provided support to the toxicity values quantitatively used and to the ranking of these two

species (as first and third most sensitive) to derive the chronic freshwater criterion. However, additional replicate level data would be helpful to fully understand the observed effects of PFOS individually and to compare across the full PFOS toxicity dataset.

4.2 Additional Analyses Supporting the Freshwater Criteria

4.2.1 Additional Analyses Supporting the Derivation of Acute Water Column Criterion for Freshwater

In addition to the acute freshwater criterion of 3.0 mg/L PFOS described above, two additional analyses supporting the derivation of the acute criterion were conducted to consider the effect of including less certain toxicity data. These additional analyses were conducted as part of a line-of-evidence discussion to better understand the influence of using less certain toxicity data in the acute dataset. The data considered to be less certain centered on the difficultly in reliably ascertaining the relative sensitivity of aquatic insects to acute exposures of PFOS based on the current toxicity literature for PFOS (see Section 3.1.1.1.6).

The two additional analyses presented below either included or excluded data from two toxicity studies (Table 4-1). The additional analyses presented here evaluated the influence of data variation on the derivation of the PFOS criteria. The inclusion of the two qualitatively acceptable studies demonstrated variability in the sensitivity of aquatic insects to acute exposures of PFOS. Therefore, EPA will continue to seek additional acute PFOS insect data to further understand the sensitivity of this taxon. Thus, the current development of an acute freshwater criterion was based on seven of the eight MDRs. 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 EPA's best estimate of the maximum concentrations of PFOS that will support protection of aquatic life from acute PFOS exposures.

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

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

Order of Additional Analyses	Purpose of Additional Analysis	Details of Additional Analysis	Acute Water Column Concentration for Additional Analysis (mg/L)	Study
1	To explore the impact of using qualitative insect data to fulfill missing aquatic insect MDR	Used author-reported LC_{50} of 1.18 mg/L for yellow fever mosquito (*Aedes aegypti*)	0.72	Olson (2017)
2		Used author-reported LC_{50} of 182.12 mg/L for chironomid (*Chironomus plumosus*)	3.1	Yang et al. (2014)

*Final derived acute freshwater criterion was 3.0 mg/L PFOS.

In the first additional analysis, the toxicity data for the invasive pest species yellow fever mosquito (*Aedes aegypti*) from Olson (2017) were used to derive an exploratory acute criterion magnitude. Including the author-reported LC_{50} of 1.18 mg/L from Olson (2017) in the derivation resulted in a freshwater FAV of 1.446 mg/L and an acute water column concentration of 0.72 mg/L (Table 4-2; FAV of 1.446 mg/L÷2 = 0.72 mg/L). Including the LC_{50} of 1.18 mg/L for *A. aegypti* decreases the criterion magnitude by a factor of 4.2 below EPA's recommended estimate of the maximum concentration of PFOS that will support aquatic life from exposure of 3.0 mg/L. EPA concluded that the inclusion of a qualitative LC_{50} for *A. aegypti* in the agency's acute criterion dataset is unwarranted given that: 1) *A. aegypti* is an invasive pest species; 2) the study was missing important exposure details; and 3) the author-reported LC_{50} and concentration-response curve could not be assessed by EPA on a statistical basis since model parameters were not provided, and there were insufficient treatment level data to independently calculate toxicity values (see Sections 3.1.1.1.6 and G.2.1.5). Additionally, EPA expects that the chronic water column criterion of 0.0084 mg/L, which is over two orders of magnitude lower than this

calculated acute water column value, will likely be the driving magnitude for permits and assessments. Until such a time when additional toxicity data on aquatic insects are available to fully understand the potential acute effects of PFOS on aquatic insects, especially considering the comparison between qualitative data for midge and mosquito, which indicated very different sensitivities among insects, EPA concluded that the acute criterion derived from the current acute dataset will adequately protect 95% of the species 99% of the time.

In the second analysis, the qualitative LC_{50} value for chironomid of 182.12 mg/L from Yang et al. (2014) was used (Table 4-2). Including this value increases the "N' in the criterion calculation by one and results in an alternative freshwater FAV for PFOS of 6.157 mg/L (Section 3.2.1.1; U.S.EPA 1985) and an acute water column concentration of 3.1 mg/L PFOS (rounded to two significant figures). This second analysis indicated that the qualitative chironomid LC_{50} for *Chironomus plumosus* had very little influence on the magnitude of the freshwater CMC for PFOS. Additionally, this test was considered for qualitative use since the test organisms were from a problematic source (from the Beijing City Big Forest Flower Market with no details of pervious exposures to PFOS or other chemicals provided) and no further quantification of previous exposure to contaminants or husbandry was provided (Yang et al. 2014). Therefore, EPA determined to proceed with the criterion derivation as previously described in Section 3.2.1.1. Again, additional toxicity data on aquatic insects are needed to fully understand the potential acute effects of PFOS on aquatic insects and modify the acute criterion if necessary. EPA will continue to seek additional acute PFOS insect data to further understand the sensitivity of this taxon.

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

MDR Group[4]	Genus	Species	Acute Criterion	Additional Analyses	
			GMAV (mg/L PFOS)[1]	First[2]	Second[3]
F	*Aedes*	Yellow fever mosquito, *Aedes aegypti*	-	1.18	-
B	*Pimephales*	Fathead minnow, *Pimephales promelas*	6.950	6.950	6.950
A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	7.515	7.515	7.515
G	*Ligumia*	Black sandshell, *Ligumia recta*	13.5	13.5	13.5
E	*Neocaridina*	Japanese swamp shrimp, *Neocaridina denticulata*	15.61	15.61	15.61
C	*Xenopus*	African clawed frog, *Xenopus laevis*	15.99	15.99	15.99
G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	16.5	16.5	16.5
D	*Moina*	Cladoceran, *Moina macrocopa*	17.20	17.20	17.20
C	*Hyla*	Gray treefrog, *Hyla versicolor*	19.88	19.88	19.88
G	*Dugesia*	Planaria, *Dugesia japonica*	22.48	22.48	22.48
B	*Danio*	Zebrafish, *Danio rerio*	24.44	24.44	24.44
D	*Daphnia*	Cladoceran, *Daphnia carinata*	42.30	42.30	42.30
		Cladoceran, *Daphnia magna*			
		Cladoceran, *Daphnia pulicaria*			
C	*Ambystoma*	Jefferson salamander, *Ambystoma jeffersonianum*	47.40	47.40	47.40
		Small-mouthed salamander, *Ambystoma texanum*			
		Eastern tiger salamander, *Ambystoma tigrinum*			
C	*Anaxyrus*	American toad, *Anaxyrus americanus*	56.49	56.49	56.49
E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	59.87	59.87	59.87

MDR Group[4]	Genus	Species	Acute Criterion	Additional Analyses	
			GMAV (mg/L PFOS)[1]	First[2]	Second[3]
H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	61.8	61.8	61.8
G	*Elliptio*	Eastern elliptio, *Elliptio complanata*	64.35	64.35	64.35
C	*Lithobates*	American bullfrog, *Lithobates catesbeiana*	109.24	109.24	109.24
		Green frog, *Lithobates clamitans*			
		Northern leopard frog, *Lithobates pipiens*			
		Wood frog, *Lithobates sylvatica*			
G	*Physella*	Bladder snail, *Physella acuta*	172.1	172.1	172.1
		Snail, *Physella heterostropha pomilia*			
F	*Chironomus*	Midge, *Chironomus plumosus*	-	-	182.12
Acute Water Column Concentration (mg/L)			**3.0**	**0.72**	**3.1**

[1] GMAVs as presented in Table 3-3 in Section 3.1.1.1. Genera presented in order of rank according to the criterion derivation.
[2] Additional analysis with the inclusion of qualitative yellow fever mosquito data.
[3] Additional analysis with the inclusion of qualitative chironomid (*C. plumosus*) data.
[4] MDR Groups identified by list provided in Section 2.10.1 above.

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

In addition to EPA's recommended chronic freshwater criterion of 0.0084 mg/L PFOS described above in Section 3.2.1.1, six 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 on the magnitude of the chronic 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 in Hazelton et al. (2012) and Bots et al. (2010) (see Section 3.1.1.3.2 and 3.1.1.3.3, respectively) and (2) the uncertainty in the chronic toxicity values given the level of data presented in the papers (see Appendices C.2.2 and C.2.3).

The six additional analyses presented below either changed the toxicity value or excluded data from two toxicity studies (Table 4-3). 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, EPA decided to retain the damselfly and fatmucket 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 the damselfly and fatmucket may serve as 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 EPA's best estimate of the maximum concentrations of PFOS that will support protection of sensitive aquatic life from unacceptable chronic exposures.

Table 4-3. Additional Analyses Supporting the Derivation of the Chronic Water Column Criterion for Freshwater.

*Presented in the order that is summarized in 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 0.0316 mg/L	0.0069	Bots et al. (2010)
2		Used 60-day NOEC of 0.1 mg/L damselfly instead of 150-day MATC of 0.0316 mg/L	0.0069	
3		Used 320-day NOEC of 0.01 mg/L damselfly instead of 150-day MATC of 0.0316 mg/L	0.0063	
4	To explore the impact of using the MATC for fatmucket	Removed MATC of 0.01768 mg/L for fatmucket	0.0079	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 0.001768 mg/L for fatmucket and 150-day MATC of 0.0316 mg/L for damselfly	0.0053	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	0.0070	Hazelton et al. (2012)

*Final derived chronic freshwater criterion was 0.0084 mg/L PFOS.

In the first additional analysis, instead of using the 150-day MATC of 0.0316 mg/L for *Enallagma cyathigerum* as described in the final criterion description above in Section 3.2.1.3, the 10-day MATC of 0.3162 mg/L was used (Table 4-4; Bots et al. 2010), yielding a freshwater FCV for PFOS of 0.006932 mg/L. This chronic water column concentration of 0.0069 mg/L

PFOS (rounded to two significant figures) is slightly lower (more protective) than the final chronic value of 0.0084 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, EPA has concluded that the 150-day MATC should be used quantitatively to derive the chronic freshwater criterion.

In the second analysis, instead of using the 150-day MATC of 0.0316 mg/L for *Enallagma cyathigerum*, the 60-day NOEC of 0.1000 mg/L from the same test was used (Table 4-4; Bots et al. 2010), also yielding an FCV of 0.06932 mg/L (Section 3.2.1.3; U.S.EPA 1985). Similar to the first analysis, there is little 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, EPA has concluded that the 150-day MATC should be used quantitatively to derive chronic freshwater criterion.

In the third analysis, instead of using the 150-day MATC of 0.0316 mg/L for *Enallagma cyathigerum*, the 320-day NOEC of 0.0100 mg/L from the same test was used (Table 4-4; Bots et al. 2010), yielding an FCV of 0.006334 mg/L. This analysis indicated that there is about a 1.3-fold difference (lower) 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), EPA has determined that the 150-day MATC should be used quantitatively to derive chronic freshwater 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 (*Lampsilis 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 4-4). This additional analysis placed the GMCV of 0.06329 mg/L for *Daphnia* among the four most sensitive genera, and yielded an FCV of 0.007895 mg/L (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, but would eliminate mollusks from the chronic PFOS dataset. 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 (*Enallagma cyathigerum*) and MATC for fatmucket (*Lampsilis 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 4-4). 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) and 10-fold difference in Bots et al. (2010) 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 0.005272 mg/L. Similar to the previous additional analysis looking at the influence of changing the chronic value for *E. cyathigerum* to the 300-day survival NOEC, the calculated chronic criterion magnitude was reduced 1.6-fold. EPA decided to retain the damselfly and fatmucket values as presented in Section 3.1.1.3 above in the current derivation, to ensure representation and protection of these sensitive taxa.

Lastly 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 4-4), particularly since 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 0.007055mg/L. This additional analysis indicated that the estimated toxicity value from *L. siliquoidea* has a modest influence on the calculated chronic criterion. Since the estimated toxicity value had a modest influence on the recommended CCC value, the author-reported MATC was used instead.

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

MDR Group[7]	Genus	Species	Chronic Criterion	Additional Analysis					
			GMCV (mg/L PFOS)[1]	First[2]	Second[2]	Third[2]	Fourth[3]	Fifth[4]	Sixth[5]
F	*Chironomus*	Midge, *Chironomus dilutus*	0.009676	0.009676	0.009676	0.009676	0.009676	0.009676	0.009676
G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	0.01768	0.01768	0.01768	0.01768	-	-	0.0123
F	*Enallagma*	Blue damselfly, *Enallagma cyathigerum*	0.03162	0.3162	0.1	0.001	0.03162	-	0.03162
B	*Danio*	Zebrafish *Danio rerio*	0.03217	0.03217	0.03217	0.03217	0.03217	0.03217	0.03217
D	*Daphnia*	Cladoceran, *Daphnia carinata* Cladoceran, *Daphnia magna*	0.06329	0.06329	0.06329	0.06329	0.06329	0.06329	0.06329
A	*Salmo*	Atlantic salmon, *Salmo salar*	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	-[6]	> 0.1
B	*Pimephales*	Fathead minnow, *Pimephales promelas*	0.1555	0.1555	0.1555	0.1555	0.1555	0.1555	0.1555
E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	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
H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	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
C	*Xenopus*	African clawed frog, *Xenopus laevis* Clawed frog, *Xenopus tropicalis*	0.8872	0.8872	0.8872	0.8872	0.8872	0.8872	0.8872
C	*Lithobates*	Northern leopard frog, *Lithobates pipiens*	1.316	1.316	1.316	1.316	1.316	1.316	1.316
G	*Physella*	Snail, *Physella heterostropha pomilia*	8.831	8.831	8.831	8.831	8.831	8.831	8.831
Chronic Water Column Concentration			**0.0084**	**0.0069**	**0.0069**	**0.0063**	**0.0079**	**0.0053**	**0.0070**

[1] GMCVs as presented in Table 3-7 in Section 3.1.1.3. Genera presented in order of rank according to the criterion derivation. Order of GMCVs were not changed for the additional analyses.
[2] Additional analysis with changes to toxicity value for *E. cyathigerum.*
[3] Additional analysis with the exclusion of *L. siliquoidea.*
[4] Additional analysis with the exclusion of *L. siliquoidea* and *E. cyathigerum.*
[5] Additional analysis with the changes to toxicity value for *L. siliquoidea*.

[6] Not ranked among the four most sensitive taxa so not included in the chronic freshwater criterion calculations per the decision rule for greater than toxicity values (see Section 2.10.3.2).
[7] MDR Groups identified by list provided in Section 2.10.1 above.

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

EPA conducted two additional analyses of the freshwater criteria by reducing the limited toxicity datasets to organisms that are resident to, or have been introduced and have established populations in the conterminous U.S. These analyses were conducted to determine the sensitivity of the criteria calculations to 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.

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

Four species were removed in the analysis of a freshwater acute water criterion with native, reproducing, or established organism in the conterminous U.S.: Japanese swamp shrimp (*Neocaridina denticulata*), planarian (*Dugesia japonica*), zebrafish (*Danio rerio*) and cladoceran (*Daphnia carinata*). Removal of these species truncated the freshwater acute dataset to 22 species (Table 4-5). The freshwater acute dataset still retained one missing MDR group (an insect), which was addressed through inclusion of qualitative data. The Japanese swamp shrimp ranked fourth (Table 3-3) and all other species mentioned above (planarian, zebrafish, and cladoceran) were not among the four most acutely sensitive species. The acute water column concentration was 2.8 mg/L PFOS (Table 4-6) when using the reduced dataset which was slightly lower than the recommended CMC of 3.0 mg/L. This value is lower than all of the GMAVs in Table 3-3. EPA decided to retain the full acute dataset and associated acute criterion for PFOS of 3.0.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 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	6.950	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	6.950
2	7.515	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	7.515
3	13.5	G	*Ligumia*	Black sandshell, *Ligumia recta*	13.5
4	15.99	C	*Xenopus*	African clawed frog, *Xenopus laevis*	15.99
5	16.5	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	16.5
6	17.20	D	*Moina*	Cladoceran, *Moina macrocopa*	17.20
7	19.88	C	*Hyla*	Gray treefrog, *Hyla versicolor*	19.88
8	47.40	C	*Ambystoma*	Jefferson salamander, *Ambystoma jeffersonianum*	51.71
				Small-mouthed salamander, *Ambystoma texanum*	30.00
				Eastern tiger salamander, *Ambystoma tigrinum*	68.63
9	56.49	C	*Anaxyrus*	American toad, *Anaxyrus americanus*	56.49
10	59.87	E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	59.87
11	61.8	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	61.8
12	64.35	G	*Elliptio*	Eastern elliptio, *Elliptio complanata*	64.35
13	80.92	D	*Daphnia*	Cladoceran, *Daphnia magna*	48.87
				Cladoceran, *Daphnia pulicaria*	134
14	109.24	C	*Lithobates*	American bullfrog, *Lithobates catesbeiana*	133.3
				Green frog, *Lithobates clamitans*	113

Rank[a]	GMAV (mg/L PFOS)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOS)
				Northern leopard frog, *Lithobates pipiens*	72.72
				Wood frog, *Lithobates sylvatica*	130
15	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 4-6. 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 = 15						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Pimephales*	6.950	1.94	3.76	0.063	0.250
2	*Oncorhynchus*	7.515	2.02	4.07	0.125	0.354
3	*Ligumia*	13.5	2.60	6.77	0.188	0.433
4	*Xenopus*	15.99	2.77	7.68	0.250	0.500
		Σ (Sum):	**9.33**	**22.28**	**0.63**	**1.54**

S^2 = 14.990 **S = slope**
L = 0.845 **L = X-axis intercept**
A = 1.711 **A = lnFAV**
FAV = 5.535 **P = cumulative probability**

Acute Water Column Concentration = 2.8 mg/L PFOS (rounded to two significant figures)

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

Three species were removed from the chronic freshwater dataset that are not native or established organism in the conterminous U.S.: zebrafish (*Danio rerio*), cladoceran (*Daphnia carinata*) and the clawed frog (*Xenopus tropicalis*). One species, the zebrafish (*Danio rerio*) is not native to the conterminous U.S., although, there have been reports of zebrafish in the wild in

several different locations in the U.S. The current status of any such zebrafish populations in the U.S. is uncertain (U.S.FWS 2018). Additionally, zebrafish are an accepted test species and are used in analyses in TSCA and FIFRA (U.S.EPA 2016c). Removal of these species truncated the freshwater chronic dataset to 12 species representing 12 genera (Table 4-7). Additionally, the non-definitive value for the Atlantic salmon was removed, since the value would rank in the top four and its use is not indicative of true species sensitivity, as an unbounded greater than value. The revised freshwater chronic dataset consisted of seven of the eight MDRs. The zebrafish ranked fourth when all species were included, and the other species mentioned above (cladoceran and clawed frog) were not among the four most chronically sensitive species. Removal of the species that are not resident to North America reduced the FCV and chronic water column concentration (Table 4-8). The chronic water column concentration was 0.004001 mg/L PFOS when using the reduced dataset and was 2.1 times lower than the recommended chronic criterion of 0.0084 mg/L. The chronic water column concentration from this additional analysis was also 2.1 times lower than the lowest GMCVs (Table 4-7). Therefore, EPA decided to retain the full chronic dataset and associated chronic criterion for PFOS of 0.0084 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 4-7. 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	0.009676	F	*Chironomus*	Midge, *Chironomus dilutus*	0.009676
2	0.01768	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	0.01768
3	0.03162	F	*Enallagma*	Blue damselfly, *Enallagma cyathigerum*	0.03162
4	0.1555	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	0.1555
5	0.167	E	*Procambarus*	Crayfish, *Procambarus fallax f. virginalis*	0.167
6	0.1789	D	*Moina*	Cladoceran, *Moina macrocopa*	0.1789
7	0.25	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	0.25
8	0.5997	C	*Xiphophorus*	Swordtail fish, *Xiphophorus helleri*	0.5997
9	> 1	C	*Xenopus*	African clawed frog, *Xenopus laevis*	> 1
10	1.267	D	*Daphnia*	Cladoceran, *Daphnia magna*	1.267
11	1.316	C	*Lithobates*	Northern leopard frog, *Lithobates pipiens*	1.316
12	8.831	G	*Physella*	Snail, *Physella heterostropha pomilia*	8.831

[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 4-8. 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 = 12						
Rank	**Genus**	**GMCV (mg/L)**	**ln(GMCV)**	**ln(GMCV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Chironomus*	0.009676	-4.64	21.51	0.077	0.277
2	*Lampsilis*	0.01768	-4.04	16.28	0.154	0.392
3	*Enallagma*	0.03162	-3.45	11.93	0.231	0.480
4	*Pimephales*	0.1555	-1.86	3.46	0.308	0.555
		Σ (Sum):	**-13.99**	**53.19**	**0.77**	**1.70**

S^2 = 99.86 **S = slope**
L = -7.756 **L = X-axis intercept**
A = -5.521 **A = lnFCV**
FCV = 0.004001 **P = cumulative probability**
Chronic Water Column Concentration = 0.0040 mg/L PFOS (rounded to two significant figures)

4.4 Qualitatively Acceptable Water Column-Based Toxicity Data

Several studies were identified as either not meeting 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 are summarized below, grouped as either acute or chronic exposures and sorted by relative sensitivity of genera used to derive the criteria (following the previous study summaries included in the Effects Analysis). Qualitative study summaries within a factor of two of the final acute and chronic values were also included below and arranged according to taxonomic relatedness. NOEC and LOEC values were provided in several of the following study summaries to be representative toxicity values for comparison to the toxicity values summarized in the Effects

Analysis section above (see Section 3) and used in the criteria calculation. The toxicity values summarized here 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 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. The toxicity values summarized as part of this Effects Characterization were not used in any quantitative analysis or in the derivation of the PFOS aquatic life criterion. Detailed study summaries and corresponding tabulated data for the studies summarized below, as well as additional qualitative study summaries of less sensitive taxa, were included in Appendix G.

4.4.1 Consideration of Qualitatively Acceptable Acute Data

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

4.4.1.1.1 Most acutely sensitive genus, Pimephales

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

4.4.1.1.2 Second most acutely sensitive genus, Oncorhynchus

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

4.4.1.1.3 Third most acutely sensitive genus, Ligumia

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

4.4.1.1.4 Fourth most acutely sensitive genus, Neocaridina

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

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

4.4.1.2.1 Genus: Danio (zebrafish)

Danio rerio was evaluated by Huang et al. (2010) in a 114-hour static measured exposure to PFOS (CAS # 1763-23-1, > 96% pure). The 114-hour LC_{50} was 2.2 mg/L PFOS, and the malformation EC_{50} was 1.12 mg/L PFOS. These data were considered qualitative due to exposure duration, which was longer (at 114 hours) than fish acute toxicity test guidelines (U.S. EPA 2016b).

A sub-chronic static unmeasured test was conducted by Ulhaq et al. (2013) to determine the toxicity of PFOS to *D. rerio*. The 144-hour LC_{50} was >10 mg/L PFOS. This toxicity value was considered qualitative because of test duration, which was longer than the fish acute toxicity test guidelines (OCSPP 850.1075) recommending exposures of 96 hours (U.S.EPA 1985; U.S.EPA 2016b).

Martinez et al. (2019a) evaluated the acute effects of perfluorooctane sulfonate potassium salt on zebrafish (*Danio rerio*) over three days, yielding a LOEC value of 10.0 µM, or 5.382 mg/L PFOS (calculated using the molecular weight of 538.22 g/mol for PFOS-K) for growth as body length. The study is acceptable for qualitative use because of the short test duration, which was shorter than the 96-hour exposure period recommended by the fish acute toxicity test guidelines (U.S. EPA 1985; U.S. EPA 2016).

(Ortiz-Villanueva et al. 2018) also evaluated the acute effects of perfluorooctane sulfonate on zebrafish (*Danio rerio*) following two days of exposure. The author-reported LOEC was 2.0 µM (1.0 mg/L) for malformations, and 20 µM (10 mg/L) for survival (based on a molecular weight of 500.13 g/mol PFOS). The short test duration made the study acceptable for qualitative use only.

(Vogs et al. 2019) evaluated the acute effects of perfluorooctane sulfonic acid potassium salt on zebrafish (*Danio rerio*) embryos in a 118-hour study. An author-reported118-hr LC_{50} was 3.8 µM PFOS (or 2.045 mg/L based on molecular weight of 538.22 g/mol PFOS-K) for mortality. However, this study is acceptable for qualitative use because of the short test duration.

(Truong et al. 2014) evaluated the acute effects of potassium perfluorooctanesulfonate (PFOS-K, CAS # 2795-39-3) and perfluorooctane sulfonic acid (PFOS, CAS #. 1763-23-1) on zebrafish (*Danio rerio*) following 114 hours of exposure. The authors reported 114-hr mortality LOEC of 61.44 µM PFOS-K (or 33.07 mg/L based on a molecular weight of 538.22 g/mol) and 6.4 µM PFOS (or 3.2 mg/L based on a molecular weight of 500.13 mg/L). Both values are qualitative only because of the atypical test duration, which was longer than the 96-hour exposure period recommended by the fish acute toxicity test guidelines (U.S. EPA 1985; U.S. EPA 2016). Only the PFOS LOEC of 3.2 mg/L was within a factor of two of the FAV.

The noted toxicity values provided in each study summary above (2.2, > 10, 5.382, 10, 2.045, 3.2 mg/L, respectively), either comprising of author-reported LC_{50} or LOEC values, indicated that this genus might be more sensitive to acute exposures of PFOS than the quantitative data for the genus (with a GMAV of 24.44 mg/L). These qualitative values were either below or within a factor of two of the FAV of 6.011 mg/L. EPA, however, concluded these toxicity studies do not suggest *D. rerio* is a relatively sensitive species to acute PFOA exposures because five of the six quantitatively-acceptable acute tests for this species reported LC_{50} values (range = 3.502 – 71.12 mg/L; geometric mean = 24.44 mg/L; n = 6) that were more than two times greater than the FAV.

4.4.2 Consideration of Qualitatively Acceptable Chronic Data

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

4.4.2.1.1 Most chronically sensitive genus, Chironomus

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 in the context of other values for the midge was prudent. The EC_{10}s for *Chironomus dilutus* of 0.05896 mg/L and 0.001588 mg/L from MacDonald et al. (2004) and McCarthy et al. (2021), 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) is lower than the chronic criterion but it was only representative of a NOEC and no effects were observed at 0.0035 mg/L (as time-weighted average). The *Chironomus* GMCV is greater than the chronic freshwater criterion of 0.0084 mg/L, and thus, the species is expected to be protected.

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 the study in the context of the more robust and definitive values for midge. That is, the chronic values established for the related midge, *C. dilutus,* from MacDonald et al (2004) and McCarthy et al. (2021) are more reliable and definitive values representing the sensitivity of the genus in the chronic criteria dataset.

4.4.2.1.2 Second most chronically sensitive genus, Lampsilis

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

4.4.2.1.3 Third most chronically sensitive genus, Enallagma

There were no qualitatively acceptable chronic tests with apical endpoints for this genus.

4.4.2.1.4 Fourth most chronically sensitive genus, Danio

Du et al. (2009) investigated the effect of PFOS (> 99% purity) on the survival, growth and hepatotoxicity of *Danio rerio* female fry exposed via renewal, unmeasured conditions for 70 days. The 70-day MATC for increased malformation and decreased survival of F1 fish was reported as 0.0224 mg/L PFOS. An independently-calculated EC_{10} could not be determined as the treatment level data required for analysis appears to have been lost (personal communication with Bingsheng Zhou, corresponding study author). The author reported MATC of 0.0224 mg/L for increased F1 malformation and decreased survival was similar to the independently-calculated EC_{10} of 0.01650 mg/L for F1 survival from Wang et al. (2011) and EC_{10} of 0.06274

for mean body length from Guo et al. (2019), which were used quantitatively in the freshwater chronic criterion.

Cui et al. (2017) investigated the toxic effects of PFOS (> 96% purity) to *Danio rerio* in a near full life-cycle (unmeasured) static renewal test**.** Breeding trials were also carried out to produce F1 offspring (F0 females were paired with F0 males from the same treatment group). Malformation and survival rate of both generations were evaluated, and the study authors indicate that F1 offspring derived from the parental fish exposed to 0.25 mg/L were observed to have severe deformities (including uninflated swim bladders, bent spine, pericardial edema, yolk sac edema, and necrosis) and low survival rates. A MATC of 0.1118 mg/L was calculated from the author-reported values for effects on altered sex ratio (female dominance) and low F1 offspring survival. However, an independently-calculated toxicity value could not be determined with the data provided in the paper. The author-reported MATC was used qualitatively since EPA was unable to independently verify the reported toxicity value with the data provided in the paper. The author reported MATC of 0.1118 mg/L for decreased F1 offspring survival was substantially higher than the independently-calculated EC_{10} of 0.0165 mg/L from Wang et al. (2011) and EC_{10} of 0.06274 mg/L from Guo et al. (2019).

Chronic effects of PFOS in *Danio rerio* were investigated by Shi et al. (2009). No effects on survival were observed (with a survival NOEC > 0.40 mg/L). The study author reported NOEC, LOEC, and MATC for growth as both total body length and weight were 0.20, 0.40, and 0.2828 mg/L, respectively. This 15-day growth MATC of 0.2828 mg/L was roughly one order of magnitude higher than the FCV of 0.008398 mg/L and higher than the EC_{10}s calculated for both quantitative studies used for criteria derivation. Shi et al. (2009) was for a shorter exposure duration (15 days in a rapid early-life stage test compared to 150 days in a full life-cycle test and

21 day early-life stage test) with a less sensitive endpoint (growth compared to reproduction). The SMCV of 0.03217 mg/L is expected to be more representative of the sensitivity of this species.

Danio rerio embryos were also investigated by Keiter et al. (2012) in a long-term flow-through measured study with PFOS-K (potassium salt, CAS # 2795-39-3, ≥ 98% purity). This test was considered qualitative as there were complications with the test design (see full description in detailed study summary in Appendix G.3.2.4) and a poor concentration-response relationship with the endpoints evaluated. There was also a wide (> 100x) difference between the NOEC and LOEC for the study. For example, the NOEC and LOEC for F1 and F2 180-day male lengths and weights, and F2 180-day female weights, was 0.0006 and 0.1 mg/L. The LOEC for the remaining growth endpoints (lengths and weights across all generations) was < 0.0006 mg/L. In contrast, the MATC for F2 180-day survival was 0.1732 mg/L. The MATC of 0.1732 mg/L is substantially higher than EC_{10}s from the Wang et al. (2011) and Guo et al. (2019) studies used quantitatively to derive the chronic criterion.

Chen et al. (2016) evaluated the estrogenic effects of PFOS (> 96% purity) to *Danio rerio* via renewal, unmeasured tests. Body length was measured on individual fish while body weight was obtained by pooling samples of 10 fish per sample and calculating averages. The 42 dpf LOEC based on an increase in condition index (beneficial effect) was 0.250 mg/L PFOS. These data are classified as qualitative because there was only one exposure concentration. The LOEC of 0.250 mg/L was one order of magnitude higher than the SMCV of 0.03217 mg/L for *D. rerio* that is expected to representative of the sensitivity of this species.

Jantzen et al. (2017) evaluated the effects of PFOS on the morphometric, behavioral and gene expression in *Danio rerio* exposed via 5-day static, unmeasured exposures (OECD Method

212). The five-day (plus nine days for observation) NOEC, LOEC, and MATC for growth as total body length were 0.02, 0.2, and 0.06325 mg/L, respectively. The LOEC was associated with only a 3.8% decrease in growth compared to control. This study was considered for qualitative use due to the short exposure duration and small (negligible) effect. The MATC of 0.06325 mg/L was similar to the SMCV for *D. rerio*.

Sharpe et al. (2010) examined the bioaccumulation and toxicity of PFOS 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 effects on fecundity of PFOS isomers. The 96-hour test was used quantitatively to derive the acute water column criterion (see Appendix A). The 48-hour tests were used qualitatively and are summarized in Section G.2.2.3. A 14-day chronic exposure was conducted to examine PFOS accumulation and changes in isomer profiles in response to maternal transfer. A 21-day exposure was also conducted to test the potential of PFOS to reduce fecundity.

Fecundity was reduced 34% relative to control in fish exposed to 0.5 mg/L PFOS for 14 days and 47% in fish exposed 21 days. The results of this study were considered qualitative as the tests consisted of just one experimental concentration of 0.5 mg/L and because one of the two control replicates was lost, which the study authors note was due to unusual aggression among the test organisms. The author-reported LOEC of 0.5 mg/L was one order of magnitude higher than the SMCV for *D. rerio*.

Chen et al. (2013) examined the behavioral effects of zebrafish resulting from prolonged chronic exposure to PFOS. Adult *Danio rerio* (US-AB strain) used for spawning were maintained following standard protocols. At approximately three months of age, F0 adults from the same treatment group were bred. Embryos (F1) hatched from these adults were monitored for

developmental progression, and hatched larvae were monitored for 8 dpf for malformation and mortality. Second generation (F1) zebrafish were maintained in water free from PFOS. F1 larvae hatched from F0 adults from the 21-120 dpf and 1-120 dpf groups showed much higher rates of mortality and malformation than the control and the 1-20 dpf groups. The author-reported LOEC was 0.250 mg/L. The results of this study were considered qualitative as the test consisted of one experimental concentration (of 0.250 mg/L) prohibiting point estimation. The author-reported LOEC of 0.250 mg/L was one order of magnitude higher than the SMCV for *D. rerio*.

(Bao et al. 2019) evaluated the chronic effects of perfluorooctane on juvenile zebrafish (*Danio rerio*), via a 21-day study. An endpoint for fecundity was not reported by the authors, and there were no significant differences between the exposure concentrations in terms of growth length or weight (NOEC > 0.2 mg/L PFOS). No mortality was observed. Independently calculated EC_{10}s could not be calculated as EPA was unable to fit a model with significant parameters. Therefore, given EPA was unable to independently calculate toxicity values based on the level data provided in the paper by the study authors, the test duration was a partial-life cycle test as opposed to the preferred life-cycle test for which there were studies on this species (Wang et al. 2011), and the author-reported toxicity values results in a NOEC > 0.2 mg/L this study was used qualitatively to derive the draft chronic water column criterion.

The noted toxicity values provided in each study summary above (0.0224, 0.1118, 0.2828, <0.0006, 0.250, 0.06325, 0.5, 0.250, and > 0.2 mg/L), comprising of a mix of author-reported NOEC, LOEC, and MATC values, indicated varying sensitivity to chronic exposure of PFOS for this genus when compared to the quantitative data for the genus (with an SMCV/GMCV of 0.03217 mg/L). However, it was difficult to compare these papers since the present studies either: (1) only included one exposure concentration prohibiting definitive point

estimation; (2) were of unacceptably short chronic exposure duration; (3) involved the assessment of different endpoints; and (4) included complications from test designs. The SMCV of 0.03217 mg/L that was used quantitatively in the chronic criterion derivation is expected to be more robust than any of the individual toxicity values reported in these qualitative studies, and therefore, is expected to be adequately protective of this genus.

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

4.4.2.2.1 Genus: Daphnia (cladoceran)

Jeong et al. (2016) conducted a 25-day chronic life-cycle renewal, unmeasured test of PFOS-K (potassium salt, purity 99%) with *Daphnia magna*. The NOEC based on reproduction in the F0 generation was 0.010 mg/L. The 25-day LOEC was 0.100 mg/L. The calculated MATC was 0.03162 mg/L, and the independently-calculated EC_{10} was 0.0041 mg/L (Appendix G). Independent statistical analyses were conducted using data that were estimated (using Web plot digitizer) from the figures presented in the paper. This independently-calculated EC_{10} value was not considered reliable, however, as this value was much lower than the author reported NOEC, because the test concentrations were widely spaced (with each treatment group increasing by one order of magnitude and ranging between 0.0001 and 10 mg/L). Furthermore, the data presented in the paper were control normalized, and there was no consistent concentration-response relationship. While the toxicity value from the study was within a factor of two of the FCV of 0.008398 mg/L indicating that this species might be more sensitive to chronic exposures of PFOS than the quantitative data for the genus indicates, it is still greater than the FCV by an order of magnitude. Additionally, while the SMCV of 1.267 mg/L is substantially greater than the chronic value from the study, the preponderance of other acceptable chronic values for *D. magna* precludes a major change in species sensitivity.

4.4.2.2.2 Genus: Oryzias (medaka)

Ji et al. (2008) evaluated the chronic toxicity of PFOS to the Japanese medaka, *Oryzias latipes*, via unmeasured renewal exposures. The author-reported 14-day NOECs for F0 (parental generation) adult survival, condition factor and adult male GSI and HSI were all > 1 mg/L PFOS, whereas the 14-day adult female HSI and GSI MATC and LOEC were 0.3162 and < 0.01 mg/L PFOS, respectively. For the F1 (progeny generation), the MATCs for percent hatchability, time to hatch, and swim-up success were all 0.3162 mg/L PFOS, and the larval growth (as organism weight and length) and non-apical GSI LOECs were < 0.01 mg/L PFOS. In the latter (F1 generation), the reduction in fish weight at 0.01 mg/L was only 12% compared to controls, thus, the concentration-response curve for weight was shallow: 1.0 mg/L yielded a 29% reduction in weight. Many of these toxicity values, particularly those for apical endpoints, suggested that this genus is likely less sensitive than the one of the four most sensitive genera that drive the chronic criterion, which is 0.0084 mg/L. The magnitude of the chronic criterion is expected to be protective of the low LOEC for larval growth in the study of < 0.01 mg/L, which appears to be the most sensitive endpoint in the study. The results of the apical endpoints evaluated in this study were considered to be qualitatively acceptable primarily because of a lack of replication during the egg stage of the F1 generation.

4.5 Evaluation of the Acute Insect Minimum Data Requirement through Interspecies Correlation Estimates (ICE)

The acute dataset for PFOS contained 18 genera (Table 3-3) representing seven of the eight taxonomic MDR groups. The missing MDR was a representative from an insect family. Evaluation of qualitatively acceptable insect data (i.e., Yang et al. 2014) relative to the acute criterion magnitude was the primary line of evidence used to inform insect sensitivity to acute PFOS exposures (see section 3.1.1.1.6). Acute insect LC_{50} data were estimated using web-ICE

and compared to the acute criterion as a secondary line of evidence to evaluate insect sensitivity to acute PFOS exposures.

EPA's web-ICE tool is described in detail in Appendix L.1. Briefly, ICE models 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). ICE models can be used 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). This analysis focused on all possible ICE models that used insects as a predictor species (i.e., y-axis) and a corresponding surrogate input species (i.e., x-axis) for which a SMAV (see Table 3-3) was available. These models are shown in Table 4-9 along with use classifications for each individual model based on a host of statistical metrics described by (Willming et al. 2016) see box one of Appendix L.1 for additional discussion on model use criteria).

Table 4-9. All ICE models available in web-ICE v3.3 for predicted insect 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)	R2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µg/L)	Surrogate model maximum value (µg/L)	Cross-validation Success (%)	Taxonomic Distance	Use Classification
Atherix variegata	*Oncorhynchus mykiss*	0.94	0.73	2	0.91	0.0439	0.08	0.61	59.27	100	6	Accepted
Chironomus plumosus	*Americamysis bahia*	0.64	1.1	9	0.65	0.0026	0.97	0.01	5083	45	5	Rejected
Chironomus plumosus	*Daphnia magna*	0.63	1.05	19	0.5	0.0002	1.14	0.13	39000	29	5	Rejected
Chironomus plumosus	*Oncorhynchus mykiss*	0.78	0.3	21	0.5	0.0001	1.04	0.82	140000	35	6	Rejected
Chironomus plumosus	*Pimephales promelas*	1.03	-0.46	15	0.64	0.0001	0.99	2.27	97000	35	6	Rejected
Chironomus tentans	*Daphnia magna*	0.83	0.94	7	0.79	0.0011	1.03	0.32	472000	33	5	Rejected
Chironomus tentans	*Oncorhynchus mykiss*	1.11	-0.64	5	0.81	0.005	0.95	11.24	905704	29	6	Accepted qualitatively
Chironomus tentans	*Pimephales promelas*	1.21	-1.04	5	0.8	0.006	1.34	19.63	766452	57	6	Rejected
Claassenia sabulosa	*Americamysis bahia*	0.34	0.4	3	0.77	0.049	0.04	0.04	8.85	100	5	Rejected
Claassenia sabulosa	*Oncorhynchus mykiss*	0.42	-0.43	7	0.55	0.0213	0.23	0.61	1638	67	6	Rejected
Claassenia sabulosa	*Pimephales promelas*	0.33	-0.62	6	0.63	0.0182	0.22	1.24	110000	75	6	Rejected
Paratanytarsus dissimilis	*Daphnia magna*	0.57	2.17	8	0.41	0.0441	1.96	0.66	1190000	50	5	Rejected
Paratanytarsus dissimilis	*Lithobates catesbeianus*	0.92	0.67	4	0.84	0.0093	0.99	2.50	3019983	67	6	Rejected
Paratanytarsus dissimilis	*Oncorhynchus mykiss*	0.8	1.45	8	0.83	0.0002	0.54	0.61	1330000	70	6	Accepted
Paratanytarsus dissimilis	*Pimephales promelas*	0.8	1.27	10	0.8	0	0.52	1.24	1430000	75	6	Accepted
Paratanytarsus parthenogeneticus	*Daphnia magna*	0.93	0.74	5	0.98	0	0.04	9.91	14500000	100	5	Accepted
Paratanytarsus parthenogeneticus	*Oncorhynchus mykiss*	0.86	1.45	4	0.78	0.0193	1.1	32	9800000	50	6	Rejected
Paratanytarsus parthenogeneticus	*Pimephales promelas*	1.05	0.22	4	0.97	0.0002	0.13	92	10600000	83	6	Accepted
Pteronarcella badia	*Americamysis bahia*	0.72	0.83	4	0.83	0.0112	0.4	0.12	7300	50	5	Accepted
Pteronarcella badia	*Oncorhynchus mykiss*	0.59	-0.21	15	0.48	0.0018	0.88	0.61	1100000	47	6	Rejected
Pteronarcella badia	*Pimephales promelas*	0.28	-0.06	8	0.7	0.0023	0.09	1.24	110000	100	6	Rejected
Pteronarcys californica	*Daphnia magna*	0.63	0.72	24	0.54	0	0.94	0.15	68300	42	5	Rejected
Pteronarcys californica	*Oncorhynchus mykiss*	0.63	0.05	44	0.25	0.0003	1.7	0.61	70500	35	6	Rejected

Table 4-10 shows model outputs from all the rejected, qualitatively acceptable, and acceptable ICE models listed in Table 4-9. PFOS acute values are typically reported as mg/L and are, therefore, often greater than the toxicity values used to develop an ICE model, meaning the input PFOS LC_{50} value of the surrogate was typically outside the model domain. In these cases, the input toxicity value could be entered as µg/L and model would be allowed to extrapolate beyond its range or the input toxicity value could be a "scaled" mg/L value (i.e., estimate the value as mg/L). Table 4-10 includes a column to denote whether the input toxicity data were µg/L or a "scaled" mg/L value for individual models. Please see Appendix L.2.1 for further discussion on the selection process for identifying whether a µg/L or a "scaled" mg/L value was used as the input toxicity value for individual models.

Within Table 4-10, bolded and underlined values in the "Estimated Toxicity" column represent the estimated LC_{50} values from the acceptable models. Only estimated toxicity values from acceptable models were used to develop the estimated insect SMAVs reported in Table 4-10. When more than one acceptable ICE model was available for an individual predicted insect species, all the acceptable estimated toxicity values (i.e., LC_{50} values) were taken together as a geometric mean to represent the estimated SMAV.

Table 4-10. ICE-estimated Insect Species Sensitivity to PFOS. Values in bold and underlined are used for estimated insect SMAVs.

Common Name	Predicted Species	Surrogate Species	µg/L or mg/L input	Estimated Toxicity (mg/L)	95% Confidence Intervals (mg/L)	SMAV
Snipefly	*Atherix variegata*	*Oncorhynchus mykiss*	mg/L	**<u>36.89</u>**	8.72 - 155.96	36.89
Midge	*Chironomus plumosus*	*Americamysis bahia*	µg/L	3.01[c]	0.25 - 36.42	NA
		Daphnia magna	µg/L	11.06[bc]	1.48 - 82.86	
		Oncorhynchus mykiss	µg/L	2.23[c]	0.59 - 8.42	
		Pimephales promelas	µg/L	3.13[c]	0.74 - 13.23	
Midge	*Chironomus tentans*	*Daphnia magna*	µg/L	71.65[ac]	6.41 - 801.48	NA
		Oncorhynchus mykiss	µg/L	4.59[a]	0.44 - 48.06	
		Pimephales promelas	µg/L	4.3[ac]	0.32 - 57.74	
Stonefly	*Claassenia sabulosa*	*Americamysis bahia*	µg/L	0.048[bc]	0.002 - 1.13	NA
		Oncorhynchus mykiss	µg/L	0.016[bc]	0.002 - 0.11	
		Pimephales promelas	µg/L	0.0045[c]	0.001 - 0.01	
Midge	*Paratanytarsus dissimilis*	*Daphnia magna*	µg/L	71.3[ac]	3.92 - 1295.81	28.72
		Lithobates catesbeianus	µg/L	249.84[ac]	9.89 - 6311.19	
		Oncorhynchus mykiss	µg/L	**<u>36.72</u>**	9.95 - 135.48	
		Pimephales promelas	µg/L	**<u>22.47</u>**	7.56 - 66.77	
Midge	*Paratanytarsus parthenogeneticus*	*Daphnia magna*	µg/L	**<u>138.7</u>**	83.27 - 231.04	51.00
		Oncorhynchus mykiss	µg/L	60.97[ac]	3.75 - 990.7	
		Pimephales promelas	µg/L	**<u>18.75</u>**	7.15 - 49.19	
Stonefly	*Pteronarcella badia*	*Americamysis bahia*	mg/L	**<u>21.36</u>**	4.03 - 113.19	21.36
		Oncorhynchus mykiss	µg/L	0.13[c]	0.028 - 0.58	
		Pimephales promelas	µg/L	0.01[c]	0.005 - 0.02	
Stonefly	*Pteronarcys californica*	*Daphnia magna*	µg/L	5.33[c]	0.64 - 44.56	NA
		Oncorhynchus mykiss	µg/L	0.34[c]	0.08 - 1.41	

[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.

Overall, acceptable ICE models and empirical acute PFOS LC_{50} values as model input data were available to support the estimation of SMAVs for four individual insect species (Table 4-10). Estimated insect SMAVs ranged from 21.36 mg/L for the stonefly, *Pteronarcella badia*, to 51.00 mg/L for the midge, *Paratanytarsus parthenogeneticus.* All four of the estimated insect SMAVs (Table 4-10) were greater than the FAV (i.e., 6.011 mg/L; section 3.2.1.1) by more than a factor of 3.6. Further, qualitatively acceptable empirical insect toxicity data (i.e., Yang et al.

2014; see section 3.1.1.1.6) suggested insects are not among the most sensitive genera to acute PFOS exposures. Estimated insect SMAVs and qualitatively acceptable insect data were considered together as multiple lines of evidence to conclude there is variability in the sensitivity of aquatic insects. EPA will continue to seek additional acute PFOS insect data to further understand the sensitivity of this taxon. And for the time the current development of an acute freshwater criterion was based on seven of the eight MDRs.

4.6 Acute-to-Chronic Ratios

The 1985 Guidelines allow the use of a 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, the calculations of ACRs are as follows. Sixteen ACRs for seven 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-Chronic Ratios (SMACRs) were also calculated as the geometric mean value of individual ACRs. In the case of a single ACR within a species, that ACR was the SMACR.

The ACRs ranged from 4.110 to 13,674 across all species (a factor of 3,327), 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 13,674 from paired tests conducted by Lu et al. (2015) appears to be an outlier. Excluding the 13,674 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 4.11 to 1,030. This range was greater than a factor of 10 with no relationship between ACR and SMAV apparent. The Guidelines do not provide for calculation of a FACR under these circumstances. However, if one were calculated as the geometric mean of the SMACRs excluding the outliers, it would be 122.2, representing the geometric mean of the eight SMACRs highlighted in bold font in Appendix I.

4.7 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 4-11). 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 4.7.1 and Section 4.7.2.

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. While tissue concentrations from the toxicity literature were limited, overall, translated tissue concentrations for invertebrate whole-body, fish whole-body and fish muscle were consistent with tissue-based PFOS concentrations from chronic toxicity studies with direct aqueous exposure. However, tissue concentrations from toxicity studies focused on maternal transfer indicated that the tissue criteria may be under protective and that a reproductive tissue criterion may be needed to ensure protection from PFOS through this exposure pathway (Hazelton et al. 2012; Wang et al. 2011). Hazelton et al. (2012) examined the effects of PFOS on

glochidia viability and metamorphic success with the highest test concentration (0.0695 mg/L) causing an estimated 34.5% reduction in metamorphosis success (see Appendix C.2.2 for more details). Wang et al. (2011) saw a decrease in the survival of the F1 generation from exposed adults, with an EPA independently-calculated an EC_{10} value of 0.0165 mg/L (see Appendix C.2.4 for more details). Nevertheless, BAF data for reproductive tissues are currently limited; and therefore, a reliable reproductive tissue criterion cannot be derived at this time (see Appendix Q).

As for other tissue types and taxa with limited data, tissue concentrations from available toxicity studies suggest that the translated tissue concentrations for fish liver and reproductive tissues may be under protective. While no amphibian tissue criteria are available, tissue concentrations from two amphibian toxicity tests indicate that the fish tissue criteria may not be protective of amphibians. However, tissue data for these tissues and taxa are limited and additional data are needed.

Table 4-11. Comparison of Empirical Tissue Concentrations to Chronic Tissue Criteria and Additional Tissue Values.

Species	Endpoint	Measured Tissue Concentration (mg/kg ww)[1]	Chronic Tissue Values[2] (mg/kg ww)	Tissue Type
Quantitative Studies				
Fatmucket (*Lampsilis siliquoidea*)	Probability of successful metamorphosis of glochidia	LOEC: 0.248	0.937	Invertebrate Whole-body (adult)
Zebrafish (*Danio rerio)*	F1 survival	LOEC: 4.0	6.75	Whole-body (adult)
Fathead minnows (*Pimephales promelas*)	Fecundity	NOEC: 8.7 – LOEC 19.6	43.30[3]	Gonad concentrations (adult male)[3]
		NOEC: 34.8 – LOEC 82.6	43.30	Gonad concentrations (adult female)
	Growth (weight in F1)	LOEC: 37.9	43.30[3]	Gonad concentrations (adult F0 male)[3]
		LOEC: 37.4	43.30	Gonad concentrations (adult F0 female)
		LOEC: 84.5	20.68	Liver (adult F0 male)
		LOEC: 68.2	20.68	Liver (adult F0 female)
Northern leopard frog (*Lithobates pipiens*)	Length at metamorphosis at GS 42[4]	LOEC: 18.81	None Available	Whole-body (before metamorphosis)
		LOEC: 13.89		Whole-body
Qualitative Studies				
Red worms (*L. hoffmeisteri*)[5]	Reduction in superoxide dismutase	LOEC: 1,757 (dw)	0.937	Invertebrate Whole-body
Great pond snails (*Lymnaea stagnalis*)	Survival	LOEC: 2,955	0.937	Invertebrate Whole-body
European eels (*Anguilla anguilla*)[5]	Changes in protein expression	NOEC: > 5.037	20.68	Liver
Goldfish (*Carassius auratus*)	Survival	NOEC: > 39.91	2.91	Muscle
Common carp (*Cyprinus carpio*)	Condition factor	LOEC: 35.97	20.68	Liver

Species	Endpoint	Measured Tissue Concentration (mg/kg ww)[1]	Chronic Tissue Values[2] (mg/kg ww)	Tissue Type
Zebrafish (*D. rerio*)[4]	Swimming activity	LOEC: 0.214	6.75	Whole-body

[1] Tissue concentrations are author reported values. 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 Q for details.
[3] Fish reproductive tissue value based on female reproductive tissue.
[4] Gosner stage 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 Kolross (1946).
[5] Toxicity data for non-apical endpoints.

4.7.1 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 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 produced 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 6.2 and 11.1 mg/kg wet weight, respectively, in males, and 4.0 and 7.7 mg/kg wet weight, respectively, in females. PFOS was also measured in embryos produced from exposed parents and averaged 5.75 and 11.0 ng/embryo wet weight. 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 held the resulting offspring 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 the 0.0276, 0.101, and 0.281 mg/L exposures averaged 28.6, 134, and 355 mg/L in males, and 48.5, 178, and 474 mg/L in females. Liver PFOS concentrations in the 0.0276, 0.101, and 0.281 mg/L exposures averaged 6.9, 19.0, and 109 mg/kg wet weight in males, and 32.8, 82.8, and 262 mg/kg wet weight in females. Gonad PFOS concentrations in the 0.0276, 0.101, and 0.281 mg/L exposures averaged 8.7, 19.6, and 109 mg/kg wet weight in males, and 34.8, 82.6, and 261 mg/kg wet weight in females. Embryo PFOS concentrations in the 0.0276, 0.101, and 0.281 mg/L exposures 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 of 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 gonad concentrations would be expected to fall between 8.7 and 19.6 mg/kg in males and 34.8 and 82.6 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, with a MATC of 0.06222 mg/L. The 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 concentrations would be expected to fall below the translated reproductive tissue concentration of 77.52 mg/kg ww and above the translated liver tissue concentration of 67.30 mg/kg ww. No muscle or whole-body measurements in adults are available to perform a direct comparison to the 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. Corresponding whole-body tissue concentrations measured in tadpoles exposed for 54 days (before metamorphosis) were 10.1 and 67.42 mg/kg dry weight. Whole body concentrations were also measured after 35 days and were similar to 54-day measurements in the 0.957 mg/L exposure (10.2 mg/kg dry weight), but higher in the 3.42 mg/L exposure (117.4 mg/kg dry weight). Tadpole moisture content was not reported. 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). The resulting whole-body wet weight concentrations corresponding to the NOEC and LOEC were 2.84 and 32.75 mg/kg wet weight based on day 35 measurements, and 2.82 and 18.81 mg/kg wet weight based on day 54 measurements.

In a separate study with *Lithobates pipiens*, Hoover et al. (2017), exposed juvenile (Gosner stage 26) northern leopard frogs to three PFOS concentrations (7.65, 78.1, and 884 mg/L measured PFOS, respectively) for 40 days. Survival, growth (snout-vent length), and developmental time (days to Gosner stage 40) were measured, and the most sensitive apical endpoint was time (in days) to reach Gosner stage 40, with a NOEC of 7.65 mg/L and a LOEC of 78.1 mg/L. Whole body PFOS concentrations in frogs exposed to the NOEC exposure level averaged 10.53 mg/kg dry weight after 40 days, and concentrations in frogs exposed to the LOEC exposure level averaged 49.77 mg/kg dry weight after 40 days. Tadpole moisture content was not reported in this study, so the 72.1% moisture content for fish species described above was applied to convert concentrations to wet weights. Corresponding 40-day NOEC and LOEC wet weight PFOS concentrations were 2.94 and 13.89 mg/kg wet weight, respectively.

Tissue concentration data from the Hazelton et al. (2012) and Wang et al. (2011) suggest that the tissue criteria may not be protective, as measured whole-body tissue LOECs are lower than corresponding whole body tissue criteria. However, these studies focused on exposure via maternal transfer, suggesting that a reproductive tissue criterion may be needed to ensure protection of PFOS through this exposure pathway. Nevertheless, BAF data for reproductive tissues is currently limited and therefore, a reliable reproductive tissue criterion cannot be derived at this time (see Appendix Q). Fathead minnow data reported in Ankley et al. (2005) and

Suski et al. (2021) suggest that fish liver and reproductive tissue chronic values may be protective. Female gonad tissue concentrations associated with decreased fecundity reported by Ankley et al. (2005) were higher than the reproductive tissue chronic value. Male and female liver tissue concentrations associated with reduced growth were higher than the liver chronic value, while female gonad tissue concentrations associated with reduced growth were slightly lower than the reproductive tissue chronic value. However, results of these fathead minnow studies were not directly comparable to chronic tissue criteria, because neither whole-body nor muscle tissue were measured. Finally, although no amphibian tissue criteria are available, tissue concentrations associated with the LOEC in both (Ankley et al. 2004) and (Hoover et al. 2017) are both higher than the fish whole-body tissue criterion, suggesting that the fish tissue criteria may be protective of amphibians.

4.7.2 Comparison of Qualitative Studies and Tissue-Based Criteria

Like the comparison with the quantitative studies, 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. Liu et al. (2016) exposed 4-5 cm body length red worms (*Limnodrilus hoffmeisteri*) to two aqueous concentrations of PFOS for 10 days 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 water concentrations used were not reported. 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. The tissue concentrations are not representative of what would be measured in water concentrations at the EC_{50}, which ranged from 23.81 mg/L PFOS at pH 6.2 to 39.80 mg/L PFOS at pH 8.0, nor are they representative of bioaccumulation in response to a chronic exposure.

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 9,179 mg/kg dry weight and 10,087 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 tissue criterion. Resulting wet weight PFOS concentrations at the NOEC and LOEC were 2,699 and 2,955 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 exposure concentrations. Corresponding liver tissue PFOS concentrations after 28 days were 0.0338 and 5.037 mg/kg wet weight, respectively. Significant ($p < 0.05$) changes in protein expression were reported for both exposure concentrations, but no significant effects of growth or survival were reported at either exposure concentration.

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 wet weight, respectively, at the lower exposure level, and 58.37, 69.02, and 39.91 mg/L wet weight, respectively, at the higher exposure level. No effects of mortality were observed during the test. Among the antioxidant enzyme activity endpoints, glutathione peroxidase was significantly ($p < 0.05$) lower than the control in the highest exposure concentration.

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 ($p < 0.0001$) higher than controls among fish in the 0.1 mg/L exposure after 14 days, although larger condition factors are associated with increase well-being in fish. The lowest exposure level where a significantly ($p < 0.0001$) lower condition factor than controls was observed was at the 0.5 mg/L exposure concentration.

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 0.022 and 0.214 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 activity, where fish exposed to 1.0 mg/L PFOS responded more slowly ($p < 0.05$) to a startle response, followed by a longer ($p < 0.05$) hyperactive response to the stimulus.

Relationships between tissue PFOS concentrations associated with effect concentrations and tissue criteria among qualitative studies were varied. Among invertebrates, *Lymnaea stagnalis* whole-body concentrations were well above the criterion at the NOEC for survival. The absence of wet weight measurements made comparisons less direct for *Limnodrilus hoffmeisteri*, but the measured whole-body dry weight NOEC for reduced enzyme activity reported by Liu et al. (2016) was nearly 100 times higher than the invertebrate tissue criterion, strongly suggesting that the wet weight NOEC would also be greater than the tissue criterion. Among fish, concentrations in *Anguilla anguilla* were below the tissue criterion, but no effects were observed. In *Carassius auratus*, the highest muscle tissue concentration was greater than the criterion, and no apical effects were reported. The measured liver LOEC for *Cyprinus carpio* condition factor was greater than the chronic liver value. Finally, the whole-body LOEC for *Danio rerio* was lower than the whole-body criterion, but the LOEC was based on the non-apical behavioral endpoint swimming activity. Overall, tissue concentration data from qualitative studies either suggest the tissue criteria would be protective, or do not provide any evidence that the PFOS tissue criteria would not be protective of aquatic species.

4.8 Effects on Aquatic Plants

Available data for aquatic plants and algae were reviewed to determine if aquatic plants were likely to be adversely affected by PFOS and if they were likely to be more sensitive to PFOS than aquatic animals (see Appendix E). Toxicity values for freshwater plants were well above the freshwater chronic criterion as effect concentrations for freshwater plants and algae ranged from 0.19 to 252 mg/L relative to animal chronic values of 0.001588 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.

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

The PFOS aquatic life criteria 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 freshwaters. The freshwater acute water column-based criterion magnitude is 3.0 mg/L, and the chronic water column-based criterion magnitude is 0.0084 mg/L (8.4 µg/L). The chronic freshwater criterion also contains tissue-based criteria expressed as 6.75 mg/kg wet weight (ww) for fish whole-body, 2.91 mg/kg ww for fish muscle tissue and 0.937 mg/kg ww for invertebrate whole-body tissue. These PFOS aquatic life criteria are expected to be protective of freshwater aquatic life, such as fish and aquatic invertebrates on a national basis. Although empirical PFOS toxicity data for estuarine/marine species were not available to fulfill the eight MDRs directly, EPA included an acute aquatic life benchmark for estuarine/marine environments in Appendix L, using available estuarine/marine species toxicity data and a NAM application of ORD's peer-reviewed webICE tool. 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 PFOS exposures. EPA conducted additional analyses supporting the derivation of the water column criteria for PFOS (all summarized above in Sections 4.2 and 4.3) 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 (2000e)
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 (2000b)
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, 2015 #325}
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**	-	Yang et al. (2019)
Cladoceran (0-24 hr), *Daphnia magna*	S, U	48 hr	PFOS-K Unknown	7.6	22	EC50	27	-	**27**	48.87	3M Company 2000

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), *Daphnia pulicaria*	S, U	48 hr	PFOS-K 95%	-	21	EC50 (immobility)	134	-	**134**	134	(Boudreau 2002; Boudreau et al. 2003a)
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)
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	(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)
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)
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*	S, U	96 hr	PFOS-K ≥97%	7.2-7.5	26	LC50	58.47	-	**58.47**	-	Hagenaars et al. (2010, 2011)
Zebrafish (adult), *Danio rerio*	R, M	96 hr	PFOS-K 98%	-	26	LC50	22.2	-	**22.2**	-	Sharpe et al. (2010)

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
Zebrafish (3 mo., 2.2 cm), *Danio rerio*	R, U	96 hr	PFOS-K Unknown	-	23	LC50	17.0	-	**17.0**	-	Wang et al. (2013a)
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. (2016); Du et al. (2017)
Zebrafish (embryo, 1 hpf), *Danio rerio*	R, U	96 hr	PFOS Unreported	-	26	LC50	34.2	38.82	**38.82**	24.44	(Stengel et al. 2017)
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 (2000d)
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 Unknown	-	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 Unknown	-	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 Unknown	-	21	LC50	79	78.33	78.33[d]	-	Tornabene et al. (2021)
Gray treefrog (larva, Gosner stage 40), *Hyla versicolor*	S, U	96 hr	PFOS Unknown	-	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 Unknown	-	21	LC50	144	154.8	154.8[d]	-	Flynn et al. (2019)

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
American bullfrog (larva, Gosner stage 26), *Lithobates catesbeiana*	S, U	96 hr	PFOS Unknown	-	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 Unknown	-	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 Unknown	-	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 Unknown	-	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)
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)

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
Jefferson salamander (larva, Harrison stage 40), *Ambystoma jeffersonianum*	S, U	96 hr	PFOS Unknown	-	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 Unknown	-	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 Unknown	-	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 Unknown	-	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.

[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 lifestages.

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. C-R models developed by EPA that were used to determine acute toxicity values used for 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, 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 concentration-response data in the publication/supplemental materials and/or did not provide concentration-response data upon EPA request. In such cases, 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: *Pimephales* (fathead minnow)

3MCompany (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 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-hr 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 (2000d) 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 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

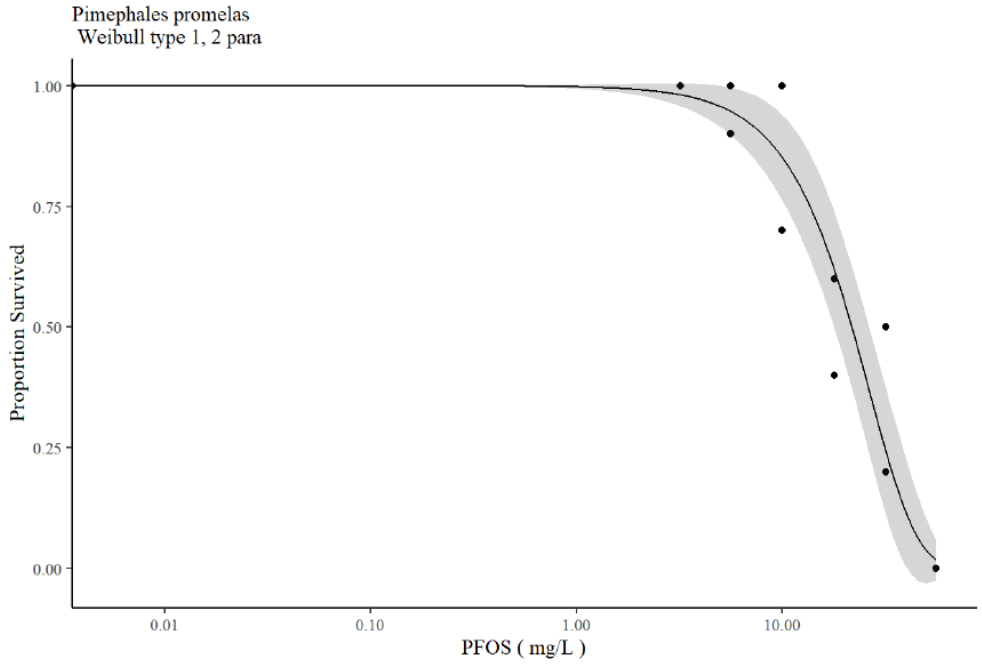
filled with 15 L of test solution with an observed dissolved oxygen of 7.7 - 8.4 mg/L, temperature of 22 ± 2ºC, pH of 8.2 - 8.5 and a total hardness of 131 mg/L as $CaCO_3$. Fathead minnows were subjected to a 16-hr:8-hr photoperiod at 391 lux. Sand and 0.45 um filtered well water from a 40 m deep well on site served as both the culture water and the testing media. The authors reported an LC_{50} of 9.5 mg/L PFOS. EPA's independently-calculated 96-hour LC_{50} was 9.0196 (7.146 - 10.89) mg/L and was used quantitatively to derive the draft acute water column criterion for freshwater.

A.2.1.1 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:

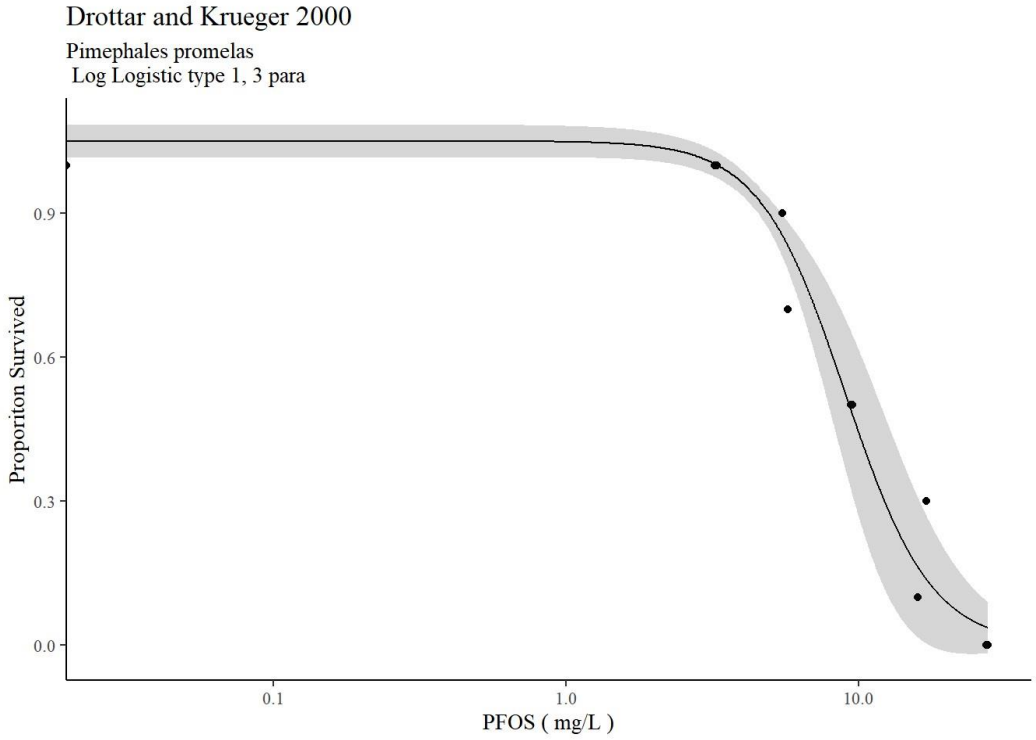


A.2.1.2 Drottar and Krueger (2000d) Concentration Response Curve – Pimephales (fathead minnow)

Publication: Drottar and Krueger (2000d)
Species: Fathead minnow, *Pimephales promelas*
Genus: *Pimephales*
EPA-Calculated LC_{50}: 9.012 (95% C.I. 7.146315 - 10.892956) 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.2.2 Second 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-hr:12-hr, 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. 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. 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 draft acute water column criterion.

In addition to the two additional analyses presented in Section 4.2.1 above, a third additional analysis was conducted to determine the influence of the LC_{50} value from Sharpe et al. 2010. In this calculation, the toxicity data for aquatic insects (consisting of data for yellow fever mosquito (*Aedes aegypti*) and a chironomid (*Chironomus plumosus*) were not used and the LC_{50} for rainbow trout (*Oncorhynchus mykiss*) from Sharpe et al. (2010) was also removed given that the biomass loading rate (see Section 3.1.1.1.2 and A.2.2) was higher than that stated in OECD Guidelines of 0.8 g/L (OECD 1992). In this scenario the alternate SMAV for *O. mykiss* (22.59 mg/L) would not rank among the four most acutely sensitive genera. The third analyses yielded a freshwater FAV for PFOS of 7.420 mg/L, again calculated following the procedures described in the 1985 Guidelines (U.S.EPA 1985). The FAV was then divided by two (see Section 3.2.1.1) to calculate an acute water column concentration, of 3.7 mg/L PFOS, which was 1.5 times higher than the reported LC_{50} from the Sharpe et al. (2010) rainbow trout study. EPA retained the rainbow trout value for the purposes of the current derivation, to ensure protection of sensitive salmonid species as a group, which includes commercially and recreationally important species, as well as endangered species. The availability of toxicity data for these taxa developed using more standardized and better documented procedures would reduce the uncertainty in the analysis. Additionally, this third analysis lacked the MDRs for aquatic insects.

(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 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-hr:8-hr, light:dark photoperiod were 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 criterion. The independently-calculated 96-hour LC_{50} value was 22.59 mg/L. This independently-calculated LC_{50} value of 22.59 (14.53 – 30.65) mg/L was used quantitatively 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 draft acute water column criterion.

