FINAL

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

for

PERFLUOROOCTANOIC ACID

(PFOA)

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ACRONYMS

8:2 FTAC	fluorotelomer acrylate
8:2 FTMAC	fluorotelomer methacrylate
8:2 FTOH	fluorotelomer alcohol
8:2 FTS	fluorotelomer stearate
8:2 HMU	aliphatic diurethane ester
ACR	Acute-to-Chronic Ratio
ADP	Action Development Process
AFFF	aqueous film-forming foam
AIC	Akaike information criteria
ASTM	American Society for Testing and Materials
AWQC	National Recommended Ambient Water Quality Criteria
BAF	bioaccumulation factor
BMF	biomagnification factors
C8/C8-PFPIA	Bis(perfluorooctyl) phosphinic acid
C8-PFPA	Perfluorooctyl phosphonic acid
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
C-F	carbon fluorine
C-R	concentration-response
CC	Chronic Criterion
CCC	Criterion Continuous Concentration
CMC	Criterion Maximum Concentration
CWA	Clean Water Act
DER	Data Evaluation Record
dpf	days post fertilization
diPAPs	polyfluoroalkyl phosphoric acid diesters
drc	dose-response curve
dw	dry weight
ECF	Electrochemical fluorination
ECOTOX	ECOTOXicology database
EC_x	Effect concentration at x percent level
EPA	U.S. Environmental Protection Agency
EU	European Union
FACR	Final Acute-to-Chronic Ratio
FAV	Final Acute Value
FCV	Final Chronic Value
FTEOs	fluorotelomer ethoxylates
FTCAs	fluorotelomer carboxylates
FTOH	fluorotelomer alcohol
GLI	Great Lakes Initiative
GMAV	genus mean acute value
GMCV	genus mean chronic value
GSD	genus sensitivity distribution
GSI	gonadal somatic index
hpf	hours post fertilization

HSI	hepatic somatic index
ICE	Interspecies Correlation Estimation
IC_x	Inhibitory concentration at x percent level
K_d	partitioning coefficients
K_{OC}	Organic carbon water partitioning coefficient
K_{OW}	n-octanol-water partition coefficient
LC_x	Lethal concentration at x percent level
LOECs	Lowest Observed Effect Concentrations
LOQ	limit of quantification
MATC	Maximum Acceptable Toxicant Concentration
MC	Maximum Criterion
MDRs	minimum data requirements
NAMs	New Approach Methods
NOECs	No Observed Effect Concentrations
NPDES	National Pollutant Discharge Elimination System
OECD	Organization for Economic Cooperation and Development
OCSPP	Office of Chemical Safety and Pollution Prevention
ORD	Office of Research and Development
OW	Office of Water
ppt	parts per thousand
PFAAs	perfluoroalkyl acids
PFAS	per- and polyfluorinated substances
PFCAs	Perfluorocarboxylic acids, perfluoroalkyl carboxylates or perfluoroalkyl carboxylic acids
PFdiCAs	Perfluoroalkyl dicarboxylic acids
PFdiSAs	Perfluoroalkane disulfonic acids
PFECAs	Perfluoroalkylether carboxylic acids
PFESAs	Perfluoroalkylether sulfonic acids
PFO	Perfluorooctanoate
PFOA	Perfluorooctanoic acid, pentadecafluoro-1-octanoic acid, pentadecafluoro-n-octanoic acid, octanoic acid, pentadecafluoro-, perfluorocaprylic acid, pentadecafluorooctanoic acid, perfluoroalkyl carboxylic acid or perfluoroheptanecarboxylic acid
PFOAAms	perfluorooctaneamido quaternary ammonium salt
PFOS	Perfluorooctane sulfonic acid or perfluorooctane sulfonate
PFOSI	Perfluorooctane sulfinic acid
PFPAs	Perfluoroalkyl phosphonic acids
PFPIAs	Perfluoroalkyl phosphinic acids
PFSAs	Perfluoroalkane sulfonic acids or perfluorokane sulfonates
PFSIAs	Perfluoroalkyl sulfinic acids
pKa	acid dissociation constant
QACs	quaternary ammonium polyfluoroalkyl surfactants
SMACR	species mean acute-to-chronic ratio
SMAV	species mean acute value
SMCV	species mean chronic value
SOP	standard operating procedure

SSD	species sensitivity distribution
TMDLs	Total Maximum Daily Loads
TSCA	Toxic Substances Control Act
U.S.	United States
web-ICE	Web-based Interspecies Correlation Estimation
WQS	water quality standards
ww	wet weight
WWTPs	wastewater treatment plants

NOTICES

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

Mention of trade names or commercial products does not constitute endorsement or recommendation for use. This document can be downloaded from:

https://www.epa.gov/wqc/aquatic-life-criteria-perfluorooctanoic-acid-pfoa.