A.2.2.1 Sharpe et al. (2010) Concentration Response Curve - Oncorhynchus (trout)

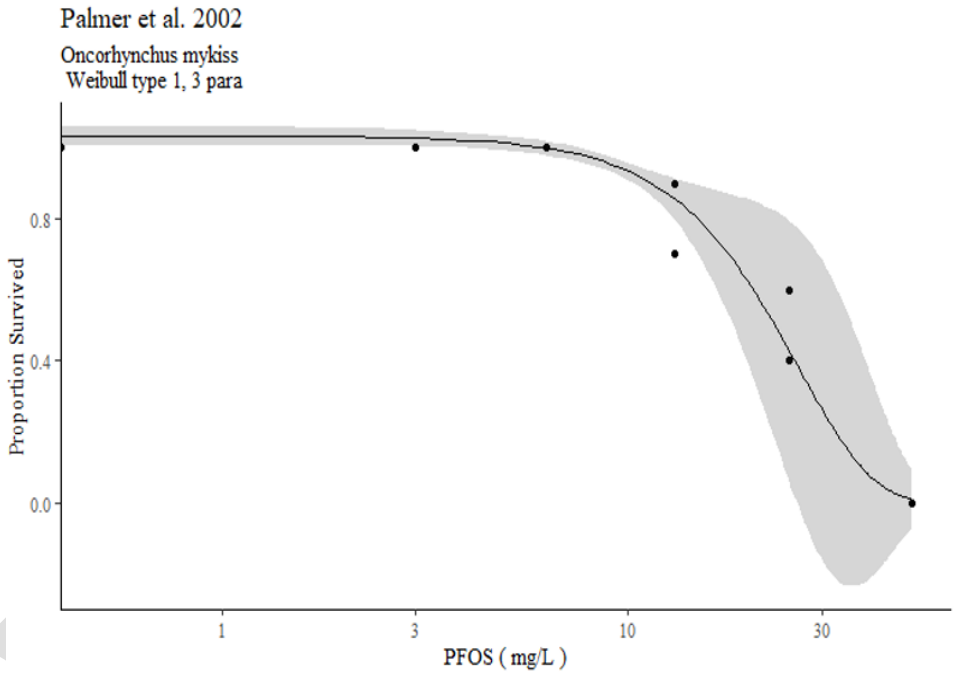
Publication: Sharpe et al. (2010)
Species: Rainbow trout, *Oncorhynchus mykiss*
Genus: *Oncorhynchus*
EPA-Calculated LC_{50}: Not calculable, concentration-response data not available

A.2.2.2 Palmer et al. (2002) 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.2.3 Third 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 3rd and 6th most sensitive genera values (respectively) in the PFOS freshwater acute toxicity database (The *L. siliquoidea* results are reported below). Acute toxicity was observed under static conditions over a 24-hour period (< 24-hour old glochidia) or a 96-hour period (4–6-

week-old juveniles). The tests followed the ASTM E2455-06 (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 < 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 dissolved oxygen 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}s are not used quantitatively in species mean acute values (SMAVs). The 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 draft acute water column criterion.

A.2.3.1 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.2.4 Fourth 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),

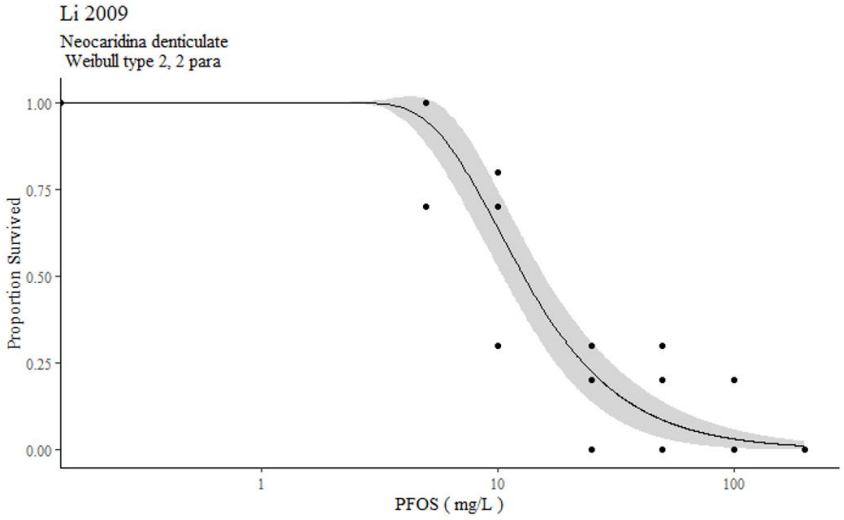
10.32 (7.788 – 12.85) mg/L, respectively. These independently-calculated LC_{50} values were used to calculate the GMAV value (as the geometric mean of the three LC_{50} values previously mentioned) of 15.61 mg/L and was used to derive the freshwater aquatic life criteria.

A.2.4.1 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:

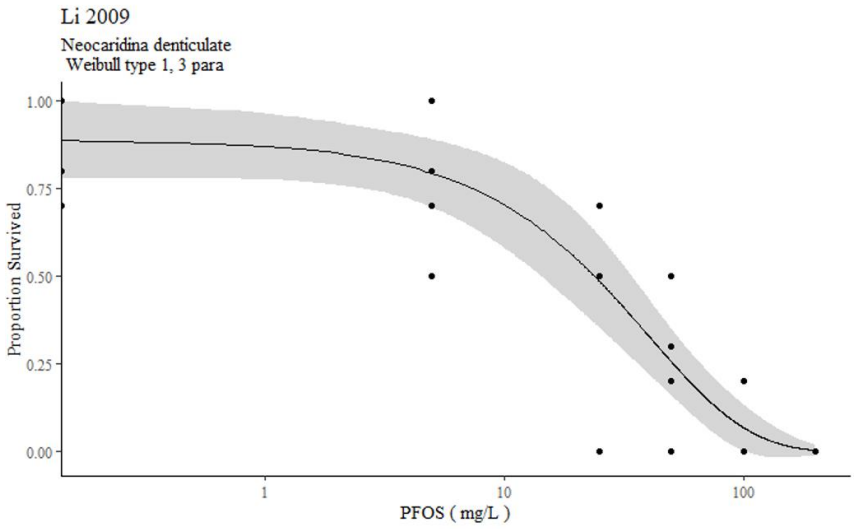
Parameter	Estimate	Std. Error	t-stat	p-value
b	-1.5141	0.1920	-7.8879	$3.091\ e^{-15}$
e	10.1360	1.0252	9.8865	$< 2.2\ e^{-16}$

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



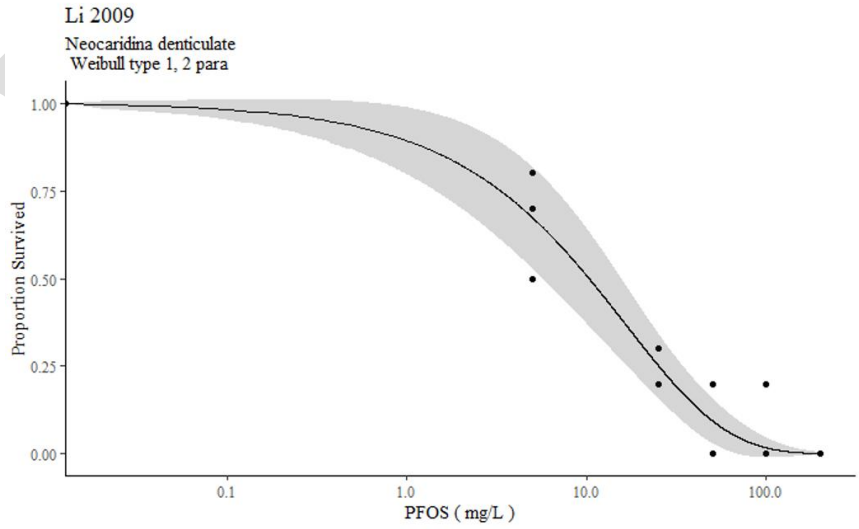
Concentration-Response Model Estimates:

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.2.5 Fifth Most Sensitive Freshwater Genus for Acute Toxicity: *Xenopus* (frog)

Palmer and Krueger (2001), associated with Wildlife International, conducted three 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-hr:8-hr (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 $CaCO_3$, dissolved oxygen of 7.8-8.1 mg/L and a 12-hr:12-hr 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. All data are deemed quantitative and the independently-calculated toxicity values were utilized

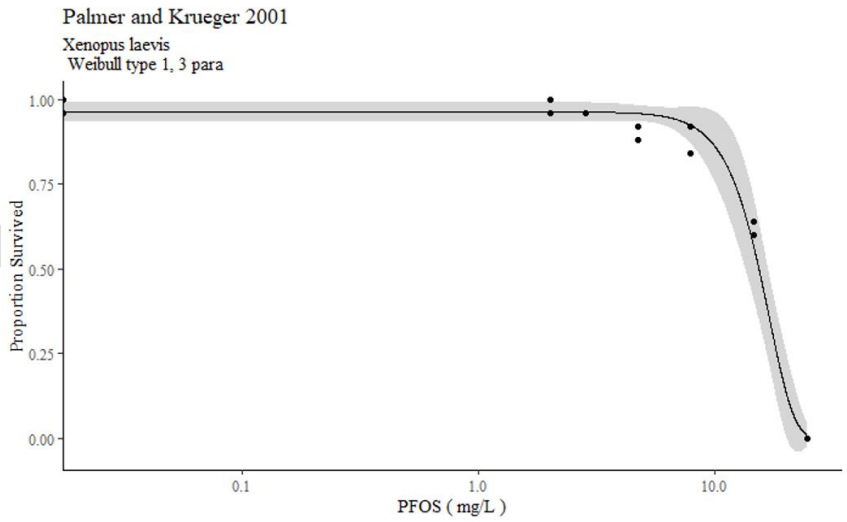
to derive the PFOS aquatic life criteria. No additional quantitative, acute toxicity data were available for this species. Therefore, these independently-calculated LC_{50} values were used to calculate the GMAV value (as the geometric mean of the three LC_{50} values previously mentioned) of 15.99 mg/L and was used to derive the freshwater aquatic life acute criterion.

A.2.5.1 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:

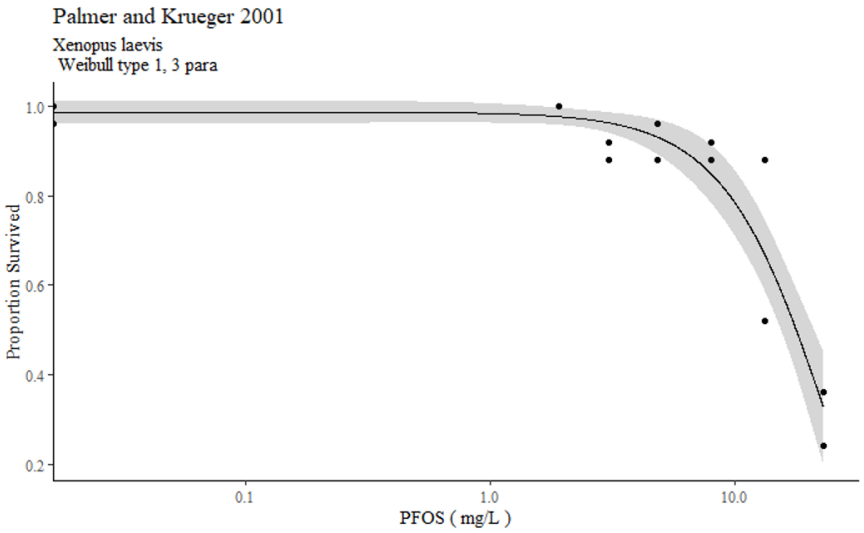
Parameter	Estimate	Std. Error	t-stat	p-value
b	4.1306	1.0464	3.9475	$7.897\ e^{-5}$
d	0.9633	0.0149	64.7242	$< 2.2\ e^{-16}$
e	16.9770	0.7991	21.2452	$< 2.2\ e^{-16}$

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



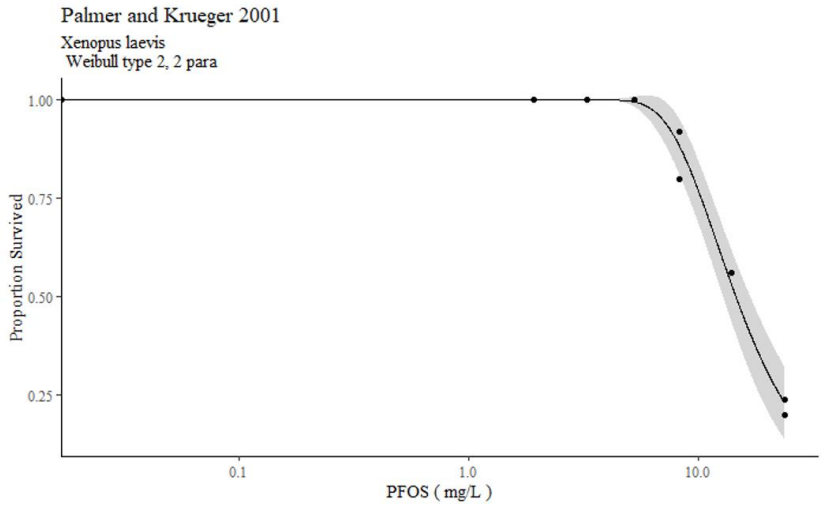
Concentration-Response Model Estimates:

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.2.6 Sixth 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 6th most sensitive genus value in the PFOS freshwater acute toxicity database. Hazelton et al.'s experimental design and study conditions for *L. siliquoidea* were reported above under the description of the third 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. The independently-calculated toxicity values could not be calculated at this time given the data presented in the paper. The study author reported values were used quantitatively to derive the draft acute water column criterion.

A.2.7 Seventh 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 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. Photoperiod for *M. macrocopa* was assumed by the reviewers to have been 16-hr:8-hr

(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 dissolved oxygen) 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 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 EPA as 17.20 (13.73 – 20.66) mg/L for *M. macrocopa.* The independently-calculated acute toxicity value was quantitatively used in the derivation of the PFOS acute criterion.

A.2.8 Eighth 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 (U.S. EPA 2002; ASTM 2017). 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 40 was run to determine if toxicity varied between life stages. Before test initiation larvae were acclimated to test conditions (21℃ and 12-hr:12-hr 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 Gosner stage 26 larva, and five to six replicates for each treatment for Gosner stage 40 frogs. No mortality occurred in the controls of the Gosner stage 26 test and two of the six frogs died in the controls of the Gosner stage 40 test. The author reported 96-hour LC_{50}s were 79 and 24 mg/L PFOS for Gosner Stage 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 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 acute freshwater criterion for PFOS.

A.2.9 Ninth 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 study author reported 96-hour LC_{50} was 17 mg/L (C.I. 16-18). The independently-calculated toxicity value could not be calculated at this time given the level of data that was presented in the paper. The study author reported value was used quantitatively to derive the draft acute water column criteria.

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 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 study author reported 96-hour LC_{50} was 23 mg/L (C.I. 20-25). The 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 PFOS 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 study author reported 96-hour LC_{50} was 29.46 mg/L (C.I. 25.80-33.12). The independently-calculated toxicity value could not be calculated at this time given the level of data that was presented in the paper. The study author reported value was used quantitatively to derive the draft 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 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 aquatic life criterion.

A.2.10 Tenth Most Sensitive Freshwater Genus for Acute Toxicity: *Danio* (zebrafish)

The acute effects of PFOS on the zebrafish, *Danio rerio*, have been reported by numerous researchers. **Hagenaars et al. (2011)** 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 ELS test and 1, 5, 10, 25, 50 and 100 mg/L in the 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 exposure condition 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-hr:10-hr (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 study authors reported a 96-hour LC_{50} of 58.47 mg/L PFOS, based on the results of the range finding test. The study author reported value was used quantitatively to derive the draft acute water column criterion.

Sharpe et al. (2010) examined the toxicity and bioaccumulation of PFOS 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 present section, as these

results were used quantitatively to derive the acute water column criterion. The 48-hour tests were used qualitatively (see G.2.2.3) and the chronic toxicity tests were used qualitatively and are summarized below in Section 4.4.2.1.4. and G.3.2.4 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 and a 14-hr:10-hr (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 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 dose 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 (approx. 500 mg/L). Zebrafish toxicity tests were performed using food grade 2 L plastic tanks with four fish per tank, and two tanks per dose. Fish were randomly assigned to nominal doses 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 LC_{50} was measured or nominal. The adult 96-hour acute test followed OECD 203 protocol and was acceptable for quantitative use. The study author reported LC_{50} was 22.2 ± 4.6 mg/L for PFOS. The study author reported value was used quantitatively to derive the draft acute water column criterion.

Wang et al. (2013a) 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, dissolved oxygen of 7.0 - 8.6 mg/L and a photoperiod of 12-hr:12-hr 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. EPA was unable to independently calculate a 96-hour LC_{50} value based on the level data provided in the paper by the study authors. Therefore, the author-reported LC_{50} value of 17.0 mg/L PFOS was used quantitatively to derive the draft acute freshwater 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-hr:10-hr 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 exposed embryos were assigned to each treatment (three replicates) and 2 mL corresponding solution per well; the four remaining wells were assigned with control embryos. Embryos were cultured in an incubator at 28.5°C, pH of 8.3 and a 14-hr:10-hr light:dark photoperiod. Toxicological endpoints included whether embryos were clear or opaque, have edema at 4, 8, 24, 48, 72, or 96 hpf, or have 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 and this toxicity value is acceptable for quantitative use and was used to derive the freshwater water criterion for PFOS.

In a later study, **Du et al. (2016;2017**) 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-hr:10-hr 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 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. EPA was unable to independently calculate a 96-hour LC_{50} value based on the level data provided in the paper by the study authors. Therefore, the author-reported LC_{50} value of 3.502 mg/L PFOS was used quantitatively to derive the draft acute freshwater criterion.

Stengel et al. (2017) 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 as described previously (Kimmel et al. 1995; Kimmel et al. 1988; Nagel 2002; Spence et al. 2006; Wixon 2000) and have been updated 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 control 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-hr:10-hr 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} were considered quantitative and were used to derive the PFOS acute water criteria.

A.2.11 Eleventh 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°C and a 16-hr:8-hr light:dark photoperiod. The acute test protocol followed OECD guidelines (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 authors 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 acute freshwater criterion for PFOS.

3MCompany (2000) provides the results of a 48-hour static, unmeasured acute toxicity test completed with the cladoceran, *Daphnia magna,* and PFOS-K (perfluorooctancesulfonate potassium salt, CAS # 2795-39-3, unknown purity) in 1984. In-house culture of daphnids were tested in unchlorinated, carbon filtered well water under a 16-hr:8-hr light:dark photoperiod, pH 7.6, total hardness of 256 mg/L as $CaCO_3$, dissolved oxygen >70% saturation and average temperature of 22°C. Twenty daphnids (12 ± 12 hour) were placed in 250 mL beakers with 200 mL of test solution and exposed to one of six nominal test treatments (specifics not provided) for 48 hours; each test treatment was duplicated. The author reported EC_{50}, based on immobility, was 27 mg/L PFOS. EPA was unable to independently calculate a 48-hour EC_{50} value based on the level data provided in the paper by the study authors. Therefore, the author-reported EC_{50} value of 27 mg/L PFOS was used quantitatively to derive the draft acute freshwater.

Drottar and Krueger (2000b) 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 202 (1994) and U.S. EPA OPPTS Number 850.1010 (1996). 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-hr:8-hr (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, 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. Daphnids in the negative control, and the 11 and the 20 mg/L treatments appeared healthy and normal throughout the test with no mortality, immobility or overt clinical signs of toxicity. Five percent mortality was observed at 48 hours in the negative control. The study 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 draft acute water column criterion.

Boudreau (2002) 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 E729-96 (1999). 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-hr:8-hr (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 study author reported 48-hour EC_{50} for *D. magna* was 67.2 mg/L (C.I. 31.3-88.5). The study author reported 48-hour EC_{50} for *D. pulicaria* was 134 mg/L (C.I. 103-175). The independently-calculated toxicity values could not be calculated at this time given the level of data that was presented in the paper. The study author reported values were used quantitatively to derive the draft 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 EPA's Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (U.S.EPA 2002b). *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-hr:8-hr (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 dissolved oxygen) 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 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 study 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 by EPA and equaled 35.46 (28.26 – 42.66) mg/L for *D. magna.* This independently-calculated acute toxicity value was included in the derivation of the PFOS acute 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 202 (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 and conductivity and dissolved oxygen 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, dissolved oxygen and specific conductivity were 67.7±6.8% and 101.8±6.8 µS/cm. After the 48-hour testing period, dissolved oxygen and specific conductivity were 55.6±1.26% and 109.1±3.5 µS/cm. The dissolved oxygen after 48-hours of testing was lower than the test guideline recommendation of >60% (ASTM 2002; U.S. EPA 2016a and b); 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 study 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. These independently-calculated LC_{50} values were used in the GMAV calculation.

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 E729 (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; dissolved oxygen, 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-hr:12-hr (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 study author reported 48-hour LC_{50} was 78.09 mg/L (C.I. 54.38-112.13). The study author reported value was used quantitatively to derive the draft acute water column criterion.

Lu et al. (2015) conducted a 48-hour static test on PFOS (purity 98%) with *Daphnia magna*, following OECD 202 (2004c). *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 (2004c) 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-hr:8-hr (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 dissolved oxygen of 6.5 ±0.5 mg/L. 100% 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). The study author reported value was used quantitatively to derive the draft 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 202 (2004c). *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-hr:8-hr (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 study 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 draft 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 OECD Guideline 202. 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. 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 draft acute water column criterion for freshwater.

A.2.12 Twelfth 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 (U.S. EPA 2002; ASTM 2017). 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℃ and 12-hr:12-hr 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) and 46.71 (34.33 – 59.09) for Harrison stage 40, 30.00 (27.14 – 32.86) for Harrison stage 46, and 68.63 (61.90 – 75.37) mg/L for the Jefferson salamander, small-mouthed salamander (both life stages tested) and eastern tiger salamander, respectively. In general, the independently-calculated toxicity values were acceptable for quantitative use and were utilized to derive the acute freshwater criterion for PFOS. Specifically, the LC_{50} value of Harrison stage 46 of 30.00 mg/L for small-mouthed salamander was used for this species alone (as opposed to both LC_{50} values) as this life stage was determined to be the most sensitive.

A.2.13 Thirteenth 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 (U.S. EPA 2002; ASTM 2017). 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 26, defined as when larvae are free swimming and feeding. An additional acute test with Gosner stage 41 was run to determine if toxicity varied between life stages. Before test initiation larvae were acclimated to test conditions (21°C and 12-hr:12-hr 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 Gosner stage 26 larva, and six to 10 replicates for each treatment for Gosner stage 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 frogs and 56.49 (49.10 – 63.90) mg/L for GS 41 toads. Given that the GS 41 appear to be a more sensitive life-stage the LC_{50} of 56.49 mg/L was considered acceptable for quantitative use and was utilized in the derivation of the acute freshwater criterion for PFOS.

A.2.14 Fourteenth 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 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-hr:10-hr (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 study 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 96-hour study author-reported value was used quantitatively to derive the draft acute water column criterion.

A.2.15 Fifteenth 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-hr:8-hr, 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, 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. 100% survival was observed at 24 hours in the negative control. The study author reported 24-hour LC_{50} was 61.8 mg/L. The study author-reported value was used quantitatively to derive the draft acute water column criterion.

A.2.16 Sixteenth Most Sensitive Freshwater Genus for Acute Toxicity: *Elliptio* (mussel)

Drottar and Krueger (2000e) 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, OPPTS Number 850.1075; OECD 203, and ASTM E729-88a (1988). *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-hr:8-hr (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. This independently-calculated acute value was acceptable for quantitative use and utilized in the derivation of the acute PFOS aquatic life criteria.

A.2.17 Seventeenth 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-hr:12-hr light:dark photoperiod. Mortality was monitored twice daily. The author reported LC_{50} value was 144 mg/L PFOS. EPA's independently-calculated 96-hour LC_{50} was 154.8 (108.7 – 200.9) mg/L PFOS and was

considered acceptable to be used quantitatively to derive the draft acute water column criterion for freshwater.

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 (U.S.EPA 2002a); ASTM 2017). The four test species (American bullfrog, *Lithobates catesbeiana*; 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-hr:12-hr 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 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. 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 acute freshwater criterion for PFOS. The author-reported LC_{50} values for green frog (113 mg/L) and wood frog (130 mg/L) were used quantitatively to derive the acute freshwater criterion for PFOS as the author-reported toxicity values were consistent with the independently-calculated LC_{50} values for other species included in the study.

A.2.18 Eighteenth 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 DO 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 study 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 draft 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-hr:12-hr (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 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 author reported 96-hour LC_{50} was reported as 161.77 mg/L. The independently-calculated toxicity value could not be calculated at

this time given the level of data that was presented in the paper. The study author reported value was used quantitatively to derive the draft 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 criteria derivation, with summaries grouped and ordered by genus sensitivity. The data available for saltwater invertebrates fulfilled three of the eight MDRs. EPA could not, therefore, develop acute estuarine/marine criteria following the 1985 Guidelines methods. In the interest of providing recommendations to states/tribes on protective values, EPA developed an estuarine/marine acute benchmark using the available empirical data supplemented with toxicity values generated through the use of New Approach Methods, specifically through the use of the EPA Office of Research and Development's peer-reviewed publicly-available webICE 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-hr:8-hr 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, 1 mg/L) or controls. The PFOS stock was made with ethanol, and ASW control samples run in parallel included ethanol at the maximal final 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 controls for a percentage of normal D-shell stage larvae, >75% (ASTM 2004a). 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 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 (2004a) protocols. Mussels were collected in the field (Sand Diego Bay, CA) and conditioned in 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-hr:8-hr 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. Controls were made in the same 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 control and filtered seawater, suggesting no adverse effects of methanol. The author reported 48-hr EC_{50}, based on normal development, is 1.1 mg/L PFOS. 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} 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 (2004) protocols. Sea urchins were collected in the field (Sand 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-hr:8-hr 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. Controls were made in the same 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 control and filtered seawater, suggesting no adverse effects of methanol. The author reported 96-hour EC_{50}, based on normal development, is 1.7 mg/L PFOS. 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 for 10 minutes after fertilization up to 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 was 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;, 2002) and (ASTM 2004a) 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-hr:8-hr 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. Controls were made in the same 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 control and filtered seawater, 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} is 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*. A stock solution of PFOS was made either with filtered sea water from the Ria of Vigo (Iberian Peninsula) 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. Mysids were exposed to one of five nominal PFOS treatments (1.25, 2.5, 5, 10 and 20 mg/L). Mysids were also collected from the same source as the dilution water 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 oxygen 6 mg/L). Twenty neonates (<24 hours old) were used per each treatment. 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 PFOSK (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, 1996) 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-hr:8-hr 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 limit of quantification (5 mg/L) and PFOS-spiked seawater, 15 mg/L. At test termination (96 hours) none of the minnows died in any of the test treatments, therefore the author reported LC_{50} was >15 mg/L. EPA was unable to independently calculate the LC_{50} value as this test only consisted of one treatment group. As such the author-reported LC_{50} >15 mg/L is acceptable for quantitative use based on the 2013 Ammonia rule which states that greater than high values can be used in the derivation of criteria.

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	8.831	**8.831**	8.831	(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 (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 (survival)	16.97	11.19	**11.19**	-	Drottar and Krueger (2000i)
Cladoceran (<24 hr), *Daphnia magna*	R, U	21 d	PFOS-K 95%	-	21	EC10 (survival)	35.36	16.35	**16.35**	-	(Boudreau 2002; Boudreau et al. 2003a)
Cladoceran (<24 hr), *Daphnia magna*	R, U	21 d	PFOS Unreported	-	21	EC10 (number of young/adult)	1.768	0.7885	**0.7885**	-	(Ji et al. 2008)
Cladoceran (<24 hr), *Daphnia magna*	R, U	21 d	PFOS-K >98%	-	20	EC10 (total neonates/female)	2.236	2.919	**2.919**	-	(Li 2010); {Lu, 2015 #325}
Cladoceran (<24 hr), *Daphnia magna*	R, M	21 d	PFOS-K 99%	7	22	EC10 (reproduction)	2.26	-	**2.26**	-	(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	0.001712	**0.001712**	-	Li (2009)

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
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)	0.82183	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**	1.267	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)
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)
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**	0.009676	McCarthy et al. (2021)
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[f]	0.01650	**0.01650**	-	(Wang et al. 2011)
Zebrafish (male, 3-5 mo), *Danio rerio*	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)

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
Fathead minnow (embryo, 48 hpf), *Pimephales promelas*	F, M	33 d	PFOS-K Unknown	6.6-7.3	22-26	EC10 (survival)	1.378	0.4408	**0.4408**	-	3MCompany (2000)
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 (2000j)
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**	0.1555	Suski et al. (2021)
Swordtail fish (juvenile female), *Xiphophorus helleri*	R, U	90 d	PFOS-K >98%	-	27	EC10 (female survival)	>0.1	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)
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)	>1	-	**>1**	>1	(Lou et al. 2013)

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
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.

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 criteria derivation, with summaries grouped and ordered by genus sensitivity. C-R models developed by EPA that were used to determine chronic toxicity values used for criterion derivation are also presented for the most sensitive genera when available. C-R models included here with study summaries were those for the four most sensitive genera (consistent with Section 3.1.1.3). When required, 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 concentration-response data in the publication/supplemental materials and/or did not provide concentration-response data upon EPA request. In such cases, 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: *Chironomus* (midge)

MacDonald et al. (2004) conducted chronic larval and 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 test and 60 days for the life-cycle test. The tests followed the general guidance given by EPA-600-R99-064 (U.S.EPA 2002) 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 and newly-hatched larvae at test initiation for the 20-day exposure in the life-cycle test. Dilution

water was reconstituted hard water consistent with ASTM (2002) with unspecified total hardness, but typically 160-180 mg/L as $CaCO_3$, with alkalinity 110-120 mg/L as $CaCO_3$, and pH 7.6-8.0. The photoperiod was 16-hrs:8-hrs, 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 (20-day exposure) employed 12 replicates of 12 midges each in five measured test solutions plus a negative control. Nominal test concentrations for the 10-day test were 0 (negative control), 0.001, 0.005, 0.010, 0.020, 0.040, 0.080, 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, 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. dilutes* 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 20-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 control (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. 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 concentration-response 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 and Table 2 of the paper stated 0.0949 mg/L. 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 PFOS draft criteria.

Independent statistical analyses were conducted for both the 10-day and 20-day exposure durations using data that were estimated (using Web plot digitizer) from the figures presented in the paper. 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 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 concentration response 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 20-day EC_{10}s for larval survival and emergence were 0.0171 and 0.0102 mg/L, respectively. The 20-day EC_{10}s were much lower than those reported in the paper of 0.0864 and 0.0893 mg/L, respectively. The 20-day EC_{10}s for survival and emergence 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 EPA's independently-calculated value. As for the emergence endpoint, there was a lack of a concentration-response 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 20-day 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 20-day exposure was more of a concern than the 10-day exposure, which had measured concentrations that were much more in line with the expected nominal concentrations. Thus, the 20-day survival and emergence endpoints were not considered for quantitative use in the derivation of the chronic 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 0.05864 mg/L. As mentioned in the Bots et al. (2010) summary and in Section 4.1.1, 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 (0.0581 – 0.0612) mg/L for 10-day growth was used quantitatively to derive the chronic aquatic life criterion. 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.

McCarthy et al. (2021) conducted a 10-day sub-chronic toxicity test and a separate 20-day (note, based on age of starting organisms, this test was actually 16 or 19 days of exposure) 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 USEPA (2000b) and since exposures of midges started on day two or four the actual exposure duration is only 16 or 19 days long. 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. 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 study 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. EPA was unable to

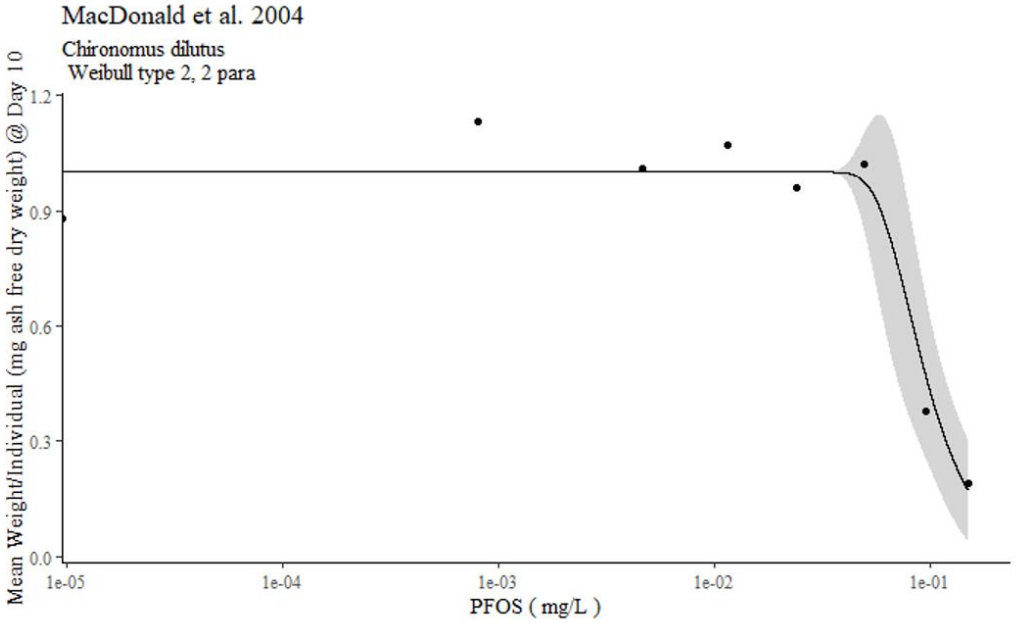
independently calculate EC_{10}s for survival and mean weight. However, the 16- to 19-day independently-calculated EC_{10} value for mean biomass was 0.0015879 (0.00118 – 0.00200) mg/L PFOS. This independently-calculated EC_{10} was acceptable for quantitative use and was utilized in the derivation of the chronic freshwater criterion for PFOS.

C.2.1.1 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. 0.0577 – 0.0602) 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:

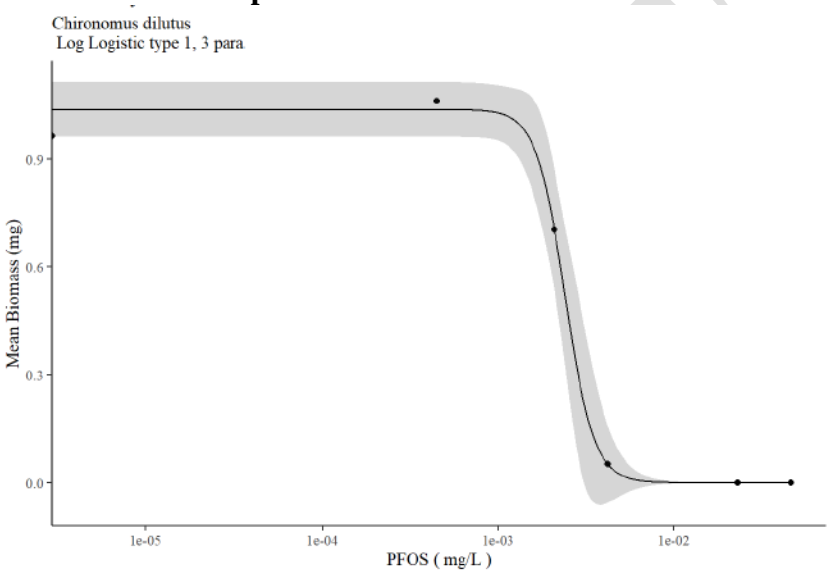


C.2.1.2 McCarthy et al. (2021) Concentration Response Curve – Chironomus (midge)

Publication: (McCarthy et al. 2021)
Species: Midge *Chironomus dilutus*
Genus: *Chironomus*
EPA-Calculated EC_{10}: 0.0015879 (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 Second 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, 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, dissolved oxygen 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, 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 2013 Ammonia Aquatic Life Criteria 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. 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), 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 and D.2.1, was used to derive a ratio of $EC_{10}/EC_{35.4}$ levels from that study, which was: $EC_{10}/EC_{35.4}$ = 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 Hazelton et al. (2012), the MATC of 0.01768 mg/L was used to derive the chronic criterion for PFOS. This MATC is currently used quantitatively to derive the draft chronic water column criterion, and 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 PFOS dataset.

C.2.2.1 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 0.0177 mg/L

C.2.3 Third 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 look at 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-hrs:8-hrs, 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 solution; 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 and the 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 (Abbot 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 was currently the only test guidelines for an emergent aquatic insect as there currently was no test guideline for odonates. Therefore, considerations regarding the use of these data for chronic 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 were not considered quantitatively in the derivation of the freshwater chronic criterion.

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. 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 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. 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 an 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 (see Section 4.1.1 below) and only the toxicity values from 10 to 200 days of exposure range were considered further for the criterion derivation.

The 150-day MATC of 0.03162 mg/L 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), which was the only other emergent insect toxicity study in the PFOS chronic dataset (see Section 4.1.1) and the test organisms at these exposure durations would likely be in similar life stages (later development and about to undergo metamorphosis). And these later toxicity values were therefore more comparable than the 10-day MATC of 0.3162 mg/L, 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 aquatic insects was likely similar (see Section 4.1.1 for more details); however additional data are needed to fully understand the effects of PFOS. The MATC for nymph survival at 150-day reported above was used quantitatively to derive the chronic water column criterion value. Additionally, 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 4.2.2).

C.2.3.1 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.2.4 Fourth Most Sensitive Freshwater Genus for Chronic Toxicity: *Danio* (zebrafish)

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% dimethyl sulfoxide (DMSO). Adult zebrafish (wild-type strain AB) were raised and kept at standard laboratory conditions of 28°C with a 14-hr:10-hr 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. 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 in the derivation of the PFOS freshwater chronic criterion. 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, EPA was unable to fit a concentration-response curve with significant model parameters for these endpoints; and therefore, were 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 components with consistent treatment groups 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, 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 4.4.2.1.1 and Appendix G).This study and the EC_{10} value for F1 survival was considered quantitatively in the derivation of the aquatic life criteria, despite the use of renewal exposure and nominal test concentrations.

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. 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) that had

the following water qualities: pH: 7.0-7.4, temperature: 28 ± 1°C and a 14-hr:10-hr light to dark cycle 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, and a total of 660 fish were divided equally among the four concentration groups. Three replicates were present for each concentration group with each containing 55 fish. Water quality throughout the experiment was maintained with standards listed above, as well as the following conditions: dissolved oxygen of 5 - 6 mg/L and total hardness of 20.0 mg/L reported as $CaCO_3$. Exposure media was completely changed every three days, and aquaria were completely 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 weight of 0.19 ± 0.03 g and a mean length of 2.5 ± 0.3 cm at test initiation. On day seven the 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. However, the 0.3 g fish weight for the 0.04 mg/L and 0.08 mg/L exposures were significantly different from the control. At days 14 and 21, fish length of only the highest concentration (0.08 mg/L PFOS) was significantly different from the control, and the same fish weight effect levels were observed at 14 and 21 days as those 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 LC_{50} value was reported due to lack of mortality (no mortality occurred for 21 days). However, an independently-calculated EC_{10} could not reliably be estimated for mean body weight as the data were sparse, was inconsistent with the author-reported toxicity values, and the confidence bands were wide. Therefore, EPA's independently-calculated EC_{10} based on mean body length

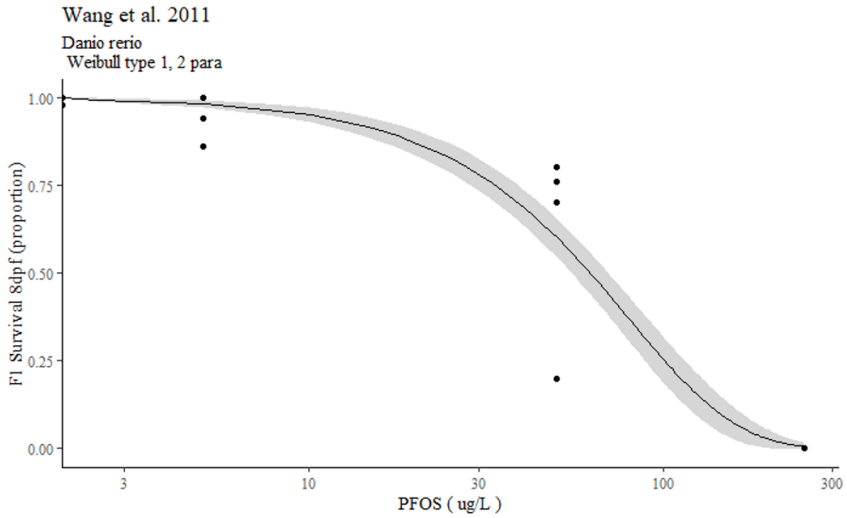
(in cm) at 21 days is 0.06274 (0.06229 – 0.06318) mg/L PFOS and was used quantitatively to derive the draft chronic water column criterion for freshwater.

C.2.4.1 Wang et al. (2011) Concentration Response Curve – Danio (zebrafish)

Publication: (Wang et al. 2011)
Species: Zebrafish, *Danio rerio*
Genus: *Danio*
EPA-Calculated EC_{10}: 0.01650 (95% C.I. 0.01267 – 0.02033) mg/L
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.4238	0.1030	13.8260	$< 2.2\ e^{-16}$
e	80.1484	4.9349	16.2410	$< 2.2\ e^{-16}$

Concentration-Response Model Fit:

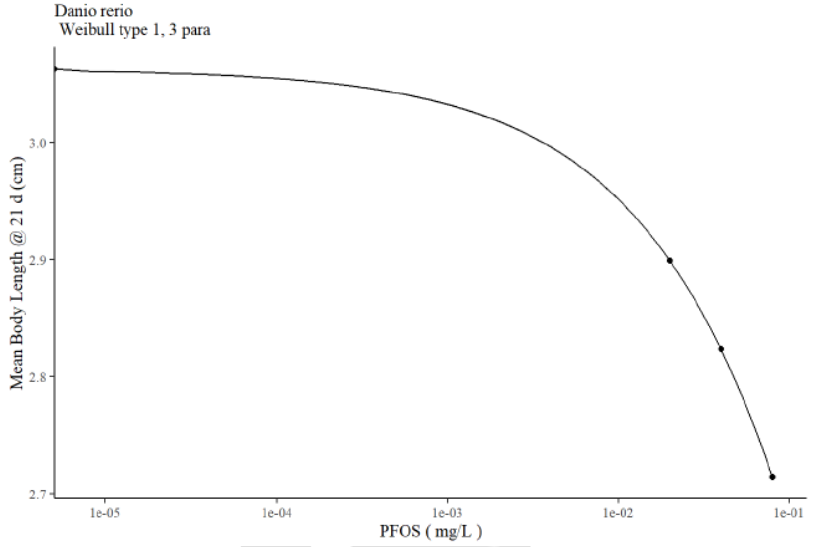


C.2.4.2 Guo et al. (2019) Concentration Response Curve – Danio (zebrafish)

Publication: Guo et al. (2019)
Species: Zebrafish, *Danio rerio*
Genus: *Danio*
EPA-Calculated EC_{10}: 0.06274 (95% C.I. 0.06229 – 0.06318) mg/L
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	0.5686	4.4315 e^{-4}	1283.1200	0.0005
d	3.0630	7.7727 e^{-5}	39406.9800	1.616 e^{-5}
e	3.2830	9.8428 e^{-3}	333.55	0.0019

Concentration-Response Model Fit:



C.2.5 Fifth 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℃ and a 16-hr:8-hr light:dark photoperiod. The chronic test protocol followed OECD guidelines (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. 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 quantitatively used to derive the chronic freshwater criterion for PFOS.

Drottar and Krueger (2000i) 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 OECD 211 (1997), U.S. EPA OPPTS Number 850.1300 (1996), and ASTM Standard E 1193-87. *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-hr:8-hr, 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). Survival 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, survival 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 survival was 11.19

(10.50 – 11.89) mg/L and was used quantitatively to derive the draft 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 E 1193-97 (1997). *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-hr:8-hr (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 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 draft chronic water column criterion.

In addition to the acute toxicity tests described above, **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 211 (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-hr:8-hr (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 dissolved oxygen) 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 survival adult 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, EPA recalculated the reproductive endpoint noted 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 calculated EC_{10} for *D. magna* was 0.7885 (0.7043 – 0.8726) mg/L. The results from this study were acceptable for quantitative use despite the nominal test concentrations, as the study design was sound, and the concentration-response curve was compelling for the tested species. The independently-calculated EC_{10} of 0.7885 mg/L was used in the aquatic life criteria derivation for *D. magna*.

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 211 (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-hr:8-hr, 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 (2.8642 – 2.9738) mg/L. The independently-calculated toxicity value (EC_{10}) based on total neonates per female was 2.919 mg/L and was used quantitatively to derive the draft 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 1993c90-1191 (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; dissolved oxygen, 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-hr:12-hr (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 respectively 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. The study author reported value was used quantitatively to derive the draft 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 211 (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-hr:8-hr (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 dissolved oxygen 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 0.001712 (0.000998 – 0.002426) mg/L for the same endpoint. Other endpoints, including growth and other reproductive endpoints, could not be independently-calculated by EPA. The independently-calculated EC_{10} from this study was acceptable for quantitative use and was used to derive the PFOS chronic water 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-hr:8-hr (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 was used quantitatively to derive the draft 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-hr:8-hr 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 draft 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-hr 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-hr old neonates, exposed in triplicate, were placed into 250 mL crystallization dishes with 100 mL of test solution. A mean temperature of 23°C, dissolved oxygen 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. 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 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 draft chronic water column criterion for freshwater.

C.2.6 Sixth 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 age 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 considered acceptable to derive the draft chronic water column criterion for freshwater.

C.2.6.1 Spachmo and Arukwe (2012) Concentration Response Curve – Salmo (salmon)

Publication: (Spachmo and Arukwe 2012)
Species: Atlantic salmon (*Salmo salar*)
Genus: *Salmo*
EPA-Calculated LC_{50}: Not calculable, concentration-response data not available

C.2.7 Seventh Most Sensitive Freshwater Genus for Chronic Toxicity: *Pimephales* (minnow)

3MCompany (2000) includes the results of an investigation of the chronic effects of PFOS-K (perfluorooctancesulfonate potassium salt, CAS # 2795-39-3, unknown purity) on the fathead minnow, *Pimephales promelas*, from a test conducted in 1978. The test methods followed those proposed for egg and fry stages of freshwater fish and EG&G Bionomics. Eggs (48 hpf) from EG&G Bionomics were exposed until 30 dph under a flow-through regime with measured PFOS concentrations. PFOS-K was dissolved in acetone to make the stock solution (19.4 mg/L) and diluted with well water to make five test concentrations and two controls (well and solvent control). The solvent control contained the same amount of acetone (43 µL/L) as in the highest test concentration. Average measured PFOS concentrations were <0.006 (solvent control), <0.006 (well water control), 0.12, 0.28, 0.45, 1.0, and 1.9 mg/L PFOS; measured on test day 3, 10, 17, 24 and 32. At test initiation sixty eggs each were assigned to each of the 14 egg cups and assigned to one of the 14 aquaria (two aquaria per test treatment). Dead eggs were counted and removed daily until hatching was complete (test day 3), afterwards 40 fry from each egg cup were transferred to their respective aquarium and exposures continued for an additional

30 days. Fry were fed brine shrimp three times daily throughout the exposure period. Observations on behavior and appearance were made daily and fry counts were made weekly. Test endpoints included hatch, survival, and growth (length and weight). Throughout the exposure period average water quality parameters varied very little between treatments (average D.O. of 8.6 mg/L, 22-26 ℃, pH 6.6-7.3). PFOS-K did not have any significant effects on hatchability of eggs or growth of fry. However, survival of fry in the 1.9 mg/L treatment was 42% less than the controls, with fish exhibiting stressful behavior (e.g., erratic swimming). The author reported 33-day NOEC and LOEC, based on survival, was 1.0 and 1.9 mg/L PFOS, respectively, with a MATC of 1.378 mg/L. The independently-calculated 33-day EC_{10} value was 0.4408 (0.3076 – 0.5738) mg/L and was considered acceptable for quantitative use in the derivation of the chronic freshwater criterion for PFOS.

The chronic effects of PFOS on the fathead minnow, *Pimephales promelas*, have been reported by several researchers. **Drottar and Krueger (2000j)**, associated with Wildlife International, conducted a 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 hours light and eight hours 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} was 0.4732 (0.3308 – 0.6156) mg/L based on survival and was used quantitatively to derive the draft 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 dissolved oxygen 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-hr:8-hr 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 was used quantitatively to derive the draft 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 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 was considered acceptable for quantitative use in the derivation of the chronic freshwater criterion for PFOS.

C.2.8 Eighth 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 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-hrs:10-hrs (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. The independently-calculated EC_{10} could not be calculated at this time given the level of data that were presented in the paper. The study author-reported LC_{20} value was used quantitatively to derive the draft chronic water column criterion.

C.2.9 Ninth Most Sensitive Freshwater Genus for Chronic Toxicity: *Moina* (cladoceran)

In addition to the acute toxicity tests described above, **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) was similar to OECD 211 (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-hr:8-hr (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 dissolved oxygen) 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. The author reported 7-day NOEC and MATC was <0.3125 mg/L for the same reproductive endpoint. In the independent verification of the toxicity values, 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. The results from this study were acceptable for quantitative use. The independently-calculated EC_{10}s of 0.1789 mg/L was used in the aquatic life criteria derivation for *M. macrocopa*.

C.2.10 Tenth 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-hr:8-hr, 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. 100% 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 NOEC and MATC were <0.25 mg/L. The study author reported value was used quantitatively to derive the draft chronic water column criterion.

C.2.11 Eleventh Most Sensitive Freshwater Genus for Chronic Toxicity: *Xiphophorus* (swordfish)

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-hr:10-hr (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 female survival was 0.5997 (0.2336 – 0.9658) mg/L, which was acceptable for quantitative use.

C.2.12 Twelfth 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, dissolved oxygen >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-hr:12-hr 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 weighted, 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 >1 mg/L PFOS. The study-author reported value was used quantitatively to derive the draft 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-hr:12-hr 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, dissolved oxygen 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 to determine weights, lengths, and sex differences amongst the organisms. Mortality data showed a NOEC value >1.10 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. This 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 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 draft chronic water column criterion for freshwater.

C.2.13 Thirteenth 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-hr:8-hr 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 study author-reported value (112-d growth MATC of 1.732 mg/L) was used quantitatively to derive the draft 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 control and exposure to 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-hr:12-hr 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 the exposure was renewal rather than the required flow-through design for chronic ALC development. Also, 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 study author-reported value (a developmental-based MATC of 0.0316 mg/L) was used quantitatively to derive the draft chronic water column criterion.

C.2.14 Fourteenth 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-hr:12-hr, 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 study 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 8.831 (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 criteria.

C.3 Comparison of Quantitative Data used to Derive Freshwater Criteria

C.3.1 Aquatic Insects

While comparing the effects of PFOS across studies presented a number of challenges, especially with differences in test species, methodologies, exposure durations, and observed endpoints, in general there appeared to be a number of similarities and few differences between the two aquatic insect toxicity studies used quantitatively to derive the PFOS chronic freshwater criterion. In both Bots et al. (2010) and MacDonald et al. (2004) effects of chronic exposures to PFOS on survival and emergence were observed in the damselfly (*Enallagma cyathigerum*) and the midge (*Chironomus dilutus*), respectively. Additionally, both studies measured effects of PFOS on growth. However, since the studies focused on very different life stages for this endpoint (on growth in emerged adult damselfly from Bots et al. 2010; and on growth in larval midge from MacDonald et al. 2004), the toxicity data for growth could not be compared.

In the early phases of both experiments, the effects of PFOS on aquatic insects did not appear to be similar. Bots et al. (2010) reported a 10-day NOEC of 0.1 mg/L, a LOEC of 1.0 mg/L, and a MATC of 0.3162 mg/L for survival. The LOEC was associated with a 79% decrease in survival compared to the control group. In contrast, MacDonald et al. (2004) reported a 10-day

NOEC of 0.0491 mg/L, a LOEC of 0.0962 mg/L, and a MATC of 0.0687 mg/L for survival. The midge LOEC was associated with an 8.2% decrease in survival compared to the control group. During the early phases and exposure durations of both experiments, it appeared that the effects of PFOS were not similar between the two species and that the midge was more the sensitive than the damselfly. However, after this initial phase, the effects of PFOS on the damselfly and the midge became more similar.

In the later phases of the tests, Bots et al. (2010) reported a 150-day NOEC of 0.01 mg/L, a LOEC of 0.1 mg/L, and a MATC of 0.0316 mg/L for survival. This 150-day LOEC was associated with a 19% decrease in nymph survival compared to the control. Similarly, MacDonald et al. (2004) reported a 20-day NOEC of 0.0217 mg/L, a LOEC of 0.0949 mg/L, and a MATC of 0.0454 mg/L for survival. The LOEC was associated with a 61% decrease in larvae survival compared to the control. EPA's independently-calculated EC_{10} was 0.0171 mg/L using estimated, treatment level summary data presented in the paper (using Web plot digitizer). This independently-calculated EC_{10} and the author reported 20-day MATC of 0.0454 mg/L for chironomid survival was similar to the author-reported 150-day survival MATC of 0.0316 mg/L for the damselfly. The greatest sensitivity of the test organisms based on the survival endpoint of each respective test likely occurred in a similar life stage (later development and about to undergo metamorphosis). And therefore, were more comparable than any of the other survival toxicity values from these studies (i.e., the 10-day values for damselfly and the 10-day values for midge), which were focused on the effects of PFOS on much less comparable instars, especially given that odonates have a much longer development and life span compared to midge. These results indicated that PFOS exposure to aquatic insects in later life stages were likely similar.

Likewise, both studies observed similar effects of PFOS on emergence. However, this endpoint was less certain compared to the survival endpoints given the level of data provided in both of the papers. Bots et al. (2010) observed that nymphs exposed to the lowest PFOS treatment group metamorphosed less (75.5%) than those in the control (92.5%) and reported a LOEC of <0.01 mg/L for metamorphic success. This less than LOEC was associated with a 18.4% decline. Metamorphic success could not be measured in the remaining PFOS treatment groups because only nymphs in the control and the lowest PFOS treatment group (or 0.01 mg/L) survived until metamorphosis. In comparison, MacDonald et al. (2004) observed a reduction in total emergence in all of the PFOS treatment groups compared to the control. The study authors reported a NOEC of <0.0023 mg/L, a LOEC of 0.0217, a MATC of 0.0071 mg/L and an EC_{10} of 0.0893 mg/L. The reported LOEC was associated with a 41.6% decline in midge emergence compared to the control. EPA's independently-calculated EC_{10} was 0.0102 mg/L using estimated, treatment level summary data presented in the paper (using Web plot digitizer). This independently-calculated EC_{10} and the author reported LOEC of 0.0217 mg/L for midge were similar to the LOEC of <0.01 mg/L reported for damselfly by Bots et al. (2010), and indicates that like the later stage, survival endpoints summarized above, PFOS may have a similar level of effect on insect metamorphosis and emergence.

These apparent similarities in the chronic effects of PFOS to aquatic insects support the toxicity values EPA used for chronic criteria derivation from the two studies, as well as the ranking of these two species (as first and third most sensitive) to derive the chronic freshwater criterion. However, additional replicate level data would have been helpful to fully understand the observed effects of PFOS individually and to compare across the full PFOS toxicity dataset.

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)
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)

[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 criteria derivation, with summaries grouped and ordered by genus sensitivity. Data for chronic PFOS toxicity were available for two saltwater invertebrate species, representing two genera and two families. The data available for saltwater fish fulfilled only two of the eight MDRs, therefore EPA could not develop chronic estuarine/marine criteria 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, with 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 liquid chromatography-tandem mass spectrometry. 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. No LC_{50} value was reported. EPA's independently calculated EC_{10} is 0.0033 (0.00330 – 0.00332) mg/L. This EC_{10} was used quantitatively to derive the draft chronic water column criterion for freshwater.

D.2.2 Second 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 *Mysidopsis 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 mg/L (the 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 the corresponding 35-day 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.2.3 Third 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 that 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. 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 EPA data quality objectives and independent calculation and verification of point estimates, the developmental stage is considered for quantitative use and the reproductive endpoint for qualitative use. The use of the reproductive endpoint could be changed based on input on clarifying questions from the study authors. The 20-day MATC (based on time to reach development stage) was 0.7071 mg/L and currently recommended by EPA as acceptable for quantitative use.

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
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 (1.5 x 10^4 cells/ml), *Chlorella vulgaris*	S, U	96 hr	PFOS-K 95%	-	23	IC50 (cell density)	81.6	Boudreau et al. (2003a)
Green alga, *Raphidocelis subcapitata* (formerly, *Selenastrum capricornutum*)	S, U	96 hr	PFOS-K 24-28%	-	23	EC50 (specific growth rate)	49.28[b]	3MCompany (2000)
Green alga, *Raphidocelis subcapitata*	S, U	4 d	PFOS-K Unknown	-	23	EC50 (cell count)	77.19	3MCompany (2000)
Green alga, *Raphidocelis subcapitata*	S, U	7 d	PFOS-K Unknown	-	23	EC50 (cell count)	76.68	3MCompany (2000)
Green alga, *Raphidocelis subcapitata*	S, U	10 d	PFOS-K Unknown	-	23	EC50 (cell count)	83.92	3MCompany (2000)
Green alga, *Raphidocelis subcapitata*	S, U	14 d	PFOS-K Unknown	-	23	EC50 (cell count)	76.78	3MCompany (2000)
Green alga, *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*)	S, M	96 hr	PFOS-K 90.49%	7.4-8.4	24	EC50 (cell density)	71	Drottar and Krueger (2000g)
Green alga (1.5 x 10^4 cells/ml), *Raphidocelis subcapitata* (	S, U	96 hr	PFOS-K 95%	-	23	IC50 (cell density)	48.2	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)
Duckweed, *Lemna gibba*	S, M	7 d	PFOS-K 86.9%	7.5	25	IC10 (frond number)	18.06	Desjardins et al. (2001)
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)

Species	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Reported Effect Concentration (mg/L)	Reference
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 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 GLP test was conducted at the Wildlife International, Ltd. in Easton, Maryland in February-March, 2000. The test followed USEPA OPPTS 850.5400 (U.S.EPA 1996) and ASTM 1218-90E (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.2.2 Green alga, Chlorella vulgaris

Boudreau et al. (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 E 1218-97a (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}=81.6 mg/L, and was quantitatively acceptable for use.

E.2.3 Green alga, Raphidocelis subcapitata

3MCompany (2000) provides 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 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 EC50, 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 and is acceptable for quantitative use.

3MCompany (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 U.S.EPA-600/9-78-018 (1978), ASTM-E-35.23 (1981), OECD 201 (1979), and ASTM STP #667. 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 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 (2000g) 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 GLP test was conducted at the Wildlife International, Ltd. in Easton, Maryland in April, 1999. The test followed USEPA OPPTS 850.5400 (U.S.EPA 1996), OECD 201 (OECD 1984), and ASTM 1218-90E (ASTM 1990) 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 99.1% recovery. 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 et al. (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 E 1218-97a (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. *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 shook 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 plant values from the study were acceptable for quantitative use.

E.2.4 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 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.2.5 Duckweed, Lemna gibba

Desjardins et al. (2001) 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 USEPA, 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 (method limit of quantitation, control), 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 is acceptable for quantitative use.

E.2.6 Watermilfoil, Myriophyllum sp.

Hanson et al. (2005) conducted a 42-day toxicity study of PFOS (potassium salt, purity not provided) with the submerged 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 concentrations 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

No data at this time.

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
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)	-	78.02[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)
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)
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)

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
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)
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)
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)
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)

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*	S, M	96 hr	PFOS-K 95%	-	20	LC50	-	150	Test species fed	Olson (2017)
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)

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 (<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	3MCompany (2000)
Cladoceran (0-12 hr), *Daphnia magna*	R, U	28 d	PFOS-K Unknown	7.6	22	MATC (reproduction)	7.0-18.0	11.22	Inability to calculate an EC10 and comments by authors	3MCompany (2000)
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)
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)
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	0.0316	Sporadic solution renewal, behavioral endpoints	(Van Gossum et al. 2009)

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 (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 (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)
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)

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 (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)
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. (2013)
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)
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)
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)

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 - 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. (2000)
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 Isomers	-	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 Isomers	-	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)
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) 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)

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, 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 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. 2014a)
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
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)

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 (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) 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 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 - (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 2016)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)

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, 1-2 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 (embryo, 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-Villanueca et al. (2018)
Zebrafish (embryo, 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-Villanueca 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 (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)
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, 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*	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, 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, <1 hpf), *Danio rerio*	R, U	96 hr	PFOS Unknown	-	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 (dechorionated embryo, 1 dpf), *Danio rerio*	R, U	30 d	PFOS Unknown	-	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)

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 (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)
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 (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)
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)
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)

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
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. 2015d
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. (2015d)
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 Unknown	8.2-8.3	-	LC50	-	31	Only one replicate per treatment	3MCompany (2000)
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)
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)

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 (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)
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)

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 (embryo, NF 10), *Xenopus laevis*	R, M	96 hr	PFOS >99%	-	24	LC50	-	>96	Non-definitive value	(San-Segundo et al. 2016)
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)
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)
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)

[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 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).

G.2 Summary of Acute PFOS Toxicity Studies Used Qualitatively in the Freshwater Aquatic Life Criterion Derivation

G.2.1 Freshwater Invertebrates

G.2.1.1 Worms (flat and annelids)

Yang et al. (2014) conducted a 96-hour measured, renewal acute test of PFOS (potassium salt, CAS # 2795-39-3, 99% purity) with the annelid worm, *Limnodrilus hoffmeisteri*. The test followed ASTM E729 (1993). *L. hoffmeisteri* (0.03 g, 0.8 cm) used for the test were obtained from Beijing City Big Forest Flower Market and allowed to acclimate for seven days before testing. Dilution water was dechlorinated tap water (pH, 7.0±0.5; dissolved oxygen, 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-hr:12-hr (light:dark) at an unreported intensity. A primary stock solution was prepared by dissolving PFOS in deionized water and cosolvent DMSO and proportionally diluted with dilution water to prepare the test concentrations. Exposure vessels were 90 cm petri dishes containing 10 mL of test solution. The test employed three replicates of 10 worms each in six test concentrations (measured in low and high treatments only) plus a negative and solvent control. Nominal concentrations were 0 (negative and solvent controls), 60.00, 72.00, 86.40, 103.68, 124.42 and 149.30 mg/L. The authors provided arithmetic mean measured concentrations before and after renewal: 51.42 and 58.94 mg/L (lowest concentration) and 127.73 and 150.84 mg/L (highest concentration). Analyses of test solutions were performed using high performance liquid chromatography with mass spectrometric detection (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 as having been measured every day during the acute test, but results are not reported. Negative control survival was 100%. Solvent control survival was 96%. The 96-hour LC_{50} was 120.97 mg/L (C.I. 103.97-140.76). The acute value was acceptable for qualitative use because the source of organisms was atypical.

Liu et al. (2016) conducted several 24-hour static, unmeasured acute tests on PFOS (>98% purity) with *Limnodrilus hoffmeisteri*. The test organisms were obtained from an aquarium market in Jinan, Shandong, China and acclimatized in a large aquarium containing river sediment (7.0-10.0 cm thickness) and aerated water for seven days before use. Only worms of uniform size were used for testing. Dilution water was carbon-filtered, dechlorinated tap water (pH 7.25±0.25, conductivity 340.67±16.4 µS/cm, total hardness 135.5±9.3 mg $CaCO_3$/L, alkalinity 40.7±5.2 mg $CaCO_3$/L, Na = 11.2±0.2 mg/L, K = 2.34±0.07 mg/L, Mg = 7.74±0.02 mg/L, Ca = 41.07±0.82 mg/L, Cl = 28.3±1.2 mg/L). No details were provided regarding photoperiod or light intensity. A primary stock solution was prepared by dissolving PFOS in DMSO. The stock solution was further diluted with dilution water to obtain different concentrations. Exposure vessel size and material type was not reported, but fill volume was 20 mL. Prior to joint toxicity and bioaccumulation of Zn and PFOS experiments, the 24-hour toxicity of PFOS to *L. hoffmeisteri* was determined at pH 5.0, 6.0, 7.0, 8.0, and 9.0 in preliminary experiments. The final pH values for the preliminary tests were adjusted to the desired values within a range of ± 0.1. The tests employed three replicates of 10 worms each and an unspecified number of test concentrations plus a negative and presumably a solvent control. The authors reported nominal test concentrations in their supplemental Table S1. The test temperature was maintained at 23±1°C. No other water quality parameters were reported as having been measured for test solutions. Survival of control animals (and presumably solvent)

were also not reported. The 24-hour LC_{50} values at pH 5.0, 6.0, 7.0, 8.0, and 9.0 were reported to be 45.26, 46.23, 60.70, 64.48, and 65.74 mg/L, suggesting a slight trend toward increasing LC50 with increasing pH for *L. hoffmeisteri.* The acute values from the study were acceptable for qualitative use because of the short test duration.

Qu et al. (2016) similarly conducted a set of 48-hour renewal, unmeasured tests on PFOS (potassium salt, 98% purity) with *Limnodrilus hoffmeisteri* at different pH levels. Test organisms were obtained from an aquarium market in Jinan, Shandong, China and acclimatized in a large aquarium containing river sediment (7.0-10.0 cm thickness) and aerated water for 10 days before use. Only worms of uniform size (body length: 3.0-4.0 cm) were used for testing. Dilution water was carbon-filtered, dechlorinated tap water (pH, 7.70 ± 0.15; conductivity, 340.6 ± 16.4 µS/cm; total hardness,135.5 ± 9.3 mg $CaCO_3$/L; alkalinity, 40.7 ± 5.2 mg $CaCO_3$/L; Na, 11.2 ± 0.2 mg/L; K, 2.34 ± 0.07 mg/L; Ca, 41.07 ± 0.82 mg/L; Mg, 7.74 ± 0.02 mg/L; Cl, 28.3 ± 1.2 mg/L). No details were provided regarding photoperiod or light intensity. A primary stock solution was prepared by dissolving PFOS in DMSO (<0.1%). The stock solution was further diluted with carbon-filtered and aerated tap water to obtain different concentrations. Exposure vessels were beakers of a size and material type not reported. Fill volume was 20 mL. The three different tests conducted at pH 6.2, 7.0, and 8.0 employed three replicates of 10 worms each in three test concentrations plus a solvent control. Nominal concentrations were 0 (solvent control), 5, 10, and 20 mg/L. The test temperature was maintained at 22 ± 1°C. No other water quality parameters were reported as having been measured for test solutions. Survival of solvent control animals were also not reported. The 48-hour LC_{50} values were 23.81 ± 1.14, 35.89 ± 0.49 and 39.80 ± 1.15 mg/L at pH 6.2, 7.0 and 8.0. Similar to the preliminary findings from Liu et al. (2016), the PFOS 48-hour LC_{50} for *L. hoffmeisteri* increased with increasing pH, but the change

was not large. The acute values from the study were acceptable for qualitative use because of the short test duration.

G.2.1.2 Mollusks

(Amraoui et al. 2018) conducted a 96-hour renewal, unmeasured toxicity test of PFOS (potassium salt, CAS # 2795-39-3, ≥98% purity) with the freshwater mussel, *Unio ravoisieri* (a non-North American species). Test organisms (6 cm) were wild-caught from Sejenane river, a tributary of Ichkeul Lake (Bizerte, northern Tunisia). The mussels were acclimated under laboratory conditions for 14 days before use. Dilution water was natural aerated freshwater from an unspecified and uncharacterized source. Use of this natural freshwater for the test reportedly provided enough food to prevent starvation during the test but not so much as to cause any discrepancy in observed effects resulting from the interaction of PFOS and food. Photoperiod was 16-hr:8-hr (light:dark) at an unreported light intensity. A stock solution was prepared by directly dissolving the powder in 100% DMSO. Stock solution was proportionally diluted with system water resulting in a final DMSO concentration of 0.01% (v/v). The test employed three replicates of five mussels each in five test concentrations plus a solvent control. Nominal test concentrations were 0 (solvent control), 10, 25, 50, 75, and 100 mg/L PFOS. Exposure vessels were 3 L tanks of unreported material, dimensions and fill volume. The test temperature was controlled at 18°C and water pH 8 in an acclimated room. No other water quality parameters were reported as having been measured in test solutions. Survival of solvent control animals appeared to be 100% (as depicted in a figure). The 96-hour LC_{50} was reported as 65.9 mg/L. The acute value was acceptable for qualitative use because the organisms were exposed using an uncharacterized natural freshwater source as dilution water.

Yang et al. (2014) conducted a 96-hour acute test of PFOS (potassium salt, CAS # 2795-39-3, 99% purity) with a non-North American snail species, *Cipangopaludina cathayensis*. The

test followed ASTM E729 (1993). The test organisms (4.0 g, 2.0 cm) were purchased from the Beijing Dahongmen Jingshen Seafood Market and were held seven days prior to testing. Dilution water was dechlorinated tap water (pH, 7.0 ± 0.5; dissolved oxygen, 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-hr:12-hr (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 three replicates of 10 snails 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), 100, 130, 169, 219.7, 285.61 and 371.29 mg/L. Mean measured concentrations before and after renewal were 86.50 and 99.85 mg/L (lowest concentration) and 328.84 and 368.24 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 as having been measured every day during the acute test, but results are not reported. Negative and solvent control survival was 100%. The 96-hour LC_{50} was 247.14 mg/L (C.I. 188.81-323.48). The acute value was acceptable for qualitative use because the source of organisms was atypical.

Olson (2017) conducted 96-hour static acute tests on PFOS (potassium salt, CAS # 2795-39-3, 95% purity) with adult and juvenile (0-3 week old) *Lymnaea stagnalis* as part of a Ph.D.

thesis at the Texas Tech University, Lubbock, TX. The test followed methodology established in Ducrot et al. (2010). Snails were fed during the acute test. The test organisms were randomly selected from the appropriate age group from a snail culture maintained at Texas Tech University. Dilution water was reconstituted laboratory (13.38 g $CaSO_4$, 5.6 g $MgSO_4$, 0.25 g KCl, and 2.95 g $NaHCO_3$ added to 50 L deionized water). Photoperiod and light intensity were not reported. Stock solutions were prepared by dissolving between 0.2000 and 0.2010 g of the chemical powder in 1 L of lab water and placing in HDPE bottles on a shaker overnight. Stock solution was then diluted as necessary to obtain the final exposure concentrations. Exposure vessels were 1 L polyethylene beakers containing 1 L of test solution. The test employed two replicates of five to 10 snails each in six test concentrations (measured in low and high treatments) plus a negative and solvent control. Nominal concentrations in the test with adults included five individuals in each replicate and were 0 (negative control), 15, 30, 60, 125, 200, and 250 mg/L. Nominal concentrations in the test with 0-3 week old snails included 10 individuals in each replicate and were 0, 12.5, 25, 50, 100, 150, and 200 mg/L. Exposure concentrations were reportedly measured initially and after three days for verification, but were not reported. Analyses of test solutions were performed HPLC/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 20°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 ≥90%. The 96-hour LC_{50} reported for adult snails was 196 mg/L. The 96-hour LC_{50} reported for juveniles was 150 mg/L. The acute values were acceptable for qualitative use because snails were fed during acute toxicity testing.

G.2.1.3 Zooplankton (rotifers and planktonic crustaceans)

3MCompany (2000) provides the results of a 48-hour static, unmeasured acute toxicity test completed with the cladoceran, *Daphnia magna*, and PFOS-Li (perfluorooctancesulfonate lithium salt, CAS # 29457-72-5). A stock solution was made with carbon-filtered well water at a test sample concentration of 1,000 mg/L and where the test sample was reported as a mixture of PFOS-Li (24.5%) in water (75.5%). Daphnids obtained from the USEPA (Duluth, MN) were used for testing and were less than 24 hours old at test initiation. Exposure vessel were 100 mL glass beakers containing 50 mL of solution and five daphnids per beaker. Each test treatment was replicated four times with nominal test concentrations (control, 100, 180, 320, 560 and 1,000 mg/L test sample). Throughout the experiment the D.O. ranged from 7.0-7.8 mg/L, pH 8.6 and a test temperature of 20.1 - 21.0°C. No immobility or mortality occurred in the control treatment and 100% was observed in treatments ≥ 320 mg/L. The author reported that the test sample containing 24.5% PFOS-Li exhibited a 48-hour EC_{50} (mortality and immobilization) of 210 mg/L, which equates to 51.45 mg/L as PFOS-Li. EPA was unable to independently calculate a 48-hour EC_{50} value based on the level data provided in the paper by the study authors. Additionally, the statistical analysis and methods used by the study authors could not be evaluated by EPA given that the details provided in the paper. Specifically, the EC_{50} was calculated using what appears to be proprietary statistical software in which few details are provided by the study authors. Therefore, the author-reported EC_{50} value was used qualitatively to derive the acute freshwater criterion for PFOS. However, this toxicity value of 51.45 mg/L is consistent with others for this species that were used quantitatively to derive the PFOS criterion.

G.2.1.4 Benthic Crustaceans

Yang et al. (2014) performed a 96-hour acute test of PFOS (potassium salt, CAS # 2795-39-3, 99% purity) with the freshwater prawn species, *Macrobrachium nipponense* (a non-North

American species). The test followed ASTM E729 (1993). *M. nipponense* (0.30 g, 4.0 cm) used for the test were purchased from the Beijing Dahongmen Jingshen Seafood Market and were held seven days prior to testing. Dilution water was dechlorinated tap water (pH, 7.0 ± 0.5; dissolved oxygen, 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-hr:12-hr (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 2 L beakers of unreported material type containing 1.5 L of test solution. The test employed three replicates of 10 prawn 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), 6.00, 10.80, 19.44, 34.99, 62.99 and 113.37 mg/L. Mean measured concentrations before and after renewal were 4.88 and 5.95 and mg/L (lowest concentration) and 97.85 and 109.22 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 as having been measured every day during the acute test, but results were not reported. Negative control survival was 100%. Solvent control survival was 96%. The 96-hour LC_{50} was 19.77 mg/L (C.I. 12.42-31.48). The acute value was acceptable for qualitative use because the source of organisms was atypical.

G.2.1.5 Aquatic Insects

Olson (2017) conducted a 48-hour static acute test on PFOS with first instar of the mosquito *Aedes aegypti* as part of a Ph.D. thesis at the Texas Tech University, Lubbock, TX. The colony was originally donated by Texas A&M and maintained in the laboratory at Texas Tech University since summer 2013. Dilution water was moderately hard reconstituted water (3 g $CaSO_4$, 3 g $MgSO_4$, 0.2 g KCl, and 4.8 g $NaHCO_3$ added to 50 L deionized water). Photoperiod was 12-14 hours light and 10-12 hours dark. Light intensity was not reported. Stock solutions were prepared by dissolving soluble amounts of powdered chemical in dilution water. Diluted stock concentrations were equal to the maximum test concentration. The stock was mixed on a shaker table at 125 rpm for at least 18 hours before being added to exposure containers and proportionally diluted. Exposure vessels were 50 mL HDPE plastic beakers containing an unspecified amount of test solution. The test employed 10 mosquito larvae each in six test concentrations plus a negative control. Replication was not reported. Nominal concentrations in the test were 0 (negative control), 0.050, 0.125, 0.250, 0.500, 1.000 and 2.000 mg/L. Experiments were conducted in incubators set to 25°C and covered with plexiglass to limit evaporation. No other water quality parameters were reported as having been measured in test solutions. Negative control survival was ≥95%. The 96-hour LC_{50} was 1.18 mg/L. The acute value was acceptable for qualitative use due to its short duration because the publication was missing some exposure details, including the purity of PFOS, it was an unmeasured test. Additionally, the author-reported LC_{50} and concentration-response curve could not be assessed by EPA on a statistical basis since model parameters and sufficient treatment level data were not provided to independently calculate toxicity values (e.g., LC_{50}). And finally, the species is an invasive, pest species to the U.S.

Yang et al. (2014) performed a 96-hour acute test on PFOS (potassium salt, CAS # 2795-39-3, 99% purity) with the midge, *Chironomus plumosus*. The test followed ASTM E729 (1993). *C. plumosus* (0.05 g, 12 cm) used for the test were purchased from the Beijing City Big Forest Flower Market and were held seven days prior to testing. Dilution water was dechlorinated tap water (pH, 7.0±0.5; dissolved oxygen, 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-hr:12-hr (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 90 cm petri dishes containing 10 mL of test solution. The test employed three replicates of 10 midges 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), 100.00, 130.00, 169.00, 219.70, 285.61 and 371.29 mg/L. Mean measured concentrations before and after renewal were 92.24 and 99.46 mg/L (lowest concentration) and 340.45 and 369.29 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, DO, and pH were reported as having been measured every day during the acute test, but results were not reported. Negative and solvent control survival was 96%. The 96-hour LC_{50} was 182.12 mg/L (C.I. 158.71-209.00). The acute value was acceptable for qualitative use because the source of organisms was atypical.

G.2.2 Freshwater Fish

G.2.2.1 Carassius auratus

Three acute PFOS studies were conducted with *Carassius auratus*, but all are classified as qualitative as indicated below. **Yang et al. (2014)** exposed *C. auratus* to the potassium salt of PFOS (CAS # 2795-39-3, 99% purity) for 96 hours using static, measured conditions (the authors note that the experiments followed ASTM 1993b88-729 (ASTM 1993). The goldfish (6.0-7.0 g) were purchased from the Beijing Chaoyang Spring Flower Market, which was considered an atypical source. The organisms were allowed to acclimate for seven days before testing, and the test was conducted at 22±2°C with a light:dark cycle of 12-hr:12-hr; with 10 fish per replicate and three replicates per concentration. Beakers used for exposure were assumed glass, but was not specified by study authors. PFOS was dissolved in deionized water and carrier solvent DMSO to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 20, 32, 51.2, 81.92, 131.07 and 209.72 mg/L PFOS. Water quality parameters reported were pH=7.0 ± 0.5, dissolved oxygen=7.0 ± 0.5 mg/L, total organic carbon=0.02 mg/L and hardness=190.0 ± 0.1 mg/L as $CaCO_3$. The supplemental data provided for the study includes a comparison of measured PFOS concentrations before and after solution renewal in the low and high acute and chronic test concentrations. PFOS concentrations in the test water did not fluctuate by more than 15% during experiments. The 96-hour LC_{50} reported for the study of 81.18 mg/L was deemed qualitative due to the atypical fish source and unknown composition of test beakers and statistical method used for calculating the LC_{50}.

The effect of PFOS (CAS # 1763-23-1, >98% pure) on oxidative stress enzyme responses of *Carassius auratus* juveniles (27.85 g) was evaluated by **Feng et al. (2015)**. The 96-hour static measured exposure was conducted at a temperature of 23°C, pH of 7.25, dissolved oxygen of 6.5 mg/L and total hardness of 174.3 mg/L as $CaCO_3$. The fish were purchased from a local aquatic

breeding base and acclimated in dechlorinated tap water for at least for 10 days, with the total mortality near zero. After acclimatization, five fish were randomly selected and placed in each glass tank (two replicates for treatments and five control replicates) containing 20 L of test solutions (nominal concentrations of 1 or 10 µmol/L PFOS) or 20 L of dechlorinated tap water. PFOS was dissolved in DMSO to prepare a 100 mmol/L stock solution. The tanks were continuously aerated, and water was refreshed to minimize the contamination from metabolic wastes. Antioxidant enzyme activity (CAT, SOD and GPx) and lipid peroxidation were adversely impacted at 10 µmol/L (5.001 mg/L) PFOS at test termination, but these data were considered qualitative because of the atypical endpoints reported and only two exposure concentrations were evaluated.

The swimming behavior of *Carassius auratus* exposed to PFOS was reported by **Xia et al. (2013)**. Juvenile goldfish (6.91 g, 6.01 cm) were exposed to the potassium salt of PFOS (>99% pure) in glass aquaria under renewal unmeasured conditions for 48 hours at 18°C and photoperiod of 15-hrs:9-hrs, light:dark. The fish were obtained from a local market in Chongqing, China and acclimated at the test temperature for three weeks in dechlorinated tap water. The PFOS stock solution (0.8 g/mL) was dissolved in DMSO, with the final exposure solutions diluted with dechlorinated tap water. The fish were divided into six groups (n=8) and were exposed to 0 (water only), 0 (DMSO vehicle control), 0.5, 2, 8 or 32 mg/L of PFOS (one replicate per concentration). The concentration of DMSO in the water did not exceed 0.004% (v/v). The contaminants were administered by replacing 50% of the water with water contaminated with the appropriate concentration of PFOS each day. After exposure to PFOS for 48 hours, the swimming performance behavior (spontaneous activity and fast-start performance) of each fish was examined. The 48-hour NOEC and LOEC for swimming behavior (motion

distance and % of actionless time) was 2 and 8 mg/L, respectively. However, the effects levels were classified as qualitative due to the non-apical endpoints reported, duration of the exposure, only eight fish per exposure concentration, and atypical source of test organisms (local market).

G.2.2.2 Cyprinus carpio

Kim et al. (2010) evaluated the effects of PFOS (100.3% purity) to biomarker responses exhibited by *Cyprinus carpio* exposed for 96 hours under flow-through measured conditions. PFOS stock solutions were prepared in N,Ndimethylformamide (<100 mg/L) and diluted with carbon-filtered and dechlorinated tap water to give nominal concentrations of 0.050, 0.500, 5.000 and 50.00 mg/L. Dechlorinated tap water was used as a control. The exposure concentrations of PFOS ranged from 90 to 124% of the nominal concentrations (or 0, 0.044, 0.620, 5.395, 48.242 mg/L), and where appropriate, the average of the PFOS measured concentrations was used to calculate endpoints when not within ± 20% of the nominal concentrations. The carp were obtained from the Chungcheongnam-do Experimental Station for Inland Waters Development and held in 2,000 L tanks with flowing dechlorinated tap water at 23 ± 2°C, which was also used in the study (pH, 6.9; alkalinity, 28.0 mg/L as $CaCO_3$; total hardness, 47.8 mg/L as $CaCO_3$). Ten juvenile fish (~12 cm; ~20 g) were held in each exposure tank (assume one replicate per concentration) under a 16-hr:8-hr (light:dark) photoperiod, with water temperature maintained at 23 ± 1°C. At test termination, the fish were removed from the tanks and evaluated for biochemical and genetic responses. DNA single-strand breaks was determined to be the most sensitive endpoint, with a 96-hour LOEC of 5.395 mg/L. This study was deemed qualitative due to the non-apical endpoints reported.

G.2.2.3 Danio rerio

Ding et al. (2012; 2013) evaluated the acute effects of PFOS-K (perfluorooctane sulfonate, potassium salt, CAS # 2795-39-3, 98% purity, purchased from Sigma-Aldrich) to

Danio rerio embryos via a 96-hour static-renewal unmeasured exposure. Adult AB strain zebrafish were cultured in aerated and biologically-filtered reconstituted freshwater at 26 ± 1°C. The day before test initiation, male and female zebrafish, at a ratio of 1:1, were placed in spawning tanks before the onset of darkness. Mating, spawning and fertilization took place within 30 minutes after light onset in the morning. Eggs were collected from spawn traps and washed with clean OECD water. Unfertilized or abnormal eggs were removed under a stereomicroscope. PFOS was dissolved in reconstituted water to achieve the desired target test concentrations; no solvents were used. Six exposure concentrations were tested with three replicates each. Graphically, these concentrations are shown as $\log_{10}$(mol/L) concentrations. These were converted to mg/L (15.74, 35.23, 125.3, 142.2, 165.9, and 207.5 mg/L, respectively) given the molecular weight of the form of PFOS-K used in the study, CAS # 2795-39-3, of 538.22 g/mol. Twenty fertilized eggs per exposure concentration were divided into a 24-well plate with one embryo per well, containing 2 mL test solution (three replicate plates per concentration x 20 embryos per concentration for a total of 60 embryos per concentration). The remaining four wells were filled with control water and one embryo each (four control embryos per plate x three plates per concentration for a total of 12 control embryos). Test solutions were half renewed every 24 hours. An embryo was considered dead when one of four end points (i.e., coagulation of the embryo, non-detachment of the tail, non-formation of somites and non-detection of the heartbeat) was observed. Mortality was monitored and documented at 72- and 96-hour post fertilization (hpf). The author-reported 96-hour LC_{50} was 0.101 mM (or 54.36 mg/L) PFOS. The presentation of the data is problematic, however, as the authors did not appear to report the control response either graphically or in the text. Specifically, for PFOS, there are six pairs of symbols (72 hour – plus sign, and 96 hour – x). Most of these symbols overlap. To

the left of these symbols is what appears to be a (minus "-") sign. Initially, it was thought that this was a poorly reproduced (plus sign) and digitized it as the 72 hour control. However, on further inspection, it was noticed that if you increase the size of the figure greatly (800%), the "symbol" is actually tilted slightly, and appears to be a discontinuous extension of the fitted PFOS toxicity curve. EPA reached out to the study authors on July 13, 2021, to request the data from the paper, but have not heard back as of April 12, 2022. Given the level of data presented in the paper and the unreported data from the control treatment, EPA changed the overall use classification of this paper from quantitative to qualitative.

In a follow-up study to one conducted earlier by the investigators, **Hagenaars et al. (2014)**, again exposed *Danio rerio* to the potassium salt of PFOS (CAS # 2795-39-3, purity ≥98%), but for six days via renewal unmeasured methodology. The objective of the study was to determine the exposure windows during early zebrafish development that are sensitive to PFOS exposure and result in impaired swim bladder inflation in order to specify the mechanisms by which this effect might be caused. Since PFOS was fully soluble in water in the tested concentrations (maximum solubility of 680 mg/L at 24-25°C), no solvents were used. Adult wildtype zebrafish (breeders) were maintained and tested in reconstituted fresh water (Instant Ocean® Sea Salt) at 28 ± 0.2°C and a 14-hr:10-hr, light:dark cycle. Male and female fish were separated in breeding tanks with a perforated bottom using a divider. The divider was removed when the lights turned on in the morning. Within 45 minutes after the lights turned on, spawning and fertilization took place and eggs were collected and then transferred to clean reconstituted fresh water with the same composition as in the breeding tanks. Seven different time windows of exposure (1-48, 1-72, 1-120, 1-144, 48-144, 72-144 and 120-144 hour post fertilization, hpf) were tested based on the different developmental stages of the swim bladder. These seven time

windows were tested at four concentrations (0.70, 1.14 3.07 and 4.28 mg/L) at a water temperature of 28.5°C, pH of 7.5, and 14-hr:10-hr (light:dark) photoperiod. Forty-two normally shaped fertilized eggs per exposure concentration were divided over a 48-well plate (sterile tissue culture plates) and each egg was placed individually in 1 mL of the test solution. The remaining six wells per plate were filled with reconstituted fresh water and used as internal negative control embryos. A plate was considered valid if no more than one internal negative control embryo showed lethal effects. Apart from the negative control embryos, a separate plate with control embryos in clean water was used in each test and this plate was used as the control for statistical comparison with exposed embryos. A test was considered valid if >90% of the controls successfully hatched and showed neither sub-lethal nor lethal effects. At six days post fertilization, effects on survival, hatching, swim bladder inflation and size, larval length and swimming performance were assessed. The reported 6-day LC_{50} and EC_{50} (uninflated swim bladder) were 6.25 and 2.29 mg/L PFOS, respectively, and were considered qualitative due to test duration.

Huang et al. (2016) later investigated the effect of PFOS (CAS # 1763-23-1, ≥98% pure) on metabolic responses of *Danio rerio* (measured metabolites (208 in total) included amino acids, biogenic amines, fatty acids, bile acids, sugars and lipids). Ethanol (0.05-0.1%) was used as the carrier solvent for PFOS, with results reported as measured values. Adult AB/Tubingen zebrafish were reared and maintained in a Zebtec aquatic system with re-circulating water (pH 7.0-7.5) and in standard laboratory conditions of 28°C and a 14-hr:10-hr (light:dark cycle). Five biological replicates comprised a treatment group and each replicate consisted of 80 zebrafish at either the embryonic (24-48 hpf) or larval (96-120 hpf) stage. All exposures were carried out statically for 24 hours. No apparent toxic effects were observed during and after the exposure

period in either the control (embryo media [HE3] and carrier control [CC]) or treatment groups at either life stage. Thus, the 24-hour LOEC for the different metabolites was >9.7 mg/L PFOS. The test was classified as qualitative due to test duration and non-apical endpoints.

Effects of PFOS on reactive oxidative stress (ROS) biomarkers of *Danio rerio* was investigated by **Shi and Zhou (2010)**. The authors subjected 4 hpf zebrafish embryos to the potassium salt of PFOS (>99% purity) for 96 hours under static unmeasured conditions. The stock solution (50,000 mg/L) was prepared by dissolving the crystals in HPLC-grade DMSO. The wild-type (AB strain) zebrafish were maintained at 28 ± 0.5°C in a 14 hour light, 10 hour dark cycle in a continuous flow-through system in charcoal-filtered tap water. Fertilized eggs were obtained from natural mating of these adult zebrafish. Zebrafish eggs were collected within four hours of spawning from several breeding tanks, pooled, washed, and then randomly transferred into a glass beaker (four replicates) containing 500 mL of exposure solution (0, 0.2, 0.4 and 1.0 mg/L) of PFOS (~300 eggs per beaker). Both the control and the treated embryos received 0.03% (v/v) DMSO. The beakers were kept in a humidified incubator at 28.5 ± 0.5°C under controlled lighting conditions (14-hr:10-hr light:dark cycle). The reported MATC (increased ROS formation) was 0.2882 mg/L PFOS (NOEC = 0.2 mg/L and LOEC = 0.4 mg/L), but the study was deemed qualitative due to the non-apical endpoint and lack of dilution water information.

Padilla et al. (2012) exposed *Danio rerio* embryos (6-8 hpf) to PFOS for six days employing renewal unmeasured procedures. The goal of this study was to describe the broad application of a zebrafish screening model to 309 chemicals, including PFOS. A stock solution of PFOS was prepared in 100% DMSO at a concentration of 20 mM, with exposure solutions of 0, 0.001, 0.004, 0.012, 0.030, 0.110, 0.320, 1.00, 2.96, 8.80, 26.6, and 80.0 µM PFOS or 0.0005,

0.002, 0.006, 0.015, 0.055, 0.16, 0.5, 1.48, 4.4, 13.3 and 40 mg/L (DMSO at 0.4% v/v) prepared in 10% Hanks' solution. Wild type adult zebrafish obtained from Aquatic Research Organisms (Hampton, NH) were held at 28°C with a 14-hr:10-hr light:dark cycle. Adult breeding fish (2-3 females per male; density=15-20 adults per tank) were kept in one of several 9 L flow-through colony tanks. Typically, adults from two to three colony tanks were mated on the same day. Two hours after light onset the adults were returned to the colony tank. All embryos were gathered from each breeder tank, pooled, and placed in a 28°C water bath for two hours, followed by two washes with 0.06% bleach (v/v) in 10% Hanks' Balanced Salt Solution for five minutes in order to remove any residual bacteria or fungi. Zebrafish embryos were exposed in 96-well plates. On day 0, approximately 6-8 hours after fertilization, zebrafish embryos were placed one embryo per well in Millipore Multiscreen Nylon mesh plates and exposed to nominal concentrations of the chemicals. All embryos and larvae were kept in a 26 ± 0.1°C incubator with a 14-hrs:10-hrs, light:dark cycle. Embryos were exposed to the chemicals for five days post fertilization (dpf) (i.e., 120 hpf) with daily dosing (i.e., complete solution change with chemical renewal every 24 hours), followed by a wash-out in Hanks' buffer for one day prior to the lethality, hatching, and malformation assessments performed on 6 dpf. The half-maximal toxicity score concentration (AC_{50}) was 16.44 mg/L. The toxicity score incorporates survival, hatchability, and malformation (maximum score of 40), and the PFOS score of 28.0 indicates observed inhibition of hatchability and malformation. Because of the limited details presented for any specific chemical, the unconventional exposure duration, and the unconventional endpoint, the study was classified as qualitative.

A 72-hour exposure of *Danio rerio* embryos to PFOS (98% purity) was conducted by **Zheng et al. (2012)** following OECD (1996) methodology. No solvent was used for PFOS

because of its high water solubility (500 mg/L). Exposure solutions were diluted from the stock solutions with embryonic water. Adult wild-type zebrafish were obtained from Model Animal Research Center of Nanjing University and kept in a semiautomatic rearing system (tap water), with five females and ten males in each 10 L tank at 28 ± 1°C. Water was exchanged at a rate of 1/3 daily and the lighting was 14-hr:10-hr (light:dark) photoperiod at 1000 lux. Spawning and fertilization took place within 30 minutes after the lights were turned on in the morning. Embryos were transferred to exposure solutions (embryo water) immediately after fertilization and examined under a stereomicroscope. Damaged or unfertilized embryos were discarded. Zebrafish embryos were exposed in 24-well cell culture plates (material not identified) with 2 mL solution per well (pH of 8.3 ± 0.2, dissolved oxygen concentration of 6.07 ± 0.24 mg/L at the beginning and end of experiments). Twenty normally shaped fertilized embryos were assigned to each treatment (0, 6.25, 12.5, 25, 50, 100, 200 mg/L) or control group. All concentrations were repeated in triple at different days with different batches of eggs. Embryos were cultured in an incubator at 28.5°C after exposure. The reported 72-hour LC_{50} and EC_{50} (malformations) were 68 and 37 mg/L PFOS, respectively. However, the data were considered qualitative because the duration is too short for an acute exposure.

Du et al. (2013) investigated the effect of PFOS (≥98% purity) on the survival, malformation and suppression of steroidogenic enzyme synthesis of *Danio rerio* embryos exposed via renewal unmeasured conditions for 120 hours. PFOS stock solutions were prepared in DMSO at a concentration of 0.1 M and stored at -20°C. They were diluted to desired concentrations in culture medium immediately before use, and the final concentration of DMSO in the culture medium did not exceed 0.1% (v/v). Wild-type adult male and female zebrafish, obtained from the Model Animal Center of Nanjing University, were maintained on a 14-hr:10-

hr light:dark cycle at 28°C under semi-static conditions with charcoal-filtered water. Spawning was induced in the morning when the light was turned on. Fertilized eggs were collected 30 minutes later and examined under the microscope. Only those that had developed normally were selected. Embryos were incubated with embryo medium in Petri dishes for subsequent experiments. Zebrafish embryos at 4 hpf were exposed (three replicates, 40 fish per replicate) to 0.100, 0.200 and 0.500 mg/L PFOS and 0.001% DMSO (control) at 28°C, with daily renewal of the embryo medium. Embryo survival and stage of embryonic development were recorded daily until test termination (120 hpf). The NOEC and LOEC for suppression of steroidogenic enzyme synthesis was reported as 0.100 and 0.200 mg/L, respectively. Since only non-apical endpoints were reported, these data are classified as qualitative. In addition, the authors noted that no effects of mortality or malformation were observed, thereby resulting in a LOEC of >0.500 mg/L. However, this was a low effect concentration compared to other acute values and therefore of little value in deriving criteria.

(Chen et al. 2014b) evaluated the effects of PFOS (≥96% purity) on malformation of *Danio rerio* statically exposed for 48 hours following procedures described by Westerfield (1993). PFOS was dissolved in 100% dimethyl sulfoxide (DMSO) to prepare PFOS stock solutions (32 mM), followed by serial dilution with embryo medium (EM) to prepare the exposure solutions (DMSO of 0.1%). The control also received 0.1% DMSO (v/v in EM). Wildtype (AB strain) zebrafish were raised and kept at standard laboratory conditions of 28°C on a 10-hr:14-hr, light:dark photoperiod in a recirculation system. 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 450-1000 µS/cm (system water). Zebrafish embryos were obtained from adults in tanks with a sex ratio of 1:1, and spawning was induced in the morning when the light

was turned on. Embryos were collected within 0.5 hours of spawning, rinsed in EM and staged under a dissecting microscope to select those with normal morphology. Embryos/larvae were waterborne exposed to nominal PFOS concentrations of 8, 16, 32 µM (4.001, 8.002, and 16.00 mg/L) in 6-well plates (20 embryos per well with 5 mL solution) from 0 to 48 hpf or 48-96 hpf. At the end of each exposure period, the embryos or larvae were rinsed three times with EM and transferred to 96-well plates (1 embryo per well with 200 µL solution) for continuous development until 120 hpf, where the incidence of various malformation was scored. Complete malformation (100%) was observed at 8.0 mg/L PFOS, but since the duration was only 48 hours, the study was deemed qualitative.

Danio rerio behavioral alterations in response to acute PFOS exposure (potassium salt, CAS # 2795-39-3, ≥98% purity) was investigated by **Spulber et al. (2014)**. PFOS was dissolved in DMSO (1 mg/mL), and further diluted in DMSO and rearing water to yield exposure concentrations of either 0.1 or 1 mg/L (0.1% DMSO). Control embryos were exposed to 0.1% DMSO in E3 water. Wildtype AB zebrafish embryos were obtained from the zebrafish core facility at Karolinska Institute. Breeding groups of adult fish (three males and two females) were housed together overnight in 10 L spawning tanks containing environmental enrichment (commercially available aquaria made of non-toxic plastic). Thirty minutes after turning the light on, the fertilized eggs were collected and stored at 28.5°C until further processing. The eggs were washed twice with fresh E3 water (pH 7.4) at room temperature and under constant illumination (approximately 500 lux). The exposure to PFOS was initiated about 2 hpf. The zebrafish larvae were then plated and maintained individually in 48-well plates (cylindrical wells, 10 mm inner diameter) in 750 µl E3 water at 28°C in a 14-hr:10-hr light:dark cycle (300 lux intensity, daylight-matching spectrum white light) until behavioral testing at six days post

fertilization (dpf). The exposure followed a static, non-replacement regime. All treatments were present in each plate, and the larvae were distributed such that an equal proportion from each group would be placed in wells at the periphery of the plate. The mortality, successful hatching, and the occurrence of embryonal malformations was assessed at the 24 (developmental failure/coagulation, light-induced coiling movements), 48 (developmental failure/coagulation, blood circulation, pericardial oedema, pigmentation), 72, and 120 hpf (hatching, eye development, swimming bladder inflation, morphological abnormalities such as scoliosis or bent spine), as well as after completing the behavioral experiments (6 dpf). The larvae displaying morphological abnormalities at 6 dpf were excluded from analyses. The behavioral NOEC and LOEC (spontaneous swimming activity) were 0.1 and 1.0 mg/L PFOS, respectively. The data were considered qualitative since only two exposures, duration and non-apical endpoints.

The acute toxic effects of PFOS (potassium salt, ≥98% purity) on sperm vitality, kinematics and fertilization success in *Danio rerio* was investigated by **Xia and Niu (2017)**. PFOS was initially dissolved in DMSO, and the stock solution (0.5 g/mL) was stored at 4°C until preparation of the final exposure solutions in dilution water (Hank's Balance Salt Solution). Adult zebrafish (AB strain) were maintained according to standard culture protocols (Westerfield 1995). The male and female fish were housed in separate aquariums for four weeks prior to the experiment. The rearing water was dechlorinated tap water, maintained at 25±1°C, dissolved oxygen level ≥6 mg/L, the pH ranged from 7.0 to 7.8, and the rearing system was maintained under a 14-hr:10-hr light:dark cycle. After the acclimation period, male and female zebrafish of uniform size (0.41 g and 0.49 g, respectively) were selected as the experimental fish. Sperm vitality and kinematics were determined with a CASA system. To avoid the possible effects of inter-male variation in sperm quality, sperm from the same fish were activated by mixing 1 µL

sperm suspension with 9 µL different activation solutions containing a range of PFOS concentrations (0, 0.1, 1 and 10 mg/L). Specifically, the concentrations of PFOS in the treatment groups were 0, 0.09, 0.9 and 9 mg/L. The concentration of DMSO in the activation solutions did not exceed 0.002% (v/v). The viabilities and kinematics of sperm exposed to the different treatments were assessed at 20, 40 60 and 80 seconds after activation at room temperature (25°C) and pH of 8. The percentage of motile sperm, the curvilinear velocity, the straight-line velocity, the angular path velocity and the mean angular displacement (MAD) of spermatozoa were calculated for each group from three recordings of at least 50 sperm (30 frames/s). A total of 24 male fish (n=24) were used for each PFOS treatment group. Eggs (approximately 200) from individual females were collected by gentle abdominal massage, mixed with 100 µL sperm suspension (sperm to egg ratio 3000:1), and immediately exposed to 50 mL solutions in 9 cm diameter petri dishes containing a range of PFOS concentrations (0, 0.09, 0.9 and 9 mg/L). After two minutes, the eggs were washed three times and then transferred to uncontaminated water without PFOS. The fertilized eggs at the gastrulation stage were examined using a stereomicroscope, and the fertilization rate was then determined six hours after exposure to spermatozoa. All of the manipulations were performed at 25°C. A total of 24 male and 24 female fish (n=24) were used for each PFOS treatment group. The NOEC and LOEC for sperm motility and fertilization success were 0.09 and 0.9 mg/L PFOS, respectively. These data were deemed qualitative due to the non-apical endpoints evaluated and duration of the exposure.

Annunziato (2018) evaluated the acute effects of perfluorooctane sulfonate (PFOS) on zebrafish (*Danio rerio*) via a 5-day static, unmeasured study. AB strain zebrafish were sourced from Zebrafish International Resource Center and followed Rutgers University Animal Care and Facilities Committee guidelines protocol 08-025. Fish were maintained at a pH of 7.2-7.7,

temperature of 27 ± 1°C and a 14-hr:10-hr light:dark cycle, and were fed twice daily a diet of artemia in the mornings and aquatox/tetramin flake mix in the evenings. The stock solution was prepared as 2,000 µM in the same water used to incubate the eggs. Twenty-five embryos (3 hpf) were exposed in 20 mL glass vials at a concentration of 0 (control), 0.02, 0.2 and 2.0 µM for five days. There were four or five replicate vials per concentration. At five days, larvae were snap frozen in liquid nitrogen, and RNA was isolated. Authors reported a 117-hr LOEC for gene expression of Leptin A mRNA of 0.02 µM, or 0.01 mg/L PFOS based on a molecular weight of 500.13 g/mol. The atypical test endpoint and test duration makes the study acceptable for qualitative use only.

Dang et al. (2018) evaluated the acute effects of PFOS (CAS # 2795-39-3, >99% purity) to *Danio rerio* via a 96-hour renewal unmeasured exposure. Stock solutions were made in DMSO, but the concentration in exposure solutions was not identified. Sixteen-week old adult zebrafish (wild type, AB strain) were maintained in a flow-through system as previously described (Dang et al., 2015; Liu et al., 2009). Sexual mature zebrafish were cultured at 28 ± 0.5°C with a 12-hr:12-hr light:dark cycle and were fed thrice daily newly hatched *Artemia* nauplii. Fertilized eggs were rapidly collected and counted from natural crosses after lights on in the morning and then were examined under a stereomicroscope. Normally developed embryos were randomly distributed in 90 mm culture dishes containing 30 mL rearing water (60 mg/L instant ocean salt in aerated distilled water) for subsequent experiments at 1-2 hours post fertilization (hpf). The experiment included two parts. First, 450 embryos from six pairs of fish were collected and cultured in three dishes, and each dish contained 150 embryos. The embryos/larvae were sampled at 2, 24, 48, 72, 96, and 120 hpf to examine the mRNA expression profiles of GH/IGF axis genes; and larvae were sampled at 72, 96, and 120 hpf to measure body

length. The body length of 20 larvae from each replicate at post-hatch along the body axis from the anterior-most part of the head to the tip of the tail was measured with digital images using the Image Pro Plus software. Twenty embryos/larvae were pooled to produce one replicate for the subsequent quantification of mRNA expressions of genes involved in GH/IGF axis and each concentration contained three replicates. Second, based on the results of the first part of the experiment, PFOS (and other chemicals) were chosen to study the responses of GH/IGF axis of zebrafish embryos/larvae at 96 hpf. A total of 1,200 embryos and four concentrations were used for toxicity testing each chemical. Each concentration contained three replicates, and each replicate contained 100 embryos. Stock solutions of PFOS were prepared in DMSO and each group contained the same concentration of DMSO or rearing water. Nominal exposure concentrations were 0.1, 1 and 10 µM PFOS (or 0.05, 0.50, and 5.0 mg/L). During the exposure period, dead larvae were removed from the culture dishes and exposure solutions were renewed at 48 hpf. Endpoints of embryo toxicity test at 96 hpf included survival rate, hatching rate, malformation incidence and body length. Heart rates were recorded at 72 hpf. For the measurement of survival, hatching and malformation incidences, all the 100 eggs were used, and for the calculations of heart rates and body length, twenty larvae from each dish, a total of sixty larvae, were used. The most sensitive endpoint was length, which was atypical for an acute exposure. The 96-hour NOEC and LOEC for growth were <0.05 and 0.05 mg/L, respectively. However, these data were considered qualitative since it was atypical for an acute assessment. In addition, there was no effect on survival, so the 96-hour LC_{50} was >5.0 mg/L PFOS.

The effects of PFOS on oxidative stress exhibited by *Danio rerio* 1 hpf embryos (wild type) was evaluated by **Sant et al. (2018)**. Stock solutions of 160, 320, and 640 mM for embryo exposures were prepared by dissolving PFOS into DMSO, and stored at room temperature in

glass bottles inside of light-prohibitive, airtight containers until use. Adult fish populations were maintained in an automated Aquaneering zebrafish system at 28.5°C and following at 14-hr:10-hr light:dark cycle daily. Breeding populations were housed at an appropriate density with a 2:1 female-to-male ratio. Embryos for experiments were collected with 1 hpf from homozygous genotyped tanks, washed thoroughly, and confirmed for fertilization prior to experimental proceedings. Pools of 10-15 mid-blastula stage embryos of each genotype were separately collected into fresh polystyrene petri dishes containing 20 mL of 0.3x Danieau's media (pH of 7.6 and 28.5°C). Polystyrene plates were used because they have lower matrix retention/adherence rates for PFOS than other materials such as glass. Stock solutions of PFOS or DMSO were added to the dishes at 0.01% v/v, resulting in exposures to 0 (DMSO control), 16, 32, or 64 µM PFOS (or 0, 8.002, 16.00, 32.01 mg/L). All exposure media was refreshed daily. At 24 hpf, all embryos were manually dechorionated using watchmakers' forceps and chorion debris was removed from dishes. All experiments were repeated 3-4 times. Embryos were imaged at 96 hpf for embryonic morphology using a FBS10 Fluorescence Biological Microscope. Embryos screened for morphological deformities were imaged at 5x magnification using transmitted light microscopy. The pericardial area NOEC and LOEC values were 8 and 16 mg/L PFOS, respectively, and were considered qualitative due to the non-apical endpoint.

Danio rerio was evaluated by **Huang et al. (2010)** in a 114-hour static measured exposure to PFOS (CAS # 1763-23-1, > 96% pure). PFOS stock solutions (32 mg/L) were prepared in DMSO, followed by serial dilution with the embryo medium (DMSO of 0.1%). The control also received 0.1% DMSO. Wild-type (AB strain) zebrafish founding stocks were obtained from the Environmental Health Sciences Center at Oregon State University. All fish were raised and kept at standard laboratory conditions of 28°C on a 10-hr:14-hr light:dark

photoperiod in a recirculation system. Zebrafish embryos were obtained from spawning adults in tanks overnight with the sex ratio of 1:1. Embryos were collected within 0.5 hours of spawning and rinsed in embryo medium (EM). Fertilized and normal embryos were staged under a stereomicroscope. To determine the LC_{50}, zebrafish embryos were exposed to 0, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/L PFOS from six to 120 hour post-fertilization (hpf). Embryos were kept in sterile 96-well plates, with one embryo per well, containing 200 µL treatment or control solutions (EM). For each exposure condition, five replicates each with 32 embryos were performed at 28±0.5°C in a light-controlled incubator. For EC_{50} determination, lower exposure concentrations of 0, 0.005, 0.05, 0.5, 2.0, and 4.0 mg/L were used. The end points of toxicity included bent spine, malformed tail, pericardial edema, yolk sac edema, uninflated swim bladder, failed hatching, single eye, and opaque head (apparent necrosis). The 114-hour LC_{50} was 2.2 mg/L PFOS, and the malformation EC_{50} was 1.12 mg/L PFOS. These data were considered qualitative due to exposure duration, which was longer (at 114 hours) than fish acute toxicity test guidelines (OCSPP 850.1075) with exposures of 96 hours. Both of these toxicity values were within a factor of two of the FAV of 2.222 mg/L and indicated that this genus might be more sensitive to acute exposures of PFOS than the quantitative data for the genus indicate. However, it is unlikely that these toxicity values would substantially change the FAV, as this genus was not among the four most sensitive genera (*Danio* GMAV of 29.31 mg/L ranks eighth), and therefore would have little impact on the acute freshwater criterion.

A sub-chronic static unmeasured test was utilized by **Ulhaq et al. (2013)** to determine the toxicity of PFOS to *Danio rerio*. PFOS stock solutions were freshly prepared in reconstituted water in concentrations below the limit for water solubility. Adult zebrafish (AB strain) were held in charcoal-filtered tap water. Breeding groups including three males and two females were

placed in 10 L glass aquaria equipped with spawning nets separating the parental fish from the eggs. Half an hour after onset of lights the eggs were collected, rinsed for removal of debris, and then only normally developed fertilized eggs at least in the four-cell stage were selected using a stereomicroscope. Within 15 minutes after collection, the zebrafish eggs were exposed to a series of concentrations of the test substance dissolved in reconstituted water (exposure medium). Fertilized eggs (4-cell stage) were randomly distributed individually into flat bottom, 48-well polystyrene plates along with 750 µL of the exposure medium. PFOS was tested at six consecutive concentrations differing by a factor of 3.3 based on logarithmic scale fitting. For each test, four 48-well plates were used, with a total of 24 embryos per concentration as well as 24 in the water control group. Each treatment group was equally distributed to each of the four well plates (i.e., six embryos/concentration/plate giving a total of 168 embryos). The plates were covered with parafilm and the embryos were exposed to the chemical until 144-hour post fertilization (hpf). Fish laboratory conditions throughout the study were kept at pH 7.2-7.6, a water temperature of 26 ± 1°C and a light cycle of 14 hours. Observations of mortality and sublethal endpoints were made after 24, 48, 120 and 144 hpf using a stereomicroscope. Sublethal endpoints such as presence of edema, malformations, not-hatched eggs, lack of circulation and reduced pigmentation were also observed. Heart rate was recorded at 48 hpf and hatching time was determined using time-lapse photography. The 144-hour LC_{50} was >10 mg/L PFOS and the EC_{50} (lethal and sublethal effects, including spinal curvatures, oedemas, uninflated swimbladder, and side-lying) was 1.5 mg/L PFOS. Both were considered qualitative data because of test duration, which was longer (at 114 hours) than fish acute toxicity test guidelines (OCSPP 850.1075) with exposures of 96 hours. Furthermore, the LC_{50} for mortality was a less certain greater than value. However, these toxicity values were within a factor of two of the FAV of

2.222 mg/L and indicated that this genus might be more sensitive to acute exposures of PFOS than the quantitative data for the genus. However, it is unlikely that these toxicity values would substantially change the FAV, as this genus was not among the four most sensitive genera (*Danio* GMAV of 29.31 mg/L ranks eighth), and therefore would have little impact on the acute freshwater criterion.

Sharpe et al. 2010 examined the bioaccumulation and toxicity of PFOS 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 48-hour tests are described in this present section, as these results were used qualitatively. The 96-hour test was used quantitatively to derive the acute water column criterion (see Appendix A) and the chronic toxicity tests were used qualitatively and are summarized in Section 4.4.2.1.4. Zebrafish were purchased from a pet store local to the University of Alberta and were reared at university facilities for six to ten months. Conditioned zebrafish water obtained from the Biological Sciences Zebrafish Facility at the University of Alberta was used to acclimate the fish in 70 L glass aquaria where they were fed powdered trout chow (Unifeed) daily, occasionally supplemented with live brine shrimp. An automated reverse osmosis system was used to maintain conditioned zebrafish water, used for acclimation and testing, at a total hardness of around 160 mg/L and a calcium carbonate hardness at 20 mg/L. Test concentrations were diluted from a 25 mg/mL stock solution in a methanol (MeOH) solvent for dosing in all experiments.

The 48-hour embryo toxicity test followed the same OECD guideline. This experiment was also performed in triplicate. Embryos for the experiment were collected one hour post-spawn and their fertilization was verified with a compound microscope. Forty fertilized embryos

were randomly selected and placed into 24-well cell plates for 72 hours at the following concentrations; control (0 mg/L PFOS), solvent control (0.65% MeOH v/v), and 1.1, 2.4, 5.3, 11.7, 25.8 and 56.8 mg/L measured PFOS concentrations. The testing water and dosages were renewed daily and mortality was measured after 48 hours to calculate a 48-hour LC_{50}. In addition to mortality observations, 24-hour checks for the presence of detached tails and somite formations were performed. At 48 hours, observers looked for the presence of a heartbeat and eyespot, and at 72 hours, the number of hatched embryos were counted.

The author-reported LC_{50} at 48 hours for the embryo toxicity ranged from 7.7 - 38.9 mg/L PFOS; however, the test results were considered to be inconsistent given the wide range of the LC_{50}s. Additionally, the study authors note that developmental deformities (i.e., delayed eyespot formation, lack of a heartbeat at 48-hours, yolk sac deformities, and a lack of cephalization) were observed in two of the three assays. However, given that the test was not of a standard duration for criteria derivation per EPA's test quality guidelines and the inconsistency in the author-reported toxicity values, the 48-hour embryo study was considered for qualitative use. However, these toxicity values were similar to the SMAV of 24.44 mg/L and support the toxicity value used for this genus in the derivation of the acute freshwater criterion.

Rainieri et al. (2017) evaluated the acute effects of PFOS (perfluorooctane sulfonate purchased from Sigma-Aldrich) on zebrafish (*Danio rerio*) in a 48-hr static measured study. A 2 mg/mL stock solution was prepared by dissolving PFOS in methanol and stored at <4°C until use. Wild type fish were obtained from AZTI Zebrafish Facility and maintained at 27°C, a 12-hr:12-hr light:dark cycle, and were fed twice daily with commercial feed. Embryos were held in culture water for seventy-two hours before testing. Twenty-five hatched embryos were exposed in 10 mL of test solution in glass petri dishes 6 cm in diameter at 27°C under a 12-hr:12-hr light-

dark photoperiod for 48 hours. Triplicate exposures ranged from 10 to 500 mg/L PFOS with a maximum of 0.45% DMSO in any exposure. Samples of each exposure solution were taken at the beginning and at the end of the test to determine PFOS concentrations. The reported 48-hr LC_{50} value was 107.6 mg/L PFOS is acceptable for qualitative use because of the short test duration.

Ortiz-Villanueva et al. (2018) evaluated the acute effects of perfluorooctane sulfonate (PFOS, purchased from Sigma-Aldrich) on wild-type zebrafish (*Danio rerio*) in a 2-day unmeasured, static-renewal study. Adult fish were maintained in reverse-osmosis water mixed with 90 µg/mL Instant Ocean salt and 0.58 mM $CaSO_4 \cdot 2H_2O$ and were fed twice daily with Tetramin flakes. A stock solution was prepared by mixing PFOS with dimethyl sulfoxide on the day of the experiment, with exposure concentrations of 0 (control), 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0, 20.0 and 200 µM PFOS (maximum of 0.2% DMSO). Six males and three females were placed in a 4 L spawning tank with a mesh bottom. At two hpf, fertilized embryos were collected, rinsed and maintained in 6-well multi-plates (10 embryos per well) at 28.5°C under a 12-hrs:12-hrslight:dark photoperiod until 48 hpf. Test solutions were added, and fish were observed for an additional 72 hours (120 hpf). Each treatment was replicated five times for a total of fifty embryos per exposure concentration. Study authors reported following protocols DAMM 7669 and 7694. The reported PFOS LOEC was 2.0 µM (1.0 mg/L) for malformations, and 20 µM (10 mg/L) for survival (based on a molecular weight of 500.13 g/mol PFOS). The short test duration made the study acceptable for qualitative use only.

(Martinez et al. 2019b) evaluated the acute effects of perfluorooctane sulfonate potassium salt (PFOS-K, ≥98% purity, CAS No. 2795-39-3, obtained from Sigma-Aldrich) on zebrafish (*Danio rerio*) in a 3-day unmeasured, static-renewal study. The stock solution was

prepared by dissolving PFOS salt in dimethyl sulfoxide and stored at -20°C. Adult, wild type zebrafish, twelve to eighteen months old, were kept at 28°C under a 12-hr:12-hr light:dark photoperiod and fed dry flakes twice daily in accordance with protocols DAMM 7669 and 7964. Eggs were collected and rinsed at two hours post-fertilization (hpf) and put in 6-well multi-plates with ten fertilized eggs per 3.0 mL of test solution with five or six replicates per test concentration. Reverse-osmosis purified water was combined with 90 µg/mL Instant Ocean and 100 µg/mL $CaSO_4 \cdot 2H_20$ to create culture medium and test concentrations of 0 (control, 0.2% DMSO), 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0, 20.0 and 200 µM PFOS. After five dpf, surviving fish were sacrificed and measured for body length, which yielded a LOEC value of 10.0 µM, or 5.382 mg/L PFOS calculated using the molecular weight of 538.22 g/mol for PFOS-K. The study is acceptable for qualitative use because of the short test duration.

Vogs et al. (2019) evaluated the acute effects of perfluorooctane sulfonic acid potassium salt (PFOS-K, ≥98% purity, CAS No. 2795-39-3, $7.6x10^{-6}$ mg/L solubility at 25°C) on zebrafish (*Danio rerio*) embryos in a 118-hour measured, static-renewal study. AB strain fish used in this study were provided by the Zebrafish Core Facility at Comparative Medicine, Karolinska Institute. Three male and three female adults were grouped, and embryos were collected in E3 medium directly after spawning. Study authors reported following OECD TG 236. A stock solution was prepared by dissolving PFOS into dimethyl sulfoxide to achieve initial measured concentrations of 0.04, 0.08 and 0.76 µM in E3 medium. Thirty embryos (2 hpf) were placed in 30 mL exposure medium in 50 mL glass petri dishes maintained at 28±1°C under dark conditions throughout the exposure (until 120 hpf). When the medium was renewed was not provided. A 118-hr EC_{50} value of 3.8 µM PFOS (or 2.045 mg/L based on molecular weight of 538.22 g/mol PFOS-K), was reported for mortality, non-inflated swim bladders, pericardial and

yolk sac edemas, and scoliosis. The atypical test duration makes the study for acceptable for qualitative use only.

Dasgupta et al. (2020) evaluated the acute effects of perfluorooctane sulfonic acid (PFOS, CAS No. 1763-23-1, 97% purity, purchased from Synquest Laboratories) on zebrafish (*Danio rerio*) via a 66-hour unmeasured, static study. A stock solution was prepared with either DMSO or NaOH and stored in 5 mL glass vials and kept at room temperature. The test solution (50 mM PFOS) was prepared by spiking stock solution into water derived from the recirculating water system used to maintain and breed adult, wildtype (5D) zebrafish. Eight embryos (6 hpf) were incubated and exposed to 10 mL of either a control or 50 µM PFOS until 72 hpf at 28°C under a 14-hr:10-hr light:dark photoperiod. At test termination there was no significant effect of survival or development on zebrafish embryos. The 66-hour NOEC of 50 µM PFOS (or 25 mg/L based on molecular weight of 500.13 g/mol PFOS), based on survival, is acceptable for qualitative use only due to the short exposure period.

Truong et al. (2014) evaluated the acute effects of potassium perfluorooctanesulfonate (PFOS-K, CAS # 2795-39-3) and perfluorooctane sulfonic acid (PFOS, CAS #. 1763-23-1) on zebrafish (*Danio rerio*) in a 114-hour unmeasured, static study. A stock solution was prepared in 100% dimethyl sulfoxide at a concentration of 20 mM and stored at -20°C until 30 minutes prior to embryo exposures. Exposure concentrations were 0 (control), 0.00614, 0.0614, 0.614, 6.144 and 61.44 µM PFOS-K and 0.0064, 0.064, 0.64, 6.4 and 64 µM PFOS. 5D wild-type zebrafish were obtained from the Sinnhuber Aquatic Research Laboratory at Oregon State University in Corvallis, Oregon. Fish were maintained at 28°C under a 14-hr:10-hr light:dark photoperiod in a synthetic fish culture water consisting of reverse osmosis water supplemented with a commercially available salt (Instant Ocean). Embryos were collected from breeding tanks using

a spawning funnel and dechorionated using pronase. Dechorionated embryos (6 hpf) were placed one embryo per well in a 96-well plate (32 replicates per concentration) filled with 90 µL embryo medium and 10 µL of stock solution (final DMSO concentration of 0.64%). The well plates were sealed and covered in foil until embryos reached 120 hpf. The reported 114-hr mortality LOEC was 61.44 µM PFOS-K, or 33.07 mg/L, based on a molecular weight of 538.22 g/mol. The 114-hr LOEC, based on mortality was 6.4 µM PFOS, or 3.2 mg/L, based on a molecular weight of 500.13 mg/L. Both values are qualitative only because of the atypical test duration.

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. Three universities participated in this study, and although zebrafish husbandry conditions varied between the laboratories, all were within OECD TG 236 guidelines. Note: only the Orebro University tested PFOS. Fish were fed ad libitum with TetraMin and newly hatched brine shrimp nauplii two times daily. Zebrafish embryos were collected and tested according to OECD TG 236, and embryo exposure started at a maximum of 4 hpf. Triplicate 100 mL glass vials covered with lids were filled with 20 mL test solution with five embryos per container. Aqueous exposures of PFOS were conducted at concentrations of 0 (control), 0.1% DMSO (negative control) and 700 ng/L maintained at 26 ± 1°C and a 10-hr:14-hr dark/light rhythm. The authors reported a 96 hr NOEC value of 700 ng/L PFOS (or 0.0007 mg/L) for hatching success, embryo mortality, and developmental deformations. Because the value represents a greater than low value (see 2013 Ammonia rule; U.S. EPA 2013) the study is only used qualitatively in the acute criterion.

G.2.2.4 Pseudorasbora parva

Juvenile female topmouth gudgeons, *Pseudorasbora parva*, were exposed to the potassium salt of PFOS (>99% purity) for 96 hours by **Xia et al. (2014)**. PFOS was initially dissolved in DMSO, and the stock solution (0.8 g/mL) was kept at 4°C until prepared for the final exposure solutions in water. Live fingerlings of *P. parva* were obtained from a local market in Chongqing, China (note: *P. parva* is not a North American species). Individuals of uniform size (0.81 g body weight and 4.03 cm body length) were selected and acclimatized in a 120 L recirculating water tank system at Chongqing Normal University for at least two weeks prior to the experiment. Fish were maintained in water at a constant temperature of 15 ± 1°C under a photoperiod of 14-hr:10-hr light:dark. The dissolved oxygen level was kept above 7 mg/L. The rearing water was dechlorinated and filtered through activated carbon. Fish were fed daily with commercial tubifex, and were used when no mortality was observed in the acclimation population. Waterborne exposures were conducted in a renewal exposure experimental apparatus, which consisted of several glass aquariums with a capacity of approximately 22 L of water. Prior to exposure, a total of 80 topmouth gudgeon were randomly divided into five groups (n=16), and were gently transferred to the aquariums. Fish were maintained at same conditions as described above for one week to eliminate stress. After the habituation period, the fish were exposed to 0 (DMSO vehicle control), 0.5, 2, 8 and 32 mg/L of PFOS (nominal concentrations) for 96 hours. The concentration of DMSO in the water did not exceed 0.004% (v/v). The toxicants were administered by replacing 50% of the water with water contaminated with the appropriate concentration of PFOS every day. Food was not provided during the course of exposure and subsequent testing. After termination of PFOS exposure, the metabolic rate and swimming performances were examined. For each treatment, eight fish (n=8) were used for RMR determination, and another eight fish (n=8) were used for swimming measurements. The

96-hour NOEC and LOEC for spontaneous swim behavior (swim distance) were reported as 0.5 and 2 mg/L PFOS, respectively, and were qualitative due to the non-apical endpoint and atypical source of test organisms (local market). However, since all 16 fish of those originally initiated were evaluated for swimming, it was assumed that no mortality occurred which gave a 96-hour LC_{50} of >32 mg/L PFOS.

Yang et al. (2014) also evaluated the toxicity of PFOS (potassium salt, CAS # 2795-39-3, 99% purity) to *Pseudorasbora parva* via 96-hour renewal measured exposures (the authors noted that the experiments followed ASTM standards and USEPA procedures for deriving water quality criteria). The topmouth gudgeon (4.0 g, 4.0 cm) were purchased from the Beijing Chaoyang Spring Flower Market, which was considered an atypical source. The organisms were allowed to acclimate for seven days before testing, and the test was conducted at 22±2°C with a light:dark cycle of 12-hr:12-hr, with 10 fish per replicate and three replicates per concentration. Beakers used for exposure were assumed glass, but glass was not specified by study authors. PFOS was dissolved in deionized water and carrier solvent DMSO to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 30, 45, 67.5, 101.25, 151.88 and 227.81 mg/L PFOS. Water quality parameters reported were pH=7.0 ± 0.5, dissolved oxygen=7.0 ± 0.5 mg/L, total organic carbon=0.02 mg/L and hardness=190.0 ± 0.1 mg/L as $CaCO_3$. The supplemental data provided for the study includes a comparison of measured PFOS concentrations before and after solution renewal in the low and high acute and chronic test concentrations. PFOS concentrations in the test water did not fluctuate by more than 15% during experiments. The 96-hour LC_{50} reported for the study of 67.74 mg/L PFOS was deemed qualitative due to the atypical fish source and unknown composition of test beakers and statistical method used for calculating the LC_{50}.

G.2.2.5 Lepomis macrochirus

3MCompany (2000) evaluated the acute effects of perfluorooctane sulfonate, DEA salt (CAS # 70225-14-8), also known as PFOS DEA salt FC-99, or 3M Sample No. 2, on bluegill sunfish (*Lepomis macrochirus*) via a 4-day static, unmeasured test. Bluegill (average length 28.6 mm, average weight 0.60 g) were sourced from Osage Catfisheries, Inc. in Osage Beach, MO. Fish were exposed in 40 L glass aquaria containing 30 L of test solution at a loading rate of 0.2 g fish/L. Dilution water was laboratory well water with hardness 255 mg/L as $CaCO_3$, alkalinity 368 mg/L as $CaCO_3$, pH 7.8 and conductivity 50 µmhos/cm. A primary stock solution prepared in deionized water at a concentration of 150 mg/L was diluted with dilution water to achieve six nominal concentrations (18, 37, 75, 160, 320, and 650 mg/L PFOA) plus a negative control. The exposure consisted of a single replicate with 10 fish for each treatment. Dissolved oxygen measured in the control and lowest treatment (18 mg/L) remained above 5.8 mg/L during the exposure, and pH ranged from 8.2 to 8.3. The author-reported 96-h LC50 was 31 mg/L but due to there being just a single replicate of ten fish in each treatment, the acute value is only being considered qualitatively.

G.2.3 Amphibians

G.2.3.1 Xenopus laevis

San-Segundo et al. (2016) evaluated the acute toxicity of PFOS (potassium salt, CAS # 2795-3-3, > 99% purity) to *Xenopus laevis* embryos via 96-hour renewal measured exposures using the FETAX assay. PFOS was dissolved in boiling distilled water to obtain a stock solution of 300 mg/L, and testing solutions were prepared in FETAX solution. *X. laevis* embryos were obtained from the broodstock at the National Institute for Agricultural and Food Research and Technology in Madrid, Spain. Frog housing and husbandry procedures were carried out as described by Martini et al. (2010). *X. laevis* mating and breeding were induced by intra-

lymphatic injections of human chorionic gonadotropin (hCG). Adults were subsequently transferred together to a spawning tank that was half filled with FETAX solution. Eggs were collected the next day and dejellied in a 2% w/v L-cysteine solution, adjusted to pH 8.1. Normally developing embryos were selected under a dissecting microscope in NF stage 10 (early gastrula). Embryos were exposed in water to nominal solutions of PFOS (0, 0.5, 6, 12, 24, 48 and 96 mg/L) at 24±1°C. Thirty total embryos for (ten per each of replicates) each treatment group were distributed into 90-mm polystyrene Petri dishes, each containing 25 mL of test solution. The experiment was conducted in triplicate and control embryos were maintained in FETAX solution. Embryo mortality and morphological abnormalities were recorded at 24, 48, 72 and 96 hours of exposure with the dissecting microscope. Coagulation of the embryo or lack of embryonic heartbeat was taken as criteria to define death and to estimate mortality. Embryonic deformities were also identified using the morphological descriptions provided by Nieuwkoop and Faber (1994), and Bantle et al. (1998). At the end of the assay (96 hours), surviving embryos were anesthetized and were subsequently photographed to measure their total length. The study author-reported 96-hour survival LOEC was > 96 mg/L PFOS. The study author-reported value was used qualitatively to derive the draft acute water column criterion as it was a greater than (>) high toxicity value compared to the other acute toxicity data for PFOS. This LOEC of > 96 mg/L was substantially higher (43.2 times) than the FAV of 6.011 mg/L and indicated that this genus may be less sensitive to acute exposures of PFOS compared to the results reported in the quantitative study by Palmer and Krueger (2001) with LC_{50} values of 15.53, 18.04, and 14.60 mg/L. Therefore, the acute freshwater criterion of 3.0 mg/L will likely be protective of this genus. Moreover, it is unlikely that these toxicity values would substantially change the FAV, as

this genus would be ranked as the tenth most sensitive genus in the acute PFOS dataset and another genus (*Lampsilis*) with a similar GMAV of 16.5 mg/L would be ranked fifth.

G.2.3.2 Bufo gargarizans

Yang et al. (2014) evaluated the acute toxicity of PFOS (potassium salt, CAS # 2795-3-3, 99% purity) to the Asiatic toad, *Bufo gargarizans* via 96-hour renewal measured exposures (the authors note that the experiments followed ASTM standards and U.S. EPA procedures for deriving water quality criteria). The tadpoles (0.048 g, 1.8 cm) were purchased from the Beijing Olympic Park, which was considered an atypical source. The organisms were allowed to acclimate for seven days before testing, and the test was conducted at 22±2°C with a light:dark cycle of 12-hr:12-hr, with 10 toads per replicate and three replicates per concentration. Beakers used for exposure were assumed glass, but glass was not specified by study authors. PFOS was dissolved in deionized water and carrier solvent DMSO to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 20, 30, 45, 67.5, 101.25 and 151.88 mg/L PFOS. Water quality parameters reported were pH=7.0 ± 0.5, dissolved oxygen=7.0 ± 0.5 mg/L, total organic carbon=0.02 mg/L and hardness=190.0 ± 0.1 mg/L as $CaCO_3$. The supplemental data provided for the study included a comparison of measured PFOS concentrations before and after solution renewal in the low and high acute and chronic test concentrations. PFOS concentrations in the test water did not fluctuate by more than 15% during experiments. The 96-hour LC_{50} reported for the study of 48.21 mg/L PFOS was deemed qualitative due to the atypical test organism source and unknown composition of test beakers and statistical method used for calculating the LC_{50}.

G.3 Summary of Chronic PFOS Toxicity Studies Used Qualitatively in the Freshwater Aquatic Life Criterion Derivation

G.3.1 Freshwater Invertebrates

G.3.1.1 Worms (flat and annelids)

Yuan et al. (2014) conducted a 10-day renewal, unmeasured chronic test on PFOS (potassium salt, >99% purity) with the planarian, *Dugesia japonica* (a non-North American species). The test organisms 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 and 50 mL fill volume. The test employed three replicates of 10 planarians each in five test concentrations: 0 (solvent control), 0.5, 1, 5, 8 and 10 mg/L PFOS. The test temperature was reported as 20°C. No other water quality parameters were reported as having been measured in test solutions. Survival of solvent control animals was not reported. The 10-day LOEC based on regeneration and decreased appearance of auricles was 0.5 mg/L (NOEC and MATC, <0.5 mg/L). The chronic value was acceptable for qualitative use because of the short test duration.

G.3.1.2 Mollusks

Hazelton et al. (2012) and Hazelton (2013) conducted a test of the effects of PFOS (acid form, >98% purity) on glochidia of *Lampsilis siliquoidea*. The test exposed brooding glochidia (in marsupia) for 36 d. Brooding female fatmucket were collected from Perche Creek, MO. Dilution water was dechlorinated tap water. Mean 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, dissolved oxygen 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. Experiments were conducted in 3.8 L glass jars of unspecified fill volume. The test employed a single replicate of four brooding females each in two measured test concentrations plus a negative control. Nominal concentrations 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). Exposures were maintained at a target temperature of 15°C and varied from 14.6 to 16.1°C during the exposure. Mussels in the negative control was >90%. The test resulted in an LOEC of 0.00452 mg/L based on reduced viability of free glochidia (NOEC and MATC <0.00452 mg/L). The chronic value was acceptable for qualitative use because there were only two test concentrations.

Olson (2017) conducted a series of chronic tests on the effects of PFOS (potassium salt, CAS # 2795-39-3, 95% purity) with juvenile, pre-adult, and adult *Lymnaea stagnalis* as part of a Ph.D. thesis at the Texas Tech University, Lubbock, TX. Chronic toxicity of PFOS to *L. stagnalis* was observed under renewal (every 3.5 days) conditions over a 21-day exposure

period. The test followed methodology established in Ducrot et al. (2010). Experimental animals were divided into four pre-adult groups of ages equal to the length of the experiment (i.e., 0-3 weeks, 3-6 weeks, 6-9 weeks, 9-12 weeks). The test series also included at 21-day test with adult *L. stagnalis*. Dilution water was reconstituted laboratory (13.38 g $CaSO_4$, 5.6 g $MgSO_4$, 0.25 g KCl, and 2.95 g $NaHCO_3$ added to 50 L deionized water). Photoperiod and light intensity were not reported. Stock solutions were prepared by dissolving between 0.2000 and 0.2010 g of the chemical powder in 1 L of lab water and placing the solutions in HDPE bottles on a shaker overnight. Stock solutions were then diluted as necessary to obtain the final exposure concentrations. Exposure vessels were 1 L polyethylene beakers containing 1 L of test solution. Number of replicates and number of snails in each replicate were not reported. Snails of various age classes were exposed to each of six test concentrations (measured in low and high treatments) plus a negative control. Nominal concentrations in the test were 0 (negative control), 1.5, 3, 6, 12.5, 25 and 50 mg/L. Exposure concentrations were reportedly measured initially and after three days for verification, but concentrations were not reported. Analyses of test solutions were performed using HPLC/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 20°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 ≥90%. The 21-day effect concentrations were as follows: 0-3 week old juveniles – the NOEC for survival, feeding rate, mass change, length change, and carbohydrate concentration was 50 mg/L (LOEC and MATC >50 mg/L); 3-6 week old juveniles – the MATC for mass and length change was 35.35 mg/L (NOEC and LOEC, 25 and 50 mg/L respectively); 6-9 and 9-12 week old juveniles – the NOEC for survival, feeding rate, mass change, length

change, and carbohydrate concentration was 50 mg/L (LOEC and MATC >50 mg/L); adult – the MATC for survival was 4.243 mg/L (NOEC and LOEC, 3.0 and 6.0 mg/L, respectively). Through this study design, all life stages were included in a 21-d (short-term) chronic toxicity test, but not as a continuation so that the same organisms were exposed to PFOS across all life stages as in a full-life cycle, or for a duration of continuous chronic exposure required to satisfy EPA requirements for an acceptable partial or early-life stage test. Thus, the chronic values from this study were acceptable for qualitative use. The qualitative values confirm the relative insensitivity of this and other snail species (i.e., *Physella heterostropha pomilia*; SMCV = 8.831) to PFOS in the chronic criteria dataset.

G.3.1.3 Zooplankton (rotifers and planktonic crustaceans)

Zhang et al. (2014) reported the results of a chronic life-cycle test of PFOS (potassium salt, CAS # 2795-39-3, ≥98% purity) with *Brachionus calyciflorus*. The full life-cycle test used renewal conditions for approximately four days. *B. calyciflorus* used for the test 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-hr: 8-hr, light:dark; 3000 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 diluted with dilution water to prepare the test concentrations. Exposure vessels and size were not reported for the four-day reproductive assay, but were likely in 6-well cell culture plates (assumed plastic) each containing at total of 10 mL of test solution.

The test employed eight test concentrations plus a negative control. Each treatment consisted of six replicates of 10 rotifers each in individual cells. Nominal concentrations were 0 (negative control), 0.125, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, and 16.0 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. Negative control survival is not provided for the life-cycle test. The *B. calyciflorus* 4-day NOEC (intrinsic rate of population increase (R) and resting egg production) was 0.125 mg/L. The 4-day LOEC was 0.25 mg/L. The calculated MATC was 0.1768 mg/L. The results from this study were acceptable for qualitative use because of the atypical concentration-response pattern.

3MCompany (2000) provides the results of a 28-daychronic toxicity test completed in 1984 with the cladoceran, *Daphnia magna*, and PFOS-K (perfluorooctancesulfonate potassium salt, CAS # 2795-39-3, unknown purity). The chronic test followed proposed standard practice for life cycle tests with *Daphnia magna* and OECD (1981). In-house culture of daphnids were tested in unchlorinated, carbon filtered well water under a 16:8 hr light:dark photoperiod, pH 7.6, total hardness of 256 mg/L as $CaCO_3$, dissolved oxygen > 70% and average temperature of 22 °C. A stock solution of PFOS was made in deionized water and diluted with test water to achieve five nominal test concentrations (0.26, 1.0, 26., 7.0 and 18.0 mg/L). The chronic test was split into two components based on the test endpoint. For mortality, five daphnids (12 ± 12 hour) were added to 250 mL beakers with 200 mL of test solution and three replicates for each test treatment. For reproductive endpoints, one daphnid was added to each beaker with seven replicates for each test treatment. The specific number of replicates and organisms per replicates

for the control was not well defined by the author, other than noting that 20 beakers in total were used for the controls. However, the specific number of organisms and replicates is further complicated by conflicting values for the number of replicates reported in the results section of the publication which do not match the stated text. Over the 28-day exposure period, test solutions were renewed and daphnids were fed three times per week. Authors reported that reproduction was a more sensitive endpoint when compared to the control with an 18% reduction in the cumulative number of live young per adult, a 55% reduction in average number of live young per brood and a 62% reduction in number of broods per adult at the highest test concentration (18.0 mg/L). The author reported 28-day NOEC and LOEC, based on all three reproductive endpoints, was 7.0 and 18.0 mg/L PFOS, respectively, with a MATC of 11.22 mg/L. EPA was unable to independently calculate an EC_{10} value based on the level data provided in the paper by the study authors. Additionally, the study authors noted that additional PFOS studies are needed on this species to understand the effects at additional concentrations. Thus, EPA assumed that the more recent tests resulted in better understanding of the effects of PFOS for this species. Therefore, the author-reported MATC of 11.22 mg/L PFOS was used qualitatively to derive the draft chronic freshwater criterion for PFOS.