FOREWORD

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

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

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

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Director
Office of Science and Technology

EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) developed the recommended perfluorooctanoic acid (PFOA) freshwater aquatic life ambient water quality criteria and an acute saltwater benchmark in accordance with the provisions of Section 304(a) of the Clean Water Act. This document provides the EPA's basis for and derivation of the national PFOA ambient water quality criteria recommendations to protect aquatic life. The EPA has derived the recommended PFOA aquatic life criteria and benchmark to be consistent with methods described in the EPA's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" (U.S. EPA 1985).

PFOA is an organic, human-made perfluorinated compound, consisting of a seven-carbon backbone and a carboxylate functional group. PFOA (and other related chemicals in the perfluorocarboxylic acids, PFCAs) is used primarily in specialized applications associated with surface coatings in a variety of industrial and commercial products. This document provides a critical review of toxicity data for aquatic life identified in the EPA's literature search for PFOA, including the anionic form (CAS No. 45285-51-6), the acid form (CAS No. 335-67-1), and the ammonium salt (CAS No. 3825-26-1). It also quantifies the toxicity of PFOA to aquatic life and provides final criteria recommendations to protect aquatic life in freshwater from the acute and chronic toxic effects of PFOA.

The Aquatic Life Ambient Water Quality Criteria for PFOA document includes water column-based acute and water column-based chronic criteria, as well as chronic tissue-based criteria for freshwaters. Quantitatively-acceptable estuarine/marine toxicity data only fulfilled three of the eight minimum data requirements (MDRs) for deriving an acute estuarine/marine criterion, and one of the eight MDRs for deriving a chronic estuarine/marine criterion per the 1985 Guidelines. The EPA did, however, include an acute aquatic life benchmark for

estuarine/marine environments in Appendix L, using available estuarine/marine species toxicity data and the New Approach Methods (NAMs) application of the EPA Office of Research and Development's (ORD) peer-reviewed web-based Interspecies Correlation Estimate tool (Web-ICE; Version 3.3; https://www.epa.gov/webice/). The estuarine/marine benchmarks are CWA Section 304(a)(2) information provided for states and authorized Tribes to consider in their state/tribal water quality protection programs. However, the acute estuarine/marine benchmark magnitude is less certain than the freshwater criteria as the benchmark was based on both direct laboratory-based and estimated PFOA acute toxicity data (Appendix L).

The final freshwater acute water column-based criterion magnitude is 3.1 mg/L, and the final chronic water column-based chronic criterion magnitude is 0.10 mg/L. The final chronic freshwater criterion also contains tissue-based criteria with magnitudes of 6.49 mg/kg wet weight (ww) for fish whole-body, 0.133 mg/kg ww for fish muscle tissue, and 1.18 mg/kg ww for invertebrate whole-body tissue. All criteria are intended to be equally protective against adverse PFOA 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 recommendations are expected to protect the freshwater aquatic community.

Table Ex-1. Recommended Perfluorooctanoic acid (PFOA) Ambient Water Quality Criteria for the Protection of Aquatic Life in Freshwater.

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	3.1 mg/L	0.10 mg/L	1.18 mg/kg ww	6.49 mg/kg ww	0.133 mg/kg ww
Duration	One-hour average	Four-day average	Instantaneous[3]		
Frequency	Not to be exceeded more than once in three years on average	Not to be exceeded more than once in three years on average	Not to be exceeded[6]		

[1] All five of these water column and tissue criteria are intended to be independently applicable and no one criterion takes primacy. All of the above recommended criteria (acute and chronic water column and tissue criteria) are intended to be protective of aquatic life. These criteria are applicable throughout the year.

[2] Tissue criteria 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 PFOA over time and space in aquatic life population(s) at a given site.

[4] Criterion Maximum Concentration; applicable throughout the water column.

[5] Criterion Continuous Concentration; applicable throughout the water column.