Jeong et al. (2016) also conducted a chronic life-cycle 25-day renewal, unmeasured test of PFOS (potassium salt, purity 99%) with *Daphnia magna*. The multi-generational exposure study was conducted in two sets with five continuous exposure generations and three discontinuous exposure generations. The initial generation (F0) was shared for continuous and discontinuous exposure sets. In the continuous exposure set, the exposure conditions were not changed during five generations (F0–F4). *D. magna* used for the test were originally obtained from the Korea Institute of Toxicology and cultured in the laboratory according to EPA-821-R-

02-012 (USEPA 2002; an acute toxicity testing protocol). Daphnids were less than 24 hours old at test initiation. Dilution water was the same used for daphnid culture and was USEPA (2002) hard reconstituted water with a hardness of 170 ± 5 mg/L as $CaCO_3$, alkalinity of 110 ± 5 mg/L as $CaCO_3$, and pH 7.8. Photoperiod was 16-hr:8-hr (light:dark) at an unreported light intensity. A primary stock solution of PFOS was said to be prepared in methanol (0.1% maximum level) at 10,000 mg/L, which seemed high. The primary stock was proportionally diluted with dilution water to achieve the test concentrations. Exposure vessels were 30 mL polypropylene beakers containing 20 mL of test solution. The test employed 20 replicates of one daphnid each in five nominal test concentrations plus a solvent control. Nominal concentrations were 0 (solvent control), 0.0001, 0.010, 0.100, 1.000, and 10.00 mg/L. The reported test temperature was 20°C. No other water quality parameters were reported as having been measured in test solutions. Solvent control survival was not reported. The most sensitive apical endpoint was reproduction (number of offspring), but it was not observed in all generations. In the first generation, the offspring number significantly decreased at concentrations above 0.100 mg/L. As the generation number increased, the degree of the adverse effect tended to diminish, implying adaptation. The impairment of offspring reproduction was greatest during re-exposure among all of the exposed generations. The *D. magna* 25-day NOEC (reproduction – F0 generation) was 0.010 mg/L. The 25-day LOEC was 0.100 mg/L. The calculated MATC was 0.03162 mg/L, and independently-calculated EC_{10} was 0.0041 mg/L. Independent statistical analyses were conducted using data that were estimated (using Web plot digitizer) from the figures presented in the paper. However, this independently-calculated toxicity was not considered reliable as this EC_{10} was much lower than the author reported NOEC and any other reproductive toxicity value for this species (ranging between 0.001712 and 16.35 mg/L; see C.2). In addition to not being able to calculate

an EC_{10} for this study, the test concentrations were widely spaced (with each treatment group increasing by one order of magnitude and ranging between 0.0001 and 10 mg/L), the data presented in the paper was control normalized, and there was no consistent concentration-response relationship. Therefore, the study author reported value was used qualitatively to derive the draft chronic water column criterion. The toxicity value was within a factor of two of the FCV of 0.008398 mg/L.

G.3.1.4 Benthic Crustaceans

G.3.1.5 Aquatic Insects

Olson (2017) conducted a chronic, approximately 42-day renewal test of PFOS with first instar of mosquito *Aedes aegypti* as part of a Ph.D. thesis at the Texas Tech University, Lubbock, TX. The colony were donated by Texas A&M and had been maintained in the laboratory at Texas Tech University since summer 2013. Dilution water was moderately hard reconstituted water prepared according to USEPA (2002; 3 g $CaSO_4$, 3 g $MgSO_4$, 0.2 g KCl, and 4.8 g $NaHCO_3$ added to 50 L deionized water). Photoperiod was 12-14 hours light and 10-12 hours dark. Light intensity was not reported. Stock solutions were prepared by dissolving soluble amounts of powdered chemical in dilution water. Diluted stock concentrations were equal to the maximum test concentration. The stock was mixed on a shaker table at 125 rpm for at least 18 hours before being added to exposure containers and proportionally diluted. Exposure vessels were 50 mL HDPE plastic beakers containing an unspecified amount of test solution. The test employed 10 mosquito larvae each in six test concentrations plus a negative control. The number of replicates was not reported. Nominal concentrations in the test were 0 (negative control), 0.050, 0.125, 0.250, 0.500, 1.000, and 2.000 mg/L. Experiments were conducted in incubators set to 25°C and covered with plexiglass to limit evaporation. No other water quality parameters were reported as having been measured in test solutions. Negative control survival was ≥ 95%.

The *A. aegypti* 42-day NOEC (average time to emergence) was 0.05 mg/L. The 42-day LOEC was 0.125 mg/L. The calculated MATC was 0.079 mg/L, and an EC_{10} could not be independently-calculated. The results from this study were considered acceptable for qualitative use to derive the freshwater acute criteria. Additional data had been requested from the study authors to help with independent verification of the toxicity values.

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-hr:8-hr light: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 non-apical endpoints.

Stefani et al. (2014) conducted a chronic (10 generation) test of PFOS (form and purity not reported) with midge, *Chironomus riparius*. The 10 generations (each approximately 20 to 28 days) were tested under static conditions. The 10 generations (each approximately 20 to 28

days) were tested under static conditions. The test followed OECD (2004a); OECD (2004b); OECD (2010), and a specific protocol for multigenerational assays using *C. riparius* developed and published by Nowak et al. (2008); Nowak et al. (2006); Nowak et al. (2007a); Nowak et al. (2007b); Nowak et al. (2009) and Vogt et al. (2007a); Vogt et al. (2010); Vogt et al. (2007b). The specific protocol for multigenerational assays was designed to highlight neutral evolutionary responses caused by exposure to contaminants. A native population collected in the Lambro River (Milan, Lombardy, Italy) was used as a starting population for the test. *C. riparius* used to initiate the test were L1 (first instar) larvae. Dilution water was reconstituted water according to U.S. EPA (2000b) - hardness not specified, pH 7.8-8.2. Photoperiod was 16-hr:8-hr, light:dark with an intensity 500 - 1,000 lux. Treatments with two replicates each (i.e., two cages with five vessels each per treatment and 60 larvae per vessel, or approximately 300 larvae per treatment) were tested, by spiking 15 L of test water with 150 µL of methanolic solution at 1 g/L of PFOS to achieve a nominal concentration of 0.01 mg/L. Exposure vessels were glass tanks (19 cm x 19 cm x 18 cm) containing and unspecified amount of test solution and 1 cm thick layer of formulated sediment (75% of the volume constituted by 250-300 µm grain size aquarium quartz sand and 25% of the volume by 63-250 µm grain size natural sediment collected in an unimpacted river, sieved and sterilized). The measured exposure concentration diminished significantly over the course of the exposure (mean concentration at beginning of experiment: 0.0089±0.21 mg/L and concentration at end of experiment: 0.0016±0.2 mg/L), meaning later generations were exposed to less PFOS than earlier generations. The reported time-weighted measured concentration was 0.0035 mg/L. Mass-balance evaluations at the end of a generation showed that most of the PFOS was detected in the sediment (36% of the added amount). Since 45% of the PFOS added to the test vessel was lost (the authors hypothesize because of air

stripping), only roughly 17% of the PFOS added to the test vessel ended up in the water. Test temperature was controlled at 20 ± 1°C, and dissolved oxygen remained above 66% saturation. No other water quality parameters were reported as having been measured in the test solutions. Controls were considered acceptable because they fulfilled the validity criteria for mortality according to OECD guideline 218 (OECD 2004a). In the control group, most vessels in all generations reached the emergence of at least 70% individuals. The NOEC and LOEC based on reproduction and emergence 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 are acceptable for qualitative use because of the use of only a single test concentration, the lack of observed effects in the one exposure concentration that was considered a greater than low value, and lack of details pertaining to the characteristics of the sediment used in the exposure, particularly considered the difference between measured concentrations over the exposure duration. This particular study provided few details pertaining to the effects of chronic PFOS exposure on midge. Therefore, it was determined that the EC_{10} of 0.05896 mg/L from MacDonald et al. (2004) that was used quantitatively in the chronic criterion derivation was more robust than the toxicity values reported in Stefani et al. (2014) and the chronic freshwater criterion of 0.0084 mg/L would likely be protective of this genus.

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 midge, *Chironomus riparius*. The test was done under static conditions for 10 generations, each approximately 36 days (or 1/10 of this year-long, 10 generation test). The test followed OECD 218 and 233 (OECD 2004a, 2010), with slight variations. *C. riparius* used for testing were from in-house cultures originating from a native population collected in the Lambro River (Milan, Lombardy,

Italy). *C. riparius* used to initiate the test were first instar larvae. Dilution water was reconstituted water according to U.S. EPA/600/R-711 99/064 (U.S. EPA 2000b) - hardness not specified, pH 7.8-8.2. Photoperiod was 16-hr:8-hr (light:dark). Light intensity was not reported. A single treatment of 0.01 mg/L (nominal) and solvent control with 10 replicates of 60 larvae each were tested. PFOS was dissolved in pure methanol (> 99%) in order to achieve stock solutions at 1 g/L of PFOS. Each stock solution was then diluted in reconstituted water in order to achieve the nominal concentration of 0.01 mg/L. Exposure vessels were glass tanks (19 cm x 19 cm x 18 cm) containing 1 L of test solution and 1 cm of formulated sediment (75% of the volume aquarium quartz sand and 25% of sterilized natural sediment). The measured exposure concentration diminished significantly over the course of the exposure, meaning later generations were exposed to less PFOS than earlier generations. The reported time-weighted measured concentration was 0.004 mg/L. PFOS was observed to sorb sediment. To check the potential presence of PFAS in the fish food used in the test, PFOS concentrations were determined in the larvae of the control after 30 days exposure. Measured concentrations of PFOS in larvae tissue were always below detection limits (2 ng/g wet weight). Water temperature, dissolved oxygen and pH were measured every three to five days in two to three replicates per treatment. Test temperature was controlled at 20.1 ± 0.7°C, and dissolved oxygen remained equal to or above 66% saturation. pH stayed within the range of 7.8-8.2. Each generation test was considered valid if emergence in the control was ≥ 0% in at least six replicates (i.e., vessels) of the 10 included. Emergence in the control groups by generation was as follows: 88 (primary emphasis for criteria development), 71, 53, 61.6, 78.6, 91.9, 62, 53.5, 79.1, 75.5. Thus, generations 1, 2, 5, 6, 9, and 10 met control survival acceptability. 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 acceptable for qualitative use because of the use of only a single test concentration, the lack of consistent observed effects in both the control and the treatment groups across the generations, the LOEC was considered a less than low value that is not very robust since there was only one treatment concentration, and lack of details pertaining to the characteristics of the sediment used in the exposure, particularly considered the difference between measured concentrations over the exposure duration. This particular study provided few details pertaining to the effects of chronic PFOS exposure on midge. Therefore, it was determined that the EC_{10} of 0.05896 mg/L from MacDonald et al. (2004) that was used quantitatively in the chronic criterion derivation was more robust than the toxicity values reported in Marziali et al. (2019) and the chronic freshwater criterion of 0.0084 mg/L would likely be protective of this genus.

G.3.1.6 Microcosm Data Summaries

Mixed species exposures are typically not used quantitatively in EPA aquatic life criteria documents, but two well-designed microcosm experiments provided results that are supportive of the preliminary SMCVs for *Moina macrocopa* and *Daphnia magna* representative of other cladocerans and copepods.

Sanderson et al. (2002) evaluated the chronic effects of PFOS on the invertebrate community in a 35-day indoor microcosm study. Indoor 30 L transparent polyvinyl chloride aquariums were filled with sediment and water from natural ponds at the University of Roskilde, Copenhagen, Denmark. Microcosms were allowed to stabilize for four weeks before PFOS-K (potassium salt, CAS # 2795-39-3, purity unreported) additions were made. Each measured treatment (nominal: 0, 1, 10 or 30 mg/L) had five replicate aquariums. Measured concentrations remained relatively stable over the exposure period; the 1, 10 and 30 mg/L nominal concentrations were 1.33, 12.3 and 33.9 mg/L at test initiation, but decreased slightly to 1.08,

11.7 and 29.8 mg/L at day 35. PFOS was added only once to each aquarium as a sub-surface injection. Test endpoints focused on the zooplankton community (overall abundance and number of taxa) and abundance of individual species (*Cyclops diaptomus*, *Daphnia magna*, *Cyclops canthocamptus staphylinus*, and total *Rotifer sp*.). Aquariums were under constant aeration and maintained an oxygen concentration of 6 mg/L over the entire study. Microcosms had a 12-hour light period (2,852 lumens), and were maintained at 18°C. The pH averaged 8.3 over the exposure period (range 8.28-8.37). Zooplankton community abundance and structure was affected at 10 and 30 mg/L. Effects on rotifers were variable (i.e., some species decreased and others increased). The authors noted that due to variability between replicates, it was not possible to determine with statistical confidence whether or not treatment related effects were present at 1 mg/L. The most sensitive species was the copepod, *C. diaptomus*, which was eliminated in the 10 and 30 mg/L by day 14. The MATC for the study, 3.162 mg/L, based on zooplankton abundance was acceptable for qualitative use because the value was from a non-definitive microcosm test.

Boudreau et al. (2003b) examined the ecotoxicological impact associated with PFOS exposure across multiple levels of biological organization using 35- to 42-day exposures in outdoor microcosms. The studies were conducted as part of a Master's thesis at the University of Guelph, Ontario, Canada (**Boudreau et al. 2002**) and later published in the open literature. Four nominal concentrations of PFOS (potassium salt, purity of 86%) were tested: 0.3, 3, 10, or 30 mg/L. The persistence of PFOS in the outdoor microcosms was evaluated over 285 days. The measured concentrations, as calculated by the time-weighted average (TWA) over 285 days, changed by ≤6.0% from nominal concentrations (the TWA for the nominal 3 mg/L exposure was 2.8 mg/L, for the nominal 10 mg/L exposure, 9.8 mg/L, and for 30 mg/L, 30.1 mg/L). For this

reason, nominal values were used in all analyses. Fifteen 12,000 L microcosms were used for this analysis, with three replicates for each treatment and controls. Water for the microcosms was provided from a spring-fed irrigation pond and was circulated among microcosms for 10 days prior to study initiation at a rate equivalent to 11,000 L per day. This circulation period enabled establishment and equilibrium of the indigenous animals and plants in the microcosms. The microcosms were open to allow for aerial colonization of flying insects and the lining was allowed to accumulate periphyton and algae. This design feature increased variability in the organisms and invertebrate and primary producer community. One day prior to treatment, circulation from the irrigation pond was terminated producing isolated systems and water and zooplankton samples were collected as a pre-treatment reference. Water quality varied little across all treatments: temperature varied from 15.9-20.5°C, D.O. from 7.2-8.8 mg/L, pH from 8.3-8.6, and hardness from 294-300 mg/L as $CaCO_3$. Zooplankton populations were significantly affected at 10 and 30 mg/L, and a community-level no observed effect concentration ($NOEC_{community}$) of 3.0 mg/L was determined over 35 days. The most sensitive taxonomic groups, Cladocera and Copepoda, were virtually eliminated at 30 mg/L by seven days. The zooplankton communities from both 0.3 and 3 mg/L treatments showed little change from the control over time. Based on these results, the MATC for zooplankton community abundance was 5.478 mg/L and was acceptable for qualitative use because the value was from a non-definitive microcosm test.

G.3.2 Freshwater Fish

G.3.2.1 Anguilla anguilla

Roland et al. (2014) evaluated the chronic effects of the potassium salt of perfluorooctane sulfonate (PFOS, CAS # 2795-39-3, 98% purity) to juveniles of the European eel, *Anguilla anguilla*. A set of 162 juvenile female eels (138.3 g) were purchased from a Dutch

eel farm (Zon-Aquafarming, Helmond, The Netherlands) and were randomly distributed at a fixed number into 18 tanks filled with 70 L tap water. Fish were acclimated to laboratory conditions in aerated water at 20±2°C under a 12-hr:12 hr light:dark photoperiod for two months before the experiment. Eels were exposed to nominal PFOS concentrations of 0, 0.001 and 0.010 mg/L (mean measured concentrations of 0.00001, 0.00081, 0.011 mg/L, respectively) at 20°C and 12-hr:12-hr light:dark photoperiod during 28 days while the control fish were kept in clean water. Each treatment included six replicate tanks with nine fish per tank. One third of the water was renewed every 72 hours. Water samples were taken at days 0, 7, 14 and 28 after the beginning of the exposure for PFOS measurements (absolute PFOS recoveries in the concentration range of 0.00001 and 0.0001 mg/L were in the range of 50-90%). Animals were fed daily during exposure and no mortality was recorded during the experiment. The 28-day survival NOEC and LOEC were 0.011 and >0.011 mg/L PFOS, respectively, but were considered qualitative data since the test was not an acceptable early life-stage (ELS) test (used juveniles not embryos) and *A. anguilla* is not a North American species. In addition, the proteomic change NOEC and LOEC were <0.00081 and 0.00081 mg/L PFOS, respectively, also qualitative (non-apical endpoint).

G.3.2.2 Oncorhynchus mykiss

Benninghoff et al. (2011) evaluated the chronic effects of perfluorooctane sulfonic acid potassium salt (PFOS-K, CAS # 2795-39-3, purchased from Sigma Aldrich in St. Louis, MO) on rainbow trout (*Oncorhynchus mykiss*) juveniles in a 15-day static, unmeasured study. Mount Shasta strain rainbow trout were hatched and reared in the Sinnhuber Aquatic Research Laboratory at Oregon State University in Corvallis, OR. Fish were maintained at 12°C and a 12-hr:12-hr light:dark cycle in a 375 L flow-through tank filled with carbon filtered water. Two weeks before testing, fish were fed a semipurified casein-based diet with menhaden oil at a rate

of 2% body weight. A stock solution was prepared by dissolving PFOS in dimethyl sulfoxide (DMSO) that was then added to oil in the fish diet. Eleven-month-old fish weighing approximately 70 g were placed in treatment groups, six fish per group, and were fed either 0.1, 1 or 5 mg/kg body weight/day PFOS laced food five times per week for 15 days: correlating to a diet concentration of 5, 50 or 250 ppm PFOS per day. Four replicates were included for each dietary treatment, along with a negative control group, and a vehicle control group (treated dietarily with 0.5 ppm DMSO). A positive control group (treated dietarily with 5 ppm estradiol) was also included with an additional twelve fish. Fish were sacrificed and weighed on day 15. The authors reported a NOEC of 250 ppm PFOS test diet for growth (weight). The lack of description of the dilution water and the test methodology (dietary exposure) makes the study acceptable for qualitative use only.

Benninghoff et al. (2012) also evaluated the chronic effects of perfluorooctane sulfonic acid potassium salt (PFOS-K, purchased from Fluka Chemical Corp in St. Louis, MO) on rainbow trout (*Oncorhynchus mykiss*) in an 8-month unmeasured study. Mount Shasta strain rainbow trout were hatched and reared in the Sinnhuber Aquatic Research Laboratory at Oregon State University in Corvallis, OR. Fish were raised in a 375 L tank filled with carbon filtered tap water and maintained at 12°C and a 12-hr:12-hr light:dark cycle. Fry (15 week post spawn) were exposed to a cancer causing agent for 30 minutes, then fed a semi purified casein-based diet for one month. Fish were fed experimental diets containing 100 ppm PFOS (approximately 25 mg/kg body weight/day) five days per week for a period of six months. Fish were sacrificed at test termination (12.5 months post spawn) and examined for tumor presence. Both survival and tumor incidence LOEC were both observed in the 100 ppm PFOS test diet (2.5 mg PFOS/kg

body weight/day). The lack of description of the dilution water, mixture based exposure, and the test methodology (dietary exposure) makes the study acceptable for qualitative use only.

G.3.2.3 Cyprinus carpio

Hagenaars et al. (2008) evaluated the effects of PFOS (98% purity) to gene expression, condition factors and energy storage endpoints exhibited by *Cyprinus carpio* exposed for 14 days under renewal unmeasured conditions. Juvenile carp (3.72 g and 5.18 cm) were acclimatized for three weeks in plastic 20 L aquaria prior to treatment. The water used during acclimatization and treatment was filtered and aerated (source not provided). Every 48 hours, the water was totally renewed with nominal PFOS concentrations. The fish were exposed to a 14-hr:10-hr light:dark cycle and fed 2% of body weight. After acclimatization, fish were exposed to PFOS at nominal concentrations of 0.1, 0.5 and 1 mg/L during two weeks while the control carp were kept in clean water during exposure. For each exposure concentration as well as for the controls, three aquaria were used resulting in three full biological replicates for each exposure condition. Twelve carp were housed in each aquarium. Relative condition factor (RCF) was determined in all fish at day 0, 7 and 14. After 14 days, fish were sacrificed by decapitation. The liver was immediately removed, weighed for hepatosomatic index (HSI) calculation, frozen in liquid nitrogen, and stored at -80°C. From each aquarium, six livers were randomly selected for use in the microarray analysis, four to determine the energy reserves and two to measure PFOS concentrations. The two most sensitive endpoints were reduced hepatosomatic index (HSI) and liver glycogen, with 14-day chronic values (MATCs) of 0.2236 and 0.7071 mg/L PFOS, respectively. This study was classified as qualitative due to non-apical endpoints, nominal water concentrations and lack of exposure detail.

G.3.2.4 Danio rerio

A sub-chronic static unmeasured test was utilized by **Ulhaq et al. (2013)** to determine the toxicity of PFOS to *Danio rerio*. PFOS stock solutions were freshly prepared in reconstituted water in concentrations below the limit for water solubility. Adult zebrafish (AB strain) were held in charcoal-filtered tap water. Breeding groups including three males and two females were placed in 10 L glass aquaria equipped with spawning nets separating the parental fish from the eggs. Half an hour after onset of lights the eggs were collected, rinsed for removal of debris, and then only normally developed fertilized eggs at least in the four-cell stage were selected using a stereomicroscope. The zebrafish eggs, within 15 minutes after collection, were exposed to a series of concentrations of the test substance dissolved in reconstituted water (exposure medium). Fertilized eggs (4-cell stage) were randomly distributed individually into flat bottom, 48-well polystyrene plates along with 750 µL of the exposure medium. PFOS was tested at six consecutive concentrations differing by a factor of 3.3 based on logarithmic scale fitting. For each test, four 48-well plates were used, with a total of 24 embryos per concentration as well as 24 in the water control group. Each treatment group was equally distributed to each of the four well plates (i.e., six embryos/concentration/plate, for a total of 168 embryos). The plates were covered with parafilm and the embryos were exposed to the chemical until 144 hours post fertilization (hpf). Exposure conditions throughout the study were kept at pH 7.2-7.6, a water temperature of 26±1°C and a light cycle of 14 hours. Observations of mortality and sublethal endpoints were made after 24, 48, 120 and 144 hpf using a stereomicroscope. Sublethal endpoints such as presence of edema, malformations, not-hatched eggs, lack of circulation and reduced pigmentation were also observed. Heart rate was recorded at 48 hpf and hatching time was determined using time-lapse photography. The 144-hour LC_{50} was >10 mg/L PFOS and the

EC_{50} (lethal and sublethal effects) was 1.5 mg/L PFOS (both were qualitative data because of test duration and unmeasured static test).

The effects of PFOS on pancreatic organogenesis in *Danio rerio* 3 hpf embryos (wild type) were evaluated by **Sant et al. (2017)**. PFOS stock solutions (160-640 mM) for embryo exposures were prepared by dissolving PFOS into DMSO and stored at room temperature in glass bottles inside of light-prohibitive containers until use. Transgenic zebrafish of the Tg(ins:GFP) and Tg(ptf1a:GFP) strains were each obtained as a heterozygous population from the University of Massachusetts Medical School and bred in house to homozygosity. The Tg(ins-GFP) strain expresses green fluorescence in the insulin-producing beta cells, allowing for visualization of pancreatic islets. The Tg(ptf1a:GFP) strain expresses green fluorescence in the exocrine pancreas tissues, and also in the retina and parts of the brain. Adult fish were housed in an Aquaneering zebrafish system maintained at 28.5°C and a 14-hr:10-hr light:dark cycle. Breeding populations were housed in tanks containing roughly 15 males and 30 females. Embryos were collected from breeding tanks 0-1 hour post fertilization (hpf), washed, and housed with no more than 25 other embryos in glass 100 mm petri dishes containing 0.3x Danieau's medium (pH 7.6) throughout the experiments. At 3 hpf, embryos staged at the midblastula transition were exposed to PFOS solutions with a total of 0.01% DMSO v/v in a total of 20 ml of 0.3x Danieau's medium. Final concentrations of PFOS were 0 (DMSO control), 16, 32, or 64 µM (or 8.002, 16.00, and 32.01 mg/L), and were refreshed daily to mimic subchronic developmental exposures. All embryos were manually dechorionated using watchmaker's forceps at 24 hpf and debris removed from dishes prior to refreshing exposures. Experiments were replicated 3-4 times on groups of 8-12 embryos per concentration. The authors

reported a 7-day chronic value (islet morphological anomalies) of 11.31 mg/L PFOS. The chronic value was classified as qualitative due to duration and an unmeasured chronic exposure.

Du et al. (2009) investigated the effect of PFOS (> 99% purity) on the survival, growth and hepatotoxicity of *Danio rerio* female fry exposed via renewal unmeasured conditions for 70 days. The PFOS stock solution (50,000 mg/L) was prepared in HPLC-grade DMSO and stored at 4°C. Adult zebrafish (AB strain) were maintained in charcoal-filtered, recirculating, aerated tap water with a 12-hr:12-hr light:dark cycle and a temperature of 27±0.5°C. Fertilized eggs were collected and the fry were maintained until 14 days post-fertilization (dpf) for subsequent experiments. Zebrafish fry were randomly distributed into 20 L glass tanks for control and exposure groups. There were three replicates for each group, with each group containing about 50 fry in each tank. The fry were exposed in a renewal system and the water was half-renewed every other day. Both the control and exposure groups received DMSO (0.002%, v/v), with nominal PFOS concentrations of 0.01, 0.05 and 0.25 mg/L. The exposure regime included a PFOS exposure period (70 d) and recovery in clean water (30 d). Fish were sampled after 40 and 70 days of exposure to determine lengths and weights, histological examination of the testis and liver, liver VTG gene expression and whole body T3 measurement. The gonad weights of the females were recorded after 70 days of exposure and after 30 days of recovery. After 70 days of exposure, the remaining fish were transferred to a 20 L glass tank and reared in dechlorinated municipal tap water to allow for 30 days of recovery, while a subset of the exposed female fish was placed into clean water and paired with unexposed male fish. The hatching rates, malformation and survival in the F1 embryo-larvae were assessed. The 70-day MATC for increased malformation and decreased survival of F1 fish was reported as 0.0224 mg/L PFOS. This MATC was associated with a mean percent of malformation of 0, 37.5, and 100% in the

0.01, 0.05, and 0.25 mg/L treatment groups, respectively. However, an independently-calculated EC_{10} could not be determined as the treatment level data needed for this analysis appear to have been lost (personal communication with Bingsheng Zhou, corresponding study author). Instead, only the independently-calculated EC_{10} for male growth as weight of 0.001990 mg/L could be determined. This EC_{10} was substantially lower than the author reported MATC of 0.0224 mg/L and the MATC of 0.0158 mg/L observed for the same endpoint in Wang et al. (2011). Therefore, the independently-calculated EC_{10} of 0.001990 mg/L was not used in the derivation of the freshwater chronic criterion since the curve fitting for this study was questionable given that the independently-calculated EC_{10} for male weight does not appear to be reasonable, was substantially lower than the MATC from this and other studies, and was not consistent with the observed toxicity of PFOS for this species. Additionally, the endpoint of male growth was difficult to tie to a population effect compared to the survival endpoint that was used to derive the freshwater chronic criterion. However, the author-reported MATC of 0.0224 mg/L for increased F1 malformation and decreased survival was similar to the independently-calculated EC_{10} of 0.01650 mg/L for F1 survival from Wang et al. (2011), which was used quantitatively in the freshwater chronic criterion.

Cui et al. (2017) investigated the toxic effects of PFOS (> 96% purity) to *Danio rerio* in a near full life-cycle (unmeasured) static renewal test. The PFOS stock solution was prepared by dissolving PFOS in DMSO. Wild-type zebrafish (AB strain) were raised under standard laboratory condition of 28°C (water temperature) with a 10-hr:14-hr light:dark photoperiod in a recirculating system according to standard zebrafish breeding protocol. Water supplied to the system was filtered by reverse osmosis (pH 7.0-7.5), and Instant Ocean salt was added to maintain the conductivity to 450-1,000 mS/cm. The adult fish were fed twice daily with live

Artemia and dry flake diet. Zebrafish embryos were obtained from adults in tanks with a sex ratio of 1:1, and spawning was induced in the morning when the light was turned on. Embryos were collected within one hour after spawning and rinsed in embryo medium. The fertilized embryos were staged using a stereomicroscope according to the standard method. High-quality embryos at 8-hour post fertilization (hpf) were divided into four groups: vehicle control (0.01% DMSO, v/v), and PFOS at 0.02, 0.1, and 0.5 mM (or 0, 0.01, 0.05, and 0.25 mg/L given the molecular weight of the form of PFOS used in this study, CAS # 1763-23-1, of 500.13 g/mol). Embryos were first exposed to PFOS in a petri dish (100/group) for five days without media change, and all embryos hatched and survived in this stage. After five days, fish were transferred into 2 L tanks until 30 dpf. After 30 dpf, fish were raised in 10 L tanks (30/tank) until the end of experiment. Throughout the whole exposure period, 50% water was renewed with freshly prepared solutions every five days. Each tank was checked for fish morbidity on a daily basis and water quality was monitored on a weekly basis. Feeding was initiated at 5 dpf. Between five and 14 dpf, fish were fed three times daily with standard larval diet, and after 14 dpf, they were fed twice daily with freshly hatched live *Artemia*. Equal amounts of feed were given among different groups each day. All experiments performed in this study were repeated three times with embryos derived from three different parental stocks. At the end of chronic exposure (180 dpf), fish from each group ($n > 30$) were checked for their sex, body weight, and length (measured from snout to the fork point of caudal fin). Condition factor (K) was tabulated to determine overall fitness. Breeding trials were also carried out to produce F1 offspring (F0 females were paired with F0 males from the same treatment group). Malformation and survival rate of both generations were evaluated and the study authors indicate that F1 offspring derived from the parental fish exposed to 0.25 mg/L were observed to have severe deformities (including

uninflated swim bladders, bent spine, pericardial edema, yolk sac edema, and necrosis) and low survival rates. A MATC of 0.1118 mg/L was calculated from the authors reported values for effects on altered sex ratio (female dominance) and low F1 offspring survival. However, an independently-calculated toxicity value could not be calculated with the data provided in the paper. The author-reported MATC was used qualitatively since EPA was unable to independently verify the reported toxicity value with the data provided in the paper, since only the growth data were provided, and because there was insufficient information, including the treatment level data, on the reproductive effects observed in the study. Further, the author-reported MATC of 0.1118 mg/L for decreased F1 offspring survival was higher than the independently-calculated EC_{10} of 0.0165 mg/L for F1 survival from Wang et al. (2011) (which was used quantitatively in the freshwater chronic criterion) and the MATC of 0.0224 mg/L for increased F1 malformation and decreased survival from Du et al. (2009) (which was used qualitatively as supporting information for this species), indicating that the author-reported toxicity value for this study may not be representative of the effects of PFOS on zebrafish.

Effects of PFOS on disruption of the hypothalamus-pituitary-thyroid axis in *Danio rerio*, was investigated by **Shi et al. (2009)**. The authors subjected blastula stage zebrafish embryos to the potassium salt of PFOS (> 99% purity) for 15 dpf under renewal unmeasured conditions. The stock solution was prepared by dissolving the crystals in HPLC-grade DMSO. Adult zebrafish (AB strain) embryos were collected at 2 hpf and embryos that had developed normally and reached the blastula stage were selected for subsequent experiments. Approximately 400 normal embryos were randomly distributed into glass beakers containing 500 mL of PFOS exposure solution (0, 0.10, 0.20, and 0.40 mg/L) with three replicates for each exposure concentration. During the experimental period, 50% of the exposure solution was renewed daily. The control

and exposure embryos received 0.003% (V/V) DMSO. The larvae were randomly sampled until 15 dpf and immediately frozen in liquid nitrogen and stored at -80°C for further gene expression and thyroid hormone assays. The body length of the larvae was also measured. The 15-day chronic value, MATC, was 0.2828 mg/L PFOS and is considered qualitative as this study was a rapid early-life stage test focused on the developmental toxicity of PFOS starting with embryos that had developed normally to the blastula stage and lasting through 15 dpf with limited procedural detail provided in the paper. No effects on survival were observed (with a NOEC > 0.40 mg/L). The study author-reported NOEC, LOEC, and MATC for growth as both total body length and weight were 0.20, 0.40, and 0.2828 mg/L, respectively. This 15-day growth MATC of 0.2828 mg/L was roughly one order of magnitude higher than the FCV of 0.008398 mg/L and suggested that this genus may be less sensitive to chronic exposures of PFOS than the quantitative study by Wang et al. (2011) with an EC_{10} of 0.0165 mg/L. However, the toxicity value from Shi et al. (2009) was for a much shorter exposure duration (15 days in a rapid early-life stage test compared to 150 days in a full life-cycle test) and a less sensitive endpoint (growth compared to reproduction). Therefore, the chronic freshwater criterion of 0.0084 mg/L would likely be protective of this genus.

Danio rerio embryos were also investigated by **Keiter et al. (2012)** in a long-term flow-through measured study with PFOS (potassium salt, CAS # 2795-39-3, ≥98% purity). PFOS was delivered to the test vessels without use of a carrier solvent. A first stock solution of PFOS (300 mg/L) was prepared by dissolving 1.5 g PFOS in 5 L of deionized water with overnight magnetic stirring. The solution after the first dilution step, hereafter named second stock solution (0.016, 2.6 and 7.8 mg/L), was freshly prepared four times a week by diluting the first stock solution (300 mg/L) with deionized water. Nominal concentrations of PFOS in the test vessels were

0.0006, 0.1, 0.3 mg/L. The test was initiated with fertilized zebrafish eggs obtained from non-exposed adults reared in the laboratory of Aquatic Ecology and Toxicology, Heidelberg University, Germany. Throughout the study, the fish were maintained in a light-isolated room with an artificial 14-hr:10-hr light:dark period. Adult fish were fed twice daily with freshly hatched *Artemia* nauplii complemented with TetraMin flake food. Larvae were initially fed twice daily with liquid starter food followed by Sera micron powder food and freshly hatched *Artemia* nauplii. Tap water and deionized water were mixed until conductivity (600-750 µS), hardness (276±17.8 mg/L as $CaCO_3$) and pH (8.0-8.2) were stably balanced. The water mix was supplied from an aerated reservoir and used to culture all embryos and fish. The final test water was routinely characterized for pH (8.25-8.75) and total hardness (167-356 mg/L). Temperature (26.0±1.0°C) and dissolved oxygen (6.45-10.97 mg/L) was checked weekly.

During the course of the study, fish were continuously exposed to PFOS with each treatment group replicated twice holding a starting number of 80 fish per replicate (160 individuals per treatment). At 2-4 hours post fertilization (hpf), eggs were transferred to glass dishes and exposed to the different treatments at 26±1°C under semi-static conditions (complete renewal of solutions after 24 hours) until 48 hpf, when they were transferred to respective test vessel. Whole-glass tanks, adjusted for a 10 L working volume were utilized as test vessels. A flow-through system with a three-fold water exchange per day was applied throughout the study in order to provide adequate supply of fresh test solution. External aeration by pressurized air was installed for each test vessel. Test solutions were daily refilled into light-isolated 10 L glass bottles located above the test vessels. Each test solution was constantly held in motion by magnetic stirring. Peristaltic pumps were used for a continuous delivery of test solution (50 mL/hour) from each glass bottle to paired test vessels serving as replicates A and B for each

treatment group. For each test vessel, a water flow rate of 1.25 L/hour was adjusted by means of rotameters.

Each replicate of the F1 and F2 generations was sub-sampled at 30 and 90 dpf. At 30 dpf, the number of fish in each replicate test vessel was reduced by 35 individuals for measurements of length and weight. At 90 dpf, each replicate was further reduced by 25 individuals for measurement of length, weight and vitellogenin (Vtg), as well as for histological evaluation of liver, thyroid and gonads. For each replicate test vessel, a total of 10 males and 10 females were retained for reproduction experiments and breeding of the F2 and F3 generations. After termination of the breeding experiments (approximately at 180 dpf), remaining adults were sampled following the exact procedure as described above for sub-sampling at 90 dpf. Post-hatching survival was documented for the F3 generation at 14 dpf, when the experiment was terminated without subsequent sampling. Breeding experiments for evaluation of fecundity and fertilization rate were performed with F1 and F2 adults starting at approximately four months of age. Breeding trials for each treatment group were repeated six to seven times (F1) and nine to ten times (F2) with a minimum of one week of recovery in between to avoid stress related bias. At the day before spawning, five individuals of either sex from each test vessel were randomly selected and transferred to breeding tanks prior to the onset of darkness. The spawning facility was constructed of six breeding tanks which were held together under un-exposed semi-static conditions with constant air supply (7.37-8.10 mg/L) and heating (25.0±1.0°C). The bottom of the breeding tanks was covered by a stainless steel grid (mesh size 1.25 mm) to allow the eggs to pass through into separate spawning trays and thus to avoid cannibalism by parental fish. About 20-30 minutes after the onset of light, spawning trays were removed and the eggs were collected and any further debris was removed. Eggs were counted and visually inspected under a stereo

microscope and transferred to petri dishes containing freshly prepared artificial water according to ISO (1996) (maximum 100 eggs/200 mL water). The eggs were incubated at 26.0±1.0°C overnight, after which coagulated and fertilized eggs were counted.

The chronic value (MATC F2 180-day survival) presented for this study was 0.1732 mg/L PFOS, and was considered qualitative as this study had a poor concentration-response relationship and test design complications. These test design complications included a pseudo-replication issue with this study that needs to be considered. There were four PFOS treatment groups with 160 fish per treatment, separated over two replicate tanks (80 fish per tank). A decrease in fish density was observed shortly after swim-up in one of the two replicates of the PFOS 0.1 mg/L treatment where the survival rate was 5%, which may indicate a problem with the test organisms. The NOEC and LOEC for F1 and F2 180-day male lengths and weights, and F2 180-day female weights, was 0.0006 and 0.1 mg/L. The LOEC for the remaining growth endpoints (lengths and weights across all generations) was < 0.0006 mg/L. The lowest LOEC values were less than the FCV of 0.008398 mg/L, suggesting this genus may be more sensitive to chronic exposures of PFOS than indicated by the quantitative study by Wang et al. (2011) with an EC_{10} of 0.0165 mg/L. However, this study had poor concentration-response relationships, across both endpoints and generations, and had test design complications, including a pseudo-replication issue. Therefore, the EC_{10} from Wang et al. (2011) that used quantitatively in the chronic criterion derivation was determined to be more robust than the toxicity values reported in Keiter et al. (2012), and the chronic freshwater criterion of 0.0084 mg/L is expected to be protective of this genus.

Chen et al. (2016) evaluated the estrogenic effects of PFOS (> 96% purity) to *Danio rerio* via renewal unmeasured tests. The stock solutions of PFOS (2.5 g/L) were prepared by

directly dissolving PFOS in 100% DMSO and storage at 4°C. The working solution of PFOS (0.250 mg/L) was prepared by a series of dilutions of the 2.5 g/L stock solution with system water, resulting in a final DMSO concentration of 0.01% (treatments and control). Adult zebrafish (wild type AB strain) were raised and kept at standard laboratory conditions of 28±1°C with a 14-hr:10-hr 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 450 to 1000 mS/cm (system water). The adult fish were fed twice daily with live *Artemia* and dry flake food. Zebrafish embryos were obtained from spawning adults (sex ratio of 1:1) in tanks overnight. Embryos were collected within one hour after spawning and rinsed in embryo medium (EM). Fertilized and normal embryos were staged under a stereomicroscope. The embryos were divided into two treatment groups: DMSO vehicle control (0.01% v/v) and PFOS (0.250 mg/L). Embryos at 8 hpf were first exposed to PFOS in a Petri-dish (100 embryos/50 mL EM) till 5 dpf, and all embryos hatched and survived to this stage. At 5 dpf, the fish were transferred into 2 L tanks for the period of 5-30 dpf. The fish were fed three times with zebrafish larval diet between five and 14 dpf and after 14 dpf, they were fed twice daily with freshly hatched live *Artemia*. The fish were raised in 9 L tanks (30 fish per tank) after 30 dpf until the end of the experiment. Half the solution volume was renewed every five days. There were three replicates from embryos derived from different parental lineages. To cover the entire sex differentiation period, fish were sampled at 21, 35, and 42 dpf for measurements of general growth, sex hormone levels and related gene expression. For general growth measurements, 80 fish were used. The body length was measured on individual fish while the body weight averages were obtained by pooling samples of 10 fish per sample. For the measurements of estradiol (E2) and testosterone (T), 60, 40, and 20 fish were

collected on 21, 35, and 42 dpf, respectively. Whole fish tissue homogenates were used for the hormone measures due to difficulty in obtaining sufficient serum volume from juvenile fish. Similarly, whole fish tissue homogenates of 40 pooled juvenile fish from each of these three sampling time periods were used for RNA extraction. All experiments were repeated three times. After 150-day PFOS exposures, fish were anesthetized and sexed, body length, body weight and gonad weights were measured. Sperm samples were collected from adult males for quality analysis, and serum samples from adult fish (15 fish per sex with blood sample pooled from five fish serving as one replicate) were collected for sex hormone measurements. The 42 dpf (increased condition index) and 150 dpf (increased estradiol in male/females and testosterone in males) LOECs were both 0.250 mg/L PFOS, but these data are classified as qualitative because there was only one exposure concentration. The LOEC of 0.250 mg/L was one order of magnitude higher than the FCV of 0.008398 mg/L and indicated that this genus may be less sensitive to chronic exposures of PFOS than the quantitative study by Wang et al. (2011) with EC_{10} of 0.0165 mg/L for F1 survival. However, it was difficult to compare these two papers since different endpoints were assessed and the present study only included one exposure concentration, which provides limited understanding of the magnitude of effects compared to the control. Therefore, the chronic freshwater criterion of 0.0084 mg/L would likely be protective of this genus.

Jantzen et al. (2017) evaluated the effects of PFOS on the morphometric, behavioral and gene expression in *Danio rerio* exposed via 5-day static unmeasured exposures (OECD Method 212). The AB strain of zebrafish (Zebrafish International Resource Center, Eugene, OR) were used for all experiments. Breeding stocks were bred and housed in recirculating systems under a 14-hr:10hr light:dark cycle. System water was obtained by carbon/sand filtration of municipal

tap water and water quality was maintained at a pH between 7.2 and 7.7, and water temperature between 26 and 28°C. Zebrafish embryos were exposed at 3 hpf to PFOS at concentrations reported by the authors of 0, 0.02, 0.2 or 2.0 μM (or 0, 0.02, 0.2, and 2.0 mg/L using a molecular weight of 500.13 μg/μmol for PFOS) for 120 hours (4 replicates, 24-35 fish per replicate). All compounds were dissolved in water. After this time, fish were transferred to non-treated system water and fed two times daily with Zeigler Larval AP50. Therefore, the only exposure was through the water from 3 hpf to 120 hpf (5 days), which corresponds to embryonic to yolk sac larval exposure. At 120 hpf, morphometric measurements were recorded and gene expression analyzed. The OECD protocol was to extend the study beyond the exposure timepoints which allowed for removing any chemical exposure from 120 hpf to 14 dpf. Morphometric measurements were also taken at 7 dpf and 14 dpf. At 14 dpf, gene expression data and swim activity endpoints were collected. Each treatment compound and corresponding control group was set up as individual experiments, and the sample size was dependent on number of embryos produced from the stock breeding sets. No experiment had mortality greater than 20% of the starting sample size. The five day (plus nine days for observation) NOEC, LOEC, and MATC for growth as total body length were 0.02, 0.2, and 0.06325 mg/L, respectively. The LOEC was associated with a 3.79% decrease in growth compared to control. This study was considered for qualitative use due to the short exposure duration of five days and since it was an early-life stage test focused on the developmental toxicity of PFOS. The MATC of 0.06325 mg/L was similar to the FCV of 0.008398 mg/L and supports the quantitative study by Wang et al. (2011) with EC_{10} of 0.0165 mg/L. Therefore, the chronic freshwater criterion of 0.0084 mg/L would likely be protective of this genus.

Sharpe et al. (2010) examined the toxicity and bioaccumulation of PFOS 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 chronic toxicity tests are described in this present section, as they were used qualitatively (also see Section 4.4.2.1.4). The 48-hour tests were used qualitatively (see G.2.2.3) and the 96-hour tests were used quantitatively and are summarized above in A.2.10. Zebrafish were purchased from a pet store local to the University of Alberta and were reared at university facilities for six to ten months. Conditioned zebrafish water obtained from the Biological Sciences Zebrafish Facility at the University of Alberta. Fish were acclimated to and kept in 70 L glass aquaria where they were fed powdered trout chow (Unifeed) daily, occasionally supplemented with live brine shrimp. An automated reverse osmosis system was used to maintain conditioned zebrafish water, used for acclimation and testing, at a total hardness of around 160 mg/L and a calcium carbonate hardness at 20 mg/L. Test concentrations were diluted from a 25 mg/mL stock solution in a methanol (MeOH) solvent for dosing in all experiments.

A 14-day experiment was conducted to examine PFOS accumulation and changes in isomer profiles in response to maternal transfer. Over a period of 14 days, eight tanks (two controls: 0 mg/L, two solvent controls: 0.01% MeOH v/v, and four treatments: 2 mg/L PFOS) received daily water changes with daily dose renewals. After 14 days, fish were transferred to breeding cages in conditioned zebrafish (control) water to spawn. The next day, following spawning, adults were sacrificed and stored as -80°C, and eggs were collected and stored at -80°C for further analysis.

A 21-day experiment was conducted to test the potential of PFOS to reduce fecundity. Over a period of 21 days, five tanks (1 control: 0 mg/L, 1 solvent control: 0.01% MeOH v/v, and three treatments: 0.5 mg/L) received daily water changes with daily dose renewals. Fish were spawned prior to exposure (day 0), and on days 14 and 21 of the exposure period, where adult fish were removed from their tanks and held in breeding cages (in conditioned zebrafish water) to spawn. Fish that spawned on day 14 were returned to their experimental tanks after spawning, and fish that spawned on day 21 were sacrificed after spawning. Eggs from all tanks were counted from day 0, 14, and 21, and eggs spawned from fish held in treatment tanks were compared to eggs spawned from fish held in control tanks. Results for this experiment are reported qualitatively because data from one control tank were lost due to unusual fish aggression.

Results for the fecundity study showed at 34% reduction in fecundity relative to control in fish exposed to 0.5 mg/L PFOS for 14 days and a 47% reduction in fish exposed 21 days. In the maternal transfer experiment, approximately 10% (wt) of the burden for PFOS was transferred from adult fish to their embryos. Resulting egg concentrations were 116 ± 13.3 mg/L PFOS, which were significantly higher than whole body PFOS concentrations of 72.1 ± 7.6 mg/L. Similar isomer patterns were observed in adults and eggs, suggesting isomer fractionation is more likely to occur from either biomagnification or isomer-specific accumulation instead of maternal transfer. Results from the fecundity study indicates an author-reported NOEC of < 0.5 mg/L and LOEC of 0.5 mg/L which was associated with a 34% reduction in fecundity relative to control in fish exposed for 14 days. This study was considered for qualitative use as a result of this test consisting of one experimental concentration (of 0.5 mg/L) and because one of the two control replicates was lost, which the study authors note was due to unusual aggression among

the test organisms. The author-reported LOEC of 0.5 mg/L was one order of magnitude higher than the FCV of 0.008398 mg/L and indicated that this genus may be less sensitive to chronic exposures of PFOS than the quantitative study by Wang et al. (2011) with EC_{10} of 0.0165 mg/L for F1 survival. However, it was difficult to compare these two papers since the present study only included one exposure concentration, which provides limited understanding of the magnitude of effects compared to the control. Therefore, the chronic freshwater criterion of 0.0084 mg/L would likely be protective of this genus.

Chen et al. (2013) examined the behavioral effects of zebrafish resulting from prolonged chronic exposure to PFOS. Adult *Danio rerio* (US-AB strain) used for spawning the test organisms were maintained following standard protocols. A stock solution of 0.5 mM (250.07 mg/L) PFOS was dissolved in 100% dimethyl sulfoxide. Fertilized embryos were collected within one hour after spawning, rinsed with embryo medium, then staged and inspected with a Nikon stereomicroscope. Testing concentrations included four treatments: a vehicle control (0.01% dimethyl sulfoxide) and three 0.5 µM (0.2501 mg/L) PFOS treatments that were maintained for different periods of time, to assess the effects of PFOS exposure during different zebrafish life stages. All groups began with 8 hpf embryos. Of the three groups exposed to PFOS, one group was exposed from 1-20 dpf, the second from 21-120 dpf, and the third from 1-120 dpf.

Testing began with 100 embryos placed into Petri dishes (one Petri dish per group) filled with 150 mL of treatment water. Each petri dish received a daily media renewal. On day 5, hatched embryos were transferred to 3.75 L stainless steel tanks (one tank per group) for 15 days, with each tank receiving a water renewal every three days. On day 21, 30 fish from each group were transferred to three replicate tanks (10 fish per tanks), for a total of 12 tanks (three tanks per

group). Solutions and water were renewed every three days. During renewal, the tanks were monitored for morbid fish and water quality. Temperature was maintained at 28±0.5 °C, pH was measured at 6.8-7.6, conductivity ranged from 450-1,000 µS/cm, and ammonia ranged from 0-2 mg/L. A 14-hr:10-hr light:dark cycle was maintained throughout the study period. Before 60 dpf, nitrite concentrations were measured at 0.073±0.12 mg/L, and after 61 dpf, nitrite concentrations ranged from 1.02±0.65 mg/L. Feeding methods varied as the fish aged. Beginning at 5 dpf, animals were fed zebrafish larval diets three times per day. From 15 dpf to 96 dpf, zebrafish were fed live brine shrimp three times a day, which was reduced to two times a day at 97 dpf until the end of the test.

Following the 120-day exposure period, swimming behavior was measured in F0 adults as follows: 12 fish from each treatment group were placed in their own individual 1.75 L tank filled with 1.5 L of water. The test organisms were fasted during the behavioral experiments and given two hours to acclimate before the trials began. The position of each fish was recorded every 0.2 seconds to calculate distance moved, which were then averaged for each 30 second interval during the test. Experiments lasted 30 minutes, and the final 16 minutes were analyzed. This period included 12 minutes of movement analysis described above, plus four minutes of startle response analysis. To induce a startle simulation, an electromagnetic solenoid was attached to the bottom of each tank and programmed to tap each tank at the same time.

At approximately three months of age, F0 adults from the same treatment group were bred. Embryos (F1) hatched from these adults were monitored for developmental progression, and hatched larvae were monitored for 8 dpf for malformation and mortality. Embryos were monitored in 6-well plates with 5 mL of water per well. This experiment was repeated five times

using 20 embryos per replicate per group. Whole body tissue concentrations were analyzed from 40 pooled embryos as one sample which was replicated four times per group.

Second generation (F1) zebrafish were maintained in water free from PFOS. Larvae aged 4 dpf that were free from malformations were subjected to an assessment involving light and dark cycles for a period of 50 minutes. Six fish per treatment group, including the control, were placed in 24-well plates inside a ZebraLab behavior monitoring station. Larvae were allowed to acclimate for 20 minutes before stimulus testing began. For fifty minutes, fish were exposed to alternating cycles of light and dark periods. Each light and dark period was ten minutes each. At the end of the experiment, the average activity of 18 fish per each treatment group was calculated.

Movement speeds in adult male and female F0 fish exposed to PFOS from 1-120 dpf were significantly higher ($P<0.05$) than control fish. PFOS whole body tissue residues in the F1 larvae from the 21-120 dpf group and the 1-120 dpf group were not statistically different from each other. However, concentrations in both groups were statistically significantly higher ($P<0.001$) than the control group and 1-20 dpf group. Additionally, F1 larvae hatched from F0 adults from the 21-120 dpf and 1-120 dpf groups showed much higher rates of mortality and malformation than the control and the 1-20 dpf groups. Finally, during the F1 larvae light/dark test, it was found that the basal swim rate of the 1-20 dpf group and the 21-120 dpf group was statistically significantly higher ($P<0.05$) than that of the control in both light and dark conditions, while the 1-120 dpf group showed a statistically significantly lower ($P<0.001$) basal swim rate during the light cycles and higher ($P=0.028$) basal swim rate during the dark cycles when compared with the control group. The author-reported NOEC was < 0.250 mg/L and the LOEC was 0.250 mg/L for mortality and malformation. This study was considered for qualitative

use because the test consisted of one experimental concentration (of 0.250 mg/L). The author-reported LOEC of 0.250 mg/L was one order of magnitude higher than the FCV of 0.008398 mg/L and indicated that this genus may be less sensitive to chronic exposures of PFOS than the quantitative study by Wang et al. (2011) with EC_{10} of 0.0165 mg/L for F1 survival. However, it was difficult to compare these two papers since the present study only included one exposure concentration, which provides limited understanding of the magnitude of effects compared to the control and different endpoints were assessed. Therefore, the chronic freshwater criterion of 0.0084 mg/L would likely be protective of this genus.

Tse et al. (2016) evaluated the chronic effects of perfluorooctane sulphonic acid (PFOS, purchased from Sigma-Aldrich) on zebrafish (*Danio rerio*) in a 6-day unmeasured, static-renewal study. AB wild-type zebrafish were obtained from Zebrafish International Resource Center and were kept in a stand-alone system at 28°C under a 14-hr:10-hr light-dark photoperiod. The study was approved and followed guidance as given by the Animal Experimental Committee at Hong Kong Baptist University. The test consisted of a control and PFOS treatment. The PFOS treatment was prepared by dissolving PFOS into dimethyl sulfoxide and diluting the stock solution with egg medium (E3) to a concentration of 0.5 µg/L. Wild-type AB embryos at the 1-4 cell stage were exposed to PFOS in 2 mL of E3 medium in a 6-well plate for six days with daily renewals. Test temperature was maintained at 28°C. On day six, organisms were sacrificed and measured for liver enlargement and taken for genetic analysis. All PFOS-exposed fish showed increased liver size in comparison with the control, and all showed significant differences in gene expression of LPL, IGFBP1, PITPNA, DDX5, LECT2, MMP9, APOE, AGT, APOA1, GALE and GMDS. The 6-day LOEC for all non-apical, sublethal

endpoints included in the study was 0.5 µg/L PFOS (or 0.00050 mg/L). The lack of apical endpoints and atypical duration makes the study acceptable for qualitative use.

Bao et al. (2019) evaluated the chronic effects of perfluorooctane sulfonate (PFOS purchased from Dr. Ehrenstorfer in Augsburg, Germany) on juvenile zebrafish (*Danio rerio*), via a 21-day unmeasured, static-renewal study. Wild-type AB strain zebrafish were also obtained from Dr. Ehrenstorfer for the study. PFOS was dissolved with dimethyl sulfoxide (DMSO) to obtain nominal test concentrations of 0 (solvent control), 0.002, 0.02 and 0.2 mg/L. Female zebrafish approximately four months old were acclimated to laboratory conditions for two weeks in a recirculating water system under a 12-hr:12-hr light:dark photoperiod, temperature of 28 ± 1 ºC, pH of 7.0 - 7.5 and conductivity of 500 to 800 µS/cm. Eighty zebrafish were divided into two subgroups for analysis, with test initiation of one group at 8:00 am and the other group at 7:00 pm. Within those subgroups, the 40 fish were divided equally among the concentrations, and each fish was considered a single biological replicate. Six fish were used for growth and fecundity testing, while the other four were sacrificed for genetic analysis. Fish were randomly placed into four glass aquaria measuring 18 x 13 x 15 cm^3 and half of the exposure water was replaced every day. Before sampling, each female was matched with a male zebrafish, and fertilized eggs were collected at either 8:30 am or 7:30 pm according to subset type. An endpoint for fecundity was not reported by the authors, and there were no significant differences between the exposure concentrations in terms of growth length or weight (NOEC > 0.2 mg/L PFOS). No mortality was observed. Independently-calculated EC_{10}s could not be calculated as EPA was unable to fit a model with significant parameters. Therefore, given EPA was unable to independently calculate toxicity values based on the level data provided in the paper by the study authors, the test duration was a partial-life cycle test as opposed to the preferred life-cycle test

for which there were studies on this species (Wang et al. 2011), and the author-reported toxicity values resulted in a NOEC > 0.2 mg/L, this study was used qualitatively to derive the draft chronic water column criterion.

Huang et al. (2021) used PFOS as a positive control in a series of short-term, 4-day tests as part of a comparative developmental toxicity assessment against sodium *p*-perfluorous nonenoxybenzene sulfonate (OBS). Normally developing zebrafish embryos (6 hours post fertilization, hpf) from adult wild-type (AB strain) breeding stock were used for the experiments. A stock solution of PFOS-K (≥98% purity, CAS # 2795-39-3) purchased from Sigma-Aldrich (Oakville, ON, Canada) was prepared at 2 g/L in DMSO and diluted with culture medium to achieve the desire concentration of 20 mg/L. The final DMSO concentration in the treatment and control groups was 0.1% (v/v). In the developmental toxicity assay, zebrafish embryos were randomly allocated to 6-well plates at a density of 30 per well containing 5 mL of exposure solution. Each treatment contained three replicates with 30 embryos per replicate. The embryos were maintained at 28.5°C with 14-hr:10-hr light:dark photoperiod for four days. The exposure solutions were renewed daily, and dead embryos were gently removed. Coagulation of the embryo, failure of somite development, lack of heartbeat and non-detachment of the tail from the yolk sac were identified as dead embryos. Several types of malformations were looked for in the embryos. Hatching rate was determined as the percentage of hatched embryos at 48 hpf and 72 hpf. In the bioconcentration, physiological and biochemical assay, zebrafish embryos were randomly exposed to a 1 L glass beaker filled with 400 mL of exposure solution that had 300 individuals for four days. Each treatment contained three replicates with 300 embryos per replicate. At the end of exposure, visually normal larvae were harvested and divided into several subsets for PFAS determination, locomotor behavior assessment, dopamine concentration

measurement, transcriptome and qRT-PCR analysis, Western blot analysis, and immunostaining of cilia. The remaining larvae served as backup samples. Finally, for the angiogenesis assay, embryos at 6 hpf were assigned to a Petri dish filled with 100 mL exposure solution that had 25 individuals. The embryos were exposed for four days to evaluate any effects on embryonic vasculature. After exposure for four days, 36 zebrafish larvae were randomly selected from each treatment group for locomotor behavior assay. Measured concentrations of PFOS were comparable to nominal concentrations (range: 21.88-22.33 mg/L). After four days of exposure to 20 mg/L PFOS, mean whole-body burden of PFAS (poly- and perfluoroalkyl substances) in zebrafish larvae was 464.7 mg/kg wet weight. PFOS at this concentration induced a series of malformations, mainly including pericardial edema, curved tail, spine curvature and shortened body. A significant 44.4% reduction in hatching rates at 48 hpf was observed compared to the negative control group but hatching rates at 72 hpf reached 100% in all treatment groups. Examination of genes related to the cell cycle revealed that PFOS treatment resulted in a significant reduction in the mRNA levels of *ccne1*, *cdk2*, *cdk6* and *pcna* genes in zebrafish larvae, but did not affect the transcript expression of *ccnd1* and *myca*. Locomotor behavior was also reduced by exposure to 20 mg/L PFOS, which also significantly reduced dopamine content in zebrafish larvae by at least 15 times compared to the control group. The transcription of several crucial genes involved in neuronal development was also significantly reduced in the PFOS treatment group. Given the lack of apical responses in the study and single PFOS treatment, this value is considered qualitative only.

The effects on zebrafish, *Danio rerio*, embryonic metabolism, pancreas development, and adiposity due to developmental and sub-chronic PFOS exposures and their persistence into later larval and juvenile periods were evaluated by **Sant et al. (2021)**. Stock solutions were prepared

by dissolving PFOS (Sigma-Aldrich, St. Louis, MO, Catalog # 77283) into DMSO, and stored at room temperature in amber glass bottles inside of light-prohibitive containers until use. Wildtype AB zebrafish were originally obtained from Boston Children's Hospital (Boston, MA). Embryos <1 hour post fertilization (hpf) used for experimentation were collected from breeding tanks of adults. Embryonic exposures to either 0 (0.01% v/v DMSO), 16, or 32 µM PFOS for biochemical analyses were performed on wild-type (AB) embryos. Successful embryos (3 hpf) were randomized and individually transferred to wells of 24-well polystyrene plates, and exposure media was prepared daily in 0.3X Danieau's medium prior to this transfer. One mL of the assigned exposure medium was added to each well containing one embryo. Exposure media was refreshed daily to model subchronic exposures throughout development, and embryos were maintained in a dedicated incubator at 28.5°C. Experiments were repeated three times each for the biochemical assays and fatty acid analysis, each with 4-6 samples per exposure group each containing 15 pooled embryos (60-80 embryos per treatment). At four days post fertilization (dpf), larvae were rinsed thoroughly and transferred to 1.5 mL polypropylene microcentrifuge tubes. For protein, cholesterol, glucose, and triglyceride quantification, 15 larvae were pooled per sample. For fatty acid analysis, 20 larvae were pooled per sample. Concentrations of lauric (C12:0) and myristic (C14:0) saturated fatty acids were increased by PFOS at 4 dpf, and PPAR gene expression was reduced. The 4 dpf LOEC based on these apical endpoints was 16 µM PFOS (8.002 mg/L PFOS based on a molecular weight of 500.13 g/mol) and is acceptable for qualitative use only.

A 10-day subchronic unmeasured test was conducted by **Zhu et al. (2021)** to investigate the effect of PFOS exposure on hepatocellular carcinoma (HCC) progression in transgenic zebrafish *Tg (fabp10:rtTA2s-M2*; *TRE2:EGFP-KRAS^{G12V})*. PFOS-K (potassium salt, purity

>98%) purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan) was dissolved into dimethyl sulfoxide (DMSO) to expose male *kras*^{V12} transgenic zebrafish to 500 μg/L PFOS for 10 days under renewal conditions. Both treated and control groups received 0.01% (v/v) DMSO previously shown not to cause a significant toxicological response in zebrafish. The concentration of PFOS selected for use in the study was based on preliminary experimentation (4 day tests) conducted over a range of concentrations to elicit an increase in liver size and fluorescence intensity. For the definitive experiment, adult transgenic zebrafish (90 dpf) were maintained in 5 L glass beakers with 3 L charcoal-filtered tap water at 28°C in the dark. During the exposure period, the water chemistry was recorded (dissolved oxygen: 8.06 ± 0.05 mg/L; water temperature: 28.0 ± 0.5°C and pH: 7.21 ± 0.09). Zebrafish were fed with *Artemia* nauplii two times every day. Treatments included three replicate beakers each containing eight zebrafish. Exposure solutions were changed daily. After 10 days of exposure, zebrafish, were euthanized, weighed, and dissected. Zebrafish livers were observed and photographed by fluorescence microscopy. The livers were then separated and weighed to calculate the hepatosomatic index (HSI). Six livers from each treatment were randomly selected and fixed in 4% paraformaldehyde solution for histological examination, and three livers from each treatment were randomly selected for transcriptomics analysis. No significant effect on HSI was observed in zebrafish exposed to 0.5 mg/L PFOS, and livers showed normal hepatocyte nuclear structure compared to control livers. Cytoplasmic vacuolation was evident in the PFOS group compared with the control, but PFOS had no significant effect on liver enlargement. Gene expression level of *cyp24a1* was significantly upregulated by 8.2 fold and expression of *cyp27b1* was significantly downregulated by 4.8 fold after exposure of zebrafish to PFOS for 10 days. The results indicated that the synthesis of calcitriol was reduced, and the degradation of calcitriol was enhanced,

resulting in a decrease in intracellular concentration. Additionally, three of the top 16 enriched pathways (p value < 0.05) were related to lipid metabolism in zebrafish liver after exposure to 0.5 mg/L PFOS for 10 days, including PPAR signaling pathway, adipocytokine signaling pathway and fatty acid metabolism pathway. Given the lack of apical responses in the study and single PFOS treatment, this value is considered qualitative only.

G.3.2.5 Pimephales promelas

Oakes et al. (2005) exposed fathead minnows to PFOS in an outdoor microcosm experiment. The University of Guelph Microcosm Facility is located at the Guelph Turfgrass Institute (ON, Canada) and consists of 30 artificial ponds of approximately 12,000 L. The microcosms were constructed below grade to a depth of 1.2 meters using galvanized steel panels lined with food-grade polyvinylchloride. Each microcosm had a diameter of 3.9 meters, was filled with water to a depth of approximately one meter, and was flush with ground level. The water supply for the microcosms was an irrigation pond supplied by a well located on site. Sediment trays containing a 1:1:1 (v/v/v) mixture of sand, loam, and organic matter, as well as potted macrophytes (*Myriophyllum spicatum*) were added to each microcosm. Prior to being treated with PFOS, water was circulated among all microcosms for two weeks at a flow rate of approximately 12 m^3/d, ensuring homogeneous water chemistry, zooplankton, and algae assemblages. PFOS (89% purity) stock solutions were premixed in 40 L Rubbermaid® containers before introduction to the microcosms by subsurface injection. Water samples from each microcosm were obtained using a metal depth-integrating water-column sampler at one hour and 1, 2, 4, 7, 14, 21, and 28 days after PFOS addition to calculate time-weighted mean PFOS concentrations. All treatment concentrations were based on the PFOS anion (without K^+). Microcosms were treated in triplicate at nominal concentrations of 0.3, 3, 10, and 30 mg/L PFOS. Three additional microcosms served as controls and did not receive any PFOS. Fathead

minnow (6.1 cm, 2.0 g) were purchased from Silhanek Baitfish Farms (Bobcageon, ON, Canada) and acclimated in the adjacent irrigation pond for 10 days prior to PFOS exposure (under a natural photoperiod). Fish were held in two wooden frames with 5 mm aperture polyvinylchloride mesh cages. Each microcosm held two cages, with each PFOS concentration replicated in three microcosms. Cages were divided into four quadrants, and each quadrant contained two female and one male fathead minnow for a total of 24 fish per microcosm. Fish were initially sexed prior to exposure based on size and presence of secondary sex characteristics. Sexes were subsequently confirmed at the conclusion of the exposure after the fish were killed. A 15 cm piece of 10 cm polyvinyl chloride pipe cut in half lengthwise served as a breeding substrate within each quadrant and was examined for egg deposition daily. Both egg production and oviposition (spawning) frequency were recorded and used for the subsequent calculation of egg and oviposition frequency per female, per microcosm, and cumulatively per dose. At the conclusion of the 28-day exposure, measurements of total length, total weight, gonad weight, and liver weight were taken, and gonadosomatic indices (GSI), liver-somatic indices (LSI), and condition factor (K) were calculated. Mean water-quality parameters (collected mid-depth) sampled over the course of the exposure include dissolved oxygen (8.7 mg/L), temperature (18.0°C), pH (9.2), and alkalinity (114.5 mg $CaCO_3$/L). The 28-day LC_{10} was 3.5 mg/L PFOS (qualitative) based on the time weighted average over the course of the experiment. Even though the microcosm experiment contained sediment, algae, macrophyte and zooplankton, the results of this study should be compared with other single species studies.

G.3.2.6 Pseudorasbora parva

Yang et al. (2014) evaluated the toxicity of PFOS (potassium salt, CAS # 2795-39-3, 99% purity) to *Pseudorasbora parva* via 30-day renewal measured exposures (the authors note that the experiments followed ASTM standards and USEPA procedures for deriving water

quality criteria). The topmouth gudgeon (4.0 g, 4.0 cm) were purchased from the Beijing Chaoyang Spring Flower Market, which was considered an atypical source. The organisms were allowed to acclimate for seven days before testing, and the test was conducted at 22±2°C with a light:dark cycle of 12-hrs:12-hrs, and 10 fish per replicate with three replicates per concentration. Beakers used for exposure were assumed glass, but glass was not specified by study authors. PFOS was dissolved in deionized water and carrier solvent DMSO to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 30, 45, 67.5, 101.25, 151.88 and 227.81 mg/L PFOS. Water quality parameters reported were pH=7.0 ± 0.5, dissolved oxygen=7.0 ± 0.5 mg/L, total organic carbon=0.02 mg/L and total hardness=190.0 ± 0.1 mg/L $CaCO_3$. The supplemental data provided for the study included a comparison of measured PFOS concentrations before and after solution renewal in the low and high acute and chronic test concentrations. PFOS concentrations in the test water did not fluctuate by more than 15% during experiments. The 30-day survival EC_{10} reported for the study of 2.12 mg/L PFOS was deemed qualitative due to the atypical fish source, not a valid ELS test (started with older unspecified life stage), and unknown composition of test beakers.

G.3.2.7 Spinibarbus sinensis

The toxicity of PFOS (potassium salt, >99% purity) to the qingbo, *Spinibarbus sinensis* was evaluated by **Xia et al. (2015a)** via a 30-day renewal unmeasured test. PFOS was initially dissolved in DMSO, and the stock solution (0.5 g/mL) was kept at 4°C until preparation of the final exposure solutions in water. Juveniles of uniform size (2.77 g, 5.62) were obtained from local farmers in Chongqing Municipality, China. The fish were housed in a 120 L recirculating water tank system at Chongqing Normal University for two weeks prior to the experiment. The rearing water was dechlorinated and filtered through activated carbon. During this time, the temperature of the water was maintained at 22 ± 1°C, the dissolved oxygen level was kept above

7 mg/L, and the photoperiod was 15 hours light and nine hours dark. The fish were fed commercial tubifex twice daily. A semi-static exposure experiment apparatus was used for waterborne PFOS exposure. The apparatus consisted of ten glass aquariums each with a capacity of approximately 22 L of water. Prior to exposure, the fish were gently transferred to the aquariums. They were maintained at conditions similar to that described above for one week to eliminate stress effects and were fed at the level of maintenance ration daily. After that, the water temperature was increased or decreased by 1°C/day until it reached the prescribed temperature (18 and 28°C). Once the water temperature reached the prescribed values, the toxicants were administered. The fish were then exposed to a range of PFOS concentrations (0, 0.32, 0.8, 2 and 5 mg/L) under two different temperatures (18 and 28°C) for 30 days. A total of 160 fish were used in the experiment (one replicate per concentration, 16 fish per replicate). The concentration of DMSO in the water did not exceed 0.001 % (v/v). During the experimental period, 50% of the exposure solution was renewed daily. After termination of PFOS exposure, the fish were fasted for 48 hours, and then their behavior, metabolic characteristics and aerobic swimming performance were examined. First, individual fish were moved into the behavior observation system in which their spontaneous swimming behavior (SSB) and social interactions (SI) with fish were assayed using the Noldus video tracking software. Second, the fish were individually transferred to continuous-flow respirometer chambers for routine metabolic rate (RMR) determination. Finally, individual fish were placed in a swim tunnel respirometer, and the aerobic swimming performance and the oxygen consumption rate MO_2 during swimming were determined using the critical swimming speed method. Subsequently, the metabolic scope (MS), factorial metabolic scope (F-MS) and energetic cost of transport (COT) were calculated. The fish were kept in a fasted state over the course of the entire experimental tests. The 30-day chronic

MATCs determined at 18°C (% mobile, % highly mobile, swim distance, swim speed, freq. highly mobile, % social, resting) and at 28°C (critical swim speed) were both 0.5060 mg/L PFOS. These data were considered qualitative because the test was not replicated, qingbo is a non-North American species, the test was unmeasured, water quality details were not reported (assumed that dilution water was the same as holding water, but this was not specified), and the biomass loading density was higher than recommended for both test temperatures.

In a companion study, **Xia et al. (2015b)** again evaluated the 30-day toxicity of PFOS (potassium salt, >99% purity) to *Spinibarbus sinensis*. PFOS was initially dissolved in DMSO, and the stock solution (0.5 g/mL) was stored at 4°C until preparation of the final exposure solutions in water. Juvenile fish of uniform size (2.69 g, 5.39 cm) were obtained from local farmers in Chongqing Municipality, China. Prior to the experiment, the fish were reared in a 120 L recirculating water tank system for two weeks. The rearing water was dechlorinated and filtered through activated carbon. Water temperature was maintained at 22 ± 1°C, water oxygen content was kept above 7 mg/L, pH ranged from 6.8 to 7.5 and the rearing system was maintained under a 15 hours light and nine hours dark cycle. The fish were fed to satiation daily with commercial *Tubifex spp*. After the acclimation period, healthy fish of similar sizes were selected for the study. Juvenile southern catfish, *Silurus meridionalis* were used to provide the predator stressor in this study. A semi-static exposure experiment apparatus consisted of 10 glass aquaria, each with a capacity of approximately 22 L. Prior to exposure, the juvenile qingbo were randomly selected and divided into 10 groups (n=16 for each group) and were gently transferred to the aquariums. Fish were maintained at conditions similar to those described above for one week to eliminate stress effects. For each group, the same amount of food (approximately 10% of body weight) was provided daily during this period and during subsequent processing. After

that, the water temperature was increased or decreased by 1°C/d until it reached a prescribed temperature (18 or 28°C). The selected temperatures were chosen based on the habitat/ season temperature. For qingbo, the habitat temperature is approximately 18°C in spring and autumn and approximately 28°C in summer. Once the water temperature reached the prescribed values, the toxicants were administered. Fish were exposed to a range of PFOS concentrations (0, 0.32, 0.8, 2 and 5 mg/L) under the two different temperatures (18 and 28°C) for four weeks. The concentration of DMSO in the water did not exceed 0.001% (v/v). During the experimental period, 50% of the exposure solution was renewed daily. After termination of PFOS exposure, feeding was withheld for 48 hours and the antipredator behavior and fast-start performance of the fish were examined successively. Again, the 30-day chronic value (decreased maximum linear acceleration) was 0.5060 mg/L PFOS at each temperature and the data are classified as qualitative (non-apical endpoints reported and unmeasured exposure). In addition, control survival was not reported; however, the study was still classified as qualitative because the study authors did not indicate any problems with exposure related mortality.

G.3.2.8 Oryzias latipes

Ji et al. (2008) evaluated the chronic toxicity of PFOS to the Japanese medaka, *Oryzias latipes*, via unmeasured renewal exposures. Solvent-free stock solutions of PFOS (200 mg/L) were prepared by dissolving the solid in MilliQ® water via sonication. Chemical measurements were not made, and nominal concentrations were used throughout the present study. The medaka were maintained in the laboratory for several years at 25°C, a 16-hr:8-hr light:dark photoperiod and fed with *Artemia* nauplii (< 24 hours after hatching) twice daily. For the F0 fish exposure study, breeding medaka pairs (~ 2.5 cm) were maintained at 25 ± 1°C for at least seven days in 1 L beakers filled with dechlorinated tap water, which was prepared by serial filtration through a sediment and two granular activated carbon filters. Thirty-six mating pairs that spawned more

than eight eggs per breeding and bred more than five times per week were selected and randomly separated into four groups. Nine mating pairs were assigned to each treatment group and the control. The following PFOS concentrations were used for definitive tests: 0.01, 0.1, and 1 mg/L, based on the preliminary range-finding results using adult medaka. The exposure duration for F0 fish was limited to 14 days, during which the fish were fed *Artemia* nauplii (< 24 hours after hatching) *ad libitum* twice daily. The exposure medium was renewed at least three times per week. Dead fish were removed as soon as possible. Eggs were counted every day, and the eggs spawned on the seventh day were saved for the F1 generation exposure study. On day 14, all surviving fish were euthanized, and body length and weight were measured, from which the condition factor (K) was calculated. The gonads and livers were also measured, and the gonadosomatic index (GSI) and the hepatosomatic index (HSI) were calculated. For the F1 fish exposure study, fertilized eggs collected from F0 fish exposed to each concentration of PFOS and the control were randomly separated into groups of 25 eggs each and then assigned to varying concentrations of PFOS (0, 0.01, 0.1, or 1 mg/L), with only one replicate per treatment. Because eggs were compiled into a single replicate for the hatching stage, results reported beyond hatching (even when larvae/juveniles were separated into replicates) are based on pseudo-replication. During the egg stage for the F1 generation, investigators maintained all possible combinations of F0 x F1 exposure concentrations for a given compound. Exposure was initiated in 50 mL beakers less than 12 hours after fertilization. The developing embryos were observed daily under a stereoscopic microscope, and dead embryos were removed. This procedure was repeated until all living embryos had hatched. Hatching was defined as the disruption of the chorion. Newly hatched larvae were then randomly transferred to 100 mL beakers and observed daily for swim-up success and survival for an additional two weeks. Larvae were fed *Artemia*

nauplii *ad libitum* twice daily. After 14 days, replicates with five fry each were randomly selected from each treatment group and transferred to 1 L beakers for the 100-day post hatch observation. All survivors were sacrificed 100 days after hatching, and body length and weight were measured. The gonads and livers were weighed to determine GSI and HSI.

The F0 (parental generation) adult survival, condition factor and adult male GSI and HSI 14-day NOECs were all > 1 mg/L PFOS, whereas the 14-day adult female HSI and GSI MATC and LOEC were 0.3162 and < 0.01 mg/L PFOS, respectively. For the F1 (progeny generation), the percent hatchability, time to hatch, and swim-up success, the MATCs were all 0.3162 mg/L PFOS, and the EC_{10} for larval growth (as organism weight and length) and LOEC for survival were 0.0013 mg/L and 0.01 mg/L PFOS, respectively. The reduction in organism weight at 0.01 mg/L was only 12%, however, and the concentration-response curve for weight was shallow: 1.0 mg/L yielded a 29% reduction in weight. Many of these toxicity values, particularly those for apical endpoints, suggested that this genus is no more sensitive than the most sensitive genus that was used to derive the criterion, which had a GMCV of 0.009676 mg/L. EPA notes, however, that the LOEC for larval growth was actually < 0.01 mg/L, because the effects were observed in the lowest concentration tested. Therefore, larval growth reported by Ji et al. (2008) may be a relatively sensitive endpoint compared to the freshwater chronic criterion of 0.0084 mg/L and indicated that this genus might be among the more sensitive genera for chronic exposures of PFOS. However, the apical endpoints were considered to be qualitatively acceptable in the criterion derivation because there was a lack of replication during the egg stage of the F1 generation. Although Ji et al. (2008) reported F1 growth as a relatively sensitive endpoint, the chronic criterion was expected to be protective of this genus based on quantitative data for other fish species.

Kang et al. (2019) evaluated the chronic effects of perfluorooctane sulfonic acid (PFOS, ≥98% purity, CAS No. 1763-23-1 purchased from Sigma Aldrich, St. Louis, MO) on Japanese medaka (*Oryzias latipes*) in a 21-day unmeasured, static-renewal study. A stock solution was prepared by dissolving PFOS into dimethyl sulfoxide and stored at 4°C. The 1 mg/L working solution was prepared by diluting the stock solution in fish culture water (carbon-filtered dechlorinated tap water). Adult fish (16 ± 2 weeks, 0.38 ± 0.06 g wet weight) were obtained from the fish culture facility at the Korea Institute of Technology in Jinju, Gyeongnam, South Korea. Fish were acclimated for seven days in carbon-filtered dechlorinated tap water at 25°C with a 14-hr:10-hr light:dark photoperiod. Eight male and eight female fish were introduced to a 20 L glass tank filled with 15 L of test solution at concentrations of 0 (control), 0 (solvent control) and 1 mg/L PFOS. Fish were fed brine shrimp and Tetramin daily, and the test solutions were renewed twice weekly. Authors reported following the exposure protocol given by OECD 229, with conditions maintained the same as during the acclimation period. Eggs were harvested and counted twice daily at 7, 14 and 21 days. Significant reduction in fecundity was shown for all time periods, and spawning became limited at day four. The 21-day fecundity LOEC was 1.0 mg/L PFOS and is acceptable for qualitative use.

G.3.3 Amphibians

G.3.3.1 Bufo gargarizans

Yang et al. (2014) evaluated the chronic toxicity of PFOS (potassium salt, CAS # 2795-3-3, 99% purity) to the Asiatic toad, *Bufo gargarizans* via 30-day renewal measured exposures (the authors note that the experiments followed ASTM standards and USEPA procedures for deriving water quality criteria). The tadpoles (0.048 g, 1.8 cm) were purchased from the Beijing Olympic Park, which was considered an atypical source. The organisms were allowed to acclimate for seven days before testing, and the test was conducted at 22 ± 2°C with a light:dark

cycle of 12-hr:12-hr. There were 10 fish per replicate and three replicates per concentration. Beakers used for exposure were assumed glass, but was not specified by study authors. PFOS was dissolved in deionized water and carrier solvent DMSO to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 1.5, 1.95, 2.54, 3.30, 4.28 and 5.57 mg/L PFOS. Water quality parameters reported were pH=7.0 ± 0.5, dissolved oxygen=7.0 ± 0.5 mg/L, total organic carbon=0.02 mg/L and total hardness=190.0 ± 0.1 mg/L as $CaCO_3$. The supplemental data provided for the study included a comparison of measured PFOS concentrations before and after solution renewal in the low and high acute and chronic test concentrations. PFOS concentrations in the test water did not fluctuate by more than 15% during experiments. The 30-day EC_{10} (survival) reported for the study of 2.00 mg/L PFOS was deemed qualitative due to the atypical test organism source, unknown composition of test beakers and a non-North American species (the test with this species is currently considered for qualitative use as details related to the source of the test organisms were limited (obtained from Beijing Olympic Park) and there was no mention of any potential previous exposure to PFAS or any other contaminant).

G.3.3.2 Lithobates pipiens

Foguth et al. (2020) evaluated the chronic effects of perfluorooctanesulfonate (PFOS) on northern leopard frogs (*Lithobates pipiens*, formerly, *Rana pipiens*) via a 116-day measured, outdoor mesocosm study. Six partial egg masses were collected from an ephemeral wetland at the Purdue Wildlife Area. Eggs were grown outdoors until reaching Gosner stage (GS) 25 in twelve 150 L cattle tanks. Empty tanks were filled with 100 L well water, 100 g leaf litter (predominately oak), and 5 g rabbit chow. Mesocosms were also inoculated with periphyton and phytoplankton (i.e., algal food resources for leopard frog larvae), as well as zooplankton from the wetland where eggs were collected. After setting for twelve days, four control mesocosms and

four treatment mesocosms were spiked with a 0 (control) and 12.8 ppb measured PFOS, respectively. After seven days, 25 tadpoles at GS 25 were added to each mesocosm to initiate the experiment. Water quality conditions during the test were reported as pH of 7.41 to 8.54 s.u., DO of 2.1 to 9.4 mg/L and temperature of 13.1 to 29.8°C. After 30 days, eight tadpoles were removed from each mesocosm, sacrificed and measured for snout-vent length and mass. Organisms that reached GS 46 were sacrificed and measured for the same endpoints. The test was terminated at day 116, and tadpoles that had not begun metamorphosis were sacrificed and measured. Survival was calculated as survival to GS 46 and as total survival. The reported survival and growth (length and weight) NOEC were both 12.8 ppb PFOS (or 0.0128 mg/L). The study is acceptable for qualitative use only, because of the test design (outdoor mesocosm exposure with algal and zooplankton communities present).

Brown et al. (2021) evaluated the chronic effects of PFOS-K (≥98% purity, CAS # 2795-39-3, purchased from Sigma-Aldrich) on northern leopard frogs (*Lithobates pipiens*) in a 10-day unmeasured, static-renewal study. Six egg masses were collected from ponds at the Purdue Wildlife Area in West Lafayette, Indiana. Tadpoles were maintained in outdoor wading pools and were fed rabbit chow ad libitum. A 500 mg/L stock solution was used to prepare PFOS exposure solutions of 10 ppb and 100 ppb. Twenty tadpoles of a median Gosner stage (GS) of 26.5 and median weight of 0.109 g were exposed to PFOS in 15 L tubs filled with 7.5 L solution made from filtered, ultraviolet-irradiated aged well water with a pH of 7.9, dissolved oxygen of 7.4 mg/L and specific conductivity of 579 µS/cm at 22°C under a 14:10 light-dark photoperiod. Tadpoles were given a one-day acclimation period prior to testing. During the exposure tadpoles were fed rabbit chow ad libitum and the experimental units were checked daily for mortality. A complete water change was done on day five and fresh chemical treatments were applied. After

10 days, tadpoles were transferred to clean water in the same 15-L bins. Tadpoles were held in clean water for seven days before parasite (*Echinoparyphium*) exposure to examine tadpole susceptibility to parasitic infection after PFOS exposure. PFOS-exposed tadpoles were assigned to individual 1-L cups filled with 0.5 L of clean water. Each tadpole was exposed to 50 trematode cercariae for six hours. The authors reported a NOEC value of 100 ppb (or 0.1 mg/L) for development stage, snout-vent length, weight, survival and parasite susceptibility. The study is acceptable for qualitative use only because of the short test duration.

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. Frog egg masses were collected from an ephemeral pond at the Purdue Wildlife area in West Lafayette, Indiana. Egg masses were held in covered 190-L outdoor tubs containing 80 L of well water. Once hatched, the larvae were fed ad libitum with Purina rabbit chow. A control (four replicates) and five replicates of measured exposure concentrations of 0.00006, 0.001 and 0.016 mg/L were set up as 180-L plastic wading pools filled with 75 L well water which contained 25 frogs in each. The stock solution was made by dissolving 0.5 g PFOS into 1 L of reverse osmosis Milli-Q water in polycarbonate bottles. Sediment was collected from the upper 5 to 8 cm of a permanent pond in the same wildlife area. The sediment was air dried for eight days, with 10.1 kg of the dried homogenized sediment placed in each experimental unit. Sediment was spiked with the assigned PFOS dose by adding the appropriate volume of stock solution to 6 L of water, stirred for five minutes, and then allowed to equilibrate for seven days. Once equilibrated, 75 L of water was added to the experimental chamber and allowed to sit for an additional three days. The water was then inoculated with algae and zooplankton from local

pond water and allowed to establish for five days, after which the Gosner stage 25 frog larvae were added to each tank. Reported average water quality conditions include pH of 7.8 and temperature of 26.2°C. 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 EPA was unable to fit a model with significant parameters. Therefore, given this was an outdoor mesocosm with spiked sediment and the addition of algal and zooplankton communities and 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 draft chronic water column criterion.

G.4 Summary of Plant PFOS Toxicity Studies Used Qualitatively in the Freshwater Aquatic Life Criterion

G.4.1 Cyanobacteria, Anabaena sp.

Rodea-Palomares et al. (2012) examined the toxicity of PFOS (98% purity) with the bioluminescent cyanobacterium, *Anabaena sp*. (CPB4337 strain) following the OECD Guidelines No. 23 (OECD 2000). The inhibition of constitutive luminescence was examined over a 24-hour test period. Very little detail was provided about the exposure details (i.e., test media, test vessel, cell density per replicate, water quality parameters). PFOS was dissolved in the exposure media with no solvent and was measured in the highest test concentration and one concentration near the reported EC_{50}. The cyanobacteria were exposed to five to seven test concentrations (specifics not provided) with replicate samples. Each test was repeated three times. The reported EC_{50} was 16.29 mg/L based on bioluminescence inhibition and was considered to be acceptable for qualitative use, based on the short test duration and lack of exposure details. However, the authors in a later publication tested the cyanobacterium again to

PFOS and it may be assumed that some of the missing test information for this earlier publication was the same.

Rodea-Palomares et al. (2015) conducted a similar 24-hour static, unmeasured test on PFOS (potassium salt, 98% purity) with the bioluminescent cyanobacterium, *Anabaena sp*. (CPB4337 strain). The test was performed in 1.5 mL of cyanobacterial growth media (AA/8+N, Allen and Arnon 1955) in transparent microtiter plates. The pH of the growth media was 7.8. The plates were incubated at 28°C under continuous illumination on a rotary shaker. The cyanobacteria in the log-growth phase were exposed to seven concentrations of PFOS-K which ranged from 0-200 mg/L. The description of how the test solutions were prepared was not provided, but it does not appear that a solvent was used. Each test was repeated three times. The reported EC50 was 83.51 mg/L based on bioluminescence inhibition and was considered to be acceptable for qualitative use, given the short test duration.

G.4.2 Green alga, Chlorella vulgaris

Xu et al. (2017) similarly conducted a 96-hour static acute algal growth inhibition test on PFOS (potassium heptadecafluoro-1-octanesulfonate; 98% purity) with *Chlorella vulgaris*. Toxicity was determined from the logarithmic growth phase of *C. vulgaris* inoculated into 100 mL conical flasks. At the beginning of the experiment, the density of the algae cells was approximately $7.0x10^5$ cells/mL in a total volume of 50 mL. The green alga was exposed to one of five nominal concentrations (0, 40, 80, 120, 160, 200 mg/L PFOS) that were verified by UHPLCMS/MS confirmation methods. Measured concentrations were not reported. Water quality conditions were also not reported by the authors other than photoperiod which consisted of a 12-hr:12-hr light:dark cycle. A number of endpoints were measured and included reactive oxygen species (ROS) production, catalase (CAT) activity, chlorophyll *a* and *b* content, superoxide dismutase (SOD) activity, cell permeability, and malondialdehyde (MDA) content.

The LOEC for decreased chlorophyll *a* content (40 mg/L) was considered qualitative for use because the study was lacking experimental details regarding nutrient medium, water quality, and exposure vessels.

G.4.3 Green alga, Raphidocelis subcapitata

Boltes et al. (2012) conducted a 72-hour static toxicity test on PFOS (potassium salt, CAS # 2795-39-3, 98% purity) with *Raphidocelis subcapitata* (identified as *Pseudokirchneriella subcapitata* in the test). The exposure included an unknown number of nominal PFOS concentrations. However, the toxicity test followed the algal growth inhibition test described in OECD TG 201 (OECD 2011). Algal beads of *R. subcapitata*, dissolving matrix and growing media were purchased from MicroBioTest Inc. (Belgium). Each test concentration had four replicates, with an unknown density of algal cells in the log growth phase incubated in plastic 96-well plates containing a small amount of test solution (200 µL). Details for how the test solutions were prepared were not provided, but it appeared no solvent was used. The only test condition reported was temperature (22 ± 2°C) with a continuous source of illumination. Optical density was recorded at 72 hours to calculate an EC_{50} (based on growth inhibition) of 35 mg/L. The limited exposure details and short exposure duration prevented the study from being used quantitatively.

Rosal et al. (2010) performed a 72-hour static, measured growth inhibition test with PFOS-K (98% purity) on the green alga, *Raphidocelis subcapitata* following OECD TG 201 Protocol (Note: the species name has changed since paper publication and is no longer *Pseudokirchneriella subcapitata*). While limited details are provided about the exposure, the authors stated they were following the OECD protocol. Green alga was cultured in 96-well microplates with a total volume of 200 µL. No solvents were used to make test solutions. Specific test concentrations were not provided, but the authors noted that nominal and measured

concentrations did not have significant deviations. The 72-hour growth inhibition EC_{50} of 35.0 mg/L PFOS was acceptable for qualitative use only because of the short test duration.

G.4.4 Green alga, Scenedesmus obliquus

Liu et al. (2008, 2009) published two studies examining the toxicity of PFOS to the green alga, *Scenedesmus obliquus*. In Liu et al. (2008), a 72-hour exposure was conducted to evaluate the effects on PFOS (acid form, CAS # 1763-23-1, purity not reported) at the cellular level, measured by flow cytometry. The test followed OECD (2002) methodology with *S. obliquus* that were obtained from the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences (Beijing). The number of test treatments was not provided but based on figures in the study, test concentrations ranged from approximately 60-350 µM PFOS (or 30-175 mg/L PFOS when converted based on a molecular weight of 500.13 g/mol). The authors did not provide details regarding how the PFOS treatments were prepared. Experiments were initiated by inoculating equal cell numbers of $1x10^4$ cells/mL into flasks containing a total volume of 20 mL of algal cell suspension per flask (with three replicates per each treatment). The algal test media was prepared according to OECD (2002) using deionized water and analytically pure chemicals, adjusted to pH 7.5 ± 0.2. Algal cells were incubated at 22 ± 1°C under cool-white lights (6,000 lux) with a 10-hrs:14-hrs light:dark cycle. The 72-hour IC_{50} (growth inhibition) of PFOS was 77.8 mg/L and 99.9 mg/L based on fluorescence and optical density at 650 nm, respectively. The plant values from the study were acceptable for qualitative use because of the short exposure duration (less than 96 hours) and the missing exposure details.

Under similar conditions **Liu et al. (2009)** conducted a 72-hour toxicity test on PFOS (≥98% purity based on dry mass) with the alga, but with lower test concentrations (0, 10, 20, 30, 40 mg/L). PFOS alone exhibited no inhibition on the growth rate of *Scenedesmus obliquus* within the concentration range of 10-40 mg/L. The PFOS concentration applied was in the range

where PFOS was previously found to not show growth inhibition but disturb the algal membrane properties in *S. obliquus* (Liu et al. 2008). Cells in exponential phase of growth collected from stock cultures were used for experiments. Experiments were initiated by inoculating equal cell numbers of $1x10^4$ cells/mL into flasks, containing a total volume of 200 mL of algal cell suspension per flask (with three replicates per each exposure treatment). The same growth media was used as in the previous experiment under the same test conditions. The PFOS stock solution was prepared in methanol and the working solution was obtained by 1,000 times of dilution of the stock solution into algal culture medium. The 72-hour NOEC (based on growth) was 40 mg/L and was acceptable for qualitative use because of the short exposure duration (less than 96 hours) and the missing exposure details.

G.4.5 Duckweed, Lemna gibba

Boudreau et al. (2002) performed a 7-day static acute algal growth inhibition test on PFOS (potassium salt, 95% purity) with duckweed, *Lemna gibba*. 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 test followed protocols found in ASTM E1415-91 (ASTM 1991), Greenberg et al. (1992) and Marwood et al. (2001). All treatment concentrations were based on the PFOS anion (without K) and solutions were prepared in laboratory-grade distilled water. Duckweed was obtained from laboratory culture maintained according to Marwood et al. (2001), and originally acquired from University of Waterloo. Toxicity testing consisted of five test treatments plus a negative control (0, 10, 20, 40, 80 and 160 mg/L) in 10 mL of Hunter's growing media in 60 x 15 mm polyethylene disposable petri dishes. There were four replicates per treatment, but the number of plants and fronds per plant were not reported. Tests were continuously illuminated with cool-white fluorescent light between 5,800 and 6,200 lux and incubated at 25±1°C. Endpoints used to determine inhibition of growth

were mean frond number and biomass, measured as wet weight. The most sensitive endpoint, wet weight, had a reported NOEC of 6.6 mg/L and an IC_{50}=31.1 mg/L. The plant values were acceptable for quantitative use.

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)
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)
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. (2001)
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)

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
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)
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 (2000c)
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 galloprovincialis*	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)

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
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)
White sunset shell (15.0-20.3 mm), *Soletellina alba*	S, M	28 d	PFOS-K Unknown	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 Unknown	8	19	30	MATC (growth - weight)	0.22-0.28	0.2482	Other PFAS measured in the sediment and water	Simpson et al. (2021)
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 (2000f)
Copepod (adult), *Nitocra spinipes*	S, M	10 d	PFOS-K Unknown	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 Unknown	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 Unknown	-	25	32	MATC (reproduction)	0.1-0.25	0.1581	Difficult to determine test methodology	Han et al. (2015)
Amphipod (adult), *Melita plumulosa*	S, M	10 d	PFOS-K Unknown	-	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 Unknown	8	19	30	NOEC (survival)	0.85->0.85	0.85	Other PFAS measured in the sediment and water	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
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)
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	Preus-Olsen et al. (2014)

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
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 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	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).

H.2 Summary of Acute PFOS Toxicity Studies Used Qualitatively

H.2.1 Saltwater Invertebrates

H.2.1.1 Echinoderms

Mhadhbi et al. (2012) conducted a 48-hour static, unmeasured acute test with PFOS (98% purity) on the sea urchin, *Paracentrotus lividus* (a non-North American species). A stock solution of PFOS was made either with filtered sea water from the Ria of Vigo (Iberian Peninsula) for low exposure concentrations, or with DMSO for high PFOS concentrations (at a final maximum DMSO concentration of 0.01% (v/v) in the test medium). However, they did not indicate what was considered a high test concentration. If a DMSO was used a solvent control was also included with the test. Sea urchins embryos were exposed to one of six nominal PFOS treatments (0.5, 1, 2, 5, 10 and 20 mg/L). Four hundred fertilized eggs (within 30 minutes of fertilization) were transferred to glass vials containing 10 mL of test solutions with four replicates per PFOS treatment and five replicates per control. Vials were incubated at 20°C in the dark for 48 hours. At test termination samples were fixed in formalin and 35 larvae per vial was measured for growth (length). The 48-hour EC_{50} (growth inhibition) was 20.0 mg/L and was acceptable for qualitative use due to the atypical acute endpoint and short test duration.

Anselmo et al. (2011) conducted a 16-day renewal toxicity test on the potassium salt of heptadecafluorooctane sulfonic acid (PFOS, CAS #: 2795-39-3, ≥98% purity) with the sea urchin, *Psammechinus miliaris* (a non-North American species). Acute endpoints were extrapolated from this longer test at 72 hpf, after feeding had been initiated. Sea urchins were collected from the Eastern Scheldt (The Netherlands) and maintained for at least two months before use. The eggs and sperm used in the study were collected from the freshly dissected gonads of a single pair of adult individuals. Fertilized eggs were randomly divided into glass beakers containing 500 mL of fresh seawater or aged artificial seawater (this specific water used

was not defined), and spiked with the appropriate test concentrations or a solvent control DMSO at 0.1% v/v. The number of organisms per beaker was defined as ± 0.5 larvae per mL, with two samples of 20 larvae each at each test measurement (16 and 72 hpf). Beakers were held at 19±1°C with a photoperiod of 16-hr:8-hr (light:dark); no other water quality parameters were reported. A primary stock solution was prepared with DMSO, and nominal test concentrations were 93, 186, 372 and 743 nM PFOS (or 0.0501, 0.1001, 0.2002, 0.3999 mg/L PFOS, when converted based on a molecular weight of 538.22 g/mol). While test concentrations were not measured in the solutions, measured larval tissue concentrations demonstrated a concentration-response relationship. At 72 hpf, the highest test concentration 0.3999 mg/L PFOS-K had no effect on morphological abnormalities. The 3-day NOEC of 0.3999 mg/L PFOS was acceptable for qualitative use because of the short test duration and atypical endpoint.

Anselmo et al. (2012) also exposed *Psammechinus miliaris* to PFOS for 85 minutes under static, unmeasured conditions. Again, sea urchins were collected from the Eastern Scheldt (The Netherlands) and maintained for at least two months before use. The eggs and sperm used were collected from the freshly dissected gonads of a single pair of adult individuals. Tests were initiated with larvae 18-20 hours post-fertilization (hpf) in the gastrula stage. PFOS stock solutions were made with DMSO (0.5% v/v) and diluted 20 times in artificial sea water (Instant Ocean) and 25 µl of each dilution was added to 225 µl of ASW present in the respective well. All nominal test concentrations (DMSO control, 0.2, 2, 20, 80, 160 and 320 µM PFOS, or 0.1076, 1.076, 10.76, 43.06, 86.12, and 172.2 mg/L PFOS when converted based on a molecular weight of 538.22 g/mol) were tested in triplicate and each test was replicated twice. The only water quality parameter reported was temperature, 19 ± 1°C. A total of 12-15 larvae at the gastrula stage were placed in each well of a 24 well plate. The 24 well plate was then placed on a

rocking shaker (30 rpm) for 45 minutes exposure, then calcein-AM was added to obtain a final concentration of 2.5 µM covered with foil and incubated for an additional 40 minutes. The accumulation of the cellular fluorescent calcein was a measure of cellular efflux pump inhibition and calcein accumulation increased in a concentration-dependent manner. The 172.2 mg/L PFOS concentration was noted as being acutely toxic to the sea urchin and not used in the curve fitting. No additional details were provided about the toxic response. The 85-minute IC_{50} based on cellular efflux pump inhibition was 1.399 mg/L PFOS-K. The value was acceptable for qualitative use only, due to the atypical endpoint and exposure duration.

H.2.1.2 Mollusks

Drottar and Krueger (2000c) reported the results of a 96-hour static, measured test on the effects of PFOS (potassium salt, 90.49% purity) on the eastern oyster, *Crassostrea virginica*. The GLP test was conducted at Wildlife International, Ltd. in Easton, MD in October, 1998, using a protocol based on procedures outlined in U.S. EPA, OPPTS Number 850.1025; and ASTM E729-88a (1988). Oysters (27.8-41.5 mm) were obtained from P. Cummins Oyster Company, Baltimore, MD and held for 12 days in the same water used for testing before exposure. The unfiltered natural seawater used for testing was collected at Indian River Inlet, Delaware and diluted to a salinity of approximately 20 ppt with well water. This water was supplemented with an algal suspension continuously during holding and testing to supplement the oyster diet and enhance their condition and growth. Test chambers were 52 L polyethylene tanks containing 40 L of consistently aerated test solutions. A primary stock solution was prepared in dilution water at 9.1 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. The test employed one replicate of 20 oysters each in five measured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 1.2,

2.0, 3.3, 5.5 and 9.1 mg/L. Mean measured concentrations were less <0.115 mg/L, 0.36, 0.40, 1.3, 1.9, and 3.0 mg/L, respectively. There was poor percent recovery across treatments (only 29-39% of nominal). Analyses of test solutions were performed at Wildlife International, Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Dissolved oxygen ranged from 6.1-7.7 mg/L; pH ranged from 7.5-8.1; test temperature ranged from 22.0-22.7°C across all treatments. There was no mortality in the oysters in the negative control and across all treatments and all appeared healthy and normal. The focus of the acute exposure was shell growth. There was significant inhibition of shell growth in the highest test concentration, but that concentration failed to reduce at least a 50% inhibition in growth. Thus, the 96-hour EC_{50} was >3.0 mg/L. This acute value was acceptable for qualitative use because of the lack of replication, atypical endpoint for an acute exposure and the poor percent recovery of the test material in the test exposures.

Aquilina-Beck et al. (2020) conducted short-term, sublethal exposures of two and seven days to examine the effects of a technical mixture of PFOS (linear and branched isomers) in adult Eastern oysters, *Crassostrea virginica*. Adult oysters (70-100 mm in length) were collected in the fall and winter seasons from a reference site commonly used as a control site at the mouth of Leadenwah Creek at North Edisto River on Wadmalaw Island, SC. Seawater for testing was collected from Charleston Harbor estuary, filtered (5 µm), UV-sterilized, activated carbon filtered (5 µm), and diluted with deionized water to 20 ppt salinity. Cleaned oysters were placed in a controlled laboratory aquatic recirculating system to acclimate for 14 days to test conditions (25°C, 20 ppt salinity, and 16-hour light:dark cycle). Oysters were fed 10 mL of commercial shellfish diet daily until the day of exposure. Oyster length and width were measured and recorded before exposures. For the 48 hour static acute exposure, a primary stock solution of

PFOS (Santa Cruz Biotechnology, CAS # 1763-23-1, purity ≥97%) was prepared in deionized water at 10,000 mg/L. Individual oysters were exposed to 1 L of PFOS treatment in seawater at 0 (control), 3, 30, and 300 mg/L in a glass beaker. After 48 hours, oysters were shucked and whole tissue wet weight (ww) was recorded. The hepatopancreas (HP) was dissected, weighed, and divided into three sections: two of which were frozen in liquid nitrogen for downstream biomarker analysis, and the third section was processed immediately for lysosomal destabilization. PFOS 48-hour exposure experiments and biomarker analyses were repeated three times with four or six replicates per treatment group, twice in the fall and once in the winter. A combined total of 16 oysters per treatment group were tested. Water quality parameters were recorded at the beginning and end of the treatment and were within acceptable test conditions (mean values ± standard deviation: Temp.: 24.9 ± 0.4°C; D.O.: 4.3 ± 2.2 mg/L; Salinity: 20 ± 0.2 ppt; and pH: 7.5 ± 0.3). Biomarker analysis (lysosomal destabilization, lipid peroxidation, and glutathione assays) in oyster tissue revealed no significant damage to lipid membranes or the glutathione phase II enzyme system up to 300 mg/L PFOS; however, significant cellular lysosomal damage was observed at 3 mg/L (LOEC; lowest treatment level). Apical measurements (survival, growth) were not reported or measured during either test. Thus, the 48-hour LOEC for cellular lysosomal damage was 3 mg/L. The value is considered qualitatively based on atypical test endpoints.

H.2.1.3 Crustaceans

Drottar and Krueger (2000f) conducted a static, measured 96-hour acute toxicity test with PFOS (potassium salt, 90.49% purity) on the mysid, *Americamysis bahia* (formerly known as *Mysidopsis bahia*). The GLP test was conducted at Wildlife International, Ltd. in Easton, MD in March 1999, using a protocol based on procedures outlined in U.S. EPA, OPPTS Number 850.1035; and ASTM E729-88a (1988). Mysids were obtained as juveniles from in-house

cultures and were fed brine shrimp daily to prevent cannibalism. The unfiltered natural seawater used for testing was collected at Indian River Inlet, Delaware and diluted to a salinity of approximately 20 ppt with well water. Test chambers were 2 L polyethylene buckets containing approximately 1 L test solutions. A primary stock solution was prepared in dilution water at 8.2 mg/L and was mixed for ~22 hours prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the four additional test concentrations. The test employed two replicates of 10 mysids each in five measured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 1.1, 1.8, 3.0, 4.9 and 8.2 mg/L. Mean measured concentrations were less <0.115 mg/L, 0.57, 1.1, 1.9, 3.0, and 5.4 mg/L, respectively. There was poor test material percent recovery across treatments (at 96 hours only 36-71% of nominal). Analyses of test solutions were performed at Wildlife International, Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Dissolved oxygen ranged from 6.6-8.8 mg/L; pH ranged from 8.1-8.2; test temperature ranged from 23.5-25.4°C across all treatments. There was no mortality in the negative control and the two lowest test concentrations and mysids appeared healthy and normal. The 96-hour LC_{50} was 3.6 mg/L PFOS and was acceptable for qualitative use

H.2.2 Saltwater Fish

H.2.2.1 Gadus morhua

Preus-Olsen et al. (2014) conducted a flow-through pulsed exposure with PFOS (technical grade) on the Atlantic cod (*Gadus morhua*). Juvenile fish were exposed to one of three nominal PFOS treatments (0, 0.1 and 0.2 mg/L PFOS) for one hour per day for five consecutive days and then further challenged to three different CO_2 levels [normocapnia, moderate (0.3%) and high (0.9%)]. The focus of this assessment was on the normal CO_2 levels, normocapnia. Juvenile fish were purchased from Atlantic Cod Juveniles (Risa, Norway) and acclimated to

laboratory conditions (10°C, 12-hr:12-hr light:dark photoperiod) in flow-through tanks with circulating seawater for 14 days. After acclimation, 120 fish were transferred to each tank and exposed to one of three concentrations of PFOS, afterwards groups of 40 fish per treatment were exposed to one of the three CO_2 conditions. At 3, 6 and 9 days after CO_2 exposure, five fish were sampled for PFOS burden, steroid hormone and gene expression. Throughout the exposure pH was maintained at 7.7, and salinity was 33.8 ppt in the normocapnia condition. While water PFOS concentrations were not measured, tissue concentration in the sampled fish increased with increasing PFOS concentrations. Mortality and growth data were not provided by the study authors, but they noted that no significant differences in survival and growth maintenance (length, weight, condition factor) between exposure groups and sampling days were observed. The 5-day NOEC, 0.2 mg/L, based on survival and growth, was acceptable for qualitative use only, due to the pulsed exposure regime.

H.2.2.2 Psetta maxima

Mhadhbi et al. (2012) conducted a 6-day renewal, unmeasured acute test with PFOS (98% purity) on the turbot, *Scophthalmus maximus*, (formerly, *Psetta maxima*; a non-North American species). A stock solution of PFOS was made either with filtered sea water from the Ria of Vigo (Iberian Peninsula) 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, they did not indicate what was considered a high test concentration. If a DMSO was used a solvent control was also included. Fish were exposed to one of ten nominal PFOS treatments (0.015, 0.03, 0.075, 0.15, 0.3, 0.325, 0.6, 1.2, 2.5 and 5 mg/L). Turbot eyed eggs from a single stock of adults were supplied by a nearby fish hatchery (PESCANOVA Insuina) and acclimated to laboratory conditions before use. At 72 hours post-fertilization (hpf), the floating fertilized eggs were collected and the non-fertilized eggs at the bottom discarded. Embryos that

had reached the blastula stage were used for testing. Fifty normal embryos were added to glass beakers containing 500 mL of test solution. Each treatment had four replicates and were incubated in the dark for six days at 18°C with no food or aeration provided. Dead embryos and larvae were removed daily. Endpoints included dead embryos, malformation, hatch success at 48 hours and larvae survival (missing heartbeat and a non-detached tail) at six days. The 6-day LC_{50} of 0.11 mg/L PFOS was acceptable for qualitative use because of the atypical acute test duration.

H.3 Summary of Chronic PFOS Toxicity Studies Used Qualitatively

H.3.1 Saltwater Invertebrates

H.3.1.1 Worms

Sakurai et al. (2017) report the results of a 7-day renewal, measured test of PFOS (purity not provided) with the marine sandworm, *Perinereis wilsoni.* The focus on the study was on the uptake and depuration kinetics of PFOS and was not a toxicity test. Sandworms were obtained from as aquaculture farm and acclimated to laboratory conditions for four days without feeding. About eight sandworms were held in polypropylene containers with holes in bottom and packed with gravel [from bottom to top with approximately 25 mm high layer of gravel (6–8 mm), 0.5 mm mesh polyethylene netting, and approximately 70 mm high layer of gravel, (3–6 mm)]. Dilution water (filtered natural seawater) flowed freely through the gravel and the gravel was too large to be ingested. There were 26 replicates for the exposure treatment and eight replicates for the control. A methanol stock solution of PFOS added to seawater and diluted to achieve 32 ng/L (or 0.000032 mg/L) PFOS for the exposure treatment. The control received a methanol-spiked seawater solution (0.35 mg/L), to mimic the same methanol concentration as the PFOS treatment. Organisms were not fed during the exposure period and the lighting cycle was approximately 10 hours light and 14 hours dark. Solutions were renewed daily with water levels in the tanks being varied to mimic tidal action. Measured PFOS concentrations (0.000028 mg/L)

were similar to nominal concentrations during the exposure period. Water quality conditions were monitored daily: 91% D.O. saturation, 8.1 pH, 17.1°C water temperature, and 36‰ salinity. Mortality was 2.2% and 6.8% for the exposure and control treatment respectively, and growth did not differ between treatments. As this study was focused on uptake and depuration, the toxicity endpoints were not statistically analyzed in the paper. Since there was only one exposure treatment and an atypical exposure period, the NOEC (0.000028 mg/L PFOS) based on survival was acceptable for qualitative use only.

H.3.1.2 Echinoderms

Ding et al. (2015) conducted a PFOS-K (98% purity) 21-day renewal, unmeasured toxicity test with the sea urchin, *Glyptocidaris crenularis* (a non-North American species). Adults, about 20 months old, were purchased from Dalian HaiBao Fishery Company and held in a recirculating aquaculture system for two months before use in testing. Healthy individuals, 5 cm in diameter and 80.3 g wet weight, were exposed to one of four nominal PFOS concentrations (0, 0.01, 0.1 and 1 mg/L). The number of sea urchins per treatment was not identified, nor any details about treatment replication. Sea urchins were held in 5 L plastic barrels with 3 L of test solutions for 21 days at a temperature of 13 ± 1°C, salinity of 30 ± 2 psu, pH about 8.1 and a photoperiod of 12 Test solutions were 50% renewed every two days and sea urchins were fed *Laminaria japonica* every other day. After the 21-day exposure period test organisms were then held for another seven days under the same test conditions. Mortality and sublethal toxicity effects were observed daily, enzymatic activity was measured on days 1, 3, 5, 7, 14, 21, and 28 and DNA methylation was measured on days 7, 13, 21, and 28. No mortality was observed throughout the exposure and observation period in any treatment. However, the SOD activity in the coelomic fluid decreased significantly in the lowest test treatment (0.01 mg/L PFOS) when compared to the control at 21 days. SOD activities increased in the

depuration period in all PFOS treatments, but were still significantly lower than the controls. The 21-day + 7-day observation LOEC based on SOD activity of 0.01 mg/L and the NOEC based on morality of 1 mg/L were both acceptable for qualitative use. This study was qualitative because of the lack of details about the number of organisms, the short test duration, and starting age of organisms.

Anselmo et al. (2011) performed a 16-day renewal, unmeasured PFOS-K toxicity test (≥98% purity) with embryos of the sea urchin, Psammechinus miliaris (a non-North American species). Sea urchins were collected from the Eastern Scheldt (The Netherlands) and maintained for at least two months before use. The eggs and sperm used were collected from the freshly dissected gonads of a single pair of adult individuals. Fertilized eggs were randomly divided into glass beakers containing 500 mL of fresh seawater or aged artificial seawater (this specific water used was not defined), and spiked with the appropriate test concentrations or a solvent control (DMSO at 0.1% v/v). The number of organisms per beaker was defined as ± 0.5 larvae per mL, with two samples of 20 larvae each at each test measurement (72 hpf and 16 dpf). Mollusks

Nalbantlar and Arslan (2017) conducted a 30-day renewal, unmeasured toxicity test on PFOS (analytical grade) with the Mediterranean mussel, *Mytilus galloprovincialis*. Mussels were collected from an area free of wastewater inputs off the Turkish Aegean coast. They were acclimated to laboratory conditions over six days in artificial seawater (5.1 ± 0.1 mg/L dissolved oxygen, 8.1 ± 0.1 pH and 34.5 ± 0.2 psu), which was continuously aerated at a temperature of 17.5 ± 1°C. A total of 10 mussels per replicate, with a mean body length of 6.4 cm, were added to glass aquaria with four replicates per treatment. PFOS was dissolved in DMSO and five test concentrations were selected based on 1/10 and <1/10 the 96-hour LC_{50} (2, 3, 4, 5 and 6 mg/L PFOS). The amount of DMSO used was not provided, but experiments included both a dilution

water control and solvent control. Mussels were exposed to PFOS for 30 days, with feeding (*Chlorella* sp.), and water renewals every two days. At the end of the exposure period gill and hemolymph cell samples were taken and examined for nuclear aberrations (frequency of micronuclei and binuclei). Survival of mussels were not reported for the controls or any treatment. The most sensitive endpoint was an increase in the frequency of micronuclei aberrations in the gill cells with all PFOS treatments containing significantly more aberrations than the control and solvent control. The 30-day LOEC (based on micronuclei nuclear aberrations in gills cells) was 2 mg/L PFOS and was acceptable for qualitative use, due to the atypical exposure endpoint.

Liu et al. (2014a, b, c) and Liu and Gin (2018) conducted a series of 7-day renewal, measured experiments with perfluorooctanesulfonate potassium salt (PFOS-K, 98% purity) on the green mussel, *Perna viridis* (a non-North American species). All of these studies employed a similar test design, but with each publication providing different level of test details. In **Liu et al. (2014a)**, green mussels were obtained from a local fish farm and acclimated to laboratory conditions prior to PFOS exposure. Adult organisms were exposed in 70 L polypropylene tanks in artificial seawater at a temperature of 25°C and at salinity of 30 ppt. Mussels were exposed to one of five nominal PFOS concentrations (0.0001, 0.001, 0.010, 0.1 and 1.0 mg/L) or control. Each tank contained 60-65 mussels, with two tanks per exposure concentration or control. During exposures mussels were fed with microalgae and each tank was cleaned and refilled every two days. After seven days of exposure and seven days of depuration, various biomarkers were measured. The EC_{50} (integrative genotoxicity) was reported as 0.033 mg/L PFOS and was based on three genotoxic endpoints (DNA fragmentation and single strand breaks (comet assay),

chromosomal breaks (micronucleus test) and apoptosis (DNA diffusion assay). The atypical duration and endpoint resulted in the value to be considered acceptable for qualitative use only.

In **Liu et al. (2014b)**, the oxidative damage of PFOS-K (98% purity) to green mussels was assessed after seven days under similar conditions as Liu et al. (2104a). Green mussels were obtained from a local fish farm in Singapore and acclimated to laboratory conditions for one week prior to exposures. Organisms (60-65 mm) were exposed in polypropylene tanks containing artificial seawater at a temperature of 25°C and at salinity of 25 ppt. Mussels were exposed to one of six nominal PFOS concentrations (0.0001, 0.001, 0.010, 0.1, 1.0 and 10.0 mg/L) or control. Nominal concentrations were similar to measured concentration (0.00012, 0.0011, 0.0096, 0.106, 0.968 and 10.156 mg/L, respectively) and no PFOS was detected in the controls. Each treatment was replicated with an unknown number of mussels per replicate. Again, during the exposure mussels were fed with microalgae and each tank was cleaned and refilled every two days. The most sensitive parameters to PFOS were activation of antioxidant enzymes (catalase [CAT] and superoxide dismutase [SOD]), which is an adaptive response to the excessive reactive oxygen species. Significant effects were observed at 0.968 mg/L PFOS, but not at 0.106 mg/L. The 7-day MATC (CAT and SOD activity) was 0.3203 mg/L and was acceptable for qualitative use based on the atypical endpoint and duration.

Liu et al. (2014c) utilized a similar test design and the same nominal PFOS concentrations as Liu et al. (2014a). However, in this study the test tanks were reported as 50 L in size and containing 40 mussels per each exposure tank, and the 7-day exposure was not followed by a 7-day depuration phase (similar to Liu et al. 2104b). The 7-day NOEC and LOEC based on the relative condition factor (relationship between weight and length) was 0.0096 and

0.106 mg/L, respectively. The 7-day MATC of 0.0319 mg/L was acceptable for qualitative use because of the atypical test duration.

Liu and Gin (2018) employed the same test design and measured PFOS concentrations as Liu et al. (2014a). The most sensitive biomarker endpoint reported was hemocyte cell viability with a reported NOEC and LOEC of 0.0096 and 0.106 mg/L, respectively. Again, the MATC of 0.0319 mg/L PFOS was acceptable for qualitative use because of the atypical test duration.

Simpson et al. (2021) evaluated the chronic effects of perfluorooctane sulfonic acid potassium salt (PFOS-K, purchased from Sigma Aldrich) on the deposit-feeding bivalve, *Tellina deltoidalis*, (8.1-18.9 mm shell length) using 28-day growth and survival tests. The bivalves were collected from the estuarine mud flats of the Lane Cove River, adjacent to Boronia Park, Hunters Hill, New South Wales (NSW). Clean seawater (salinity 33±2 ppt) for culturing was sourced from the southeast coast of New South Wales (NSW), Australia, and clean sediments were from Bonnet Bay, NSW. Organisms were acclimated 10 days before starting experiments. Bivalves were randomly assigned to a specific replicate of each treatment, labelled, weighed (wet mass) and sized (dimensions by scanning as below) and then distributed to the corresponding test vessel. The test was comprised of three treatments plus a solvent control. The tests were undertaken with laboratory-controlled conditions (dissolved oxygen >85% saturation, pH 8.0±0.1, salinity 30±0.5 ppt, temperature 19±1ºC and ammonia <10 mg NH_3-N/L) over 28 days. Sediments (PFOS-spiked and control) were re-homogenized immediately prior to being added to test vials (80 g whole sediment per 250 mL polypropylene vial) and then equilibrated for 48 h before tests were started. Bivalves were exposed in triplicate and fed once a week during the exposure. At the end of the exposure the sediments were gently sieved to isolate bivalves. The differences in wet mass and size (length and surface area) of bivalves measured at start and

completion of tests (for surviving organisms) were used to assess growth. Bivalve growth was expressed as average percent change in wet mass, shell length and surface area. There was no effect on survival of the bivalve in the treatment with the highest concentration of PFOS in the overlying water of 0.28 mg/L PFOS (NOEC). The author-reported 28-d growth (wet mass; most sensitive endpoint) NOEC and LOEC were 0.22 and 0.28 mg/L PFOS, resulting in a 28-d growth MATC of 0.2482 mg/L. Given the presence of other PFAS measured in the sediments and overlying water at very low concentrations, this study was considered a mixture. The other PFAS made up a much smaller percentage of the total PFAS and were generally in low concentration, and thus, the result from the study is considered qualitative use only.

Simpson et al. (2021) evaluated the chronic effects of perfluorooctane sulfonic acid potassium salt (PFOS-K, purchased from Sigma Aldrich) on the deposit-feeding bivalve, *Soletellina alba* (15.0-20.3 mm shell length) using a 28-day survival test. The bivalves were collected from the estuarine mud flats of the Lane Cove River, adjacent to Boronia Park, Hunters Hill, New South Wales (NSW). Clean seawater (salinity 33 ± 2 ppt) for culturing was sourced from the southeast coast of New South Wales (NSW), Australia, and clean sediments were from Bonnet Bay, NSW. Organisms were acclimated 10 days before starting experiments. Bivalves were randomly assigned to a specific replicate of each treatment, labelled, and then distributed to the corresponding test vessel. The test was comprised of a single treatment plus a solvent control under laboratory-controlled conditions (dissolved oxygen >85% saturation, pH 8.0 ± 0.1, salinity 30 ± 0.5 ppt, temperature 19 ± 1ºC and ammonia <10 mg NH_3-N/L) over 28 days. Sediments (PFOS-spiked and control) were re-homogenized immediately prior to use. The exposure occurred in 2 L HDPE wide-mouth bottles (Nalgene) each containing approximately 500 g of sediment and 1 L of overlying seawater. Bivalves were exposed in triplicate and fed once a week

during the exposure. At the end of the exposure bivalves were isolated gently from the sediments. Survival of bivalves in each treatment were calculated as the mean mortality of each replicate while dead organisms were determined as no movement in 1-2 min by gentle touch. There was no effect on survival of the bivalve in the treatment with a measured overlying water concentration of 0.85 mg/L PFOS (NOEC). Given the presence of other PFAS measured in the sediments and overlying water at very low concentrations, this study was considered a mixture. The other PFAS made up a much smaller percentage of the total PFAS and were generally in low concentration, and thus, the result from the study is considered qualitative use only.

Aquilina-Beck et al. (2020) conducted short-term, sublethal exposures of two and seven days to examine the effects of a technical mixture of PFOS (linear and branched isomers) in adult eastern oysters, *Crassostrea virginica*. Adult oysters (70-100 mm in length) were collected in the fall and winter seasons from a reference site commonly used as a control site at the mouth of Leadenwah Creek at North Edisto River on Wadmalaw Island, SC. Seawater for testing was collected from Charleston Harbor estuary, filtered (5 μm), UV-sterilized, activated carbon filtered (5 μm), and diluted with deionized water to 20 ppt salinity. Cleaned oysters were placed in a controlled laboratory aquatic recirculating system to acclimate for 14 days at 25°C, 20 ppt salinity, and 16-hour light: 8-hour dark cycle. Oysters were fed 10 mL of commercial shellfish diet daily until the day of exposure. Oyster length and width were measured and recorded before exposures. For the 7-day biouptake and depuration exposure, 24 oysters collected in the fall were kept in individual glass beakers containing 1-L volume of PFOS (Santa Cruz Biotechnology, CAS 1763-23-1, purity ≥97%) concentrations at 0 (control), 0.3, and 3 mg/L. Eight replicates were included for each treatment and control group. Each exposure concentration and control seawater were replaced daily for seven days. Mortality was assessed daily, and water-quality

measurements were recorded. Water-quality parameters for all treatments during exposures were within acceptable test conditions (mean values ± standard deviation: Temp.: 25.3 ± 0.5°C; D.O.: 5.1 ± 1.4 mg/L; Salinity: 20.0 ± 0.4 ppt; and pH: 7.7 ± 0.3). After seven days of exposure, half of the oysters from each treatment group were shucked and whole tissue was flash frozen in liquid nitrogen. The remaining four oysters were placed into clean 20 ppt seawater and allowed to depurate for 48 hours. At the end of 48-hour depuration, oysters were shucked, flash frozen in liquid nitrogen, and stored at -80°C. All 24 oysters were processed for chemical analysis. Additionally, 1 mL water samples were taken for PFOS analysis from each treatment group on day two of the seven day exposure and 24 hours later (day three) to represent a 1-day dose. The BAF calculated on day seven of the exposure revealed that oysters had incorporated 50 times and 116 times the level of PFOS in the 0.3 and 3 mg/L PFOS treatments, respectively. Since the study is less than 28 days and the authors did not demonstrate that steady-state was achieved, the BAFs are considered qualitative only.

H.3.1.3 Crustaceans

A 21-day renewal, unmeasured PFOS-K (>98% purity) toxicity test with the Chinese mitten crab, *Eriocheir sinensis* (a non-North American species) was evaluated by **Zhang et al. (2015)**. Healthy crabs (11.89 ± 1.2 g) were purchased from a commercial crab hatchery on Chongming Island (Shanghai, China) and acclimated to laboratory conditions for two weeks in 120 L plastic tanks. During acclimation and testing pre-aerated municipal tap water was kept at 18-22°C, 8.3-8.6 pH, 0.3‰ salinity, and >6.5 mg/L dissolved oxygen. After acclimation, 20 crabs per replicate were transferred to tanks and exposed to five nominal concentrations (0, 0.01, 0.1, 1.0 and 10 mg/L PFOS-K). There were three replicates for each treatment with a total of 60 crabs per treatment. Three crabs were randomly sampled from each replicate at day 0, 1, 4, 7, 14 and 21 and total hemocyte count, lysozyme activity levels, phenoloxidase activity levels, acid

phosphatase activity levels, alkaline phosphatase activity levels, lysozyme gene mRNA expression levels, hepatopancreas-specific C-type lectin gene expression levels and prophenoloxidase activating factor in the hepatopancreas mRNA expression levels were measured in the hemolymph. Survival on control and treatments was not provided. The total hemocyte count was the most sensitive endpoint with a reported NOEC and LOEC of 0.01 and 0.1 mg/L, respectively. The 21-day MATC, based on total hemocyte count, of 0.03162 mg/L PFOS was acceptable for qualitative use because of the short test duration and atypical endpoint.

Park et al. (2015) conducted a 7-day renewal, unmeasured toxicity test on PFOS (98% purity) with the mud crab, *Macrophthalmus japonicus* (a non-North American species). The test organisms (average length of 3 cm) were purchased from the Yeosu Aquatic Products Market (Jeonnam, Korea) and acclimated for at least seven days to laboratory conditions. Crabs were acclimated to a temperature of 20 ± 1°C, salinity of 30‰, and an alternating 12-hrs: 12-hrs light:dark schedule with daily water changes and constant aeration. Adult, non-damaged specimens were then used for testing in glass containers and natural seawater (source of dilution water not provided). A primary stock solution was prepared with analytical grade acetone. In acetone controls, solvent was added at an actual test concentration of <0.1% acetone. Fifteen crabs per replicate were exposed to one of three PFOS concentrations (0.001, 0.010 or 0.03 mg/L) or a solvent control. Each treatment was replicated three times. Exposures lasted seven days, but survival was also recorded at 96 hours. The author did not calculate an LC_{50}, but at 96 hours there was 64% survival in the highest test concentration. Crabs in the solvent control had 96% survival over the entire test duration. The 96-hour LC_{50} was >0.03 mg/L and was acceptable for qualitative use since no point estimate was calculated and only three exposures were used.

The main focus of the study was to determine the effects of PFOS on the molecular transcription of antioxidant and detoxification signaling.

Simpson et al. (2021) evaluated the chronic effects of perfluorooctane sulfonic acid potassium salt (PFOS-K, purchased from Sigma Aldrich) on the smooth sentinel crab, *Macrophthalmus* sp., (carapace width of 6-15 mm) using a 28-day survival test. The crabs were collected from the estuarine mud flats of the Lane Cove River, adjacent to Boronia Park, Hunters Hill, New South Wales (NSW). Clean seawater (salinity 33±2 ppt) for culturing was sourced from the southeast coast of New South Wales (NSW), Australia, and clean sediments were from Bonnet Bay, NSW. Organisms were acclimated 10 days before starting experiments and were held in trays (16 × 12 cm) with approximately 3 cm depth of sediment and 4 cm of seawater, under laboratory-controlled conditions. Crabs were randomly assigned to a specific replicate of each treatment, labelled, and then distributed to the corresponding test vessel. The test was comprised of a single treatment plus a solvent control under laboratory-controlled conditions (dissolved oxygen >85% saturation, pH 8.0 ± 0.1, salinity 30 ± 0.5 ppt, temperature 19 ± 1ºC and ammonia <10 mg NH_3-N/L) over 28 days. Sediments (PFOS-spiked and control) were re-homogenized immediately prior to use. The exposure occurred in 2-L HDPE wide-mouth bottles (Nalgene) each containing approximately 500 g of sediment and 1 L of overlying seawater. Crabs were exposed in triplicate and fed once a week during the exposure. Survival of crabs in each treatment were calculated as the mean mortality of each replicate while dead organisms were determined as no movement in 1-2 min by gentle touch. There was no effect on survival of the bivalve in the treatment with a measured overlying water concentration of 0.85 mg/L PFOS (NOEC). Given the presence of other PFAS measured in the sediments and overlying water at very low concentrations, this study was considered a mixture. The other PFAS made up a much

smaller percentage of the total PFAS and were generally in low concentration, and thus, the result from the study is considered qualitative use only.

Simpson et al. (2021) evaluated the chronic effects of perfluorooctane sulfonic acid potassium salt (PFOS-K, purchased from Sigma Aldrich) on the epibenthic amphipod *Melita plumulosa* from previously-established cultures. Clean seawater (salinity 33 ± 2 ppt) for culturing was sourced from the southeast coast of New South Wales (NSW), Australia, and clean sediments were from Bonnet Bay, NSW. A total of sixteen different PFOS-spiked sediments were prepared, each having varying PFOS concentrations and sediment properties, by adding a mass of PFOS-K into a 250 mL HDPE container with 5-10 mL of ethanol added to dissolve it. Filtered NSW seawater was added (10 mL/mL methanol) and PFOS-K methanol/seawater suspension immediately poured onto the top of the wet sediment and homogenized. The PFOS concentrations in all sediments and porewaters of selected sediments were determined prior to toxicity testing. The non-spiked controls were prepared in the same manner. PFOS treatments used for toxicity tests were prepared from dilutions of the PFOS-spiked sediments with clean sediment (95% <63 μm) and sand (0% <63 μm) that were then equilibrated for a further 1-2 weeks before testing. A 10-day amphipod survival-reproduction test was used to measure adult (male and female) survival and the number of embryos and <1 day old juveniles in the second brood. Sediments were re-homogenized immediately prior to being added to test vials (40 g whole sediment and 200 mL seawater per 250 mL vial) and then equilibrated for 48 hours before tests were started. Each sediment tested had four replicates. Filtered seawater (<0.45 μm, 30‰) was added, and each beaker was incubated at 21°C with aeration for 1-week prior to commencing the tests. The overlying water was not changed prior to tests commencing and was not renewed during the tests. Six gravid females (gravid for <24 hour) and six males were

randomly assigned to each beaker. Treatments were fed at a rate of 0.5 mg Sera Micron fish food per amphipod twice a week. The sediments were renewed after five days. On day 10, the adults were removed and counted (survival), and the number of embryos per female was counted by microscopy. The total number of embryos and <1-day-old juveniles were summed and expressed as a percentage of the control. Subsamples of overlying seawater were taken on Day 1, and at three or four other times during the tests were combined to analyze the time-weighted average concentration of PFOS. A 28-day test was also run for the amphipod using a single nominal PFOS-spiked sediment at 50 mg/kg. The 28-day amphipod test had multiple phases to collect test endpoints: (1) 28-day survival of adults; (2) juveniles produced during days 0-9 and 9-20; (3) adult reproduction after an initial 20-day exposure to PFOS-contaminated sediments; and (4) 10-day survival and growth (body length) of juveniles that had been born between day 9-20. For the purpose of this document, EPA considered only the results from the 10-day test in lieu the 28-d multiple phases test due to the single sediment concentration used in the latter and inability to obtain chronic point estimates from the 28-day test. The author-reported 10-day LC_{10} (adult survival) was 2.0 mg/L PFOS and 10-day EC_{10} for reproduction (number of embryos and <1 d-old juveniles in the second brood; most sensitive endpoint) was 0.9 mg/L PFOS. Given the presence of other PFAS measured in the sediments and overlying water at very low concentrations, this study was considered a mixture. The other PFAS made up a much smaller percentage of the total PFAS and were generally in low concentration, and thus, the result from the study is considered qualitative use only.

Simpson et al. (2021) evaluated the chronic effects of perfluorooctane sulfonic acid potassium salt (PFOS-K, purchased from Sigma Aldrich) on the harpacticoid copepod (*Nitocra spinipes*). Clean seawater (salinity 33 ± 2 ppt) for culturing was sourced from the southeast coast

of New South Wales (NSW), Australia, and clean sediments were from Bonnet Bay, NSW. A total of six different PFOS-spiked sediments were prepared, each having varying PFOS concentrations and sediment properties, by adding a mass of PFOS-K into a 250 mL HDPE container with 5-10 mL of ethanol added to dissolve it. Filtered NSW seawater was added (10 mL/mL methanol) and PFOS-K methanol/seawater suspension immediately poured onto the top of the wet sediment and homogenized. The PFOS concentrations in all sediments and porewaters of selected sediments were determined prior to toxicity testing. The non-spiked controls were prepared in the same manner. PFOS treatments used for toxicity tests were prepared from dilutions of the PFOS-spiked sediments with clean sediment (95% <63 μm) and sand (0% <63 μm) that were then equilibrated for a further 1-2 weeks before testing. A 10-day copepod survival-reproduction test was used to measure adult survival and reproduction and survival of offspring. The females of *N. spinipes* are iteroparous, producing several broods after only one mating encounter, and tests were initiated with gravid females collected directly from the cultures. Sediments were homogenized, but not sieved, immediately prior to being added to test vials (0.5 g sediment per 1 cm diameter 10 mL vial, with five to six replicates per sediment). Autoclaved filtered seawater (pH 8.1, salinity 30‰) was added, and each vial was incubated at 21°C overnight to allow sediments to settle. The following day, five gravid females (three to five weeks old) were randomly assigned to each vial. Treatments were fed twice a week. The tests were static. After 10 days, the total offspring (total nauplii, first juvenile life-stage, and copepodite, second life-stage) in each vial was recorded by microscopy. Subsamples of overlying seawater were taken on day one, and at three or four other times during the tests were combined to analyze the time-weighted average concentration of PFOS for two of the five spiked-sediment tests. A 28-day copepod (survival only) test was also run using the same conditions as the

standardized 10-day copepod test but was conducted in 50 mL centrifuge vials containing 2 g of sediment and 48 mL of autoclaved filtered seawater and only one nominal test concentration, 50 mg/kg PFOS spiked sediment. On day 28, the sediment was gently sieved (44 μm) and the surviving adults were counted. The author-reported 10-d reproduction (progeny count) NOEC was 2.0 mg/L, and the 28-d survival NOEC was 0.48 mg/L. Given the presence of other PFAS measured in the sediments and overlying water at very low concentrations, this study was considered a mixture. The other PFAS made up a much smaller percentage of the total PFAS and were generally in low concentration, and thus, the result from the study is considered qualitative use only.

H.3.2 Saltwater Fish

H.3.2.1 Oryzias melastigma

A series of PFOS experiments with marine medaka embryos were conducted by Huang et al. (2011), Fang et al. (2012) and Wu et al. (2012). In all of these experiments medaka were exposed to the same nominal test concentrations and under similar water quality parameters.

Huang et al. (2011) conducted an 8-day renewal, unmeasured PFOS (98% purity) toxicity test with *Oryzias melastigma* embryos. Fertilized eggs were acclimated in artificial seawater for two days to laboratory conditions (salinity of 30 ppt, 28 ± 1°C, and 14-hr:10-hr light:dark photoperiod). PFOS was dissolved in DMSO and the control and each exposure group contained 0.1% DMSO. Embryos (2 dpf) were exposed to four nominal concentrations (solvent control, 1, 4 and 16 mg/L PFOS). Three replicates of 70 embryos each were held in 90 mm petri dishes containing 20 mL of solutions for eight days with solutions renewed daily. Twelve embryos from each replicate were collected on day 2, 4, 6 and 8 and the cardiac morphology and heart rates were recorded. The distance between the sinus venosus (SV) and bulbus arteriosus (BA) region of the heart was also measured. The heart rate significantly decreased in all PFOS treatments

when compared to the solvent control at 10 days post fertilization (exposure day eight). Additionally, the SV-BA distance was increased at 10 dpf in the highest test concentration (16 mg/L PFOS) compared to other treatments. The 8-day LOEC (based on heart rate) of 1 mg/L and the 8-day MATC (based on SV-BA distance) of 8.0 mg/L were both acceptable for qualitative use (due to the short test duration).

Fang et al. (2012) also conducted an 8-day renewal, measured toxicity test on PFOS (98% purity) with embryos of marine medaka, *Oryzias melastigma*. Fertilized eggs were cultured in artificial seawater for two days (2 dpf) and then exposed to one of four nominal treatments (0.1% DMSO control or 1, 4, and 16 mg/L PFOS). Three replicates of 70 embryos each were held in 90 mm petri dishes containing 20 mL of solutions for eight days (10 dpf). Limited water quality conditions were reported by the authors (salinity of 30 ppt, 28 ± 1°C, and 14-hr:10-hr light:dark photoperiod). Test treatments were renewed daily and PFOS concentrations were measured at 4 dpf and 10 dpf by LC/MS. Measured concentrations were only reported graphically, but from visual inspection measured concentrations were close to nominal concentrations. Similarly, tissue concentrations at 4 dpf reflected a concentration response manner, with increasing tissue concentrations at higher test treatments. At 10 dpf larva mortality was recorded and the percentage of malformations observed. There was no significant effect on mortality in any concentration despite increasing embryo mortality from 2.0% in the DMSO control to 14.6% in the highest test treatment (16 mg/L PFOS). However, a significant increase in larval malformations (bent spine and edema) was observed in all PFOS treatments. Bent spine malformations were observed in 29.0% of the larva in the DMSO control, but increased to 68.2% at 1 mg/L PFOS. The atypical duration (8 days) deemed the LOEC based on malformations of 1 mg/L PFOS as acceptable for qualitative use only.

Wu et al. (2012) conducted another renewal, unmeasured toxicity test with PFOS (98% purity) on marine medaka embryos. Exposure lasted up to 21 days (time it took embryos to hatch in the DMSO controls) plus a seven day observation period in clean water with hatched larva. For each treatment (three replicates), 50-70 embryos (2 dpf) were collected from in-house cultures and distributed into 90 mm petri dishes containing 20 mL artificial seawater and at a salinity of 30‰, 28 ± 1°C under a 14-hr:10-hr (light:dark) photoperiod. In all nominal treatments (solvent control, 1, 4 and 16 mg/L PFOS), the concentration of DMSO was 0.1%. Embryos were observed and the media was renewed daily. After embryos hatched (varied across treatments), larvae were held for an additional seven days in clean 30‰ artificial seawater without food. PFOS had a significant effect on increasing the hatching rate, decreasing the hatching time and decreasing the larval survival in the observation period at 4 mg/L PFOS. The ~21-day MATC (based on larval survival) of 2 mg/L PFOS (geomean of 1 and 4 mg/L) was acceptable for qualitative use only due to the short exposure duration for an early life stage fish test.

H.3.2.2 Sebastes schlegeli

Jeon et al. (2010c) performed a 6-day renewal, unmeasured toxicity test on perfluorooctane sulfonic acid potassium salt (PFOS-K, 99% purity) with 5-month old blackrock fish, *Sebastes schlegeli* (a non-North American species). Fish were obtained from the National Fishery Science Institute (Busan, Korea) and acclimatized to four different salinities (10, 17.5, 25, and 34 ppt) for two weeks before exposure. The salinity of test solutions was adjusted by mixing filtered natural seawater and groundwater. A 3.0 mg/L PFOS stock was made with HPLC grade methanol and diluted to two PFOS concentrations (0.1 and 1 mg/L). Twenty fish were added to each tank with 180 L test solutions renewed on day two and four. Treatments and control were not replicated and fish were not fed during the exposure. Across all exposures, pH ranged from 8.0-8.2 and temperature from 8-12°C; no other water quality parameters were

reported. 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) and was acceptable for qualitative use based on the atypical test duration.

H.4 Summary of Plant PFOS Toxicity Studies Used Qualitatively

H.4.1 Bacterium, Vibrio fischeri

Rosal et al. (2010) conducted a 15-minute static, measured bioluminescence inhibition test with perfluorooctane sulfonate potassium salt (PFOS-K, 98% purity) on the bacterium, *Vibrio fischeri* following ISO 11348-3 standard protocol. While limited details are provided about the exposure, the authors stated they were following the standard protocol. The experiment used a commercially available Biofix Lumi test (Macherey-Nagel, Germany), where the bacterium is supplied freeze-dried. It was reconstituted and incubated at 3°C for five minutes before use. The experiments employed a 0.34 M NaCl (2% w/v) test medium and conducted at 18°C. No solvents were used to make test solutions. Specific test concentrations were not provided, but the authors noted that nominal and measured concentrations did not have significant deviations. The 15-minute EC_{50} based on bioluminescence inhibition was >500 mg/L. At this concentration there was 12% luminescence. The test was acceptable for qualitative use only because of the short test duration and lack of exposure details.