[6] PFOA chronic freshwater tissue-based criteria should not be exceeded, based on measured tissue concentrations representing the central tendency of samples collected at a given site and time.

Table Ex-2. Acute Perfluorooctanoic Acid (PFOA) Benchmark for the Protection of Aquatic Life in Estuarine/Marine Waters.

Type/Media	Acute Water Column Benchmark
Magnitude	7.0 mg/L
Duration	One hour average
Frequency	Not to be exceeded more than once in three years on average

1 INTRODUCTION AND BACKGROUND

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

This document provides a critical review of toxicity data identified in the EPA's literature
search for PFOA, including the anionic form (CAS No. 45285-51-6), the acid form (CAS No.

335-67-1), and the ammonium salt (CAS No. 3825-26-1). It also quantifies the toxicity of PFOA to aquatic life and provides criteria to protect aquatic life in freshwater from the acute and chronic toxic effects of PFOA.

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

1.1 Previously Derived PFOA Toxicity Values and Thresholds

Other jurisdictions (e.g., states, countries, etc.) have previously published PFOA acute and chronic criteria, benchmarks, or thresholds, including values for both freshwater and marine systems. These values focus exclusively on water column-based values only; no other jurisdiction has previously derived tissue-based values. Within the United States, no states or Tribes have CWA Section 303(c) approved PFOA water quality standards for the protection of

aquatic life. However, several states have published draft/interim acute and chronic ecological screening level values/benchmarks for the protection of aquatic life (e.g., Michigan, Minnesota, Texas, Florida, California).

These publicly available freshwater acute values range from 4.47 mg/L in Texas (TCEQ 2021) to 20 mg/L in Florida (Stuchal and Roberts 2019) (Table 1-1). The EPA's freshwater acute PFOA criterion (3.1 mg/L) is slightly lower than the range of state-derived values. No acute estuarine/marine criteria, benchmarks, or protective values have been established for PFOA, other than the benchmark provided herein.

Publicly available freshwater chronic values for other jurisdictions range from 0.22 mg/L in Australia/New Zealand (95% species protection level; CRC CARE 2017; EPAV 2016; HEPA 2020; Table 1-1) to 2.27 mg/L in Texas (TCEQ 2021), which are all higher than the EPA's freshwater chronic criterion of 0.10 mg/L, which includes more recently published data not accounted for in other previously published values.

Previously published estuarine/marine chronic values are available for Australia/New Zealand with a chronic protective value of 0.22 mg/L (95% species protection level) and California with a chronic "interim final screening level value" of 0.54 mg/L (Table 1-1).

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

State/Country of Applicability	Aquatic Life Protective Value (mg/L)	Criteria or Benchmark and Calculation Approach	Source
Freshwater Acute			
Texas	4.47	Based on NOAELs, LOAELs, or similar values from specific toxicological studies. Contact the TCEQ for more information. This is an acute surface water benchmark and does not represent a CWA Section 303(c) approved water quality standard for PFOA.	TCEQ 2021
Michigan	7.7	Calculated from a species sensitivity distribution (SSD) consisting of two species-specific values. The Final Acute Value (FAV) was based on the lowest EC_{50} divided by a safety factor of 13 (following the U.S. EPA Great Lakes Initiative [GLI; U.S. EPA 1995a]). 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 PFOA.	EGLE 2010
Minnesota	15	Calculated from a species sensitivity distribution (SSD) consisting of three species-specific values. The Maximum Criterion (MC) was based on the lowest EC_{50} divided by a safety factor of 13 (following the U.S. EPA Great Lakes Initiative [GLI; U.S. EPA 1995a]). This draft value is does not represent a CWA Section 303(c) approved water quality standard for PFOA.	STS/MPCA 2007
Florida	20	Secondary Acute Value (SAV) calculated using the U.S. EPA Great Lakes Initiative (GLI; U.S. EPA 1995a) Tier II Methodology. FAV calculated as the lowest GMAV (unspecified) divided by a safety factor of 5.2. 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)	Criteria or Benchmark and Calculation Approach	Source
Freshwater Chronic			
Australia, New Zealand	0.019 (99% species protection – high conservation value systems) 0.22 (95% species protection – slightly to moderately disturbed systems) 0.632 (90% species protection – highly disturbed systems) 1.824 (80% species protection – highly disturbed systems)	Guidelines calculated from a species sensitivity distribution (SSD) consisting of 12 species-specific values for fish, insects, crustaceans, rotifers, algae, and plants following the guidance of Warne et al. (2018) and Batley et al. (2014)	CRC CARE 2017, EPAV 2016, HEPA 2020
California	0.54 (99% species protection)	HC1 calculated from an acute and chronic NOEC-based SSD as reported in DoD-SERDP Project ER18-1614 (DoD-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 PFOA.	San Francisco Bay RWQCB 2020; DoD-SERDP 2019
Michigan	0.88	Final Chronic Value (FCV) was calculated as the FAV ÷ Final Acute: Chronic ratio (ACR) (following the GLI; U.S. EPA 1995a). 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 PFOA.	EGLE 2010