H.4.2 Cyanobacteria, Anabaena sp.

Rosal et al. (2010) also conducted a 24-hour static, measured bioluminescence inhibition test with PFOS-K (98% purity) on the cyanobacteria, *Anabaena sp*. Limited details are provided about the exposure, but the authors stated they were following the test design in Rodea-Palomares et al. (2009). The cyanobacterium *Anabaena*, CPB4337 strain, was grown at 28°C on a rotary shaker in 50 mL AA/8 media supplemented with nitrate (5 mM) in 125 ml Erlenmeyer

flasks and 10 mg/mL of neomycin sulphate. No solvents were used to make test solutions. Specific test concentrations were not provided, but the authors noted that nominal and measured concentrations did not have significant deviations. The 24-hour bioluminescence inhibition EC_{50} was 143.27 mg/L, and was acceptable for qualitative use only because of the short test duration and lack of exposure details.

H.4.3 Dinoflagellate, Pyrocystis lunula

Hayman et al. (2021) conducted a short-term, sublethal 24 hour exposure to examine the effects of PFOS on the bioluminescent dinoflagellate (*Pyrocystis lunula*) following ASTM E1924-97 (ASTM 2004). Dilution water was 0.45 µm filtered seawater collected from North San Diego Bay, CA and spiked PFOS. Spiking consisted of the addition of stock solutions of perfluorooctanesulfonic acid potassium salt (PFOS-K, 98% purity, CAS # 2795-39-3) dissolved in methanol; highest methanol concentration of 0.8% (v/v). Concentrations of PFOS for the toxicity tests were determined from a range finding study. Measured concentrations for PFOS were 0 (control and solvent control), 0.88, 1.1, 1.6, 2.0, 2.5, 10, 34, and 120 mg/L. Approximately 3,000 cells of *P. lunula* were added to 2.5 mL of test solution in acrylic test cuvettes, with six replicates per treatment concentration. *P. lunula* were exposed for 24 hours in a 19°C incubator with a reversed (e.g., dark during the typical "day" period) 12-hr:12-hr light:dark cycle. Test cuvettes were removed from the incubator after 24 hours and after being in the dark period for approximately three hours, inserted and analyzed in a specialized spectrometer (QwikLite 200 Biosensor System, Assure Controls, Carlsbad, CA) and the light output was recorded. Less light output relative to concurrently evaluated controls is indicative of an adverse effect. The 24-hr bioluminescence EC50 for *P. lunula* was determined to be 4.9 mg/L PFOS. The value is considered qualitatively only because of the short exposure period (less than 96 hours).

H.4.4 Golden brown alga, Isochrysis galbana

A 72-hour static, unmeasured algal growth inhibition test on PFOS (98% purity) with *Isochrysis galbana* was performed by **Mhadhbi et al. (2012)** following OECD (2006) test methodology. Golden brown alga were provided by Estacion de Ciencias Marinas de Toralla (ECIMAT). The cultures were maintained in 250 mL glass Erlenmeyer flasks with autoclaved filtered sea water and EDTA-free f/2 culture medium. PFOS stock solutions were prepared in DMSO and added to the dilution water with a maximum DMSO concentration of 0.01% (v/v). Nominal test concentrations were solvent control, 3.75, 7.5, 15, 30 and 60 mg/L PFOS. Each flask was inoculated at a density of 10,000 cells/mL, with the algae in the exponential growth phase. Each treatment was replicated three times. Flasks were kept at 20ºC with a 24 hour light period, and manually shaken daily. Cell counts were carried out every 24 hours, with a reported 72-hour EC_{50} based on growth inhibition of 37.5 mg/L PFOS. The short test duration (<96 hours) made the effect concentration acceptable for qualitative use only.

H.4.5 Alga, Ceratoneis Closterium

Simpson et al. (2021) evaluated the toxic effects of perfluorooctane sulfonic acid potassium salt (PFOS-K, purchased from Sigma Aldrich) on the temperate marine benthic microalga, *Ceratoneis closterium* (previously named *Nitzschia closterium*) from previously-established cultures using a 72-hr growth test. Clean seawater (salinity 33±2 ppt) for culturing was sourced from the southeast coast of New South Wales (NSW), Australia, and clean sediments were from Bonnet Bay, NSW. Toxicity was assessed by measuring the decrease in rate of growth (growth rate inhibition) of the microalga attached to the surface of a PFOS-spiked sediments in three tests, each with different sediment PFOS concentrations and sediment properties. The benthic microalgae were added to the surface of a sediment subsample and total chlorophyll extracted as a surrogate for algal cell density. To each 50 mL triplicate centrifuge

tube, 2 g of sediment was added along with overlying water consisting of 20 mL filtered seawater and nutrients (0.2 mL of 0.26 mM $NaNO_3$ and 0.2 mL of 1.3 mM KH_2PO_4). After a day overlying water was exchanged and 2-6 × 10^4 cells/mL of algae was added to initiate the test. Seawater only and sediment without algae were also tested as controls. PFOS concentrations in the overlying water were assumed based on the PFOS measured in the overlying water from other tests in the study. There was no effect on growth of the microalgae in the treatment with the highest estimated overlying water concentration of 4.16 mg/L PFOS. The author-reported 72-hour survival NOEC was 4.16 mg/L. Given the presence of other PFAS measured in the sediments and overlying water at very low concentrations, this study was considered a mixture. The other PFAS made up a much smaller percentage of the total PFAS and were generally in low concentration, and thus, the result from the study is considered qualitative use only.

H.4.6 Diatom, Skeletonema costatum

Desjardins et al. (2001) performed a static, measured 96-hour growth inhibition study on the marine diatom *Skeletonema costatum*. Protocol from U.S. Environmental Protection Agency, OPPTS Number 850.5400 was followed. Algae was cultured at Wildlife International Ltd. in saltwater algal medium. Diatoms were exposed to one control and one test concentration (3.46 mg/L PFOS nominal, 3.20 mg/L PFOS measured). Diatoms were added to sterile 250 mL Erlenmeyer flasks, filled with 100 mL of either test or control medium with a salinity of 30‰, and plugged with foam stoppers1. Test chambers were kept at 20 ± 2 °C on a mechanical shaker table set to 100 rpm, with a 14-hr:10-hr light:dark cycle. Single cell counts were taken every 24 hours throughout the duration of the test, and group cell counts were taken at 72 hours and 96 hours. While there was some minor growth inhibition in the treatment group, it was not enough to show a statistical difference when compared to the control group.

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 hr	36 d	MATC (metamorphosis success)	16.5	0.01768	**933.3**	**933.3**	Hazelton et al. (2012), Hazelton (2013)
Snail, *Physella heterostropha pomilia* (formerly, *Physa pomilia)*	PFOS-K ≥98%	S, M	R, M	96 hr	44 d	EC10 (clutch size)	161.8	8.831	**18.32**	**18.32**	Funkhouser (2014)
Rotifer, *Brachionus calyciflorus*	PFOS ≥98%	S, U[b]	R, U[b]	24 hr	Up to 158 hr	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 hr	21 d	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 hr	21 d	EC10 (survival)	58.51	11.19	**5.229**	-	Drottar and Krueger (2000a)
Cladoceran, *Daphnia magna*	PFOS-K 95%	S, U	R, U	48 hr	21 d	EC10 (survival)	67.2	16.35	**4.110**	-	Boudreau et al. (2003a)
Cladoceran, *Daphnia magna*	PFOS Unreported	S, U	R, U	48 hr	21 d	EC10 (# of young/adult)	35.46	0.7885	**44.97**	-	Ji et al. (2008)
Cladoceran, *Daphnia magna*	PFOS-K >98%	S, U	R, U	48 hr	21 d	EC10 (total neonates/female)	63.84[d]	2.919	**21.87**	-	Li (2009, 2010)
Cladoceran, *Daphnia magna*	PFOS-K 99%	S, M	R, M	48 hr	21 d	EC10 (reproduction)	78.09	2.26	**34.55**	-	Yang et al. (2014)
Cladoceran, *Daphnia magna*	PFOS 98%	S, U	R, U	48 hr	21 d	EC10 (number of offspring/brood/female)	23.41	0.001712	13,674[b]	-	Lu et al. (2015)
Cladoceran, *Daphnia magna*	PFOS-K ≥98%	S, U	R, U	48 hr	21 d	EC10 (survival)	94.58	3.596	**26.30**	-	Liang et al. (2017)
Cladoceran, *Daphnia magna*	PFOS-K 98%	S, U	R, U	48 hr	21 d	EC10 (growth-length)	22.43	0.9093	**24.67**	**17.35**	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 hr	7 d	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 hr	28 d	LC20	59.87	0.167	**358.5**	**358.5**	Funkhouser (2014)
Zebrafish, *Danio rerio*	PFOS-K unknown/ PFOS 96%	R, U	R, U	96 hr	LC	EC10 (F1 offspring: % survival)	17	0.01650	**1,030**	**1,030**	Wang et al. (2011), (Wang et al. 2013b)
Fathead minnow, *Pimephales promelas*	PFOS-K 90.49%	S, M	F, M	96 hr	47 d	EC10 (survival)	9.020	0.4732	**19.06**	**19.06**	Drottar and Krueger (2000d)

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

[b] Values appears to be an outlier and is not used 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 hr	10 d	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 hr	21 d	LC50	MATC (survival)	171.5	4.243	40.41	Olson (2017)
Midge, *Chironomus sp.*	PFOS-K (99%) / PFOS Unreported	S, M	S,M	96 hr	~36 d (1st of 10 generations)	LC50	LOEC (F1 developmental time, adult weight, exuvia length)	182.12	0.004	45,530	Yang et al. (2014); Marziali et al. (2019)
Midge, *Chironomus sp.*	PFOS-K (99%) / PFOS-K (95%)	S, M	S,M	96 hr	Life cycle (>50 d)	LC50	EC10 (total emergence)	182.12	0.089	2,039	Yang et al. (2014); MacDonald et al. (2004)
Yellow fever mosquito, *Aedes aegypti*	PFOS Unreported	S, U	R,U	48 hr	~42 d	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 hr	14 d	LC50	LOEC (decrease LSI)	2.5	1.0	2.500	Sharpe et al. (2010); Oakes et al. 2005
Zebrafish, *Danio rerio*	PFOS ≥97%	S, U	R, U	96 hr	6 d	LC50	EC50 (uninflated swim bladder)	58.47	2.29	25.53	(Hagenaars et al. 2011),Hagenaars et al. (2014)
Zebrafish, *Danio rerio*	PFOS-K 98%	R, M	R, M	96 hr	21 d	LC50	LOEC (reduced fecundity)	22.2	0.5	44.40	Sharpe et al. (2010)
Zebrafish, *Danio rerio*	PFOS 98%	S, U	R,U	96 hr	70 d	LC50	MATC (increased malformation & decreased survival of F1 fish)	3.502	0.02236	156.6	Du et al. (2016); 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 hr	96 hr	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, 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
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)
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

Author	Citation	Reason Unused
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
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. Safety 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
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 concetration; missing detail (focus is on other chemicals)
Hoff, P.T., W. Van Dongen, E.L. Esmans, R. Blust, 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)
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
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

Author	Citation	Reason Unused
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. Aquatic Toxicology. 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, T. Luckenbach	2016. Does perfluorooctane sulfonate (PFOS) act as chemosensitizer in zebrafish embryos? Science of the Total Environment. 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	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. Comparative Biochemistry and Physiology, Part C. 148: 14-22.	In vitro
Lee, W. and Y. Kagami	2010. Effects of perfluorooctanoic acid and perfluorooctane sulfonate on gene expression profiles in medaka (Oryzias latipes). Abstracts. Toxicol. Letters 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)

Author	Citation	Reason Unused
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
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
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)
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)
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)

Author	Citation	Reason Unused
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.	Injectedt 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)
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
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, 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 EPA's request for data or were unable to locate concentration-response data, the toxicity values were not independently-calculated by EPA, and the reported toxicity values were retained for criteria deviation. EPA also retained author-reported effect concentrations when data availability did not support effect concentration calculation by 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 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 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 was 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* (1985 Guidelines; Stephen et al. 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, 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, EPA was able to develop a draft 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 acute estuarine/marine benchmark recommendations 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, EPA's interest in providing useful information to states and tribes regarding protective values for aquatic life, and 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 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 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 of 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 provides 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. 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 provides a level of protection that is consistent with using measured laboratory data (Dyer et al. 2008; (Feng et al. 2013; Fojut et al. 2012a; Fojut et al. 2012b; 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).

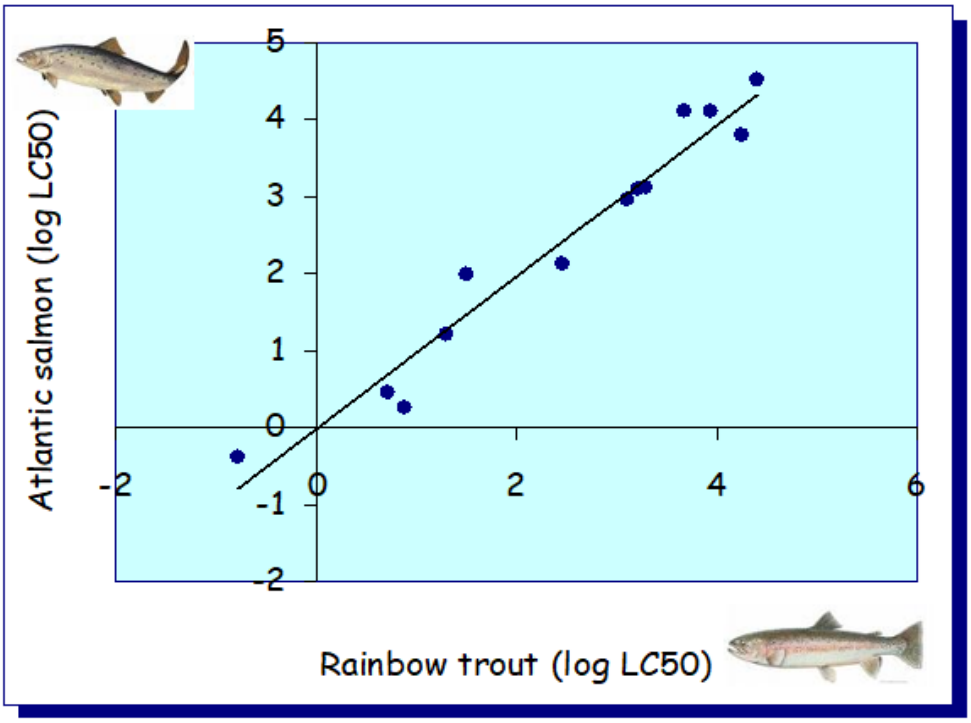


Figure L-1. Example ICE Model for Rainbow Trout (surrogate) and Atlantic Salmon (predicted).
Each model datapoint is a common chemical that was tested in both species to develop a log-linear regression.

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. in prep).
- 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 at the 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 is described in detail in Raimondo et al. (in

prep). 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 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 3104 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. in prep). 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 In prep).

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 draft 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.1were used as values for surrogate species to predict to 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.

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*	48.87	53
Fish	Zebrafish	*Danio rerio*	24.44	2 (juvenile models) 6 (embryo models)
Fish	Rainbow trout	*Oncorhynchus mykiss*	7.515	77

Broad Taxon	Species		PFOS SMAV (mg/L)	Number of ICE Models
	Common Name	Scientific		
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 Z.2., Raimondo et al. 2010, Raimondo et al. in prep). 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 and described more thoroughly in Raimondo et al. (in prep) 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) (Raimondo et al. in prep).

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	56338.67	11646.07 - 272542.13	2.4	133.3	59.73	6.99 - 510.48	2.2[a]
	Fathead minnow (*Pimephales promelas*)		8356.68	3748.61 - 18629.28	16.0		13.26	4.13 - 42.57	10.1
	Rainbow trout (*Oncorhynchus mykiss*)		15140.53	8139.36 - 28163.81	8.8		33.9	13.63 - 84.26	3.9
African clawed frog (*Xenopus laevis*)	Fathead minnow (*Pimephales promelas*)	15,990	7034.49	800.65 - 61804.35	2.3[a]	15.99	18.93	0.306 - 1170.65	1.2[ab]
Mysid (*Americamysis bahia*)	Daphnid (*Daphnia magna*)	4,914	8774.84	4988.00 - 15436.64	1.8	4.914	27.16	18.60 - 39.67	5.5
	Fathead minnow (*Pimephales promelas*)		359.91	135.34 - 957.15	13.7[c]		0.481	0.104 - 2.21	10.2[c]
	Rainbow trout (*Oncorhynchus mykiss*)		1172.37	702.88 - 1955.47	4.2[c]		2.01	1.08 - 3.75	2.4[c]
Daphnid (*Daphnia magna*)	Bullfrog (*Lithobates catesbeianus*)	48,870	81946.04	17394.84 - 386042.67	1.7	48.87	199.47	32.95 - 1207.24	4.1
	Fathead minnow (*Pimephales promelas*)		1697.85	1149.29 - 2508.22	28.8[c]		3.29	1.36 - 7.96	14.9[c]
	Fatmucket (*Lampsilis siliquoidea*)		23122.84	7634.81 - 70030.01	2.1		7.73	1.46 - 40.85	6.3[b]
	Mysid (*Americamysis bahia*)		6096.75	3829.31 - 9706.79	8.0		21.29	13.73 - 33.02	2.3
	Rainbow trout (*Oncorhynchus mykiss*)		2775.45	2007.74 - 3836.72	17.6[c]		8.83	5.26 - 14.80	5.5[c]
	Zebrafish embryo (*Danio rerio* - embryo)		3926.87	910.19 - 16941.77	12.4[c]		2.48	0.143 - 42.99	19.7[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
Rainbow trout (*Oncorhynchus mykiss*)	Bullfrog (*Lithobates catesbeianus*)	7,515	82395.25	38247.48 - 177501.32	11.0	7.515	39.73	16.29 - 96.91	5.3
	Daphnid (*Daphnia magna*)		21354.25	14550.83 - 31338.69	2.8[c]		236.65	174.72 - 320.52	31.5[c]
	Fathead minnow (*Pimephales promelas*)		2771.13	2136.90 - 3593.60	2.7		3.43	2.01 - 5.85	2.2
	Fatmucket (*Lampsilis siliquoidea*)		48028.61	3264.96 - 706515.68	6.4[ac]		13.14	1.03 - 167.64	1.7[abc]
	Mysid (*Americamysis bahia*)		6169.68	3855.10 - 9873.91	1.2		68.63	45.85 - 102.73	9.1
	Zebrafish embryo (*Danio rerio* - embryo)		10047.33	3682.52 - 27412.95	1.3		2.97	0.513 - 17.19	2.5[b]
Fathead minnow (*Pimephales promelas*)	African clawed frog (*Xenopus laevis*)	6,950	16080.14	1020.67 - 253332.73	2.3[a]	6.95	7.89	0.071 - 868.40	1.1[ab]
	Bullfrog (*Lithobates catesbeianus*)		121541.51	44334.20 - 333204.08	17.5		91.08	33.84 - 245.08	13.1
	Daphnid (*Daphnia magna*)		45004.86	29095.72 - 69612.88	6.5[c]		687.68	456.02 - 1037.02	98.9[c]
	Fatmucket (*Lampsilis siliquoidea*)		116669.9	19477.15 - 698863.06	16.8[c]		595.88	48.52 - 7317.09	85.7[abc]
	Mysid (*Americamysis bahia*)		13672.93	5348.62 - 34952.76	2.0[c]		254.88	118.25 - 549.38	36.7[c]
	Rainbow trout (*Oncorhynchus mykiss*)		14424.97	11028.30 - 18867.80	2.1		36.58	23.89 - 56.02	5.3
	Zebrafish embryo (*Danio rerio* - embryo)		27897.95	15393.52 - 50559.97	4.0		50.45	15.02 - 169.43	7.3[b]
Fatmucket (*Lampsilis siliquoidea*)	Black sandshell (*Ligumia recta*)	16,500	11412.52	2418.09 - 53863.02	1.4	16.5	8.15	0.319 - 208.15	2.0[ab]
	Daphnid (*Daphnia magna*)		22790.4	8979.24 - 57844.79	1.4		132.33	45.71 - 383.08	8.0
	Fathead minnow (*Pimephales promelas*)		717.52	149.82 - 3436.35	23.0[c]		3.21	0.065 - 158.39	5.1[abc]
	Rainbow trout (*Oncorhynchus mykiss*)		1585.37	485.38 - 5178.24	10.4[c]		44.11	9.18 - 211.95	2.7[c]
Black sandshell (*Ligumia recta*)	Fatmucket (*Lampsilis siliquoidea*)	13,500	19191.22	4438.79 - 82973.68	1.4	13.5	26.59	1.49 - 472.22	2.0[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.

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 (Inet al. in prep). 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

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
Menidia peninsulae	*Oncorhynchus mykiss*	1.01	-0.36	2	0.91	0.0421	0.35	0.82	1600.00	50	4	Accepted qualitatively
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

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	12.66[abc]	0.66 - 244.8	NA
Amphipod	*Allorchestes compressa*	*Daphnia magna*	mg/L	**1020.12**	310.42 - 3352.37	49.69
		Pimephales promelas	µg/L	**2.42**	1.29 - 4.54	
Mysid	*Americamysis bahia*	*Daphnia magna*	µg/L	**8.77**	4.99 - 15.44	8.77
		Oncorhynchus mykiss	µg/L	1.17[c]	0.70 - 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 - 10393.70	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	1.886
		Daphnia magna	µg/L	4.19[c]	1.97 - 8.91	
		Lampsilis siliquoidea	µg/L	**1.56**	0.44 - 5.55	
		Oncorhynchus mykiss	µg/L	2.01[c]	1.30 - 3.10	
		Pimephales promelas	µg/L	**2.28**	0.78 - 6.67	
Leon springs pupfish	*Cyprinodon bovinus*	*Oncorhynchus mykiss*	mg/L	27.57[a]	3.20 - 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	5.769
		Daphnia magna	µg/L	19.69[c]	9.49 - 40.84	
		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	**4.70**	2.32 - 9.52	
Pink shrimp	*Farfantepenaeus duorarum*	*Americamysis bahia*	mg/L	**6.02**	1.34 - 26.97	6.02
		Daphnia magna	µg/L	162.46[c]	14.13 - 1868.00	
		Oncorhynchus mykiss	µg/L	2.12[c]	0.38 - 11.71	
Banana prawn	*Fenneropenaeus merguiensis*	*Daphnia magna*	mg/L	**688.07**	124.18 - 3812.56	688.07
Threespine stickleback	*Gasterosteus aculeatus*	*Oncorhynchus mykiss*	mg/L	**16.46**	5.22 - 51.84	16.46
Polychaete	*Hydroides elegans*	*Daphnia magna*	µg/L	8.20[bc]	1.29 - 52.12	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

Common Name	Scientific	Surrogate	Input Unit	Estimated Toxicity (mg/L)	95% Confidence Intervals (mg/L)	SMAV
Tidewater silverside	*Menidia peninsulae*	*Americamysis bahia*	mg/L	22.65[d]	3.47 - 147.72	NA
		Oncorhynchus mykiss	mg/L	3.35[a]	0.095 - 118.60	
Mysid	*Metamysidopsis insularis*	*Daphnia magna*	mg/L	**245.18**	45.26 - 1328.00	152.2
		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**	7.20 - 45.24	18.04
Harpacticoid copepod	*Tisbe battagliai*	*Daphnia magna*	mg/L	**522.86**	103.84 - 2632.73	522.86
Calanoid copepod	*Acartia tonsa*	*Daphnia magna*	µg/L	12.66[abc]	0.66 - 244.8	NA

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. 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 draft acute benchmark for estuarine/marine aquatic life is lower than the recommended acute freshwater criterion (3.0 mg/L), suggesting that estuarine/marine species may be more 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*	1.886	1.886	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*	49.69	49.69	12	0.75
C	Mysid	*Metamysidopsis insularis*	152.2	152.2	13	0.81
H	Harpacticoid copepod	*Tisbe battagliai*	522.9	522.9	14	0.88
F	Banana prawn	*Fenneropenaeus merguiensis*	688.1	688.1	15	0.94

MDR Groups

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

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*	1.886	0.63	0.40	0.250	0.500
		Σ (Sum):	**1.85**	**1.04**	**0.63**	**1.54**

S^2 =	5.30	S = slope	
L =	-0.423	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)		

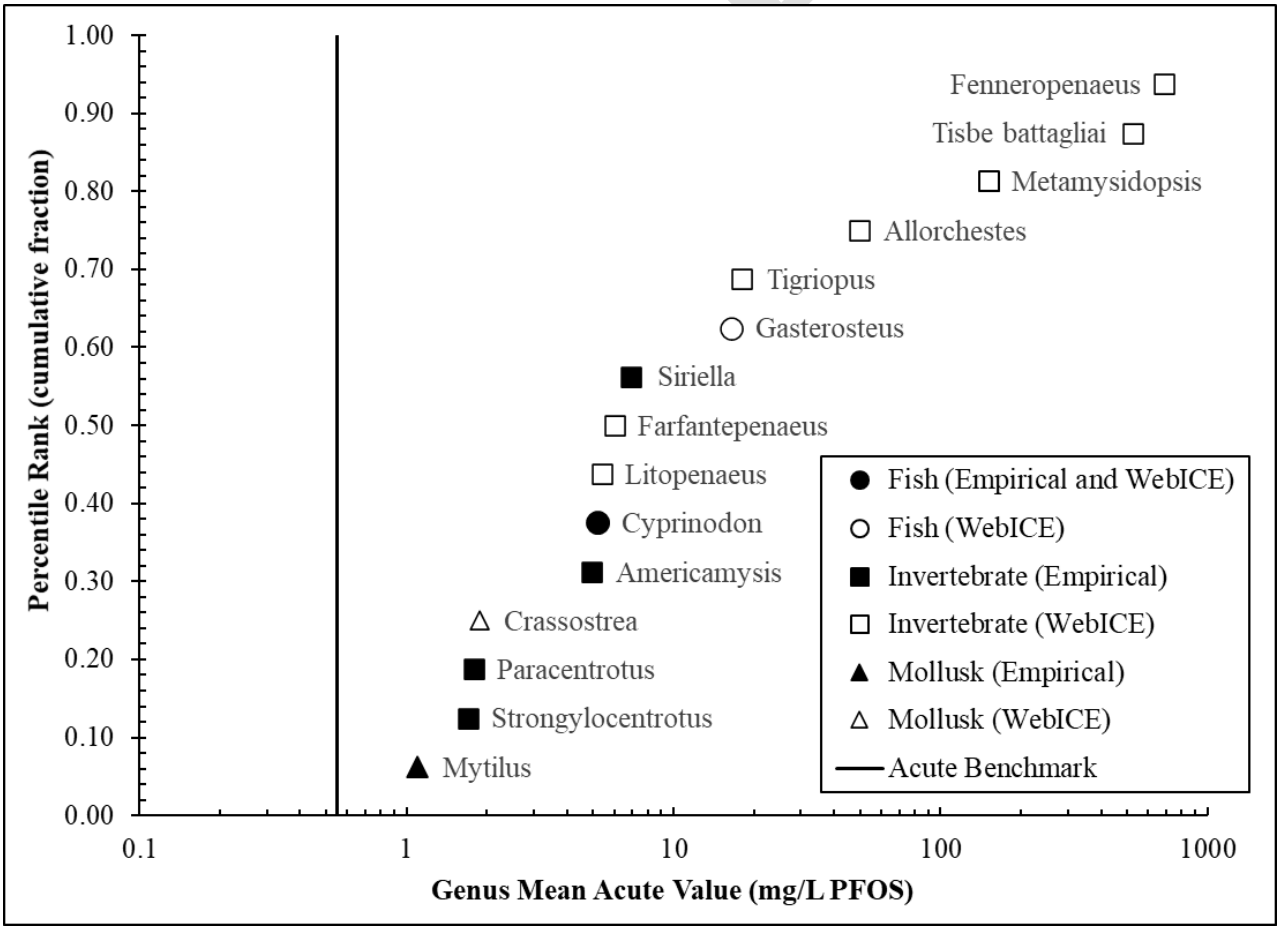


Figure L-2. Ranked Estuarine/Marine Acute PFOS GMAVs used for the Aquatic Life Acute Benchmark Calculation.

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 536.05%. 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., 536.04%) 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. Interestingly, 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.

It is unclear if ICE-estimated data were typically greater than empirical data because of a simple coincidence or a systematic mechanistic reason. A systematic mechanistic reason why ICE-estimated acute values were greater than empirical acute values could be attributed to the use of freshwater species to predict to estuarine/marine species in the ICE regressions. For example, estuarine/marine LC_{50} values from quantitatively acceptable studies (Appendix B.1) were typically lower than acute LC_{50} values for freshwater species (Appendix A.1). The apparent increase in PFOS toxicity in estuarine/marine environments relative to freshwaters may represent a unique toxicological consideration of PFOS (and possibly other PFAS) that was not a toxicological attribute of the other chemicals used to build the supporting ICE models, which would result in artificially high PFOS LC_{50} estimates for estuarine/marine species.

The estuarine/marine benchmark still 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*. EPA further evaluated the appropriateness of the estuarine/marine benchmark by comparing it to empirical, but qualitatively acceptable, data for estuarine/marine species. 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 a 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). 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

author 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, *Scophthalmus maximus*. Endpoints included dead embryos, malformation, hatch success at 48 hours and larvae 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 PFOA exposures at concentrations below the acute estuarine/marine benchmark.
3. Jeon et al. (2010c) 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 PFOA 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 PFOA benchmark.

L.2.6 ICE Regressions Supporting the Acute Estuarine/Marine Benchmark

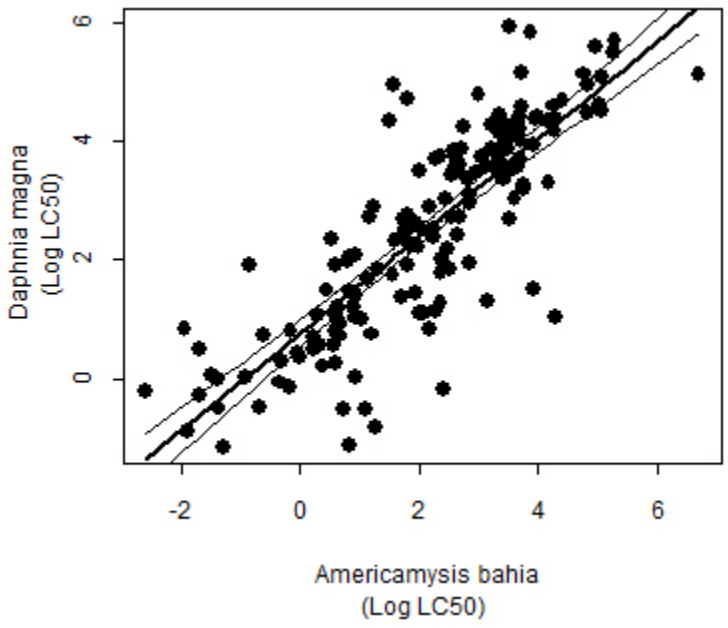


Figure L-3. *Americamysis bahia* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

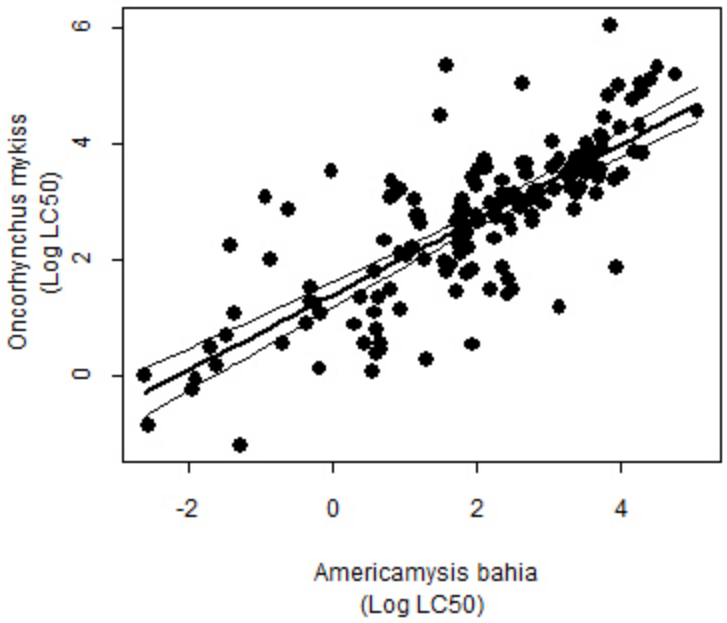


Figure L-4. *Americamysis bahia* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

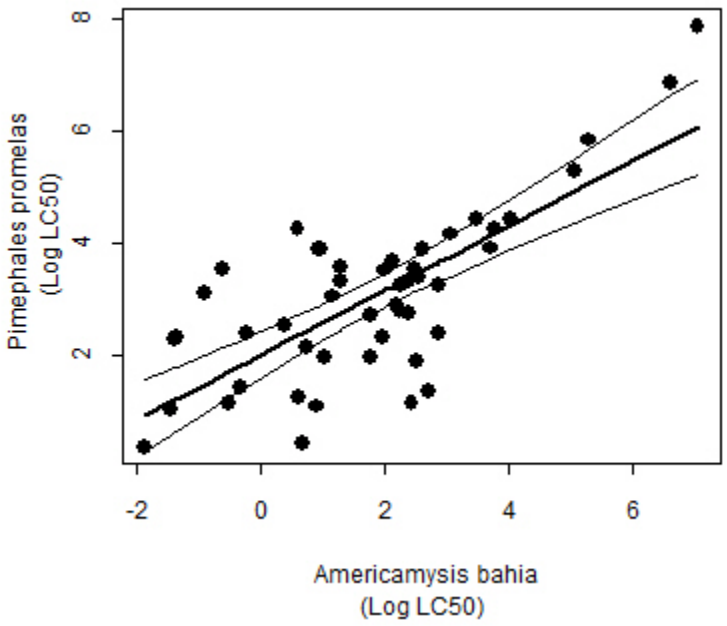


Figure L-5. *Americamysis bahia* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

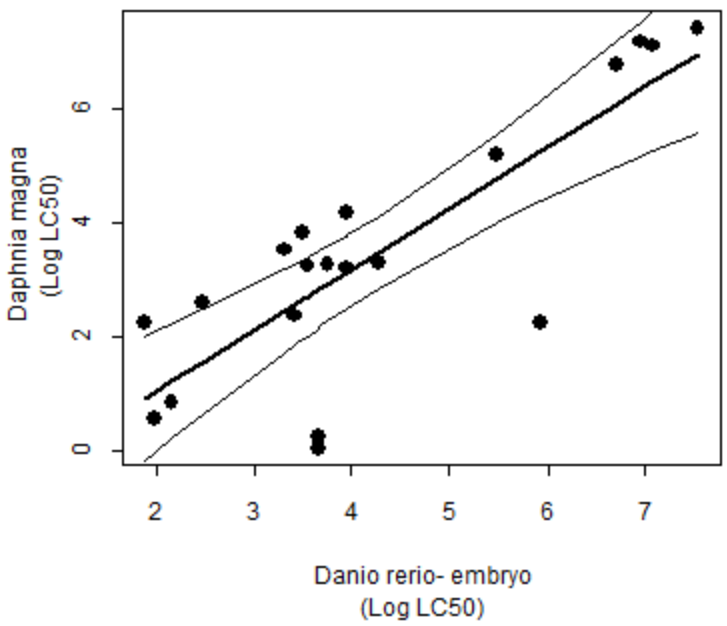


Figure L-6. *Danio rerio* -embryo (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

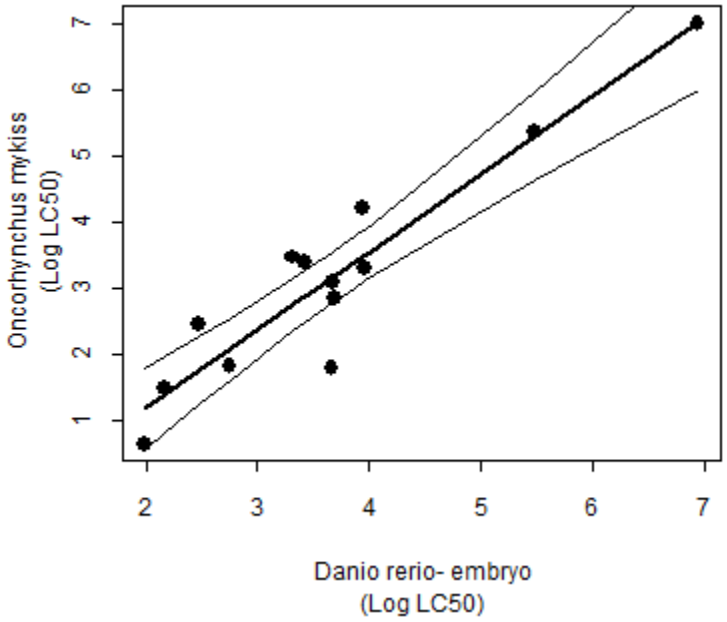


Figure L-7. *Danio rerio* - embryo (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

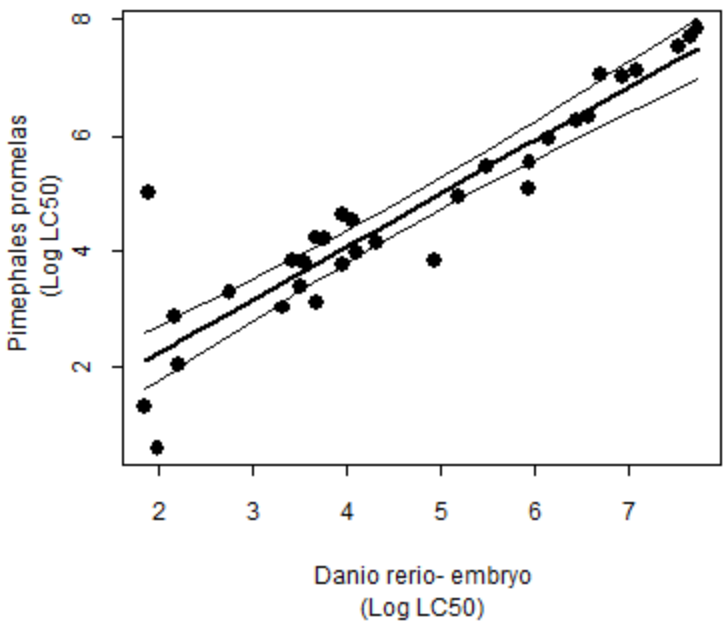


Figure L-8. *Danio rerio* - embryo (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

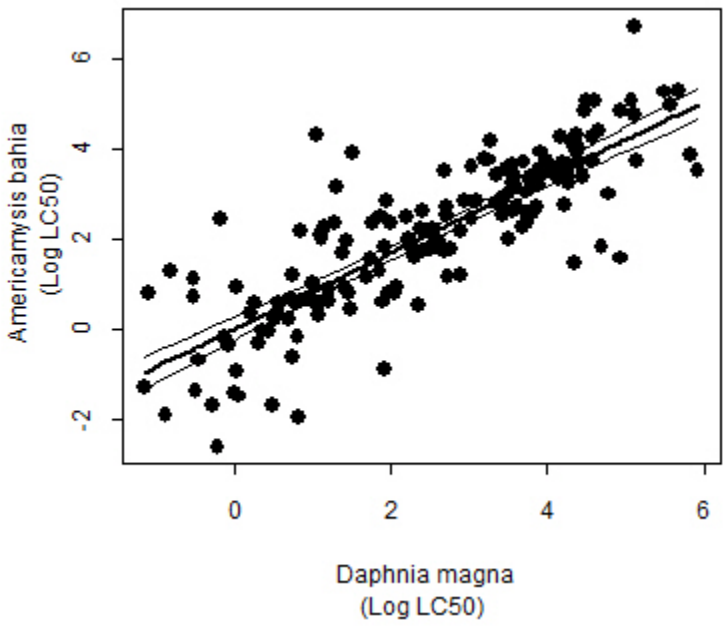


Figure L-9. *Daphnia magna* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

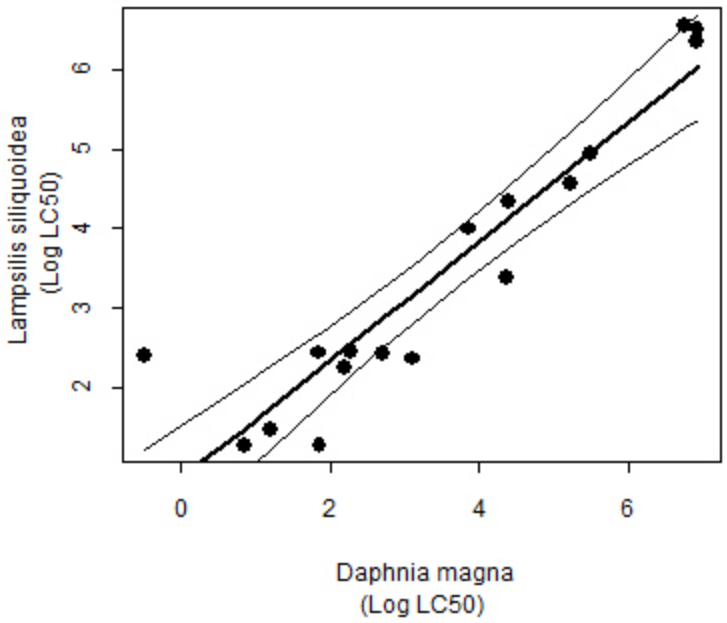


Figure L-10. *Daphnia magna* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

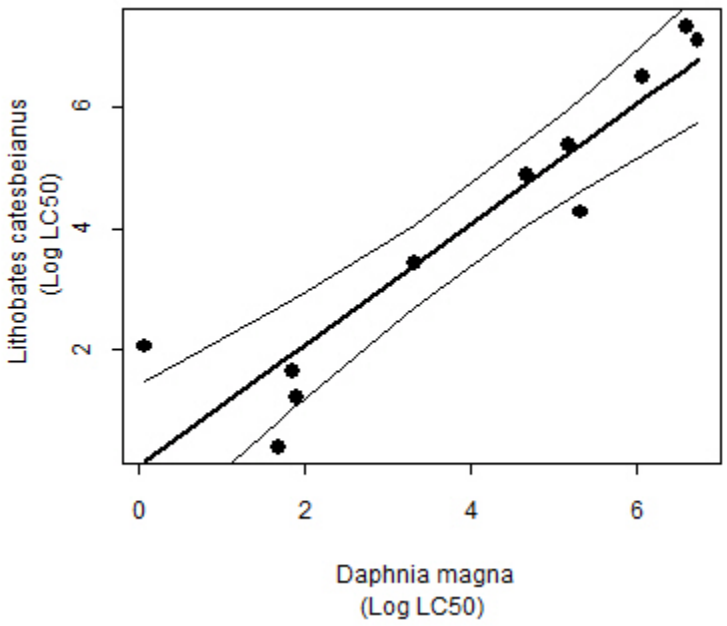


Figure L-11. *Daphnia magna* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

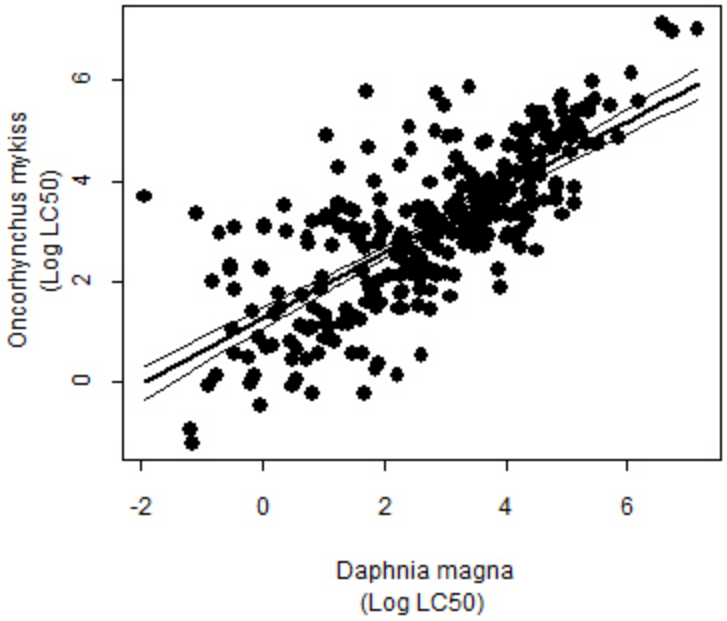


Figure L-12. *Daphnia magna* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

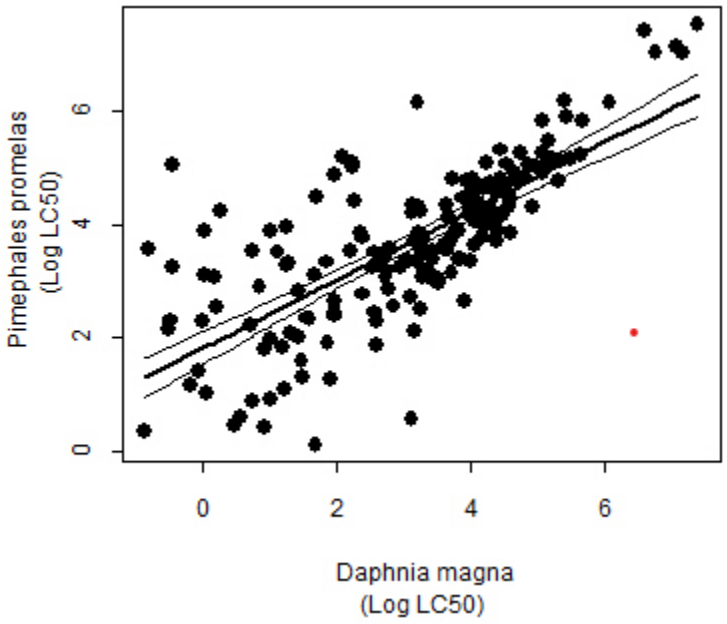


Figure L-13. *Daphnia magna* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

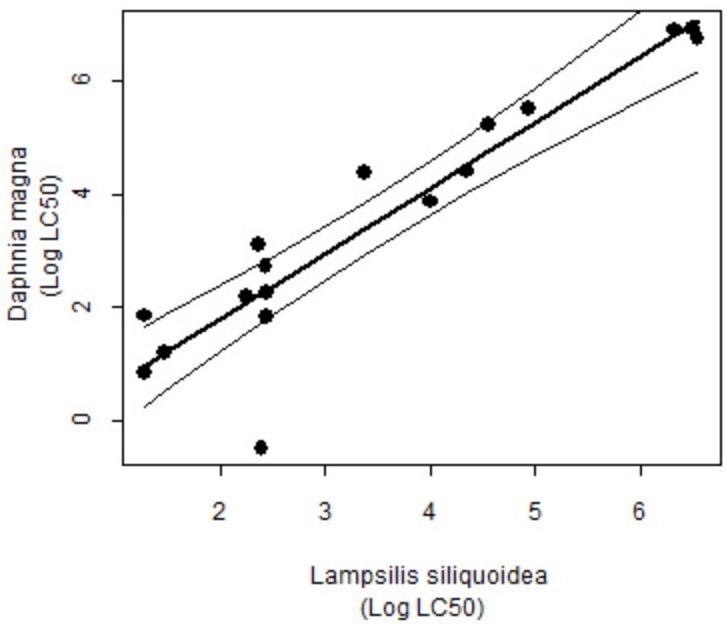


Figure L-14. *Lampsilis siliquoidea* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

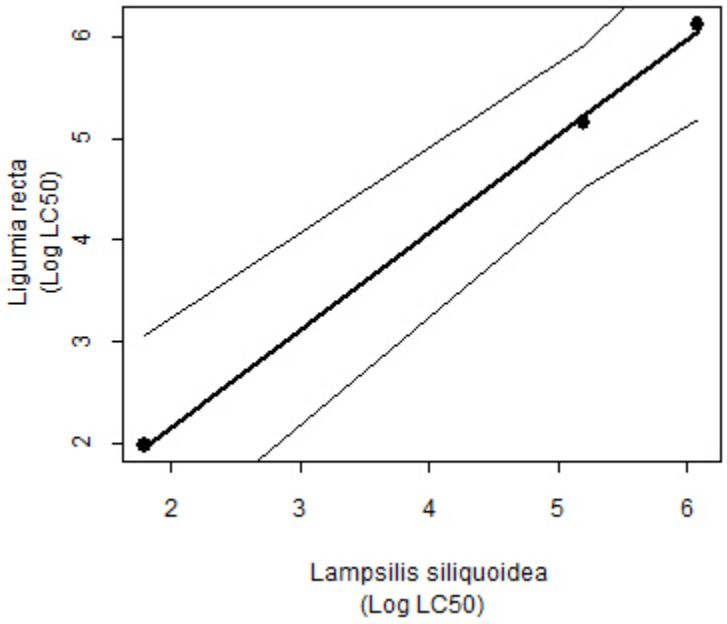


Figure L-15. *Lampsilis siliquoidea* (X-axis) and *Ligumia recta* (Y-axis) regression model used for ICE predicted values.

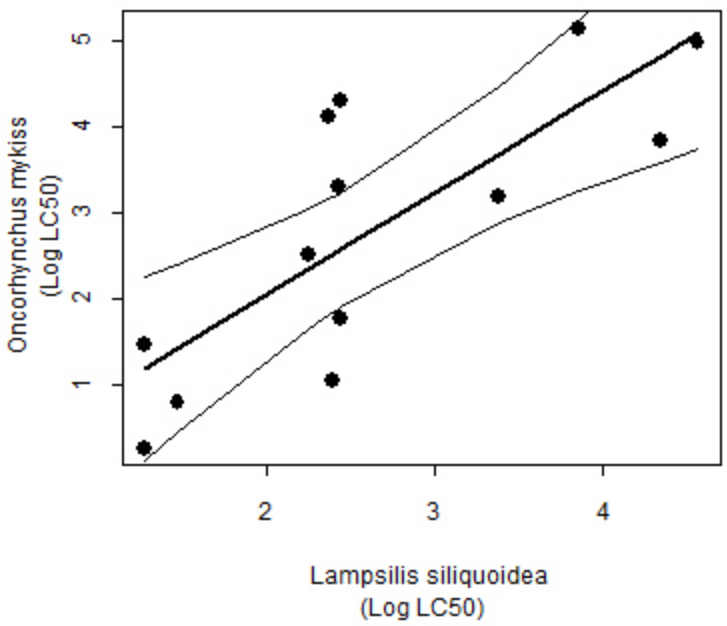


Figure L-16. *Lampsilis siliquoidea* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

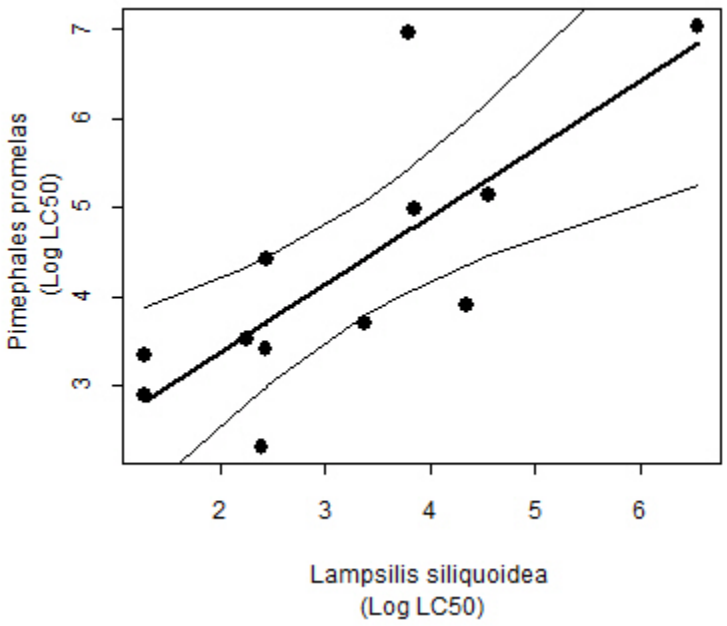


Figure L-17. *Lampsilis siliquoidea* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

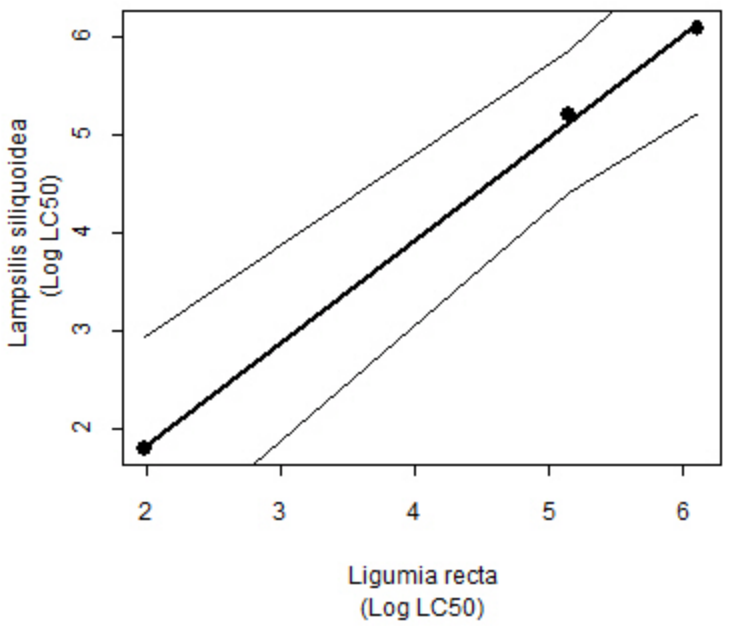


Figure L-18. *Ligumia recta* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

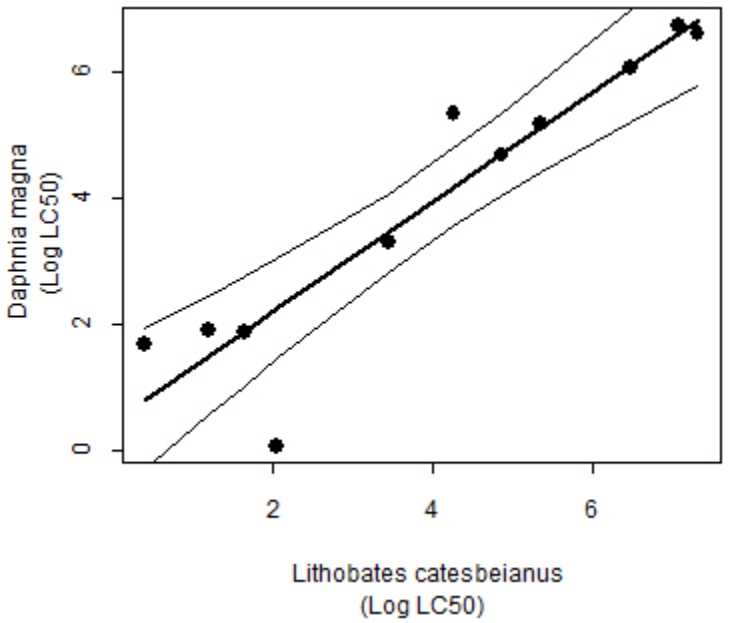


Figure L-19. *Lithobates catesbeianus* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

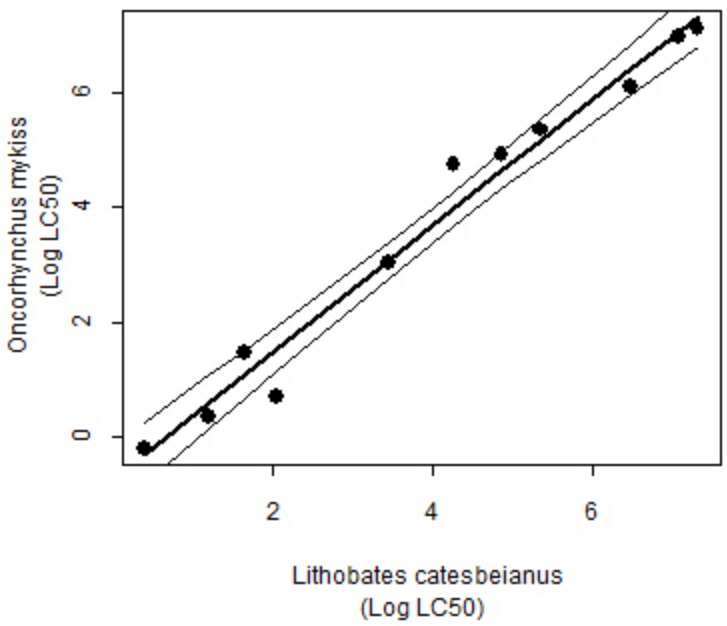


Figure L-20. *Lithobates catesbeianus* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

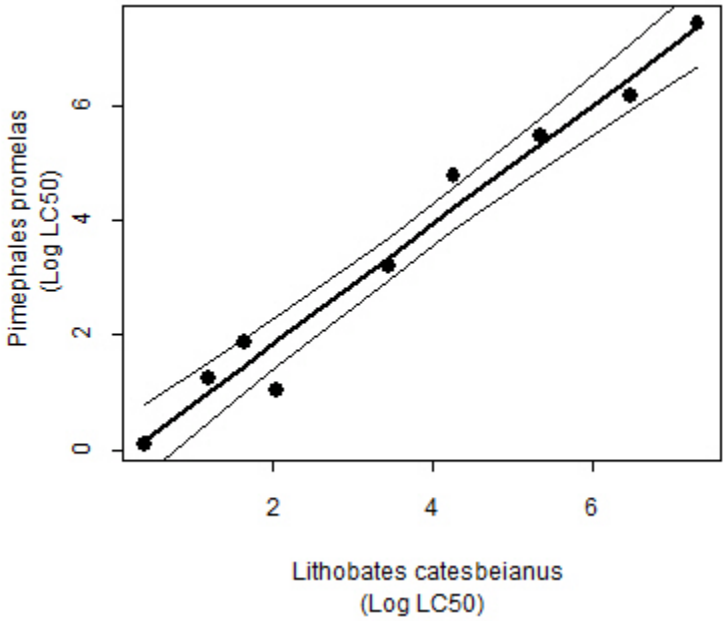


Figure L-21. *Lithobates catesbeianus* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

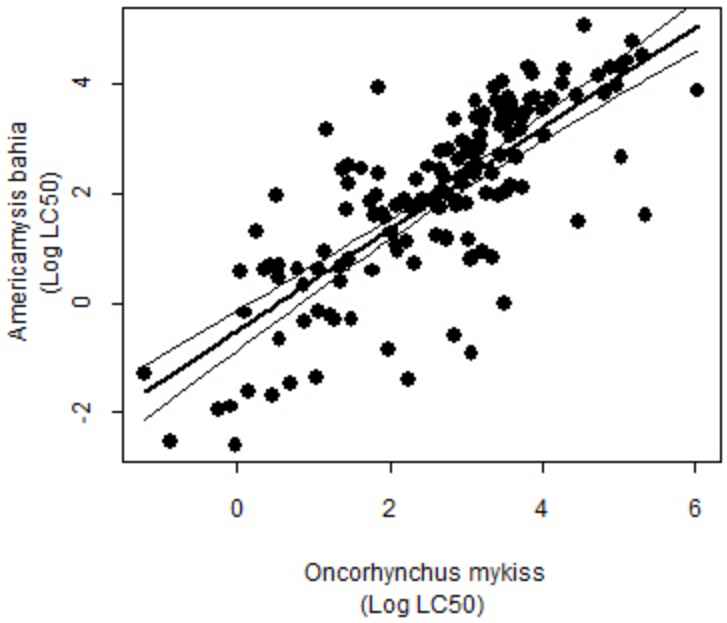


Figure L-22. *Oncorhynchus mykiss* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

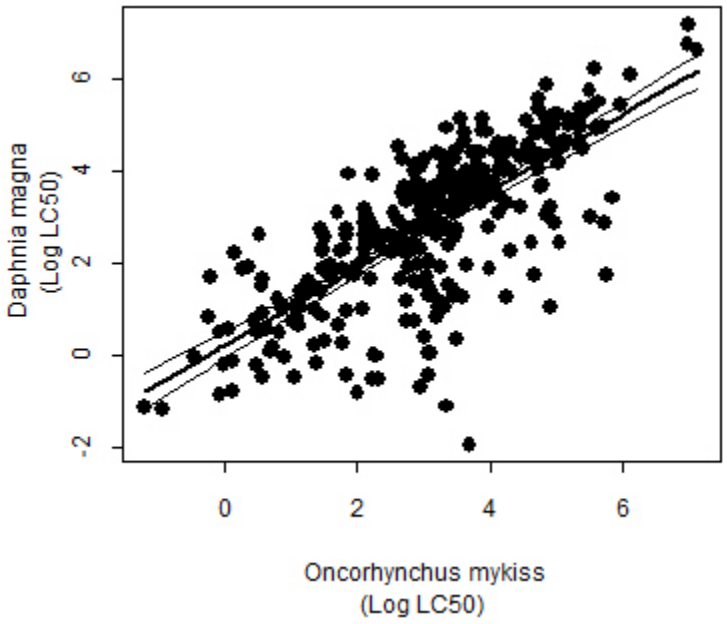


Figure L-23. *Oncorhynchus mykiss* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

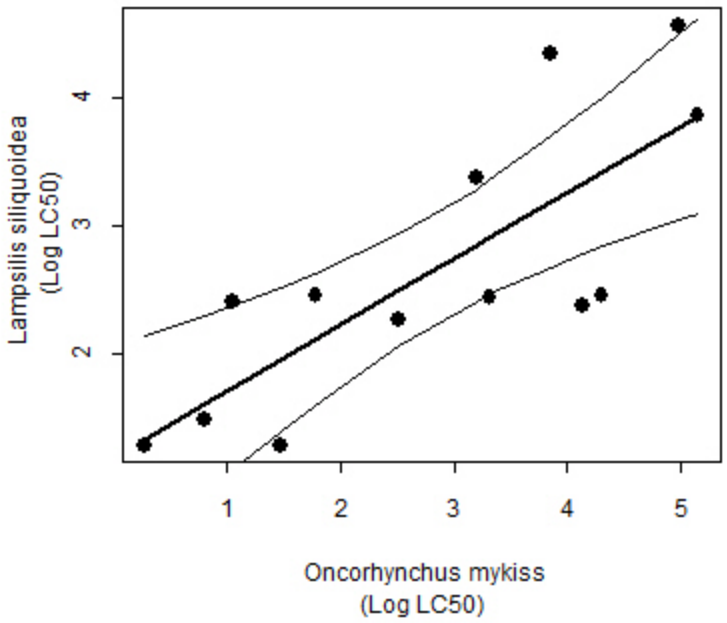


Figure L-24. *Oncorhynchus mykiss* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

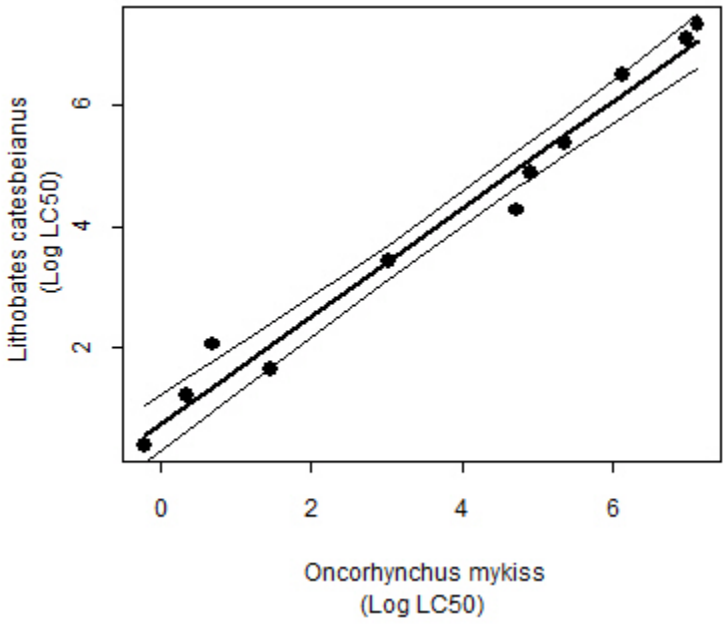


Figure L-25. *Oncorhynchus mykiss* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

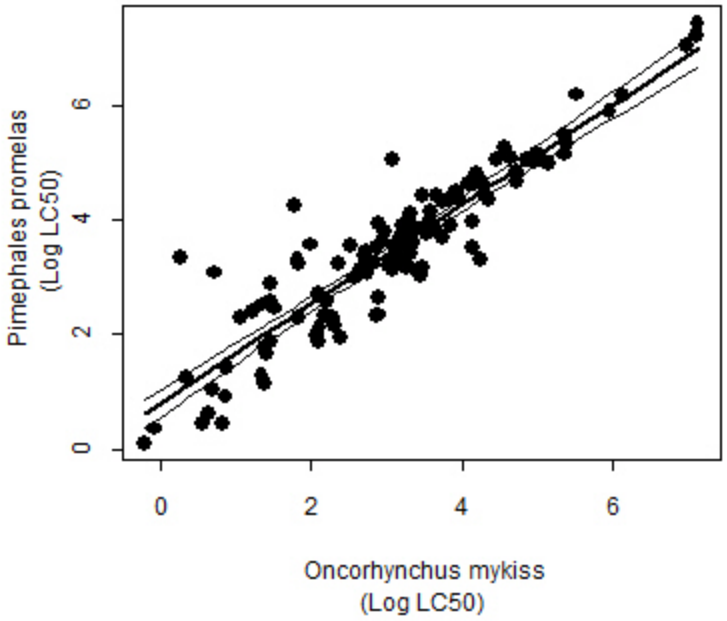


Figure L-26. *Oncorhynchus mykiss* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

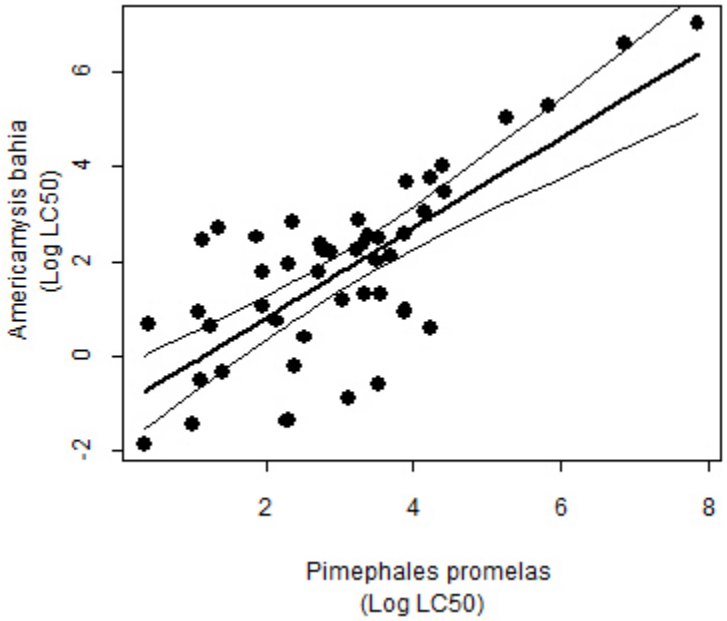


Figure L-27. *Pimephales promelas* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

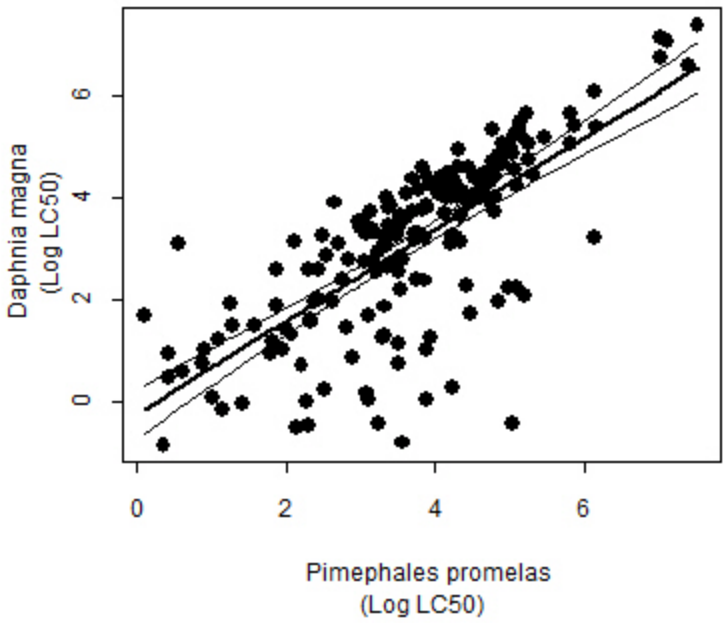


Figure L-28. *Pimephales promelas* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

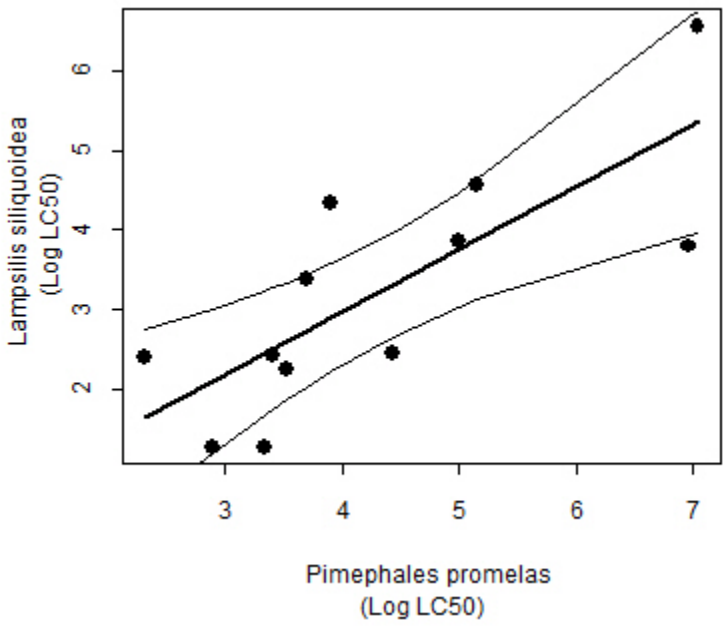


Figure L-29. *Pimephales promelas* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

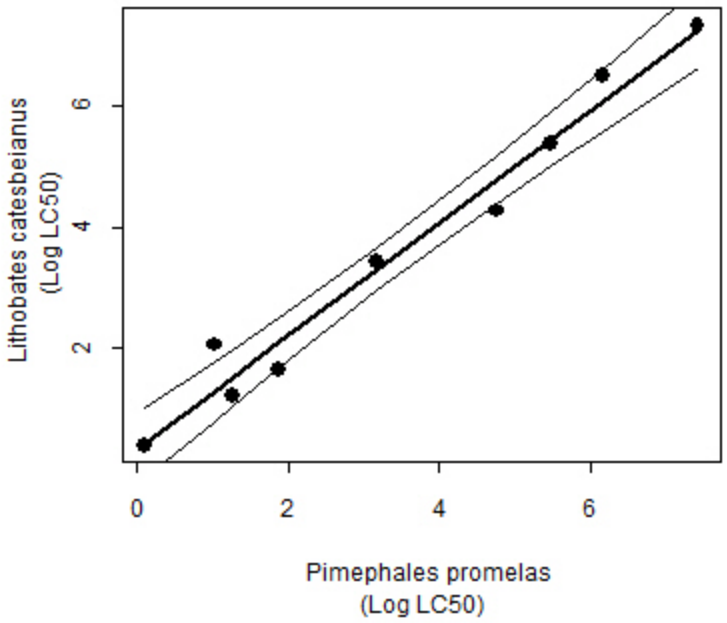


Figure L-30. *Pimephales promelas* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

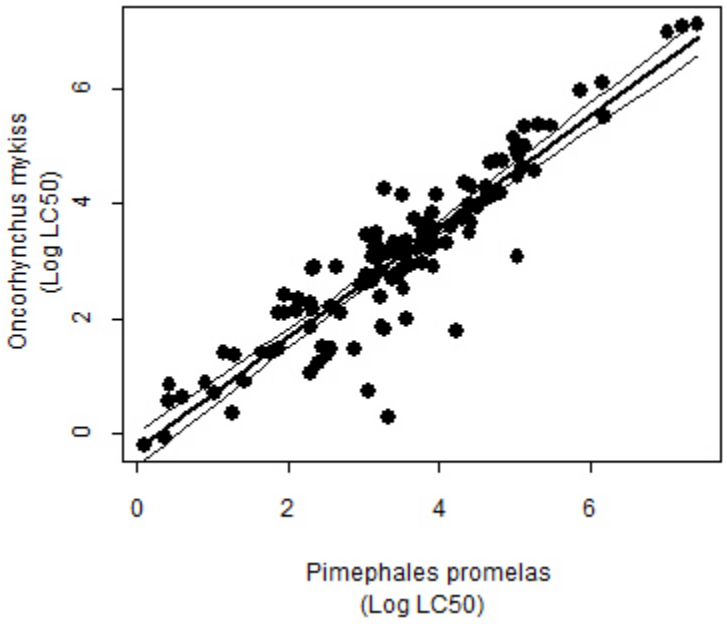


Figure L-31. *Pimephales promelas* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

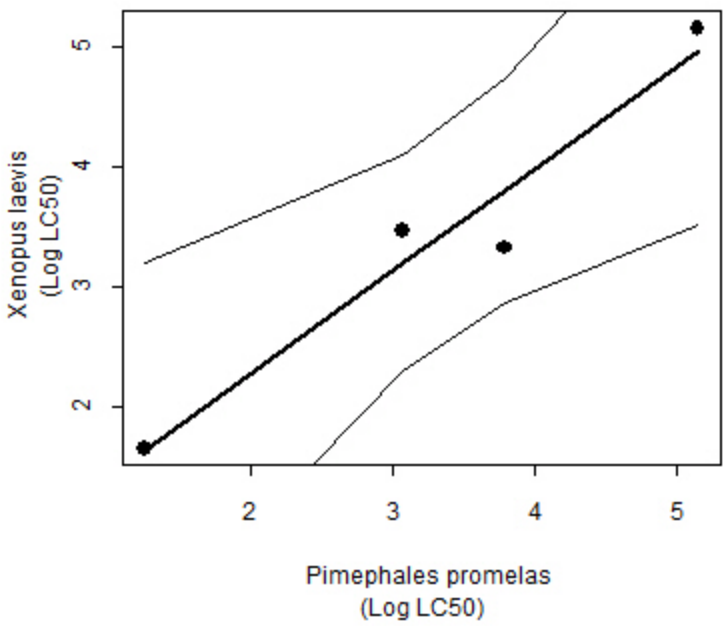


Figure L-32. *Pimephales promelas* (X-axis) and *Xenopus laevis* (Y-axis) regression model used for ICE predicted values.

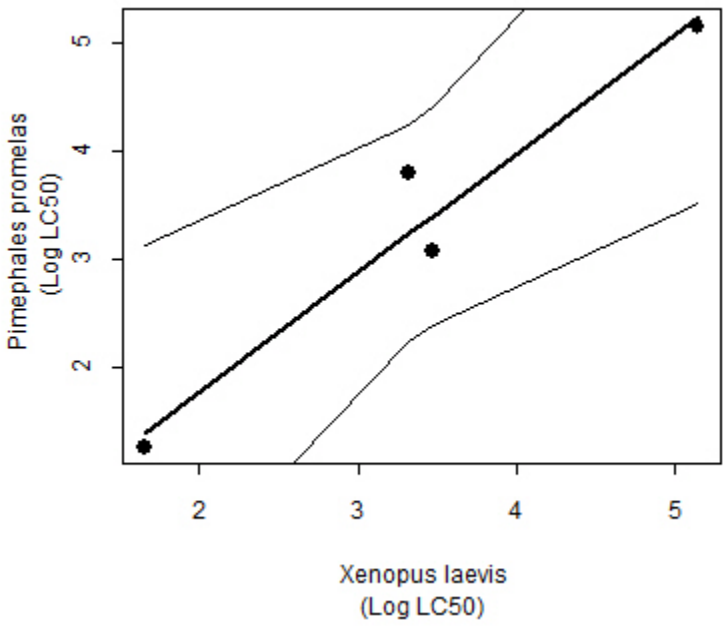


Figure L-33. *Xenopus laevis* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

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 and 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 2001b). 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 2000 a-c; Key et al. 1998; Kurume Laboratory 2002; Lange 2001; Remde and Debus 1996; Sáez 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 the sulfur-limiting conditions, PFOS did not degrade. Similarly,

Sáez 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 and PFOS has a K_{oc} of 2.57 indicated that PFOS is relatively mobile in water and the physicochemical characteristics of the sediment ultimately influence the sorption of PFOS (Ahrens et al. 2011; Beach et al. 2006; Giesy et al. 2011; 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. 2011). 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);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. 2011; 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	3MCompany (2001)
	Pond in Decatur	111	111	111	
	Waterbody in Mobile	30.3	35.5	< 25 – 41.5	3MCompany (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	Colorado Department of Public Health and the Environment (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	
	Colorado River	0.67	0.66	0.65 - 0.69	
	Coon Creek	< 0.48	< 0.48	< 0.48	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	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	
	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	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	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)
Florida	Waterbody in Pensacola	16.29	2.5 < x < 25	<25 – 29	3MCompany (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. (2006)
Georgia	Waterbody in Columbus	59.9	55	44.6 – 80	3MCompany (2001)

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	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)
	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	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
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	< 1	New Mexico Environment Department (2021)
	Animas River	0.799	0.625	< 0.89 - 1.5	
	Canadian River	0.848	0.9	< 0.89 - 1.2	
	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	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	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	
	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	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	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	
	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	

State	Waterbody[1]	Arithmetic Mean PFOS Concentration (ng/L)[2]	Median PFOS Concentration (ng/L)[2]	Range of PFOS Concentration (ng/L)	Reference
	Woonasquatucket River	14.6	14.6	5.87 – 23.2	
South Carolina	Charleston Harbor	12.0	not provided	not provided	Houde et al. (2006)
Tennessee	Waterbody near Cleveland	2.5 < x < 25	2.5 < x < 25	< 2.5 - < 25	3MCompany (2001)
	Conasauga River	<0.009[4]	<0.009[4]	<0.009[4]	Laiser et al. (2011)
Texas	Rio Grande	4.17	4.1	2.0 - 6.5	New Mexico Environment Department (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 (3MCompany 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 (3MCompany 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; 3MCompany 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 (3MCompany 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 (3MCompany 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 (3MCompany 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. (2002) 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 (3MCompany 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. (2017) 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 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 (Sinclair et al. 2006).

The New Jersey Department of Environmental Protection (NJ DEP) 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 (3MCompany 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, 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 Newsted (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 et al. 2010; Butt et al. (2010)
Faroe Islands	< 50 - 0.11 µg/kg	Butt et al. 2010; Kallenborn et al. 2010;(Butt et al. 2010)
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 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 in the particulate phase had a mean concentration of 0.64 pg/m^3 (range: 0.35-1.16) (Kim and Kannan 2007). Kim and Kanaan (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.

2005); with a range of concentrations from detected to 8.1 pg/m^3 (Martins 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 20072008).

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) (EC 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 (Martin et al. 2010). 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 (Research 2007a; Research 2007b).

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 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 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 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 (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 (Martin et al. 2010).

Appendix O Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations

A substantial number of PFOS toxicity tests reported only nominal, or unmeasured, PFOS concentrations (roughly 71% of the acute and 50% of the chronic freshwater quantitative aquatic life toxicity tests, and 43% of the acute and 33% of the chronic saltwater quantitative aquatic life toxicity tests). Therefore, EPA examined whether nominal and measured concentrations were typically in close agreement with each other among the current toxicity literature for aquatic life. The studies used in the analysis were the aquatic life toxicity tests with measured PFOS concentrations that were considered quantitatively (Appendix A through Appendix D) and qualitatively acceptable (Appendix G through Appendix H) for both freshwater and saltwater. Approximately 36% of the 180 of the freshwater toxicity tests and 49% of the 41 saltwater toxicity tests measured PFOS concentrations.

O.1 Summary of Available Freshwater and Saltwater Data for PFOS

Among the PFOS freshwater toxicity tests used quantitatively and qualitatively in the criteria derivation, 65 had measured concentrations. However, several tests only reported measured concentrations graphically, reported summary statistics of the measured concentrations (e.g., measured concentrations averaged 88% ± 7% of their nominal concentrations), reported the same measured concentrations across several tests (i.e., acute test by Hazelton et al. 2012; Hazelton, 2013), or lacked the detail to be part of this analysis. Therefore, 49 unique toxicity tests were used, yielding 477 pairs of measured and nominal concentrations. These pairs excluded controls since PFOS was rarely detected in controls. Specifically, there were only four instances where PFOS was detected in controls. However, the measured concentrations were low at 0.00008 mg/L (Keiter et al. 2012), 0.00001 mg/L (Roland et al. 2014), 0.000006-0.00006 mg/L (Foguth et al. 2020) and 0.002 mg/L (Hazelton et al. 2012). Based on current occurrence

data, PFOS concentrations in the first two studies are consistent with ambient surface water concentrations that are considered to be reference sites, which range between below detection and 0.000138 mg/L (see Section 2.4.1).

Similarly, among the PFOS saltwater toxicity tests considered for quantitatively and qualitatively use, 20 had measured concentrations. However, for similar reasons as the freshwater toxicity tests summarized above, 11 of the tests could not be part of this analysis. Therefore, nine toxicity tests were used, yielding 171 pairs of measured and nominal concentrations. Of these, 58 pairs were from two acute tests, conducted by the same investigators. Lastly, the saltwater pairs excluded controls since PFOS was not detected or not reported in controls from the saltwater studies.

O.2 Methods of Meta-Analysis to Compare of Nominal and Measured PFOS Concentrations

EPA grouped the data by classifications of water type (salt or freshwater) and experimental conditions. The experimental conditions included: (1) acute and chronic test duration; (2) whether test organisms were fed or unfed; (3) test vessel material (glass or plastic); (4) use of solvent or no solvent; and (5) the presence of a substrate. These data classifications were used to observe if differences between nominal and measured concentrations of PFOS could be linked to these experimental conditions, as has been indicated in some toxicity literature for PFAS (Boudreau et al. 2003a and b; Hansen et al. 2001; Martin et al. 2004).

Once grouped by the classifications, paired nominal and measured concentrations were compared by water type and across the experimental conditions mentioned above through linear correlation. The linear correlation evaluations were followed by comparisons of measured concentrations as a percent of nominal in relation to a threshold of greater than ± 20% or 30% to better understand the magnitude and trend of any discrepancies identified in the linear

correlations. Lastly, changes in PFOS concentration in a test solution over time were evaluated for the studies that measured PFOS concentrations in a test solution at the time it was introduced (new solution) and at a later time (old solution) in the exposure duration. All methods pertaining to these analyses are included below.

O.2.1 Linear Correlation Analysis

The linear correlation analysis plotted nominal concentrations on the X-axis and corresponding measured concentrations on the Y-axis and assessed correlation between the paired concentrations across the classifications of water type (freshwater or saltwater) and experimental conditions. Additionally, the geometric means of the ratios between measured and nominal concentrations and the median percent differences were calculated across the classifications of water type and experimental conditions. The geometric means of the ratios between measured and nominal concentrations were calculated by dividing the measured concentration by the nominal. The median percent differences were calculated as the absolute value of the difference between the nominal and measured concentration divided by the nominal concentration multiplied by 100.

O.2.2 Assessment of Measured Concentrations as a Percent of Nominal

The assessment of measured concentrations as a percent (or relative ratio) of nominal was used to identify the proportion of paired nominal and measured PFOS concentrations that were outside a threshold of greater than either ± 20% or 30%. Measured concentrations within 20% of the corresponding nominal concentrations were considered in close agreement within one another based on EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines. For example, U.S. EPA (2016c) states, "*measured concentration of test substance at each treatment level remains within plus or minus (±) 20% of the time-weighted average concentration for the duration of the test*." Similarly, U.S. EPA

(1996) states, "*In any case there must be evidence that test concentrations remained at least 80 percent of the nominal concentrations throughout the test or that mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period*." Finally, the Organization for Economic Cooperation and Development (OECD 2019) defines a stable exposure concentration as, "*A condition in which the exposure concentration remains within 80-120% of nominal or mean measured values over the entire exposure period*." Recently, in a study of key considerations for accurate exposures in ecotoxicological assessments of perfluorinated carboxylates and sulfonates, Rewerts et al. (2021) used a threshold of ± 30% to agree with nominal concentration for both stock and exposure solutions, as specified by the guidelines in the consolidated Quality Systems Manual for Environmental Laboratories set by the U.S. Department of Defense and the U.S. Department of Energy (Coats et al. 2017). The assessment of measured concentrations as a percent of nominal was used to understand the magnitude and trend of PFOS concentrations from the toxicity literature that were outside either one of these two thresholds. Similar to the linear correlations above, the assessment of measured concentrations as a percent of nominal was conducted across the classifications of water type (salt or freshwater) and experimental conditions.

O.2.3 Assessment of Measured PFOS Concentrations in Test Solution Over Time

The assessment of measured PFOS concentrations in a test solution over time compared concentrations from studies that measured PFOS in a solution at the time it was introduced (new solution) and at a later time (> 2 days) in the test (old solution). Thus, this comparison was limited to measurements made on the same solution at different times and excludes measurements on the same treatment but not the same solution. There were five freshwater studies (Drottar and Krueger 2000e; Drottar and Krueger 2000i; Sanderson et al. 2002) that allowed a direct comparison of the change in PFOS concentration in a test solution over time

(see greater detail in Appendices A through G). This assessment excludes Yang et al. (2014), which measured PFOS concentrations on the same treatment, but not the same solution and Palmer and Krueger (2001), which measured PFOS from different renewal solutions of the same treatment. Unlike the two previous analyses, this assessment was not conducted across the classifications of water type (salt or freshwater) and experimental conditions since the available dataset was relatively limited.

O.3 Results of Meta-Analysis to Compare of Nominal and Measured PFOS Concentrations

O.3.1 Examination of Freshwater and Saltwater Data with Discrepancies between Nominal and Measured Concentrations

Of the 477 freshwater pairs of measured and nominal concentrations evaluated in this meta-analysis, 85 were greater than the 20% threshold and 57 were greater that the 30% threshold described by Rewerts et al. (2021). Of these 63 pairs are from several tests and discussed in more detail in Table O-5 below. Any study not summarized in Table O-5 had nominal and measured concentrations that were within the 20% exceedance threshold.

Evaluation of saltwater data was consistent with the approach described for the freshwater data. Discussion of similar studies with large discrepancies are described in Table O-6 (also see greater detail in Appendices A through G). Of the 171 pairs of measured and nominal concentrations evaluated in this meta-analysis, 112 were greater than the 20% threshold and 77 were greater than the 30% threshold used by Rewerts et al. (2021). In this examination of the saltwater data, it was apparent that none of the experimental factors could explain the differences between nominal and measured concentrations. Instead, apparent systematic dosing issues were indicated as the cause for the observed differences. Similar to the freshwater dataset, any saltwater study not summarized in Table O-6 had nominal and measured concentrations that were within the 20% exceedance threshold.

O.3.2 Comparison of Paired Nominal and Measured PFOS Concentrations through Linear Correlation

O.3.2.1 Linear Correlation of Nominal and Measured PFOS Concentrations by Water Type

Overall, the comparison of nominal and measured concentrations from the freshwater PFOS toxicity literature indicated that measured and nominal concentrations were in close agreement. Specifically, the ratio of measured to nominal concentrations from the freshwater dataset showed little bias with a geometric mean value of 0.9676.

Figure O-1A also displays the strong correlation (0.9770) of the 477 pairs of nominal and measured concentrations, with the pairs mostly falling in a tight range (close to 1.0). The median percent difference between pairs was 6.923%.

In contrast, the comparison of measured to nominal concentrations in saltwater showed greater differences, with most measured concentrations being lower than the nominal concentrations indicated. Specifically, while Figure O-1B displays the strong correlation (0.9735) of the 171 pairs of nominal and measured concentrations, the ratio of measured to nominal concentrations from the saltwater dataset showed bias with a geometric mean value of 0.6468. Additionally, the median percent difference between measured and nominal concentration was 28.02%. These results indicate that measured and nominal concentrations from saltwater tests were not in close agreement, with most measured concentrations being lower than nominal. These disparities were examined further in the subsequent analysis below to better understand the magnitude of these apparent differences in relation to the 20% threshold (see Section O.3.3 below).

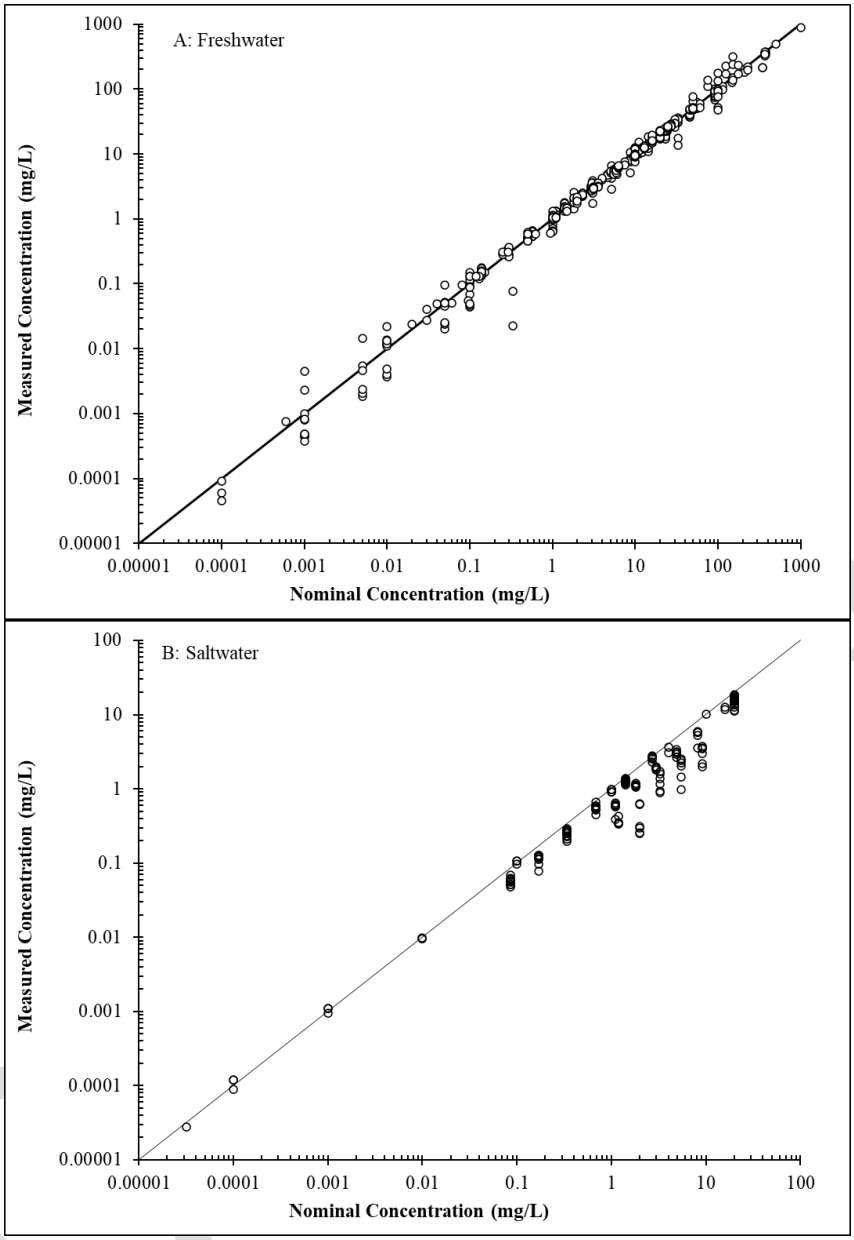


Figure O-1. Comparison of PFOS measured and nominal concentrations for freshwater (A) and saltwater (B) data.
Many points overlie each other, such that the dark areas represent multiple values, over-plotted.

O.3.2.2 Linear Correlation of PFOS Nominal and Measured Concentrations by Experimental Condition

The nominal and measured concentration pairs were further compared in terms of experimental conditions, keeping the data separated by water type. In general, strong correlations were observed across all experimental conditions in freshwater (correlations > 0.95) with only

slightly weaker correlation in saltwater tests (correlations > 0.84). Additionally, the freshwater nominal and measured concentrations were in close agreement across experimental conditions. Ratios between paired concentrations were generally in a tight range (close to 1.0) with a geometric mean ratio of > 0.90, which indicated experimental conditions play little role in the observed differences between nominal and measured PFOS concentrations in freshwater. The exception was the inclusion of substrate in freshwater tests where the geometric mean value of the ratios between measured and corresponding nominal concentrations was only 0.7455.

In contrast to freshwater, the saltwater nominal and measured concentrations were found to be in less agreement across experimental conditions compared to the freshwater dataset, with the pairs falling outside a tight range and geometric mean values of the ratios between measured and nominal concentrations ranging between 0.5056 and 0.8115 (Table O-1).

Table O-1. Comparison of Pairs Nominal and Measured PFOS Concentrations across Experimental Conditions.

Experimental Condition	Freshwater				Saltwater			
	# Paired Obs.	Correlation	Geometric Mean of Measured/ Nominal	Median Percent Difference	# Paired Obs.	Correlation	Geometric Mean of Measured/ Nominal	Median Percent Difference
Acute	211	0.9978	0.9608	5.544%	82	0.9706	0.5056	39.52%
Chronic	266	0.9570	0.9731	8.035%	89	0.9911	0.8115	19.30%
Unfed	213	0.9978	0.9663	5.556%	25	0.8430	0.7701	21.50%
Fed	264	0.9569	0.9687	8.000%	146	0.9002	0.6277	29.52%
Solvent	78	0.9981	0.9035	8.147%	31	0.9455	0.7824	20.81%
No solvent	399	0.9645	0.9807	6.552%	140	0.8621	0.6201	31.85%
Substrate	49	0.9951	0.7455	48.40%	1	-[a]		
No substrate	428	0.9826	0.9970	5.795%	170	0.9547	0.6456	28.13%
Glass	155	0.9993	1.031	6.957%	72	0.9959	0.7707	22.20%
Plastic	245	0.9567	0.9405	5.932%	99	0.9705	0.5694	36.34%
Unspecified material	77	0.9980	0.9317	9.403%	0	-[a]		

[a] Not evaluated due to a lack of data for these experiment condition categories.

O.3.2.3 Comparisons by Test Duration and Organismal Feeding Conditions

First, freshwater and saltwater nominal and measured concentrations were compared by exposure duration (i.e., acute or chronic) and organismal feeding. In the freshwater dataset, the acute pairs were the same as the unfed pairs and the chronic pairs are the same as the fed pairs, with the exception of two data points from the unfed chronic mussel test of Hazelton et al. (2012). Conversely, while pairs for saltwater acute and chronic tests were more evenly split 82 vs. 89 pairs, respectively, most pairs were from fed tests, despite being acute tests.

The comparison of nominal and measured concentrations in freshwater indicated that measured and nominal concentrations under acute, chronic, unfed, and fed conditions displayed strong correlations (> 0.95; Table O-1). Further, the freshwater nominal and measured concentrations were found to be in close agreement, with the pairs mostly falling in a tight range (close to 1.0) with geometric mean values of the ratios between measured and nominal concentrations being > 0.96, indicating that test duration and organismal feeding play little role in the observed differences between nominal and measured concentrations of PFOS in

freshwater. Additionally, the median percent differences for the pairs across these experimental conditions were equal to or ≤ 8% (Table O-1).

In contrast, the saltwater nominal and measured concentrations were in less agreement, with test pairs under acute, chronic, unfed, and fed conditions falling in a highly variable range with geometric mean values of the ratios between measured and nominal concentrations being anywhere from 0.5056 to 0.8115. Additionally, the median percent differences for the pairs across these experimental conditions were relatively high (≥ 19%; Table O-1). The saltwater comparison of nominal and measured concentrations indicates that these experimental conditions (acute/chronic and unfed/fed) may influence the observed differences between measured and nominal concentrations.

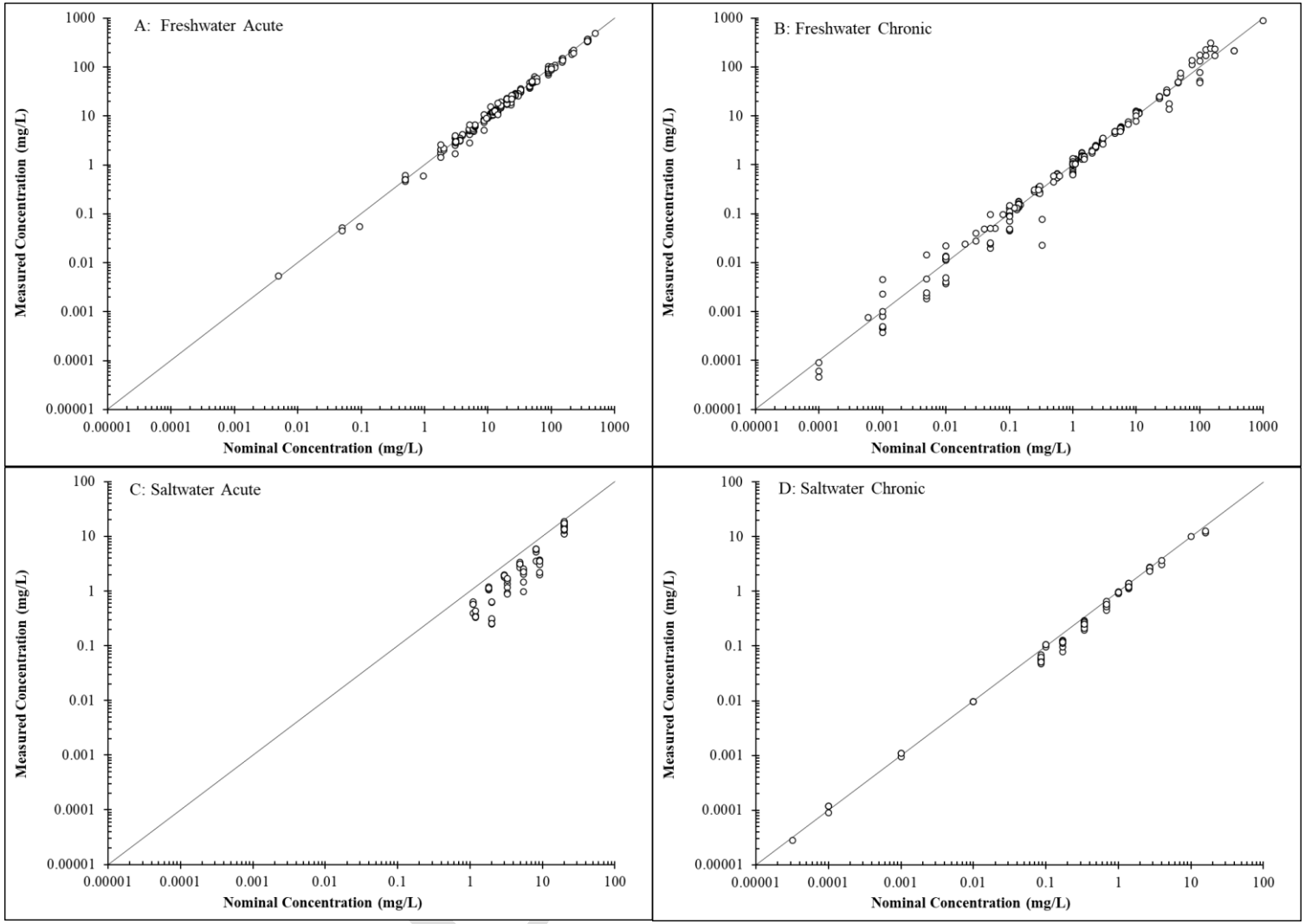


Figure O-2. Comparison of PFOS measured and nominal concentrations in freshwater (top) and saltwater (bottom) tests with acute (A and C, respectively) and chronic durations (B and D, respectively).

In the freshwater datasets acute is the same as unfed, and chronic the same as fed, with the exception of two data points. Many points overlie each other, such that the dark areas represent multiple values, over-plotted.

O.3.2.4 Comparisons by Test Vessel Material

The potential influence of additional experimental conditions related to test vessel material (e.g., plastic, glass, or unspecified test vessels) were also considered. This comparison considers the influence of the material from which the exposure chambers were constructed for freshwater and saltwater tests. The "unspecified" designation were those tests that did not specify the material used to construct the exposure chambers and appears to be similar to the other two test vessels types (Table O-1); thus, this experimental condition was not included in Figure O-3 below.

The comparison of nominal and measured PFOS concentrations in freshwater tests indicated that measured and nominal concentrations for tests using various test vessel types displayed strong correlations (> 0.95; Table O-1). Additionally, the freshwater nominal and measured concentrations were found to be in close agreement, with pairs mostly falling in a tight range (close to 1.0) with geometric mean of the ratios between measured and nominal concentrations being > 0.93. The median percent differences for the pairs across these experimental conditions were < 10% (Table O-1). These results indicate that test vessel material played little role in the observed differences between nominal and measured concentrations of PFOS in freshwater tests.

In contrast, the saltwater comparison of nominal and measured concentrations indicated that tests using various test vessel types of plastic and glass were in less agreement and nominal to measured ratios displayed higher variability than in corresponding test vessel materials from freshwater tests. The geometric mean ratio of measured and nominal PFOS concentrations from saltwater tests using glass was 0.7707. Pairs from saltwater tests conducted in plastic test vessels showed the highest degree of variability, with a geometric mean of 0.5694. Additionally, the

median percent differences for the pairs across test vessel materials were relatively high (> 22%; Table O-1).

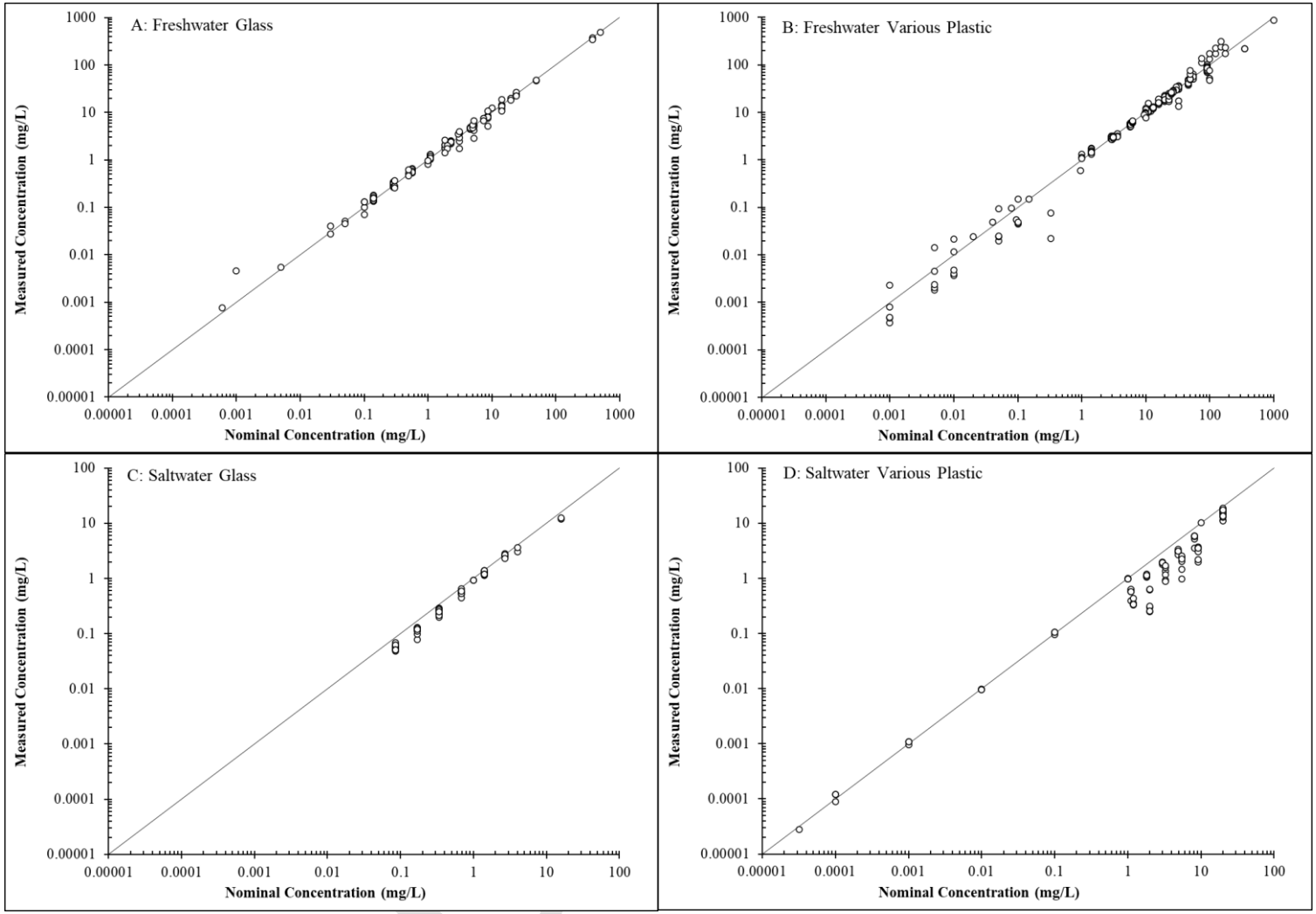


Figure O-3. Comparison of PFOS measured and nominal concentrations in freshwater (top) and saltwater (bottom) tests conducted in glass (A and C, respectively) and plastic test vessels (B and D, respectively).
Many points overlie each other, such that the dark areas represent multiple values, over-plotted.

O.3.2.5 Comparisons by Presence of Solvent

The potential influence of the use of a solvent was also considered as part of the experimental conditions that might impact potential differences between nominal and measured concentrations in the PFOS toxicity literature. Measured and nominal concentrations for freshwater tests using solvent and not using solvent both displayed strong correlations of >0.99 and > 0.96, respectively (Table O-1). The nominal and measured concentrations were in agreement in tests with solvent (geometric mean of nominal to measured ratios was 0.9035), and in even closer agreement in tests without solvent (geometric mean of nominal to measured ratios was 0.9807).

The pairs from saltwater tests with or without solvent were in less agreement than freshwater pairs, with a geometric mean of the ratios between measured and nominal concentrations being 0.7824 with solvent and 0.6201 without solvent. Further, the median percent difference for the pairs with solvent was 21% while the median percent difference for saltwater tests without a solvent was 32%.

O.3.2.6 Comparisons by Presence of Substrate

Lastly, the potential influence of the presence of a substrate was considered as part of the experimental conditions that might impact potential differences between nominal and measured concentrations in the PFOS toxicity literature. This experimental condition was considered to be potentially important since the presence of substrate could possibly increase the likelihood of disparities between measured and nominal given the potential sorption of PFOS under certain environmental conditions (see Section 2.2), but there are too few data to be conclusive. Generally, strong correlations (> 0.98; Table O-1) were observed with freshwater pairs from test with and without substrate. However, the geometric mean of the ratios between measured and nominal concentrations were 0.9970 (no substrate) or 0.7455 (substrate) indicating that the

presence of substrate may be removing the PFOS from test solutions. Similarly, the median percent differences of 5.795% for tests without substrate and 48.40% with substrate indicate disparities between measured and nominal concentrations that may be associated with the presence of substrate.

There were insufficient data for the same comparison in saltwater because only one observation of paired and nominal concentrations was available for saltwater tests with substrate. Pairs from saltwater tests without substrate were disparate, with a geometric mean of the ratios between measured and nominal concentrations being 0.6456 and a median percent difference of 28.13%.Drottar and Krueger (2000e); Drottar and Krueger (2000f)

O.3.3 Assessment of Measured Concentrations as a Percent of Nominal in Relation to the 20% Threshold

O.3.3.1 Assessment of Measured Concentrations as a Percent of Nominal based on Water Type

In general, the freshwater nominal and measured concentrations were found to be in close agreement, with limited instances (only about 18%) of measured concentrations differing from paired nominal concentrations by more than 20%. It should be noted that the majority of these are from studies detailed in Table O-5 below. A smaller portion, approximately 12%, of measured concentrations differed from paired nominal concentrations by more than 30%. (Figure O-4). The magnitude of measured concentrations as a percent (presented as relative ratio in Figure O-4 below) of nominal varied by a minimum of 6.8% to a maximum of 452% of nominal (Table O-2). In contrast, while the saltwater nominal and measured concentrations were found to be in relatively close agreement in the linear correlation, a much higher proportion (65.5%) of nominal and measured concentration pairs were outside the 20% threshold and 45% outside the 30% threshold (Figure O-4). The magnitude of measured concentrations as a percent of nominal varied by a minimum of 12.45% to a maximum of 120% of nominal (Table O-2). However, as

noted previously, the saltwater dataset was much smaller and the systematic discrepancies between measured and nominal concentrations are harder to decipher.

Table O-2. Proportion of Paired Nominal and Measured PFOS Concentrations Outside the 20% Threshold.

	Freshwater				Saltwater			
Experimental Condition	**# Paired Obs.**	**Proportion Outside +/- 20% Threshold**	**Min. of Measured Concentration as a Percent of Nominal (%)**	**Max. of Measured Concentration as a Percent of Nominal (%)**	**# Paired Obs.**	**Proportion Outside +/- 20% Threshold**	**Min. of Measured Concentration as a Percent of Nominal (%)**	**Max. of Measured Concentration as a Percent of Nominal (%)**
All	477	0.1782	6.8	452	171	**0.6550**	12.45	120
Acute	211	0.0806	54.7	142	82	**0.8659**	12.45	93.00
Chronic	266	0.2556	6.8	452	89	**0.4607**	45.76	120
Unfed	213	0.0892	54.7	452	25	**0.5200**	55.50	93.00
Fed	264	0.2500	6.8	288	146	**0.6781**	12.45	120
Solvent	78	0.1282	45	124	31	**0.5161**	55.50	93.00
No Solvent	399	0.1880	6.8	452	140	**0.6857**	12.45	120
Substrate	49	**0.7347**	6.8	288	1	-[1]		
No Substrate	428	0.1145	45	452	170	**0.6588**	12.45	120
Glass	155	0.1290	54.7	452	72	**0.5694**	45.76	103.3
Plastic	245	0.2204	6.8	288	99	**0.7172**	12.45	120
Unspecified	77	0.1429	45	136	0	-[1]		

[1] Not evaluated due to a lack of data for these experiment condition categories.
[2] Bolded values represent test conditions with a high proportion of measured concentrations that were not within 20% threshold.

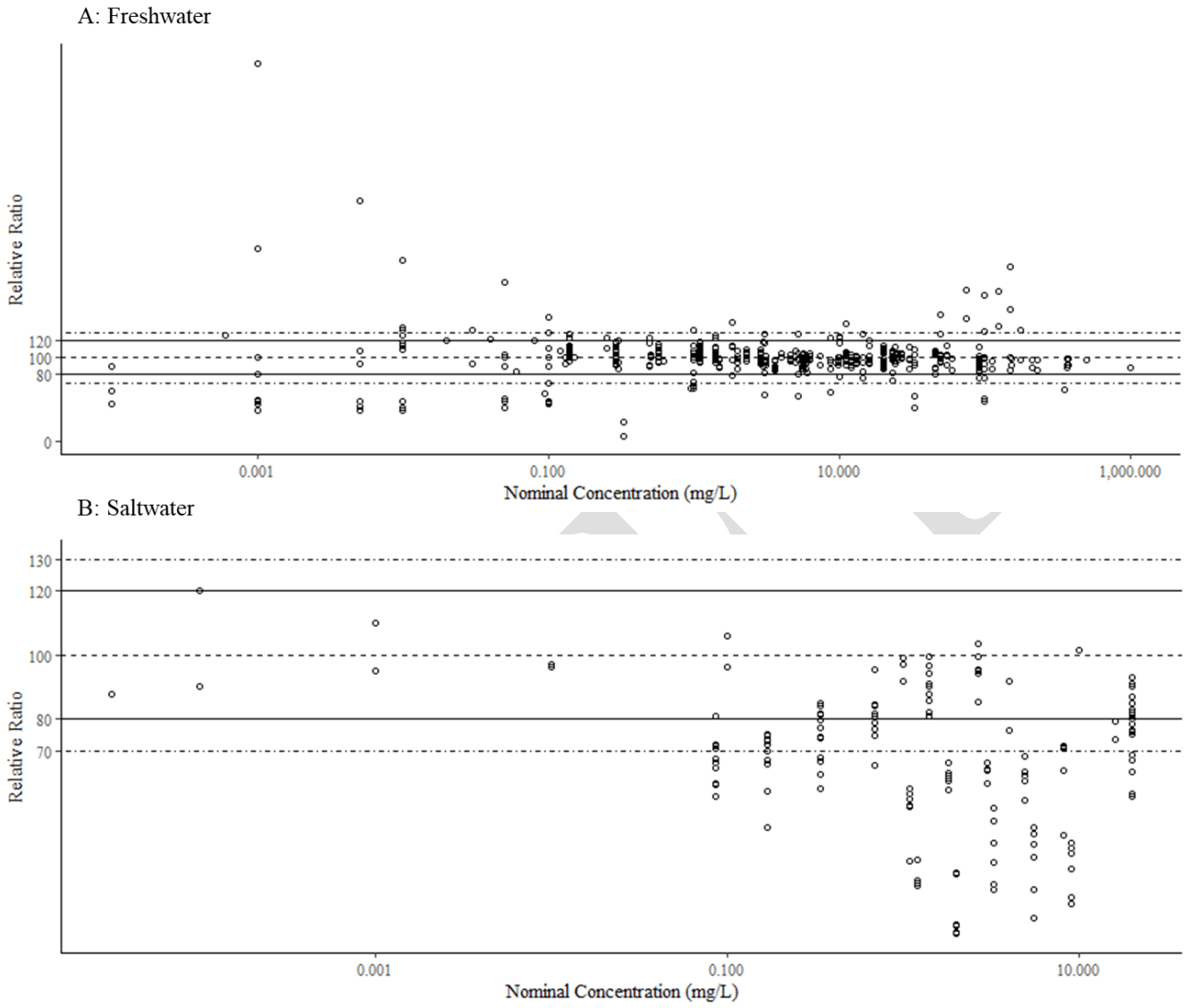


Figure O-4. Assessment of measured concentrations as a percent of nominal in relation to a 20% and 30% threshold for freshwater (A) and saltwater (B) data.
The horizontal line coding: 100% (dash line); +/- 20% (solid lines); +/- 30% (dash/dot lines). Data within these thresholds were considered to result in close agreement of measured and nominal concentrations. Many points overlie each other, such that the dark areas represent multiple values, over-plotted.

O.3.3.2 Assessment of Measured Concentrations as a Percent of Nominal based on Experimental Conditions

In freshwater across all test conditions, 18% paired nominal and measured concentrations were outside the 20% threshold. In the various subsets of conditions, the proportion of these values outside this threshold ranged from 8 to 73% (excluding test with substrate, the maximum percent of values outside the threshold was ≤ 26%). Conversely, all saltwater pairs were

observed to fall outside the 20% threshold across the experimental conditions, with all experimental conditions having > 46% of pairs outside the 20% threshold.

To examine these observed differences further, key experimental conditions with either noted differences documented in the linear correlation analysis above or previously documented potential influence from the toxicity literature (Boudreau et al. 2003a and b; Hansen et al. 2001; Martin et al. 2004) are presented below in greater detail. These experimental conditions include (1) test duration in saltwater tests; (2) test vessel material in both freshwater and saltwater tests; and (3) the presence of substrate in freshwater tests. The nominal and measured concentrations for all other experimental conditions were found to be in close agreement (Table O-2) or the observed differences were considered to be influenced by individual studies, not the individual experimental condition. Thus, these additional experimental conditions were not considered further.

O.3.3.3 Assessment based on Test Duration in Saltwater Tests

Similar to the linear correlation analysis above, the nominal and measured concentrations from acute saltwater tests were disparate, with the majority (> 86%) of measured concentrations as a percent of nominal (presented as relative ratio in Figure O-5 below) falling outside the 20% threshold and 73% outside the 30% threshold. Additionally, the magnitude of the discrepancies outside the 20% threshold ranged between 12.45 and 93.00% (Table O-2). In contrast, while the nominal and measured concentrations from chronic saltwater tests were found to be in closer agreement in the linear correlation analysis, a relatively high proportion (46.07%) of measured concentrations as a percent of nominal were outside the 20% thresholds and 19.10% outside the 30% threshold (Figure O-5B). Further, the magnitude of exceedances outside the 20% threshold ranged between 45.76 and 120% (Table O-2). Indicating that measured concentrations were

sometimes much lower than corresponding nominal concentrations in both acute and chronic saltwater tests.

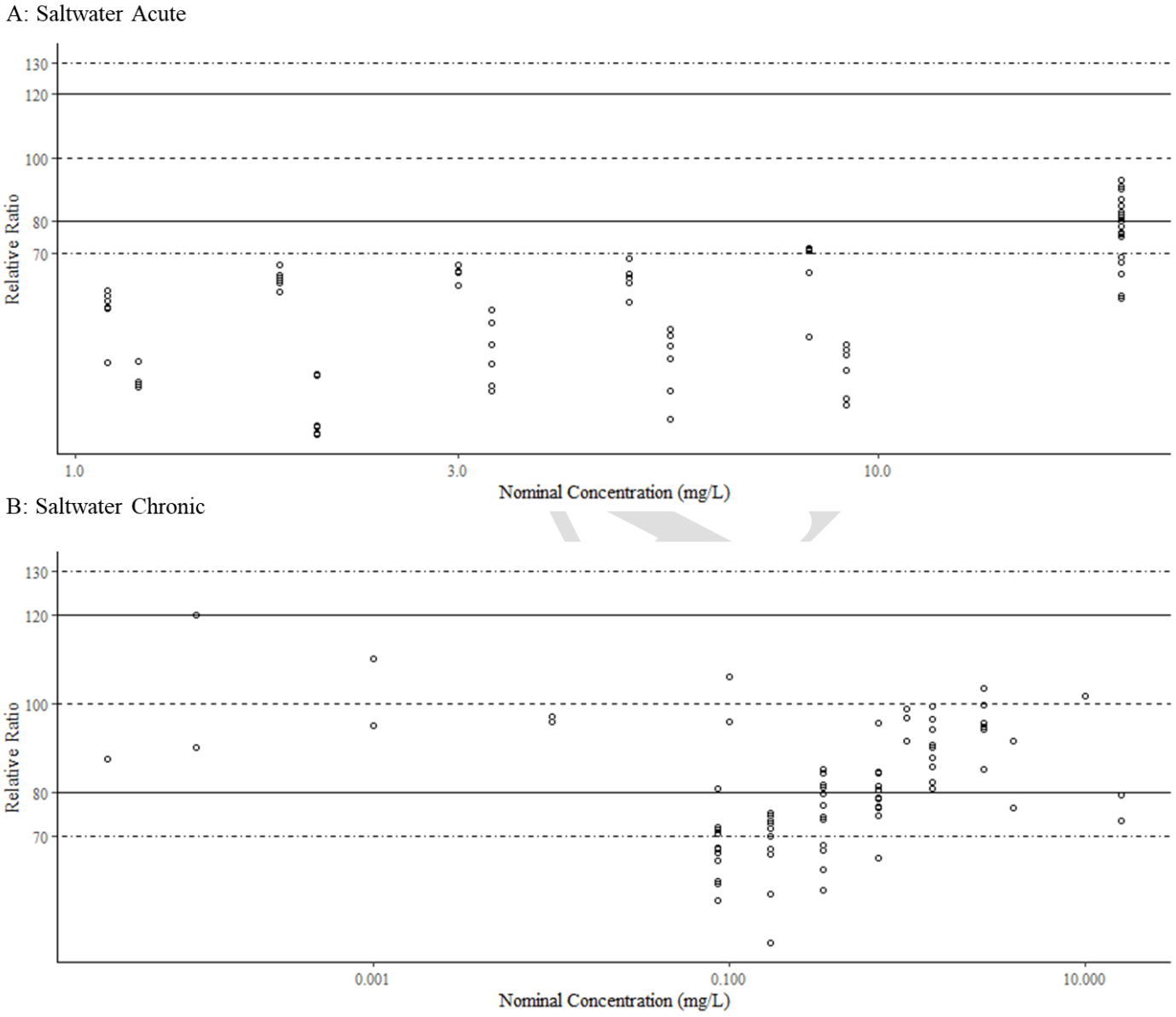


Figure O-5. Assessment of measured concentrations as a percent of nominal in relation to a 20% and 30% threshold for saltwater acute (A) and chronic (B) tests.
The horizontal line coding: 100% (dash line); +/-20% (solid lines); +/-30% (dash/dot lines). Data within these thresholds were considered to result in close agreement of measured and nominal concentrations. Many points overlie each other, such that the dark areas represent multiple values, over-plotted.

O.3.3.4 Assessment based on Test Vessel Material in Freshwater and Saltwater Tests

Similar to the linear correlation analysis above, the freshwater nominal and measured concentrations from tests using various test vessel types were found to be in close agreement, with ≤ 22% outside the 20% threshold and ≤ 17% outside the 30% threshold (Figure O-6). Tests conducted in glass test vessels had the widest magnitude of measured concentrations varied with respect to nominal by a minimum of 54.7% to a maximum of 452% of nominal (Table O-2). These results further indicate that test vessel material played little role in the observed differences between nominal and measured concentrations of PFOS in freshwater tests.

As for the saltwater nominal and measured concentrations from tests using various test vessel types, relatively high proportions (56.94 and 71.72%) of measured concentrations as a percent of nominal were outside the 20% threshold for glass and plastic test vessels, respectively (Figure O-6B). Further, tests conducted in plastic test vessels had the widest magnitude of exceedances outside the 20% threshold, which ranged between 12.45 and 120% (Table O-2). Indicating that measured concentrations were typically lower than corresponding nominal concentrations in saltwater tests. However, as previously mentioned, the saltwater data were relatively limited, and it was difficult to discern if these observed differences were the result of the test vessel material, water type, or were directly related to the systematic discrepancies in individual studies.

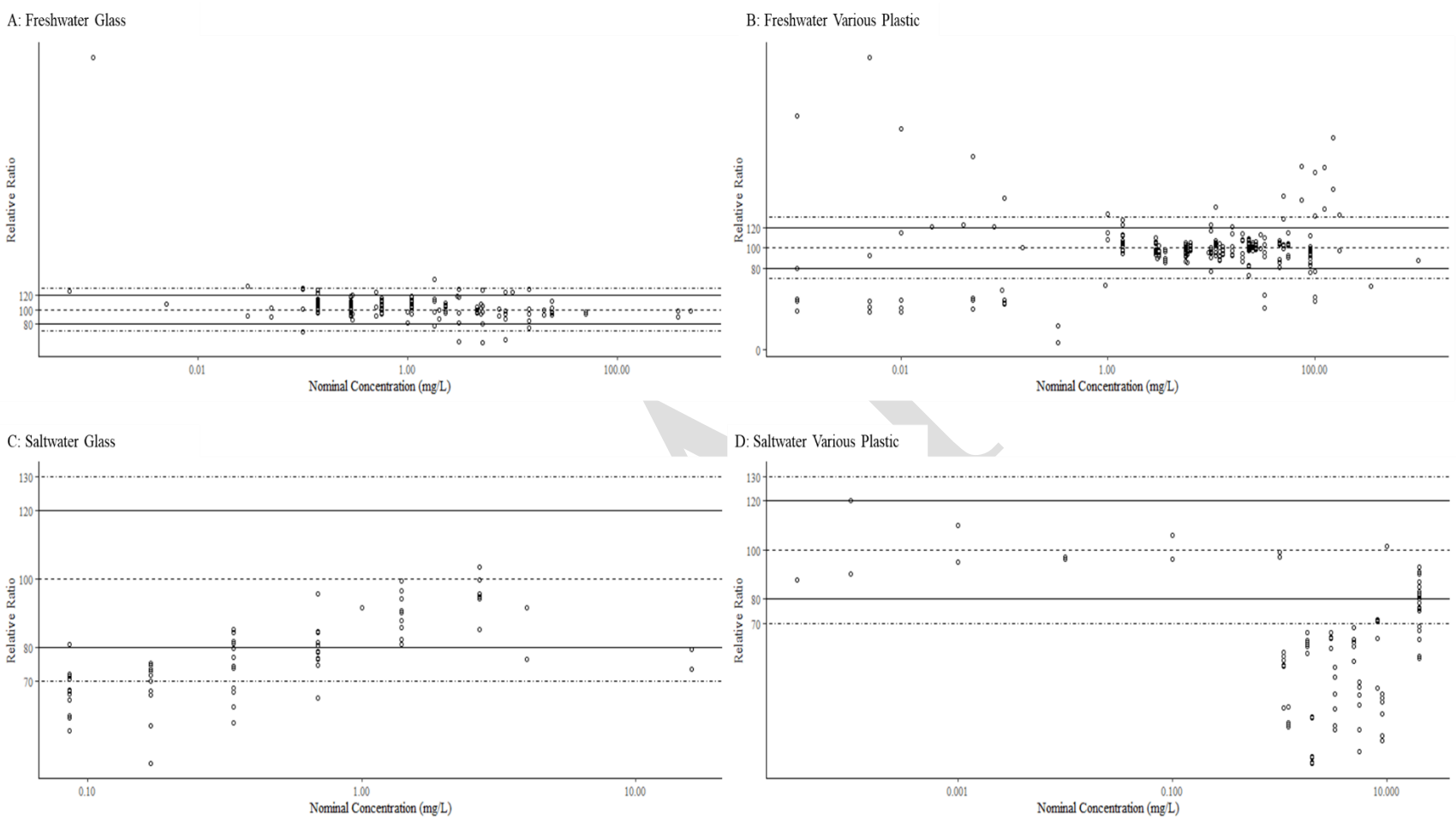


Figure O-6. Assessment of measured concentrations as a percent of nominal in relation to a 20% and 30% threshold for freshwater (top) and saltwater (bottom) tests conducted in glass (A and C; respectively) and various plastic (B and D; respectively) test vessels.

The horizontal line coding: 100% (dash line); +/-20% (solid lines); +/-30 i.e. (dash/dot lines). Data within these thresholds were considered to result in close agreement of measured and nominal concentrations. Many points overlie each other, such that the dark areas represent multiple values, over-plotted.

O.3.3.5 Assessment based on Presence of Substrate in Freshwater Tests

The nominal and measured concentrations from freshwater tests with the presence of a substrate indicate a discrepancy, with 73.47% of measured concentrations as a percent of nominal falling outside the 20% threshold and 63.27% falling outside the 30% threshold. Additionally, the magnitude of the exceedances outside the 20% threshold ranged between 6.8 and 288% (Table O-2). In contrast, the nominal and measured concentrations from freshwater tests without the presence of a substrate were found to be in much close agreement, with few (11.45%) of the measured concentrations as a percent of nominal outside the 20% threshold and 6.07% outside the 30% threshold (Figure O-7B). However, the magnitude of exceedances outside the 20% threshold ranged between 45 and 452% in freshwater tests without the presence of substrate (Table O-2). It should be noted that measured and nominal concentration pairs for studies with a substrate present were limited, especially for saltwater tests in which there was only a single pair. It is because of this data limitation that a similar assessment was not conducted for saltwater tests.

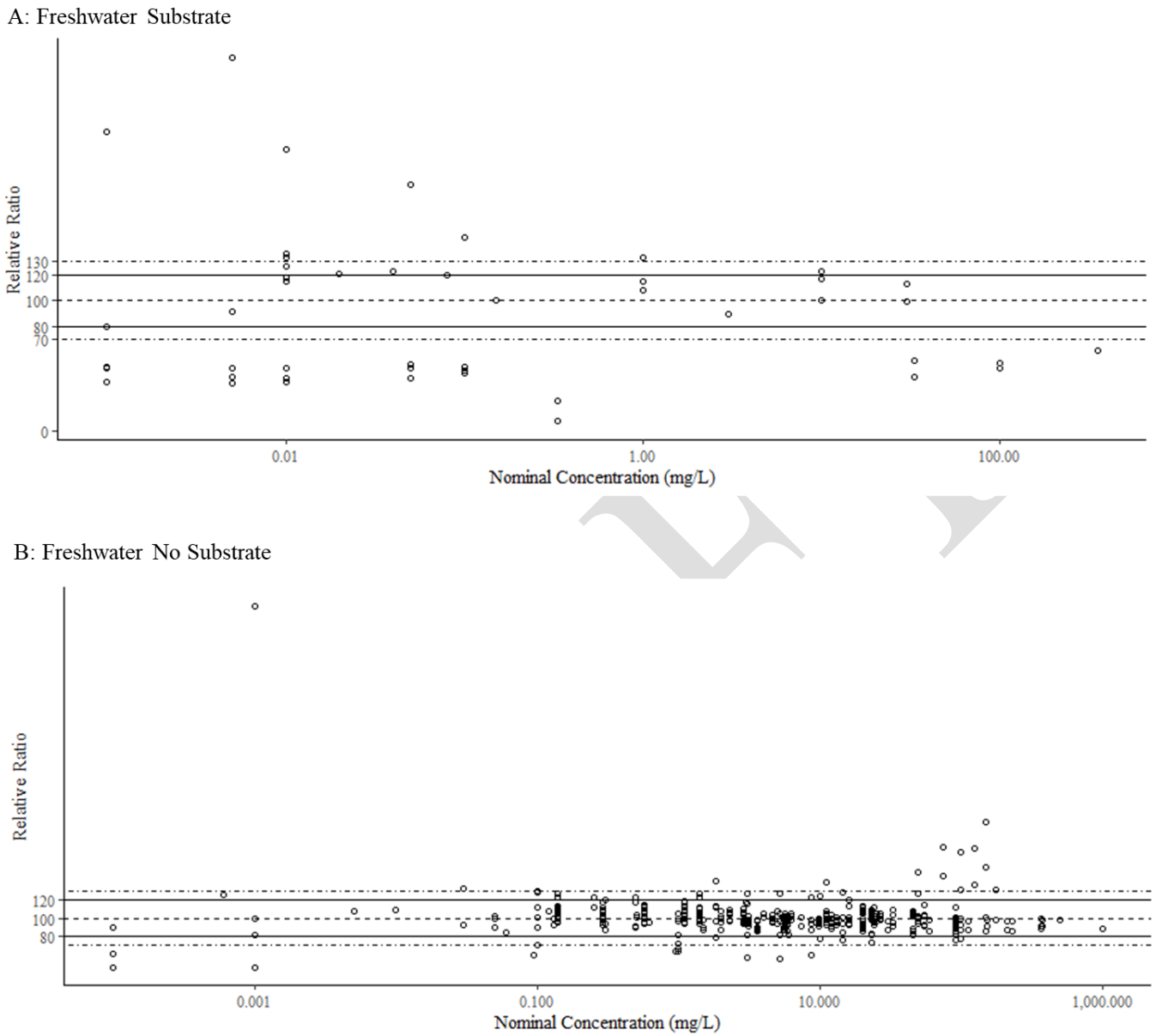


Figure O-7. Assessment of measured concentrations as a percent of nominal in relation to a 20% and 30% threshold for freshwater tests with substrate (A) and without substrate (B).
The horizontal line coding: 100% (dash line); +/-20% (solid lines); +/-30% (dash/dot lines). Data within these thresholds were considered to result in close agreement of measured and nominal concentrations. Many points overlie each other, such that the dark areas represent multiple values, over-plotted.

O.3.4 Assessment of Measured PFOS Concentrations in Test Solution Over Time

Figure O-8 compares the measured PFOS concentrations from old test solution versus the new test solution and a strong correlation (0.9938) was observed. The measured PFOS concentrations in old test solutions appear to correspond well to those in the new test solutions

with the median ratio between new and old solutions of 1% (that is, the old solution 1% lower than the new) and the geometric mean of the ratios between measured and nominal concentrations of 0.9517. These results are limited considering the level of PFOS data that is currently available. However, these results indicate that concentrations of PFOS in water were stable over time.

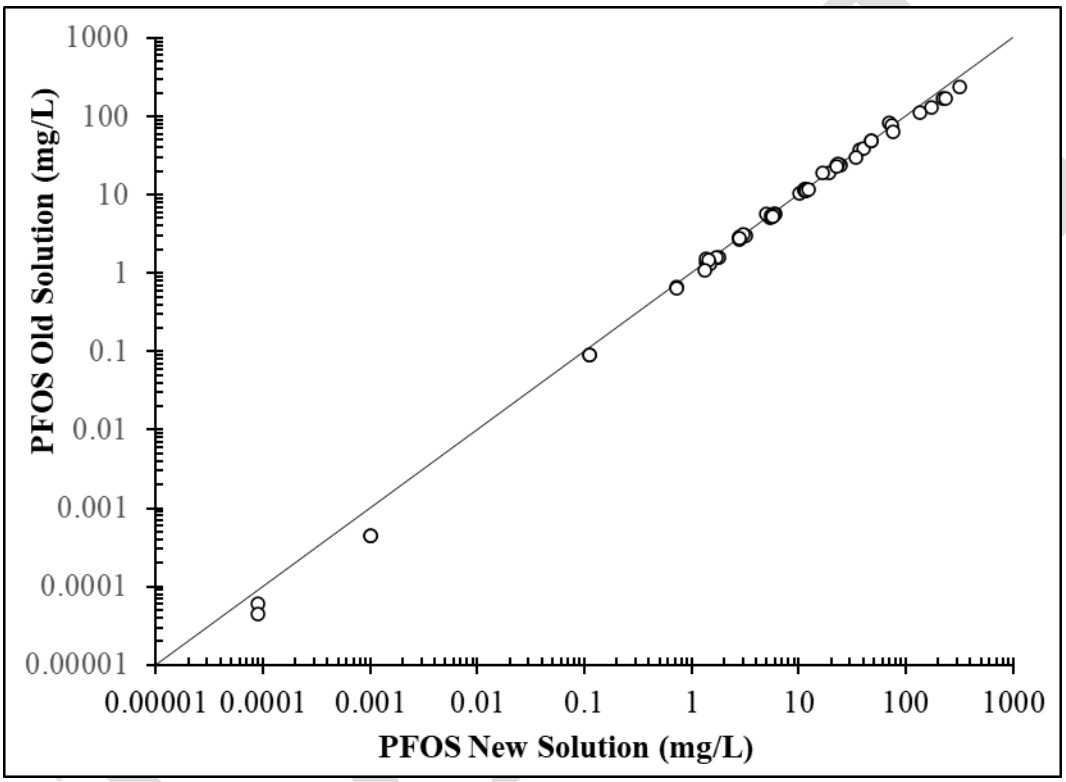


Figure O-8. PFOS exposure concentrations measured at the end of the renewal period or static test (old solutions) vs. concentrations measured in the same solution immediately after it was introduced into the exposure chamber (new solutions).

O.4 Conclusions of Measured Meta-Analysis to Compare of Nominal and Measured PFOS Concentrations

The comparison of nominal and measured concentrations in the current PFOS toxicity literature generally displayed a high degree of linear correlation and close agreement based on geometric means of the ratios and the median percent differences between measured and nominal concentrations. Assessment of measured concentrations as a percent (or relative ratio) of nominal

also indicated that PFOS concentrations were generally in close agreement, with greater than 82% of the freshwater data within 20% threshold that is consistent with the test acceptability threshold identified by EPA's OCSPP's Ecological Effects Test Guidelines. Recent PFAS literature has indicated standard variability between nominal and corresponding measured concentrations may even be as high as 30% and only 12% of paired values were outside this threshold. For example, Rewerts et al. (2021) indicated that , nominal and measured concentrations for both stock and exposure solutions should to fall within the margin of $100 \pm 30\%$, as specified by the guidelines in the consolidated Quality Systems Manual for Environmental Laboratories set by the U.S. Department of Defense and the U.S. Department of Energy (Coats et al. 2017). Further, Rewerts et al. (2021) concluded the variability between measured and nominal concentrations may be influenced by solution homogenization and subsampling procedures, noting storage container type may influence agreement between measured and nominal PFOS concentrations based on the concerns stated in previous literature. However, it should be noted that container type (as glass or plastic) did not influence the observed differences between measured and nominal PFOS concentrations in the analysis presented here.

Specifically, these analyses indicated that nominal and measured concentrations of PFOS were in close agreement across freshwater data when grouped by water type and the following experimental conditions: (1) acute and chronic test duration; (2) whether test organisms were fed or unfed; (3) test vessel material (glass or plastic); and (4) use of solvent or no solvent. Thus, the results of this meta-analysis indicate that these experimental conditions had little influence on any observed discrepancies between nominal and measured concentrations of PFOS. Instances where measured concentrations were not found to be in close agreement with nominal

concentrations (either in the linear correlation analysis or the assessment of measured concentrations as a percent of nominal related to a 20% exceedance threshold) were isolated to a few studies. In these cases, suspected dosing errors, unexplained phenomena, and/or presence of substrate may have contributed to observed differences. Therefore, dosing errors and differences in experimental design were not considered to be systemic issues across PFOS toxicity tests since discrepancies were only observed in a small subset of the observed pairs of measured and nominal concentrations. The presence of substrate caused disparities between measured and nominal concentrations. However, in these tests measured concentrations were not systematically less than or greater than nominal concentrations. Therefore, PFOS could bind to substrate, effectively removing it from the water column, or added substrate may even be acting as a source of PFOS in certain instances. However, expected discrepancies between nominal and measured concentrations in freshwater tests with substrate are of minimal concern to the final acute and chronic freshwater PFOS criteria magnitudes. No unmeasured tests with substrate were used to quantitatively derive the PFOS acute criterion magnitude. Only one unmeasured test with substrate (Spachmo and Arukwe 2012) was used to derive the chronic water column-based criterion magnitude. In this test, Spachmo and Arukwe (2012) used tank bed gravel to simulate a riverbed environment for hatching Atlantic salmon. Spachmo and Arukwe (2012) observed no effects at the single treatment concentration (i.e., 0.10 mg/L PFOS). Because no other chronic toxicity data were available for members of the genus *Salmo*, the 49-day growth-based NOEC of 0.10 mg/L served directly as the *Salmo salar* SMCV and the *Salmo* GMCV. The *Salmo* GMCV was the sixth most sensitive GMCV, which had a minimal impact on the chronic water-column criterion magnitude since it was not among the four most sensitive genera (see section C.2.6).

Compared to freshwater tests there was much less agreement between nominal and measured PFOS concentrations in saltwater tests bot as a whole and across experimental conditions. Overall, results of this meta-analysis indicated that measured concentrations from saltwater tests were systematically lower than nominal concentrations. Therefore, it could be hypothesized (but not confirmed) that the PFOS saltwater benchmark is underproductive because two unmeasured tests were included in the derivation process, including the acute 72-hr. acute test on *Paracentrotus lividus* (Gunduz et al. 2013), which was the basis of the third most sensitive estuarine/marine GMAV (see Appendix L).

Based on the results of this meta-analysis and the general close agreement between measured and nominal concentrations of PFOS, both measured and unmeasured PFOS toxicity tests were used to derive the aquatic life criteria. Additionally, use of both measured and unmeasured PFOS toxicity tests is further supported by the high stability of PFOS indicated in this meta-analysis (Section O.3.4) and current literature (see Section 1.2.1).

Table O-3. Freshwater Nominal and Measured Concentrations for PFOS.