State/Country of Applicability	Aquatic Life Protective Value (mg/L)	Criteria or Benchmark and Calculation Approach	Source
Florida	1.3	Secondary Chronic Value (SCV) calculated using the U.S. EPA Great Lakes Initiative (GLI; U.S. EPA 1995a) Tier II Methodology with acute-to-chronic ratio (ACR) of 15.3. SCV = SAV (20,000 µg/L) ÷ ACR (15.3) = 1,300 µg/L or 1.3 mg/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
Minnesota	1.7	Chronic Criterion (CC) calculated as the FAV ÷ a generic ACR following Minnesota Rules Chapter 7050. No species-specific ACRs were available at the time to calculate the FACR. This draft value idoes not represent a CWA Section 303(c) approved water quality standard for PFOA.	STS/MPCA 2007
Texas	2.77	Based on NOAELs, LOAELs, or similar values from specific toxicological studies. Contact the TCEQ for more information. This is a chronic surface water benchmark and does not represent a CWA Section 303(c) approved water quality standard for PFOA.	TCEQ 2021
Marine Chronic			
California	0.54 (99% species protection)	HC1 calculated from an acute and chronic NOEC-based SSD as reported in DoD-SERDP Project ER18-1614 (DoD-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 PFOA.	San Francisco Bay RWQCB 2020; DoD-SERDP 2019
Australia, New Zealand	0.019 (99% species protection – high conservation value systems) 0.22 (95% species protection – slightly to moderately disturbed systems)	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.	HEPA 2020

State/Country of Applicability	Aquatic Life Protective Value (mg/L)	Criteria or Benchmark and Calculation Approach	Source
	0.632 (90% species protection – highly disturbed systems)		
	1.824 (80% species protection – highly disturbed systems)		

1.2 Overview of Per- and Polyfluorinated Substances (PFAS)

Perfluorooctanoic acid (PFOA), and its salts, belong to the per- and polyfluorinated substances (PFAS) group of chemicals. PFAS are a large group of structurally diverse anthropogenic chemicals that include PFOA, PFOS, and thousands of other fully or partially fluorinated chemicals. There are many families or subclasses of PFAS, and each contains many individual structural homologues and can exist as either branched-chain or straight-chain isomers (Buck et al. 2011; U.S. EPA 2021). These PFAS families can be divided into two primary categories: non-polymers and polymers. The non-polymer PFAS include perfluoroalkyl and polyfluoroalkyl substances. Polymer PFAS include fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers (Table 1-2). Several U.S. federal, state, and industry stakeholders as well as European entities have posited various definitions of what constitutes a PFAS. OECD, an international organization comprised of 38 countries, recently published a practical guidance regarding the terminology of PFAS (OECD 2021). The OECD-led "Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance" workgroup provided an updated definition of PFAS, originally posited in part by Buck et al. (2011), as follows: "PFASs are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e. with a few noted exceptions, any chemical with at least a perfluorinated methyl group ($-CF_3$) or a perfluorinated methylene group ($-CF_2-$) is a PFAS". It is not within the scope of this framework to compare and contrast the various definitions, or the nuances associated with defining or scoping PFAS; rather the reader of this document is referred to OECD (2021) for review. Generally, the structural definition of PFAS includes chemicals that contain <u>at least one of the following three structures</u>:

- R-(CF$_2$)-CF(R$'$)R$''$, where both the CF$_2$ and CF moieties are saturated carbons, and none of the R groups can be hydrogen (TSCA draft definition);
- R-CF$_2$OCF$_2$-R$'$, where both the CF$_2$ and CF moieties are saturated carbons, and none of the R groups can be hydrogen; and
- CF$_3$C(CF$_3$)R$'$R $''$, where both the CF$_2$ and CF moieties are saturated carbons, and none of the R groups can be hydrogen.