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS-K 90.49%	96 hours	Polyethylene	None	High performance liquid chromatography with mass spectrometric detection	0 hours	5.7	5.47	Drottar and Krueger (2000d)
					48 hours	5.7	5.18	
					96 hours	5.7	5.24	
					0 hours	5.7	4.93	
					48 hours	5.7	5.70	
					96 hours	5.7	5.26	
					0 hours	11	11.4	
					48 hours	11	11.2	
					96 hours	11	10.9	
					0 hours	11	10.1	
					48 hours	11	10.5	
					96 hours	11	15.4	
					0 hours	23	19.0	
					48 hours	23	18.7	
					96 hours	23	22.9	
					0 hours	23	16.8	
					48 hours	23	18.7	
					96 hours	23	22.4	
					0 hours	46	37.2	
					48 hours	46	37.1	
					96 hours	46	48.2	
					0 hours	46	40.6	
					48 hours	46	39.5	
					96 hours	46	40.5	
					0 hours	91	69.0	
					48 hours	91	81.3	
					96 hours	91	88.2	
					0 hours	91	74.7	
					48 hours	91	77.6	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					96 hours	91	85.7	
PFOS >98%	24 hours (glochidia) and 96 hours (juvenile)	Glass (assumed)	None	High Performance Liquid Chromatography / Mass Spectrometry	0 hours	0.005	0.0054	Hazelton et al. (2012), Hazelton (2013)
					0 hours	0.05	0.0514	
					0 hours	0.5	0.456	
					0 hours	5	4.68	
					0 hours	50	47.2	
					0 hours	500	490	
PFOS-K 90.49%	48 hours	Plastic	None	High Performance Liquid Chromatography / Mass Spectrometry	0 hours	12	10.5	Drottar and Krueger (2000a)
					24 hours	12	11.5	
					48 hours	12	10.9	
					0 hours	12	10.6	
					24 hours	12	12.5	
					48 hours	12	12.0	
					0 hours	20	17.2	
					24 hours	20	22.8	
					48 hours	20	21.4	
					0 hours	20	18.1	
					24 hours	20	21.6	
					48 hours	20	18.8	
					0 hours	33	30.2	
					24 hours	33	34.0	
					48 hours	33	31.3	
					0 hours	33	34.1	
					24 hours	33	36.1	
					48 hours	33	34.0	
					0 hours	55	50.5	
					24 hours	55	57.0	
					48 hours	55	56.8	
					0 hours	55	49.9	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					24 hours	55	63.0	
					48 hours	55	56.4	
					0 hours	91	87.6	
					24 hours	91	90.1	
					48 hours	91	88.7	
					0 hours	91	102	
					24 hours	91	84.4	
					48 hours	91	92.4	
PFOS-K 99%	48 hours	Glass (assumed)	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	0 hours	20	19.8	Yang et al. (2014)
					48 hours	20	18.43	
					0 hours	377.91	372.35	
					48 hours	377.91	341.74	
PFOS-K 86.9%	96 hours	Glass	None	Liquid Chromatography / Tandem Mass Spectrometry	0 hours	1.82	2.58	Palmer and Krueger (2001)
					96 hours	1.82	1.42	
					0 hours	3.07	3.94	
					96 hours	3.07	1.72	
					0 hours	5.19	6.62	
					96 hours	5.19	2.84	
					0 hours	8.64	10.7	
					96 hours	8.64	5.09	
					0 hours	14.4	18.5	
					96 hours	14.4	10.8	
					0 hours	24	26.9	
					96 hours	24	22.3	
PFOS-K 86.9%	96 hours	Glass	None	Liquid Chromatography / Tandem Mass Spectrometry	0 hours	1.82	1.77	Palmer and Krueger (2001)
					96 hours	1.82	2.04	
					0 hours	3.07	3.59	
					96 hours	3.07	2.49	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					0 hours	5.19	5.45	
					96 hours	5.19	4.18	
					0 hours	8.64	8.43	
					96 hours	8.64	7.51	
					0 hours	14.4	14.5	
					96 hours	14.4	12.1	
					0 hours	24	23.0	
					96 hours	24	23.1	
PFOS-K 86.9%	96 hours	Glass	None	Liquid Chromatography / Tandem Mass Spectrometry	0 hours	1.82	1.77	Palmer and Krueger (2001)
					96 hours	1.82	2.08	
					0 hours	3.07	3.59	
					96 hours	3.07	2.94	
					0 hours	5.19	5.45	
					96 hours	5.19	5.05	
					0 hours	8.64	8.43	
					96 hours	8.64	8.09	
					0 hours	14.4	14.5	
					96 hours	14.4	13.5	
					0 hours	24	23.0	
					96 hours	24	24.7	
PFOS >98%	36 days	Glass	None	High Performance Liquid Chromatography / Mass Spectrometry	10, 11 days	0.001	0.00452	Hazelton et al. (2012)
					10, 11 days	0.1	0.0695	
PFOS-K 90.49%	21 days	Plastic	None	Reverse Phase High Performance Liquid Chromatography	0 days	1.4	1.78	Drottar and Krueger (2000b)
					2 days	1.4	1.58	
					11 days	1.4	1.38	
					14 days	1.4	1.36	
					18 days	1.4	1.38	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					21 days	1.4	1.50	
					0 days	1.4	1.72	
					2 days	1.4	1.56	
					11 days	1.4	1.47	
					14 days	1.4	1.32	
					18 days	1.4	1.43	
					21 days	1.4	1.45	
					0 days	2.9	3.20	
					2 days	2.9	3.01	
					11 days	2.9	2.75	
					14 days	2.9	2.85	
					18 days	2.9	2.79	
					21 days	2.9	2.81	
					0 days	2.9	3.05	
					2 days	2.9	3.07	
					11 days	2.9	2.77	
					14 days	2.9	2.71	
					18 days	2.9	2.81	
					21 days	2.9	2.82	
					0 days	5.7	5.97	
					2 days	5.7	5.65	
					11 days	5.7	5.63	
					14 days	5.7	5.36	
					18 days	5.7	5.58	
					21 days	5.7	5.24	
					0 days	5.7	5.87	
					2 days	5.7	5.72	
					11 days	5.7	5.59	
					14 days	5.7	5.39	
					18 days	5.7	5.75	
					21 days	5.7	5.37	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					0 days	11	11.5	
					2 days	11	11.6	
					11 days	11	11.3	
					14 days	11	11.2	
					18 days	11	11.8	
					21 days	11	11.5	
					0 days	11	11.5	
					2 days	11	11.8	
					11 days	11	11.3	
					14 days	11	11.6	
					18 days	11	11.6	
					21 days	11	11.3	
					0 days	23	24.2	
					2 days	23	24.0	
					11 days	23	22.8	
					14 days	23	23.6	
					18 days	23	24.8	
					0 days	23	23.1	
					2 days	23	24.6	
					11 days	23	22.5	
					14 days	23	23.1	
					18 days	23	25.0	
					0 days	46	47.3	
					2 days	46	49.1	
					0 days	46	48.0	
					2 days	46	49.4	
PFOS-K 99%	21 days	Glass (assumed)	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After renewal	2	1.98	Yang et al. (2014)
					Before renewal	2	1.74	
					After renewal	7.43	7.54	
					Before renewal	7.43	6.78	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS-K 95%	20 days	Polypropylene	None	Liquid Chromatography / Tandem Mass Spectrometry	20 day	0.001	0.0023	MacDonald et al. (2004)
					20 day	0.005	0.0144	
					20 day	0.01	0.0217	
					20 day	0.05	0.0949	
					20 day	0.1	0.149	
PFOS-K 95%	10 days	Polypropylene	None	Liquid Chromatography / Tandem Mass Spectrometry	10 day	0.001	0.0008	MacDonald et al. (2004)
					10 day	0.005	0.0046	
					10 day	0.01	0.0115	
					10 day	0.02	0.0241	
					10 day	0.04	0.0491	
					10 day	0.08	0.0962	
					10 day	0.15	0.1501	
PFOS-K 90.49%	47 days	Glass	None	HPLC/MS Verification	0 days	0.14	0.147	Drottar and Krueger (2000c)
					4 days	0.14	0.141	
					7 days	0.14	0.144	
					14 days	0.14	0.134	
					21 days	0.14	0.153	
					28 days	0.14	0.160	
					35 days	0.14	0.179	
					42 days	0.14	0.157	
					47 days	0.14	0.147	
					0 days	0.14	0.160	
					4 days	0.14	0.140	
					7days	0.14	0.148	
					14 days	0.14	0.135	
					21 days	0.14	0.143	
					28 days	0.14	0.158	
					35 days	0.14	0.173	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					42 days	0.14	0.160	
					47 days	0.14	0.155	
					0 days	0.29	0.287	
					4 days	0.29	0.270	
					7 days	0.29	0.292	
					14 days	0.29	0.269	
					21 days	0.29	0.307	
					28 days	0.29	0.343	
					35 days	0.29	0.311	
					42 days	0.29	0.319	
					47 days	0.29	0.296	
					0 days	0.29	0.277	
					4 days	0.29	0.289	
					7days	0.29	0.296	
					14 days	0.29	0.266	
					21 days	0.29	0.315	
					28 days	0.29	0.341	
					35 days	0.29	0.325	
					42 days	0.29	0.313	
					47 days	0.29	0.276	
					0 days	0.57	0.571	
					4 days	0.57	0.619	
					7 days	0.57	0.597	
					14 days	0.57	0.539	
					21 days	0.57	0.608	
					28 days	0.57	0.639	
					35 days	0.57	0.646	
					42 days	0.57	0.575	
					47 days	0.57	0.545	
					0 days	0.57	0.576	
					4 days	0.57	0.659	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					7days	0.57	0.642	
					14 days	0.57	0.535	
					21 days	0.57	0.580	
					28 days	0.57	0.617	
					35 days	0.57	0.644	
					42 days	0.57	0.576	
					47 days	0.57	0.543	
					0 days	1.1	1.14	
					4 days	1.1	1.21	
					7 days	1.1	1.13	
					14 days	1.1	1.03	
					21 days	1.1	1.19	
					28 days	1.1	1.3	
					35 days	1.1	1.3	
					42 days	1.1	1.14	
					47 days	1.1	1.13	
					0 days	1.1	1.13	
					4 days	1.1	1.25	
					7days	1.1	1.23	
					14 days	1.1	1.1	
					21 days	1.1	1.24	
					28 days	1.1	1.31	
					35 days	1.1	1.31	
					42 days	1.1	1.19	
					47 days	1.1	1.09	
					0 days	2.3	2.21	
					4 days	2.3	2.52	
					7 days	2.3	2.43	
					0 days	2.3	2.27	
					4 days	2.3	2.46	
					7days	2.3	2.38	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					0 days	4.6	4.56	
					4 days	4.6	4.79	
					7 days	4.6	4.46	
					0 days	4.6	4.4	
					4 days	4.6	4.79	
					7days	4.6	4.76	
PFOS >98%	21 days (F0) + 24 days (F1)	Glass	None	Liquid Chromatography / Electrospray Ionization / Mass Spectrometry	Not reported.	0.03	0.0276	Ankley et al (2005)
					Not reported.	0.1	0.101	
					Not reported.	0.3	0.281	
					Not reported.	1	0.818	
PFOS 98%	110 days	Glass	None	Liquid Chromatography / Electrospray Ionization / Mass Spectrometry	Averaged over 110 days	0.03	0.04	Ankley et al. (2004)
					Averaged over 110 days	0.1	0.13	
					Averaged over 110 days	0.3	0.36	
					Averaged over 110 days	1	0.97	
					Averaged over 110 days	3	3.55	
					Averaged over 110 days	10	12.5	
PFOS 98%	4 months	Not reported.	DMSO	Liquid chromatography coupled to tandem mass spectrometry with electrospray ionization	0 hours after water change	0.0001	0.00009	Lou et al. (2013)
					24 hours after water change	0.0001	0.00006	
					48 hours after water change	0.0001	0.000045	
					0 hours after water change	0.001	0.001	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					24 hours after water change	0.001	0.00045	
					48 hours after water change	0.001	0.00045	
					0 hours after water change	0.1	0.1117	
					24 hours after water change	0.1	0.0898	
					48 hours after water change	0.1	0.0891	
					0 hours after water change	1	0.716	
					24 hours after water change	1	0.661	
					48 hours after water change	1	0.632	
PFOS-K Unknown	35 days	Polyvinyl chloride	None	High-performance liquid chromatography ion chromatography	1 day	1	1.33	Sanderson et al. (2002)
					8 days	1	1.15	
					35 days	1	1.08	
					1 day	10	12.3	
					35 days	10	11.7	
					1 day	30	33.9	
					35 days	30	29.8	
PFOS-K 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	60	58.94	Yang et al. (2014)
					Before Renewal	60	51.42	
					After Renewal	149.3	150.84	
					Before Renewal	149.3	127.73	
PFOS-K 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	100	99.85	Yang et al. (2014)
					Before Renewal	100	86.5	
					After Renewal	371.29	368.24	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					Before Renewal	371.29	328.84	
PFOS-K 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	6	5.95	Yang et al. (2014)
					Before Renewal	6	4.88	
					After Renewal	113.37	109.22	
					Before Renewal	113.37	97.85	
PFOS-K 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	100	99.46	Yang et al. (2014)
					Before Renewal	100	92.24	
					After Renewal	371.29	369.29	
					Before Renewal	371.29	340.45	
	28 days	Not reported.	None (assumed)	Reversed Phase Liquid Chromatography with Electrospray ionization Mass Spectrometry	Average (See Note)	0.001	0.00081	Roland et al. (2014)
					Average (See Note)	0.01	0.011	
PFOS-K 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	20	19.2	Yang et al. (2014)
					Before Renewal	20	17.5	
					After Renewal	209.72	203.43	
					Before Renewal	209.72	183.36	
PFOS >98%	96 hours	Glass	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	See Note	0.500	0.5201352	Feng et al. (2015)
					See Note	5.001	5.0913234	
PFOS 100.3%	96 hours	Glass with Teflon steel components	N,N-dimethylformamide	Liquid Chromatography / Mass Spectrometry	See Note	0.05	0.045	Kim et al. (2010)
					See Note	0.5	0.62	
					See Note	5	5.395	
					See Note	50	48.242	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS >96%	114 hours	Not provided.	DMSO	Liquid Chromatography / Mass Spectrometry	0 days	0.5001	0.5051	Huang et al. (2010)
					5 days	0.5001	0.5064	
					0 days	2.0005	2.0403	
					5 days	2.0005	2.1603	
					0 days	4.001	4.0123	
					5 days	4.001	4.2228	
PFOS ≥98%	24 hours	Plexiglass	ETOH	Liquid Chromatography / Mass Spectrometry	Not reported.	0.0950247	0.0550143	Huang et al. (2016)
					Not reported.	0.950247	0.600156	
					Not reported.	9.50247	9.00234	
PFOS-K 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	30	29.1	Yang et al. (2014)
					Before Renewal	30	26.12	
					After Renewal	227.81	220.98	
					Before Renewal	227.81	195.2	
PFOS-K 99%	30 days	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	1.5	1.48	Yang et al. (2014)
					Before Renewal	1.5	1.34	
					After Renewal	5.57	5.73	
					Before Renewal	5.57	4.98	
PFOS-K 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	20	21.16	Yang et al. (2014)
					Before Renewal	20	17.92	
					After Renewal	151.88	150.03	
					Before Renewal	151.88	137.93	
PFOS-K 99%	30 days	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	1.5	1.47	Yang et al. (2014)
					Before Renewal	1.5	1.31	
					After Renewal	5.57	5.46	
					Before Renewal	5.57	4.85	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS-K ≥98%	14 days	Polypropylene	None	LC-MS/MS	0 hours	50	64.14	Funkhouser (2014)
					96 hours	50	75.61	
					0 hours	75	110.2	
					96 hours	75	135.4	
					0 hours	100	131.8	
					96 hours	100	174.2	
					0 hours	125	172.4	
					96 hours	125	223.9	
					0 hours	150	236.3	
					96 hours	150	313.3	
					0 hours	175	170.8	
					96 hours	175	231.3	
PFOS >98%	40 days	Plastic	None	LC-MS/MS	40 days	10	7.662	Hoover et al. (2017)
					40 days	100	76.34	
					40 days	1000	877.6	
PFOS-K 86%	285 days	Plastic	None	Ion chromatography	6-33 days	3	2.681294118	Boudreau et al. (2003b)
					6-33 days	10	9.998625	
					6-33 days	30	29.85411765	
PFOS-K ≥98%	330 days	Glass	None	LC-MS/MS	30-60 days	0.0006	0.0007568	Keiter et al. (2012)
					30-60 days	0.1	0.12907375	
					30-40 days	0.3	0.259083333	
PFOS-K 90.49%	96 hr	Polyethylene	None	HPLC/MS	0 hr	3.6	3.16	Drottar and Krueger (2000)
					48 hr	3.6	3.08	
					96 hr	3.6	3.46	
					0 hr	3.6	3.53	
					48 hr	3.6	3.22	
					96 hr	3.6	3.13	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					0 hr	5.9	6.05	
					48 hr	5.9	5.48	
					96 hr	5.9	5.7	
					0 hr	5.9	5.07	
					48 hr	5.9	5.89	
					96 hr	5.9	5.55	
					0 hr	9.9	8.99	
					48 hr	9.9	9.88	
					96 hr	9.9	9.7	
					0 hr	9.9	9.47	
					48 hr	9.9	9.33	
					96 hr	9.9	9.52	
					0 hr	16	18.2	
					48 hr	16	15	
					96 hr	16	14.8	
					0 hr	16	19.3	
					48 hr	16	15.6	
					96 hr	16	16.2	
					0 hr	27	28.5	
					48 hr	27	27	
					96 hr	27	26.8	
					0 hr	27	28.5	
					48 hr	27	27.8	
					96 hr	27	26.6	
PFOS-K 86.9%	96 hr	Polyethylene	None	HPLC	0 hr	3.1	3.15	Palmer et al. (2002)
					48 hr	3.1	2.9	
					96 hr	3.1	2.83	
					0 hr	3.1	3.02	
					48 hr	3.1	3.01	
					96 hr	3.1	2.97	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					0 hr	6.3	6.22	
					48 hr	6.3	6.16	
					96 hr	6.3	6.15	
					0 hr	6.3	6.21	
					48 hr	6.3	6.43	
					96 hr	6.3	6.6	
					0 hr	13	13.2	
					48 hr	13	12.7	
					96 hr	13	13.1	
					0 hr	13	12.1	
					48 hr	13	12.3	
					96 hr	13	12.6	
					0 hr	25	25	
					48 hr	25	24.3	
					96 hr	25	25.7	
					0 hr	25	25.7	
					48 hr	25	25.7	
					96 hr	25	26.2	
					0 hr	50	49.7	
					48 hr	50	51.1	
					96 hr	50	49.6	
					0 hr	50	49.8	
					48 hr	50	51.5	
					96 hr	50	50.8	
PFOS-K ≥98%	96 hr	Not provided	DMSO	LC-MS/MS	0 hr	20	21.8806	Huang et al. (2021)
					24 hr	20	22.3311	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS-K Not reported	33 d	Not provided	Acetone	Radiometric analysis	not reported	0.13	0.12	3M Co. (2000)
					not reported	0.25	0.28	
					not reported	0.5	0.45	
					not reported	1	1	
					not reported	2	1.9	
PFOS ≥98%	150 d post metamorphosis	Not provided	None	LC-MS	time weighted average (initiation and end)	0.06	0.05	Fort et al. (2019)
					time weighted average (initiation and end)	0.13	0.13	
					time weighted average (initiation and end)	0.25	0.31	
					time weighted average (initiation and end)	0.5	0.59	
					time weighted average (initiation and end)	1	1.05	
PFOS ≥98%	150 d post metamorphosis	Not provided	None	LC-MS	time weighted average (initiation and 150 day post metamorphosis)	0.05	0.05	Fort et al. (2019)
					time weighted average (initiation and 150 day post metamorphosis)	0.12	0.13	
					time weighted average (initiation and 150 day post metamorphosis)	0.29	0.31	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					time weighted average (initiation and 150 day post metamorphosis)	0.62	0.59	
					time weighted average (initiation and 150 day post metamorphosis)	1.1	1.05	
PFOS-K 86.9%	96 hours	Glass	None	Liquid Chromatography/Tandem Mass Spectrometry	0 hr	1.82	2.58	Palmer and Krueger (2001)
					96 hr	1.82	1.42	
					0 hr	3.07	3.94	
					96 hr	3.07	1.72	
					0 hr	5.19	6.62	
					96 hr	5.19	2.84	
					0 hr	8.64	10.7	
					96 hr	8.64	5.09	
					0 hr	14.4	18.5	
					96 hr	14.4	10.8	
					0 hr	24	26.9	
					96 hr	24	22.3	
PFOS-K 95%	21 days	Polypropylene	None	Liquid Chromatography/Tandem Mass Spectrometry	20 day	0.001	0.0023	MacDonald et al. (2004)
						0.005	0.0144	
						0.01	0.0217	
						0.05	0.0949	
						0.1	0.149	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS 98%	4 months	Not reported.	DMSO	Liquid chromatography coupled to tandem mass spectrometry with electrospray ionization (HPLC–ESI–MS/MS)	0 hours after water change	0.0001	0.00009	Lou et al. (2013)
					24 hours after water change	0.0001	0.00006	
					48 hours after water change	0.0001	0.000045	
					0 hours after water change	0.001	0.001	
					24 hours after water change	0.001	0.00045	
					48 hours after water change	0.001	0.00045	
					0 hours after water change	0.1	0.1117	
					24 hours after water change	0.1	0.0898	
					48 hours after water change	0.1	0.0891	
					0 hours after water change	1	0.716	
					24 hours after water change	1	0.661	
					48 hours after water change	1	0.632	
PFOS ≥98%	24 hours	Plexiglass	ETOH	Liquid Chromatography/Mass Spectrometry	Not reported.	0.0950247	0.0550143	Huang et al. (2016)
					Not reported.	0.950247	0.600156	
					Not reported.	9.50247	9.00234	
PFOS-K ≥98%	14 days	Polypropylene	None	LC-MS/MS	0 hr	50	64.14	Funkhouser (2014)
					96 hr	50	75.61	
					0 hr	75	110.2	
					96 hr	75	135.4	
					0 hr	100	131.8	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					96 hr	100	174.2	
					0 hr	125	172.4	
					96 hr	125	223.9	
					0 hr	150	236.3	
					96 hr	150	313.3	
					0 hr	175	170.8	
					96 hr	175	231.3	
PFOS >98%	40 days	Plastic	None	LC-MS/MS	40 days	10	7.662	Hoover et al. (2017)
					40 days	100	76.34	
					40 days	1000	877.6	
PFOS Not reported	116 d	Not provided	None (assumed)	LC/MS/MS	0 hr	0.01	0.0127	Foguth et al. (2020)
					0 hr	0.01	0.0118	
					0 hr	0.01	0.0136	
					0 hr	0.01	0.0133	
PFOS 98%	10 day	HDPE	None	LC/MS	1 day	0.33	0.0759	McCarthy et al. (2021)
					10 day	0.33	0.0225	
					1 day	33	17.7	
					10 day	33	13.5	
					1 day	100	51.6	
					10 day	100	47.6	
					1 day	350	217	
					10 day	350	216	
PFOS 98%	16 day	HDPE	None	LC/MS	10 day	0.001	0.000376	McCarthy et al. (2021)
					15 day	0.001	0.000477	
					20 day	0.001	0.000489	
					10 day	0.005	0.00182	
					15 day	0.005	0.00208	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					20 day	0.005	0.00238	
					10 day	0.01	0.00369	
					15 day	0.01	0.00406	
					20 day	0.01	0.00485	
					10 day	0.05	0.0199	
					15 day	0.05	0.0241	
					20 day	0.05	0.0253	
					10 day	0.1	0.0443	
					15 day	0.1	0.0459	
					20 day	0.1	0.0486	

Table O-4. Saltwater Nominal and Measured Concentrations for PFOS.

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS-K 90.49%	96 hours	Polyethylene	None	High-Performance Liquid Chromatography with Mass Spectrometric Detection	0 hours	1.1	0.575	Drottar and Krueger (2000f)
					48 hours	1.1	0.605	
					96 hours	1.1	0.391	
					0 hours	1.1	0.622	
					48 hours	1.1	0.64	
					96 hours	1.1	0.58	
					0 hours	1.8	1.12	
					48 hours	1.8	1.1	
					96 hours	1.8	1.04	
					0 hours	1.8	1.19	
					48 hours	1.8	1.09	
					96 hours	1.8	1.13	
					0 hours	3	1.92	
					48 hours	3	1.92	
					96 hours	3	1.79	
					0 hours	3	1.99	
					48 hours	3	1.91	
					96 hours	3	1.91	
					0 hours	4.9	3.05	
					48 hours	4.9	2.96	
					96 hours	4.9	3.11	
					0 hours	4.9	2.66	
					48 hours	4.9	3.35	
					96 hours	4.9	3.11	
					0 hours	8.2	5.82	
					48 hours	8.2	3.58	
					96 hours	8.2	5.22	
					0 hours	8.2	5.78	
					48 hours	8.2	5.85	
					96 hours	8.2	5.86	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS-K 90.49%	35 days	Glass	None	High-Performance Liquid Chromatography with Mass Spectrometric Detection	0 days	0.086	0.0694	Drottar and Krueger (2000g)
					14 days	0.086	0.0606	
					28 days	0.086	0.0515	
					7 days	0.086	0.0478	
					21 days	0.086	0.0554	
					35 days	0.086	0.058	
					0 days	0.086	0.0578	
					14 days	0.086	0.0614	
					28 days	0.086	0.0569	
					7 days	0.086	0.0619	
					21 days	0.086	0.0509	
					35 days	0.086	0.0514	
					0 days	0.17	0.125	
					14 days	0.17	0.124	
					28 days	0.17	0.122	
					7 days	0.17	0.0778	
					21 days	0.17	0.097	
					35 days	0.17	0.124	
					0 days	0.17	0.114	
					14 days	0.17	0.127	
					28 days	0.17	0.128	
					7 days	0.17	0.125	
					21 days	0.17	0.112	
					35 days	0.17	0.119	
					0 days	0.34	0.289	
					14 days	0.34	0.276	
					28 days	0.34	0.262	
					7 days	0.34	0.231	
					21 days	0.34	0.227	
					35 days	0.34	0.278	
					0 days	0.34	0.286	
					14 days	0.34	0.253	
					28 days	0.34	0.271	
					7 days	0.34	0.197	
					21 days	0.34	0.212	
					35 days	0.34	0.251	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					0 days	0.69	0.562	
					14 days	0.69	0.543	
					28 days	0.69	0.529	
					7 days	0.69	0.581	
					21 days	0.69	0.516	
					35 days	0.69	0.556	
					0 days	0.69	0.659	
					14 days	0.69	0.542	
					28 days	0.69	0.544	
					7 days	0.69	0.45	
					21 days	0.69	0.528	
					35 days	0.69	0.583	
					0 days	1.4	1.23	
					14 days	1.4	1.35	
					28 days	1.4	1.39	
					7 days	1.4	1.13	
					21 days	1.4	1.23	
					35 days	1.4	1.26	
					0 days	1.4	1.32	
					14 days	1.4	1.27	
					28 days	1.4	1.39	
					7 days	1.4	1.2	
					21 days	1.4	1.15	
					35 days	1.4	1.2	
					0 days	2.7	2.56	
					14 days	2.7	2.54	
					7 days	2.7	2.58	
					0 days	2.7	2.79	
					14 days	2.7	2.69	
					7 days	2.7	2.3	
PFOS-K Unknown	7 days	Polypropylene	Methanol	Liquid chromatograph connected to a triple-quadrupole type tandem mass spectrometer	Average of day 1, 3,5, 7	0.000032	0.000028	Sakurai et al. (2017)

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS-K 90.49%	96 hr	Polyethylene	None	High-Performance Liquid Chromatography with Mass Spectrometric Detection	0 hours	1.2	0.331	Drottar and Krueger (2000h)
					0 hours	1.2	0.353	
					48 hours	1.2	0.341	
					48 hours	1.2	0.429	
					0 hours	2.0	0.622	
					0 hours	2.0	0.633	
					48 hours	2.0	0.299	
					48 hours	2.0	0.313	
					96 hours	2.0	0.249	
					96 hours	2.0	0.257	
					0 hours	3.3	1.36	
					0 hours	3.3	1.15	
					48 hours	3.3	0.924	
					48 hours	3.3	0.878	
					96 hours	3.3	1.58	
					96 hours	3.3	1.72	
					0 hours	5.5	2.42	
					0 hours	5.5	2.53	
					48 hours	5.5	2.02	
					48 hours	5.5	2.24	
					96 hours	5.5	1.45	
					96 hours	5.5	0.970	
					0 hours	9.1	3.44	
					0 hours	9.1	3.01	
					48 hours	9.1	3.74	
					48 hours	9.1	3.57	
					96 hours	9.1	1.99	
					96 hours	9.1	2.19	
PFOS-K 98%	7 days	Polypropylene	None	Liquid Chromatography - tandem mass spectrometry	Every 24 hours	0.0001	0.00009	Liu et al. (2014a)
					Every 24 hours	0.001	0.00095	
					Every 24 hours	0.01	0.0097	
					Every 24 hours	0.1	0.096	
					Every 24 hours	1	0.989	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOS-K 98%	7 days	Polypropylene	None	Liquid Chromatography - tandem mass spectrometry	Samples used for water concentration data were taken every 2 days. Twelve samples per concentration were taken.	0.0001	0.00012	Liu et al. (2014b,c); Lui and Gin (2018)
						0.001	0.0011	
						0.01	0.0096	
						0.1	0.106	
						1	0.968	
						10	10.156	
PFOS 98%	8 days	Glass	DMSO	Liquid Chromatography - tandem mass spectrometry	2 days	1	0.916	Fang et al. (2012)
					2 days	4	3.053	
					2 days	16	11.76	
					8 days	1	0.916	
					8 days	4	3.664	
					8 days	16	12.67	
PFOS-K 86.9%	96 hr	Polyethylene	Methanol	HPLC/MS	0 day	20	16.4	Palmer et al. (2002)
					24 hr (old)	20	15.2	
					24 hr (new)	20	16.2	
					48 h (old)	20	13.4	
					48 hr (new)	20	17.4	
					72 hr (old)	20	15.2	
					72 hr (new)	20	17	
					96 hr	20	13.7	
					0 day	20	15.7	
					24 hr (old)	20	15.3	
					24 hr (new)	20	15.7	
					48 h (old)	20	11.3	
					48 hr (new)	20	18.2	
					72 hr (old)	20	18	
					72 hr (new)	20	16.6	
					96 hr	20	11.1	
					0 day	20	16	
					24 hr (old)	20	15.2	
					24 hr (new)	20	15	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
					48 h (old)	20	12.7	
					48 hr (new)	20	18.6	
					72 hr (old)	20	16.2	
					72 hr (new)	20	17.4	
					96 hr	20	13.4	
PFOS-K 98%	7 d	Polypropylene	None	LC-MS	0 day	0.0001	0.00012	Liu et al. (2013)
					0 day	0.001	0.0011	
					0 day	0.01	0.0096	
					0 day	0.1	0.106	
					0 day	1	0.968	

Table O-5. Freshwater PFOS Toxicity Studies with Systematic Discrepancies between Nominal and Measured Concentrations that were > 20%.

Study	Test Duration	Test Method	Sample Collection Frequency	PFOS Analytical Method[1]	Notes on Discrepancies
Foguth et al. (2020)	Chronic (116-d)	Static	Not reported. Assumed at the beginning of the mesocosm test after spiking with PFOS	LC-MS/MS	All measured test concentrations were higher than the nominal concentrations, by a factor of approximately 1.3, with three of the measured values in the four replicate mesocosms dosed with PFOS falling outside of the 20% exceedance threshold. The systematic discrepancies in the mesocosm test indicate an apparent dosing or perhaps test design issue related to use of the leaf litter in the mesocosms.
Funkhouser (2014)	Chronic (14-d)	Renewal (every 3-4 d)	Before and after each renewal	LC-MS/MS	All measured concentrations were higher than the nominal concentrations with all treatments falling outside of the 20% exceedance threshold. These systematic discrepancies indicate an apparent dosing issue. Particularly since these systematic discrepancies were also consistent with other tests reported in the thesis (that were not included in the meta-analysis), all of which were higher than the nominal concentrations and outside the 20% exceedance threshold.
Hoover et al. (2017)	Chronic (40-d)	Renewal (every 4 d)	End of test duration (at 40 days)	LC-MS/MS	All measured test concentrations were lower than the nominal concentrations with two of the three treatments falling outside of the 20% exceedance threshold. These systematic discrepancies indicate an apparent dosing issue.
Huang et al. (2016)	Acute (24-hr)	Static	Not reported.	LC-MS	All of the measured PFOS concentrations were lower than the nominal concentrations with two of the three measured concentrations falling outside of the 20% exceedance threshold. These systematic discrepancies indicate an apparent dosing issue
Lou et al. (2013)	Chronic (4-month)	Renewal (every 48 hr)	Before and after each renewal	HPLC–ESI–MS/MS	Nine of the twelve measured test concentrations were lower than the nominal concentrations with seven of the twelve measured concentrations falling outside of the 20% exceedance threshold. These systematic discrepancies indicate an apparent dosing issue.
MacDonald et al. (2004)	Chronic (20-d)	Renewal (every 48 hr)	End of test duration (20 days)	LC-MS/MS	All measured test concentrations were higher than the nominal concentrations, by a factor of 2, with all of the five treatments falling outside of the 20% exceedance threshold. However, similar systematic discrepancies were not observed in the 10-day test that was also reported in this paper. In the 10-day test the measured concentrations were not systematically higher or lower than the nominal concentrations and only two of the five treatments were slightly outside the 20% exceedance threshold (at 120.5 and 122.75%). The systematic discrepancies in the 20-day test indicate an apparent dosing issue.
McCarthy et al. (2021)	Chronic (10-d)	Renewal (every 48-72 hr)	At days 1 and 10	LC-MS	All measured test concentrations were lower than the nominal concentrations, by over a factor of 2, with all of the four treatments falling outside of the 20% exceedance threshold. The systematic discrepancies in the 10-day test indicate an apparent dosing or perhaps test design issue related to use of natural field-collected sediment in exposure chambers.

Study	Test Duration	Test Method	Sample Collection Frequency	PFOS Analytical Method[1]	Notes on Discrepancies
McCarthy et al. (2021)	Chronic (16-d)	Renewal (every 24 hr)	At days 10, 15 and 20	LC-MS	All measured test concentrations were lower than the nominal concentrations, by over a factor of 2, with all of the five treatments falling outside of the 20% exceedance threshold. Similar systematic discrepancies were observed in the 10-day test that was also reported in this paper. The systematic discrepancies in the 16-day test indicate an apparent dosing or perhaps test design issue related to use of natural field-collected sediment in exposure chambers.
Palmer and Krueger (2001)	Acute (96-hr)	Renewal (every 24 hr)	Before and after each renewal	LC-MS/MS	Three, independent assays of this test were conducted instead of simultaneous replicates. All measured test concentrations from the first assay were systematically higher than the nominal concentrations after renewal and systematically lower before the next renewal and all were outside the 20% exceedance threshold. These same systematic differences were not observed in the other two assays and all treatments in second and third assays were within the 20% exceedance threshold. The systematic discrepancies in the first assay indicate an apparent dosing issue.
Ankley et al. (2004)	Chronic (110-d)	Flow-through	Water samples were collected and averaged over the exposure period	HPLC–ESI–MS/MS	Three of the six treatment groups had measured concentrations higher than nominal and were outside the 20% exceedance threshold. Two of the three remaining treatment groups were also higher than the nominal concentrations and were not outside the 20% exceedance threshold. One of the treatment groups was lower than the nominal concentration and was also not outside the 20% exceedance threshold. Therefore, the observed exceedances were not considered to be systematic across the treatment groups and the three treatment groups outside the 20% exceedance threshold were fairly close to the threshold (at 133, 130, and 125%).
Drottar and Krueger (2000b)	Chronic (21-d)	Renewal (every 3 days per week)	On days 0, 2, 11,14, 18, and 21	RP-HPLC	Two of the sixty-three measured test concentrations were outside the 20% exceedance threshold. However, these differences were not considered to be systematic across the treatment groups and the two differences were fairly close to the 20% exceedance threshold (at 127 and 123%).
Drottar and Krueger (2000c)	Chronic (47-d)	Flow-through	Water samples were collected on days 0, 4, 7,14, 21, 28, 35, 42, and 47	HPLC/MS	Two of the eighty-three measured test concentrations were outside the 20% exceedance threshold. However, these differences were not considered to be systematic across the treatment groups and the two differences were fairly close to the 20% exceedance threshold (at 128 and 124%).
Drottar and Krueger (2000d)	Acute (96-hr)	Renewal (every 48 hr)	Before and after each renewal	HPLC/MS	Three of the thirty measured test concentrations were outside the 20% exceedance threshold. However, these differences were not considered to be systematic across the treatment groups and the three exceedances were fairly close to the 20% exceedance threshold (at 73, 75, and 140%).

Study	Test Duration	Test Method	Sample Collection Frequency	PFOS Analytical Method[1]	Notes on Discrepancies
Hazelton et al. (2012)	Chronic (36-d)	Renewal (every 24 hr)	on days 10 and 11	LC-MS	All measured test concentrations were outside the 20% exceedance threshold. However, the measured concentrations were not systematically higher or lower than the nominal concentrations between the two treatment groups. Specifically, the measured concentrations from the low treatment group was higher (452% higher) than the nominal concentration and the high treatment was lower (69.5%) than the nominal concentration. While there appears to be a dosing issue for the chronic test in Hazelton et al. 2012, the discrepancies are not systematic (both lower or higher than the nominal concentrations) and there is no apparent justification for the observed discrepancies.
Keiter et al. (2016)	Chronic (330-d)	Flow-through	On days 30 and 60	LC-MS/MS	Two of the three treatment groups had measured concentrations higher than nominal and was outside the 20% exceedance threshold. The remaining treatment group was lower than the nominal concentrations and was not outside the 20% exceedance threshold. While there appears to be a dosing issue in Keiter et al. 2016, the discrepancies are not systematic (all lower or higher than the nominal concentrations) and there was no apparent justification for the observed discrepancies.
Sanderson et al. (2002)	Chronic (35-d)	Static	On days 1, 8, and 35	HPLC-IC	Six out of the seven treatment groups had measured concentrations higher than nominal and two of the seven were outside the 20% exceedance threshold. Therefore, while the observed exceedances could be considered to be systematic across the treatment groups, since only two treatment groups were outside the 20% exceedance threshold (and were fairly close to the threshold at 123 and 133%).

[1] PFOS Analytic Methods: Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS), Liquid Chromatography / Mass Spectrometry (LC-MS), Liquid Chromatography Coupled to Tandem Mass Spectrometry with Electrospray Ionization (HPLC–ESI–MS/MS), Reverse Phase High Performance Liquid Chromatography (RP-HPLC), and High-Performance Liquid Chromatography with Mass Spectrometric Detection (HPLC/MS)

Table O-6. Saltwater PFOS Toxicity Studies with Systematic Discrepancies between Nominal and Measured Concentrations that were > 20%.

Studies are listed alphabetically.

Study	Test Duration	Test Method	Sample Collection Frequency	PFOS Analytical Method[1]	Notes on Discrepancies
Drottar and Krueger (2000f)	Acute (96-hr)	Static	At 0, 48, and 96	HPLC/MS	All measured test concentrations were outside the 20% exceedance threshold and were systematic across the treatment groups.
Drottar and Krueger (2000g)	Chronic (35 days)	Flow-through	At 0, 7, 14, 21, 28 and 35 days	HPLC/MS	The majority of measured test concentrations at low concentration (i.e., <1 mg/L) were slightly outside the 20% exceedance threshold although the level of quantitation was adequate for the range of concentrations tested. Possible analytic quantitation issue.
Drottar and Krueger (2000h)	Acute (96-hr)	Static	At 0, 48, and 96 hours	HPLC/MS	All measured test concentrations were outside the 20% exceedance threshold and were systematic across the treatment groups.
Palmer et al. (2002)	Acute (96-hr)	Renewal (every 24 hr)	At 0, 24, 48, 72, and 96 hours	HPLC/MS	Half of the measured test concentrations were outside the 20% exceedance threshold across the treatment groups.

[1] PFOS Analytic Methods: Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS), Liquid Chromatography / Mass Spectrometry (LC-MS), Liquid Chromatography Coupled to Tandem Mass Spectrometry with Electrospray Ionization (HPLC–ESI–MS/MS), Reverse Phase High Performance Liquid Chromatography (RP-HPLC), and High-Performance Liquid Chromatography with Mass Spectrometric Detection (HPLC/MS

Appendix P Bioaccumulation Factors (BAFs) Used to Calculate PFOS Tissue Values

P.1 Summary Table of PFOS BAFs used to calculate tissue criteria and supplemental fish tissue values

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
goldfish	*Carassius auratus*	Blood	4.048	11167	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
mandarin	*Siniperca scherzeri*	Blood	4.867	73612	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
common carp	*Cyprinus carpio*	Blood	3.860	7244	high	Xiaoqing River, China	Pan et al. (2017)
Crucian carp	*Carassius carassius*	Blood	3.984	9638	high	Tangxum Lake, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	Blood	4.301	19999	high	Xiaoqing River, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	Blood	4.352	22484	high	Gaobeidian Lake, China	Shi et al. (2020)
Crucian carp	*Carassius carassius*	Blood	4.328	21275	high	Yubei River, China	Shi et al. (2020)
Crucian carp	*Carassius carassius*	Blood	4.904	80168	high	Beijing Airport, China	Wang et al. (2016)
European perch	*Perca fluviatilis*	Blood	4.763	58000	medium	Lake Halmsjön, near Stockholm, Sweden	Wang et al. (2016)
conger eel	*Conger myriaster*	Blood	3.544	3500	medium	Tokyo Bay	Taniyasu et al. (2003)
Japanese stingfish	*Sebastiscus marmoratus*	Blood	3.712	5154	medium	Tokyo Bay	Taniyasu et al. (2003)
rockfish	*Sebastes inermis*	Blood	3.974	9423	medium	Tokyo Bay	Taniyasu et al. (2003)
black seabream	*Acanthopagrus schlegeli*	Blood	4.150	14138	medium	Osaka Bay	Taniyasu et al. (2003)
Japanese scad	*Trachurus japonicus*	Blood	4.150	14138	medium	Osaka Bay	Taniyasu et al. (2003)
White croaker	*Argyrosomus argentatus*	Blood	4.291	19540	medium	Osaka Bay	Taniyasu et al. (2003)
bluegill	*Lepomis macrochirus*	Blood	4.043	11053	medium	Lake Biwa	Taniyasu et al. (2003)
largemouth bass	*Micropterus salmoides*	Blood	5.230	169737	medium	Lake Biwa	Taniyasu et al. (2003)
carp	*Cyprinus carpio*	Blood	4.925	84211	medium	Lake Biwa	Taniyasu et al. (2003)
Lefteye flounder	*Paralichthys olivaceus*	Blood	3.750	5625	medium	Ariake Bay	Taniyasu et al. (2003)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
European chub	*Leuciscus cephalus*	Gonad	4.000	10000	high	Orge River, near Paris, France	Labadie and Chevreuil (2011)
Crucian carp	*Carassius carassius*	Gonad	3.770	5888	high	Tangxum Lake, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	Gonad	4.060	11482	high	Xiaoqing River, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	Gonad	3.904	8012	high	Gaobeidian Lake, China	Shi et al. (2020)
Crucian carp	*Carassius carassius*	Gonad	3.903	7990	high	Yubei River, China	Shi et al. (2020)
Crucian carp	*Carassius carassius*	Gonad	4.409	25645	high	Beijing Airport, China	Wang et al. (2016)
European perch	*Perca fluviatilis*	Gonad	4.204	16000	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. (2015)
chub	*Leuciscus cephalus*	Gonad	3.347	2222	medium	Roter Main, Upper Franconia, Germany	Becker et al. (2010)
common shiner	*Notropis cornutus*	Liver	4.100	12589	high	Spring/Etobicoke Creek, Toronto, Canada	Awad et al. (2011)
European chub	*Leuciscus cephalus*	Liver	4.300	19953	high	Orge River, near Paris, France	Labadie and Chevreuil (2011)
goldfish	*Carassius auratus*	Liver	3.660	4572	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
mandarin	*Siniperca scherzeri*	Liver	4.393	24718	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
common carp	*Cyprinus carpio*	Liver	3.650	4467	high	Xiaoqing River, China	Pan et al. (2017)
Bream	*Parabramis pekinensis*	Liver	3.500	3162	high	Pearl River Delta, China	Pan et al. (2014)
goldfish	*Carassius auratus*	Liver	4.300	19953	high	Pearl River Delta, China	Pan et al. (2014)
Common carp	*Cyprinus carpio*	Liver	4.400	25119	high	Pearl River Delta, China	Pan et al. (2014)
Chub	*Hypophthalmichthys molitrix*	Liver	3.900	7943	high	Pearl River Delta, China	Pan et al. (2014)
Tilapia	*Tilapia aurea*	Liver	3.500	3162	high	Pearl River Delta, China	Pan et al. (2014)
Snakehead	*Ophicephalus argus*	Liver	4.200	15849	high	Pearl River Delta, China	Pan et al. (2014)
Leather catfish	*Clarias fuscus*	Liver	3.700	5012	high	Pearl River Delta, China	Pan et al. (2014)
grass carp	*Ctenopharyngodon idellus*	Liver	4.600	39811	high	Pearl River Delta, China	Pan et al. (2014)
Crucian carp	*Carassius carassius*	Liver	3.646	4426	high	Tangxum Lake, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	Liver	3.965	9226	high	Xiaoqing River, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	Liver	4.048	11180	high	Gaobeidian Lake, China	Shi et al. (2020)
Crucian carp	*Carassius carassius*	Liver	4.031	10735	high	Yubei River, China	Shi et al. (2020)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Silver perch	*Bidyanus bidyanus*	Liver	4.415	26000	high	Shoalhaven region, Australia	Terechovs et al. (2019)
Crucian carp	*Carassius carassius*	Liver	4.923	83753	high	Beijing Airport, China	Wang et al. (2016)
European perch	*Perca fluviatilis*	Liver	4.591	39000	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. (2015)
chub	*Leuciscus cephalus*	Liver	3.659	4556	medium	Roter Main, Upper Franconia, Germany	Becker et al. (2010)
Mozambique tilapia	*Oreochromis mossambicus*	Liver	2.640	436.8	medium	aMatikulu, N2 Bridge	Fauconier et al. (2020)
Cape stumpnose	*Rhabdosargus holubi*	Liver	2.046	111.2	medium	aMatikulu, N2 Bridge	Fauconier et al. (2020)
tilapia	tilapia	Liver	3.708	5108	medium	Key River, Taiwan	Lin et al. (2014)
tilapia	tilapia	Liver	3.621	4181	medium	Key River, Taiwan	Lin et al. (2014)
tilapia	tilapia	Liver	3.533	3409	medium	Key River, Taiwan	Lin et al. (2014)
Mud carp	*Cirrhinus molitorella*	Liver	4.400	25119	medium	Pearl River Delta, China	Pan et al. (2014)
common seabass	*Lateolabrax japonicus*	Liver	3.514	3269	medium	Tokyo Bay	Taniyasu et al. (2003)
flatfish	*Pleuronectidae*	Liver	3.835	6846	medium	Tokyo Bay	Taniyasu et al. (2003)
Japanese stingfish	*Sebastiscus marmoratus*	Liver	3.646	4423	medium	Tokyo Bay	Taniyasu et al. (2003)
rockfish	*Sebastes inermis*	Liver	3.391	2462	medium	Tokyo Bay	Taniyasu et al. (2003)
common seabass	*Lateolabrax japonicus*	Liver	2.663	459.8	medium	Osaka Bay	Taniyasu et al. (2003)
Japanese scad	*Trachurus japonicus*	Liver	3.015	1034	medium	Osaka Bay	Taniyasu et al. (2003)
White croaker	*Argyrosomus argentatus*	Liver	3.207	1609	medium	Osaka Bay	Taniyasu et al. (2003)
bluegill	*Lepomis macrochirus*	Liver	4.870	74211	medium	Lake Biwa	Taniyasu et al. (2003)
largemouth bass	*Micropterus salmoides*	Liver	4.789	61579	medium	Lake Biwa	Taniyasu et al. (2003)
carp	*Cyprinus carpio*	Liver	3.022	1053	medium	Lake Biwa	Taniyasu et al. (2003)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Lefteye flounder	*Paralichthys olivaceus*	Liver	4.379	23958	medium	Ariake Bay	Taniyasu et al. (2003)
sea mullet	*Mugil cephalus*	Liver	3.699	5000	medium	Sydney Harbour, Australia	Thompson et al. (2011)
European perch	*Perca fluviatilis*	Muscle	3.531	3400	high	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. (2015)
minnow	*Hemiculter leucisculus*	Muscle	3.785	6092	high	Taihu Lake, China	Fang et al. (2014)
silver carp	*Hypophthalmichthys molitrix*	Muscle	3.246	1761	high	Taihu Lake, China	Fang et al. (2014)
white bait	*Reganisalanx brachyrostralis*	Muscle	3.452	2835	high	Taihu Lake, China	Fang et al. (2014)
Japanese crucian carp	*Carassius cuvieri*	Muscle	4.193	15599	high	Taihu Lake, China	Fang et al. (2014)
Lake Saury	*Coilia mystus*	Muscle	3.963	9190	high	Taihu Lake, China	Fang et al. (2014)
common carp	*Cyprinus carpio*	Muscle	3.882	7623	high	Taihu Lake, China	Fang et al. (2014)
Mongolian culter	*Culter mongolicus*	Muscle	4.179	15088	high	Taihu Lake, China	Fang et al. (2014)
mudfish	*Misgurnus anguillicaudatus*	Muscle	4.034	10810	high	Taihu Lake, China	Fang et al. (2014)
Chinese bitterling	*Rhodeus sinensis Gunther*	Muscle	3.809	6444	high	Taihu Lake, China	Fang et al. (2014)
Goby	*Ctenogobius giurinus*	Muscle	3.788	6144	high	Taihu Lake, China	Fang et al. (2014)
eel	*Anguilla anguilla*	Muscle	3.510	3236	high	Netherlands	Kwadijk et al. (2010)
European chub	*Leuciscus cephalus*	Muscle	3.400	2512	high	Orge River, near Paris, France	Labadie and Chevreuil (2011)
Juvenile char	*Salvelinus alpinus*	Muscle	3.274	1878	high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)
Juvenile char	*Salvelinus alpinus*	Muscle	3.016	1038	high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
Juvenile char	*Salvelinus alpinus*	Muscle	4.033	10800	high	Char Lake, Canadian High Arctic	Lescord et al. (2015)
Adult char	*Salvelinus alpinus*	Muscle	2.767	585.4	high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Adult char	*Salvelinus alpinus*	Muscle	3.653	4500	high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
Adult char	*Salvelinus alpinus*	Muscle	4.602	40000	high	Char Lake, Canadian High Arctic	Lescord et al. (2015)
Anchovy	*Engraulis encrasicolus*	Muscle	3.246	1761	high	Gironde estuary, SW France	Munoz et al. (2017)
Mullet	*Liza ramada*	Muscle	3.089	1226	high	Gironde estuary, SW France	Munoz et al. (2017)
Meagre	*Argyrosomus regius*	Muscle	3.397	2496	high	Gironde estuary, SW France	Munoz et al. (2017)
Common seabass	*Dicentrarchus labrax*	Muscle	3.513	3257	high	Gironde estuary, SW France	Munoz et al. (2017)
Spotted seabass	*Dicentrarchus punctatus*	Muscle	3.404	2535	high	Gironde estuary, SW France	Munoz et al. (2017)
Spotted seabass	*Dicentrarchus punctatus*	Muscle	3.766	5830	high	Gironde estuary, SW France	Munoz et al. (2017)
common carp	*Cyprinus carpio*	Muscle	2.730	537.0	high	Xiaoqing River, China	Pan et al. (2014)
Bream	*Parabramis pekinensis*	Muscle	2.600	398.1	high	Pearl River Delta, China	Pan et al. (2014)
goldfish	*Carassius auratus*	Muscle	3.200	1585	high	Pearl River Delta, China	Pan et al. (2014)
Common carp	*Cyprinus carpio*	Muscle	3.200	1585	high	Pearl River Delta, China	Pan et al. (2014)
Chub	*Hypophthalmichthys molitrix*	Muscle	2.800	631.0	high	Pearl River Delta, China	Pan et al. (2014)
Tilapia	*Tilapia aurea*	Muscle	2.400	251.2	high	Pearl River Delta, China	Pan et al. (2014)
Snakehead	*Ophicephalus argus*	Muscle	2.600	398.1	high	Pearl River Delta, China	Pan et al. (2014)
Leather_catfish	*Clarias fuscus*	Muscle	2.400	251.2	high	Pearl River Delta, China	Pan et al. (2014)
grass carp	*Ctenopharyngodon idellus*	Muscle	3.400	2512	high	Pearl River Delta, China	Pan et al. (2014)
Crucian carp	*Carassius carassius*	Muscle	2.870	741.3	high	Tangxum Lake, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	Muscle	3.195	1567	high	Xiaoqing River, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	Muscle	3.053	1130	high	Gaobeidian Lake, China	Shi et al. (2020)
Crucian carp	*Carassius carassius*	Muscle	3.067	1167	high	Yubei River, China	Shi et al. (2020)
Silver perch	*Bidyanus bidyanus*	Muscle	3.778	6000	high	Shoalhaven region, Australia	Terechovs et al. (2019)
Crucian carp	*Carassius carassius*	Muscle	4.701	50234	high	Beijing Airport, China	Wang et al. (2016)
chub	*Leuciscus cephalus*	Muscle	2.683	481.5	medium	Roter Main, Upper Franconia, Germany	Becker et al. (2010)
goby	*Gobio gobio*	Muscle	3.472	2963	medium	Roter Main, Upper Franconia, Germany	Becker et al. (2010)
Black Crappie	*Pomoxis nigromaculatus*	Muscle	3.200	1585	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Brown Bullhead	*Ameiurus nebulosus*	Muscle	2.900	794.3	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
Channel Catfish	*Ictalurus punctatus*	Muscle	3.500	3162	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
Common Carp	*Cyprinus carpio*	Muscle	3.900	7943	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
Largemouth Bass	*Micropterus salmoides*	Muscle	3.700	5012	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
Northern Pike	*Esox lucius*	Muscle	3.000	1000	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
Pumpkinseed	*Lepomis gibbosus*	Muscle	2.800	631.0	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
Smallmouth Bass	*Micropterus dolomieu*	Muscle	3.800	6310	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
White Crappie	*Pomoxis annularis*	Muscle	3.000	1000	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
Yellow Perch	*Perca flavescens*	Muscle	2.900	794.3	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. (2016)
Mozambique tilapia	*Oreochromis mossambicus*	Muscle	1.241	17.44	medium	aMatikulu, N2 Bridge	Fauconier et al. (2020)
Cape stumpnose	*Rhabdosargus holubi*	Muscle	0.9400	8.718	medium	aMatikulu, N2 Bridge	Fauconier et al. (2020)
Eel	*Anguilla anguilla*	Muscle	3.060	1148	medium	Schiphol Amsterdam Airport	Kwadijk et al. (2014)
Eel	*Anguilla anguilla*	Muscle	2.370	234.4	medium	Schiphol Amsterdam Airport	Kwadijk et al. (2014)
tilapia	tilapia	Muscle	2.389	245.0	medium	Key River, Taiwan	Lin et al. (2014)
tilapia	tilapia	Muscle	2.509	323.0	medium	Key River, Taiwan	Lin et al. (2014)
tilapia	tilapia	Muscle	2.328	213.0	medium	Key River, Taiwan	Lin et al. (2014)
Sprat	*Sprattus sprattus*	Muscle	2.908	808.7	medium	Gironde estuary, SW France	Munoz et al. (2017)
Mud carp	*Cirrhinus molitorella*	Muscle	3.400	2512	medium	Pearl River Delta, China	Pan et al. (2014)
sea mullet	*Mugil cephalus*	Muscle	2.196	157.1	medium	Sydney Harbour, Australia	Thompson et al. (2011)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
common shiner	*Notropis cornutus*	WB[a]	3.300	1995	high	Spring/Etobicoke Creek, Toronto, Canada	Awad et al. (2011)
medaka	*Oryzias latipes*	WB	3.740	5500	high	Seven locations across Japan	Iwabuchi et al. (2015)
Juvenile char	*Salvelinus alpinus*	WB	3.645	4415	high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)
Juvenile char	*Salvelinus alpinus*	WB	3.935	8615	high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
Juvenile char	*Salvelinus alpinus*	WB	4.477	30000	high	Char Lake, Canadian High Arctic	Lescord et al. (2015)
Juvenile char	*Salvelinus alpinus*	WB	3.949	8889	high	Small Lake, Canadian High Arctic	Lescord et al. (2015)
Grey mullet	*Mugil cephalus*	WB	2.915	821.6	high	Mai Po Marshes, Hong Kong	Loi et al. (2011)
Mozambique tilapia	*Oreochromis mossambicus*	WB	2.626	422.5	high	Mai Po Marshes, Hong Kong	Loi et al. (2011)
Ladyfish	*Elops saurus*	WB	2.741	550.9	high	Mai Po Marshes, Hong Kong	Loi et al. (2011)
Crucian carp	*Carassius carassius*	WB	3.293	1963	high	Tangxum Lake, China	Shi et al. (2015)
Crucian carp	*Carassius carassius*	WB	3.450	2818	high	Xiaoqing River, China	Shi et al. (2015)
lake trout	*Salvelinus namaycush*	WB	4.160	14453	high	Lake Superior	De Silva et al. (2011)
lake trout	*Salvelinus namaycush*	WB	4.325	21142	high	Lake Huron	De Silva et al. (2011)
lake trout	*Salvelinus namaycush*	WB	4.927	84598	high	Lake Erie	De Silva et al. (2011)
walleye	*Sander vitreus*	WB	4.678	47659	high	Lake Erie	De Silva et al. (2011)
lake trout	*Salvelinus namaycush*	WB	4.238	17293	high	Lake Ontario	De Silva et al. (2011)
Crucian carp	*Carassius carassius*	WB	4.643	43954	high	Beijing Airport, China	Wang et al. (2016)
Common carp	*Cyprinus carpio*	WB	4.070	11749	high	Baiyangdian Lake, China	Zhou et al. (2012)
European perch	*Perca fluviatilis*	WB	3.806	6400	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. (2015)
Grass carp	*Ctenopharyngodon idellus*	WB	3.901	7960	medium	Bantou Reservoir - Xiamen Sea, China	Dai and Zheng (2019)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Chameleon goby	*Tridentiger trigonocephalus*	WB	3.908	8086	medium	Gulf Park - Xiamen Sea, China	Dai and Zheng (2019)
Lake Trout	*Salvelinus namaycush*	WB	4.300	19953	medium	Lake Superior	Furdui et al. (2007)
Lake Trout	*Salvelinus namaycush*	WB	4.200	15849	medium	Lake Huron	Furdui et al. (2007)
Lake Trout	*Salvelinus namaycush*	WB	4.400	25119	medium	Lake Erie	Furdui et al. (2007)
Lake Trout	*Salvelinus namaycush*	WB	3.900	7943	medium	Lake Ontario	Furdui et al. (2007)
Lake Trout	*Salvelinus namaycush*	WB	3.800	6310	medium	Lake Michigan	Furdui et al. (2007)
herring	*Clupea harengus membras*	WB	4.320	20893	medium	Baltic Sea	Gebbink et al. (2016)
spat	*Sprattus sprattus*	WB	4.350	22387	medium	Baltic Sea	Gebbink et al. (2016)
alewife	*Alosa pseudoharengus*	WB	4.380	24000	medium	Lake Ontario	Houde et al. (2008)
rainbow smelt	*Osmerus mordax*	WB	4.653	45000	medium	Lake Ontario	Houde et al. (2008)
slimy sculpin	*Cottus cognatus*	WB	5.369	234000	medium	Lake Ontario	Houde et al. (2008)
lake trout	*Salvelinus namaycush*	WB	4.531	34000	medium	Lake Ontario	Houde et al. (2008)
Sea Bass	*Lateolabrax*	WB	2.585	384.6	medium	Omuta River mouth and estuary, Japan	Kobayashi et al (2018)
Grey mullet	*Mugil cephalus*	WB	3.016	1038	medium	Omuta River mouth and estuary, Japan	Kobayashi et al (2018)
Yellowfin goby	*Acanthogobius flavimanus*	WB	2.761	576.9	medium	Omuta River mouth and estuary, Japan	Kobayashi et al (2018)
Perch	*Esox lucius*	WB	3.370	2344	medium	Schiphol Amsterdam Airport	Kwadijk et al. (2014)
Perch	*Esox lucius*	WB	3.800	6310	medium	Schiphol Amsterdam Airport	Kwadijk et al. (2014)
Small snakehead	*Channa asiatica*	WB	3.108	1283	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
Flag-tailed glass perchlet	*Ambassis miops*	WB	2.889	774.6	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
goby	*Pomatoschistus*	WB	3.380	2400	medium	Gironde estuary, SW France	Munoz et al. (2017)
Chinese icefish	*Neosalanx tangkahkeii taihuensis*	WB	3.355	2267	medium	Lake Chaohu, China	Pan et al. (2019)
Common carp	*Cyprinus carpio*	WB	3.000	1000	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
Mullet	*Liza*	WB	0.680	4.786	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
Roach	*Rutilus rutilus*	WB	2.300	199.5	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Rudd	*Scardinius erythrophtalmus*	WB	1.900	79.43	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
European catfish	*Silurus glanis*	WB	2.000	100.0	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
Ebro chub	*Squalius laietanus*	WB	2.000	100.0	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
Bleak	*Alburnus alburnus*	WB	2.400	251.2	medium	Xerta, Ebro Delta, Spain	Pignotti et al. (2017)
grass goby	*Zosterisessor ophiocephalus*	WB	2.936	863.7	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
grass goby	*Zosterisessor ophiocephalus*	WB	2.752	565.0	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
grass goby	*Zosterisessor ophiocephalus*	WB	2.521	332.0	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
Manila clam	*Ruditapes philippinarum*	Invert[b]	3.601	3991	high	Jiaozhou Bay, China	Cui et al. (2019)
zooplankton	zooplankton	Invert	2.580	380.3	high	Taihu Lake, China	Fang et al. (2014)
zooplankton	zooplankton	Invert	2.813	650.0	high	Lake Ontario	Houde et al. (2008)
Microzooplankton	Microzooplankton	Invert	3.480	3017	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
Mesozooplankton	Mesozooplankton	Invert	3.538	3450	high	17 Sites in six major rivers, Korea	Lam et al. (2014)
zooplankton	zooplankton	Invert	3.077	1195	high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	3.363	2308	high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	3.380	2400	high	Char Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	3.388	2444	high	Small Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	4.564	36667	high	North Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	5.000	100000	high	9-Mile Lake, Canadian High Arctic	Lescord et al. (2015)
zooplankton	zooplankton	Invert	2.470	295.1	medium	Baltic Sea	Gebbink et al. (2016)
zooplankton	zooplankton	Invert	2.425	266.0	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
amphipod	*Gammarus, Hyalella*	Invert	3.779	6015	high	Welland River, Hamilton, Ontario, Canada	De Solla et al. (2012)
freshwater mussel	Unionidae	Invert	2.758	572.2	high	Taihu Lake, China	Fang et al. (2014)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
pearl mussel	Unionidae	Invert	3.005	1011	high	Taihu Lake, China	Fang et al. (2014)
chironomids	Diptera	Invert	3.845	7000	high	Meretta Lake, Canadian High Arctic	Lescord et al. (2015)
chironomids	Diptera	Invert	4.233	17115	high	Resolute Lake, Canadian High Arctic	Lescord et al. (2015)
chironomids	Diptera	Invert	5.447	280000	high	Char Lake, Canadian High Arctic	Lescord et al. (2015)
chironomids	Diptera	Invert	4.770	58889	high	Small Lake, Canadian High Arctic	Lescord et al. (2015)
chironomids	Diptera	Invert	5.386	243333	high	North Lake, Canadian High Arctic	Lescord et al. (2015)
chironomids	Diptera	Invert	5.740	550000	high	9-Mile Lake, Canadian High Arctic	Lescord et al. (2015)
worms	Capitellidae	Invert	2.961	913.93	high	Mai Po Marshes, Hong Kong	Loi et al. (2011)
Copepods	Copepoda	Invert	0.531	3.400	high	Gironde Estuary, SW France	Munoz et al. (2019)
mysids	Mysidacea	Invert	0.591	3.900	high	Gironde Estuary, SW France	Munoz et al. (2019)
white shrimp	*Palaemon longirostris*	Invert	0.531	3.400	high	Gironde Estuary, SW France	Munoz et al. (2019)
brown shrimp	*Crangon crangon*	Invert	0.591	3.900	high	Gironde Estuary, SW France	Munoz et al. (2019)
Oyster	*Crassostrea gigas*	Invert	2.086	122.0	high	Gironde Estuary, SW France	Munoz et al. (2017)
snails	*Bithynia tentaculata*	Invert	2.109	128.4	high	Hogsmill River, Chertsey Bourne River, Blackwater River	Wilkinson et al. (2018)
amphipod	*Gammarus pulex*	Invert	2.072	118.0	high	Hogsmill River, Chertsey Bourne River, Blackwater River	Wilkinson et al. (2018)
Pacific Oyster	*Crassostrea gigas*	Invert	3.808	6430	medium	Gulf Park - Xiamen Sea, China	Dai and Zheng (2019)
Pacific Oyster	*Crassostrea gigas*	Invert	3.621	4180	medium	Jimei Bridge - Xiamen Sea, China	Dai and Zheng (2019)
Ghost crab	*Ocypode stimpsoni*	Invert	3.515	3270	medium	Fenglin - Xiamen Sea, China	Dai and Zheng (2019)
Ghost crab	*Ocypode stimpsoni*	Invert	3.627	4240	medium	Jimei Bridge - Xiamen Sea, China	Dai and Zheng (2019)
Orange-striped hermit crab	*Clibanarius infraspinatus*	Invert	3.589	3879	medium	Jimei Bridge - Xiamen Sea, China	Dai and Zheng (2019)
Snail	*Gastropoda*	Invert	1.183	15.26	medium	aMatikulu N2 Bridge	Fauconier et al. (2020)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
mysid	*Mysis relicta*	Invert	3.477	3000	medium	Lake Ontario	Houde et al. (2008)
diporeia	*Diporeia hoyi*	Invert	4.505	32000	medium	Lake Ontario	Houde et al. (2008)
Snail	*Cerithidea rhizophorarum*	Invert	0.430	2.692	medium	Omuta River mouth and estuary, Japan	Kobayashi et al (2018)
waterlouse, water boatmen, amphipods, roundworm	Isopoda, Hemiptera, amphipoda, nematoda	Invert	2.974	942.0	medium	site A Stockholm Arlanda Airport	Koch et al. (2019)
Fresh water amphipods	Amphipoda	Invert	2.957	905.0	medium	site R Ronneby Airport	Koch et al. (2019)
Mayflies, Caddisflies, Dragonflies, Water boatmen, Waterlouse, Fresh water amphipods	Ephemeroptera, Trichoptera, Odonata, Hemiptera, Isopoda, Amphipoda	Invert	2.728	534.0	medium	site K the Kvarntorp area	Koch et al. (2019)
Gastropoda	Gastropoda	Invert	1.965	92.33	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
worms	Nereidae	Invert	1.893	78.25	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
worms	Sabellidae	Invert	2.562	364.6	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
Black tiger prawn	*Penaeus monodon*	Invert	2.344	220.7	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
Sand prawn	*Metapenaeus ensis*	Invert	2.457	286.4	medium	Mai Po Marshes, Hong Kong	Loi et al. (2011)
Copepods	Copepoda	Invert	3.140	1380	medium	Gironde Estuary, SW France	Munoz et al. (2017)
Mysids	Mysidacea	Invert	3.551	3560	medium	Gironde Estuary, SW France	Munoz et al. (2017)
Gammarids	*Gammarus*	Invert	3.377	2380	medium	Gironde Estuary, SW France	Munoz et al. (2017)
White shrimp	*Palaemon longirostris*	Invert	3.448	2803	medium	Gironde Estuary, SW France	Munoz et al. (2017)
Brown shrimp	*Crangon crangon*	Invert	3.852	7110	medium	Gironde Estuary, SW France	Munoz et al. (2017)
bivalve	*Mytilus galloprovincialis*	Invert	3.701	5029	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
bivalve	*Mytilus galloprovincialis*	Invert	3.436	2728	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
bivalve	*Mytilus galloprovincialis*	Invert	3.056	1137	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
crab	*Carcinus aestuarii*	Invert	3.210	1623	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
crab	*Carcinus aestuarii*	Invert	3.057	1140	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
crab	*Carcinus aestuarii*	Invert	2.742	551.5	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
bivalve	*Ruditapes decussatus*	Invert	3.025	1059	medium	M Site, Orbetell lagoon, Italy	Renzi et al. (2013)
bivalve	*Ruditapes decussatus*	Invert	3.061	1150	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
bivalve	*Ruditapes decussatus*	Invert	2.761	577.2	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. (2013)

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
bivalve	*Ruditapes decussatus*	Invert	2.593	392.2	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
prawn	*Palaemon serratus*	Invert	2.655	451.4	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
prawn	*Palaemon serratus*	Invert	2.481	302.6	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
prawn	*Palaemon serratus*	Invert	2.273	187.5	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. (2013)
rock oyster	*Saccostrea commercialis*	Invert	1.933	85.71	medium	Sydney Harbour, Australia	Thompson et al. (2011)

a – Fish whole body
b – Invertebrate whole body

P.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 P.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 Q-2, respectively. Rankings for individual BAFs were determined by Lawrence (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 (Lawrence 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 (Lawrence 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 Q-3).

P.3 PFOS BAFs References

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Appendix Q 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) includes 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., 0.0084 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:

$$BAF = \frac{C_{biota}}{C_{water}} \qquad (Eq.\ Q\text{-}1)$$

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 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 Q-1.

Table Q-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

[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 Q-2) and individual BAFs are provided in Appendix P.

Table Q-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	5,708	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 Q-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 the equation Q-2 are reported in Table Q-3. The values reported in Table Q-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 Q-3 are only presented for comparative purposes and are not recommended criteria.

Table Q-3. PFOS Concentrations for Additional Fish Tissue.[1, 2]

Category	PFOS Concentration (mg/kg ww)
Liver	20.68
Blood	52.69
Reproductive Tissue	43.30

[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 R Example Data Evaluation Records (DERs)

The PFOS toxicity literature evaluated and used to derive the draft 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 Office of Water. All studies were evaluated for data quality generally as described by U.S.EPA (1985) in the 1985 Guidelines and in EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S.EPA 2016c), and 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 R.1 shows an example fish DER and Appendix R.2 shows an example aquatic invertebrate DER.

R.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 following tabulated data table in each associated results section for all studies. 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 (°C)	*[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: o Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [1,5] 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] • 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.

R.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 (°C)	*[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*	____ Yes ____ No	
• Were replicate level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks*	____ Yes ____ No	
• If treatment and/or replicate level concentration-response data were included, how was data presented? *(check all that apply)*	____ Tables ____ Graphs ____ Supplemental Files	
• Were concentration-response data estimated from graphs study publication or supplemental materials?	____ Yes ____ No *If yes, indicate software used:*	
Should additional concentration-response data be requested from study authors?	____ Yes ____ No Requested by: Request date: Date additional data received:	
*If concentration-response data are available, complete **Verification of Statistical Results (Part C)** for sensitive species.*		

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.