It should also be noted that what defines or constitutes a PFAS may change or evolve

over time and under different purviews (e.g., federal, state, international).

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

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

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

PFOA belongs to the perfluoroalkyl acids (PFAAs) of the non-polymer perfluoroalkyl substances category of PFAS (Table 1-2). 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). Although the protonated and anionic forms may have different physiochemical

properties, the anionic form is the dominant form in the aquatic environment, including in the

toxicity tests used to derive the PFOA criteria.

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

Classification	Functional Group	Example
Perfluoroalkyl carboxylic acids (PFCAs) Or Perfluoroalkyl carboxylates (PFCAs)	-COOH	Perfluorooctanoic acid (PFOA)[2]
	-COO$^-$	Perfluorooctanoate (PFO)
Perfluoroalkane sulfonic acids (PFSAs) Or Perfluorokane sulfonates (PFSAs)	-SO$_3$H	Perfluorooctane sulfonic acid (PFOS)
	-SO$_3^-$	Perfluorooctane sulfonate (PFOS)
Perfluoroalkyl sulfinic acids (PFSIAs)	-SO$_2$H	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$_m$F$_{2m+1}$)	Bis(perfluorooctyl) phosphinic acid (C8/C8-PFPIA)
Perfluoroalkylether carboxylic acids (PFECAs)	CF$_3$(OCF$_2$)$_n$COO$^-$	perfluoro (3,5,7-trioxaoctanoic) acid
Perfluoroalkylether sulfonic acids (PFESAs)	CF$_3$(OCF$_2$)$_n$SO$_3$H	6:2 Cl-PFESA
Perfluoroalkyl dicarboxylic acids (PFdiCAs)	HOOC-C$_n$F$_{2n}$-COOH	9:3 Fluorotelomer betaine
Perfluoroalkane disulfonic acids (PFdiSAs)	HO$_3$S-C$_n$F$_{2n}$-SO$_3$H	Perfluoro-1,4-disulfonic acid

[1]: Modified from Buck et al. (2011) and OECD (2021).
[2]: At most environmentally relevant pH conditions, PFOA occurs in the anionic form.

Perfluoroalkyl carboxylic acids (PFCAs), including PFOA, consist of a general chemical

structure of C$_n$F$_{2n+1}$COOH. This chemical structure makes PFOA (see Figure 1-1) extremely

strong and stable, and resistant to hydrolysis, photolysis, microbial degradation, and metabolism

(Ahrens 2011; Beach et al. 2006; Buck et al. 2011).
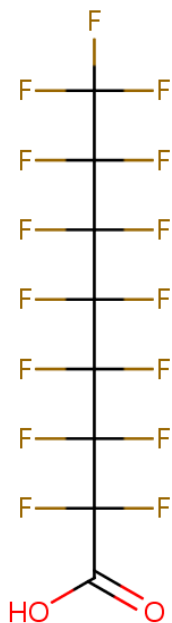
Figure 1-1. Chemical Structure of the Linear Isomer of Perfluorooctanoic acid (PFOA).
(Source: United States EPA Chemistry Dashboard; https://comptox.epa.gov/dashboard).

1.2.1 Physical and Chemical Properties of PFOA

Physical and chemical properties along with other reference information for PFOA are

provided in Table 1-4. These physical and chemical properties helped to define the

environmental fate and transport of PFOA in the aquatic environment. In the environment,

PFOA rapidly ionizes in water to its anionic form (perfluorooctanoate, PFO). PFOA is highly

stable and is resistant to hydrolysis, photolysis, volatilization, and biodegradation (UNEP 2015).

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

Property	PFOA, acidic form[1]	Source
Chemical Abstracts Service Registry Number (CASRN)	335-67-1	
Chemical Abstracts Index Name	2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid	
Synonyms	PFOA; Pentadecafluoro-1-octanoic acid; Pentadecafluoro-n-octanoic acid; Octanoic acid, pentadecafluoro-; Perfluorocaprylic acid; Pentadecafluorooctanoic acid; Perfluoroheptanecarboxylic acid;	
Chemical Formula	$C_8HF_{15}O_2$	
Molecular Weight (grams per mole [g/mol])	414.07	PubChem Identifier (CID 9554) (URL: https://pubchem.ncbi.nlm.nih.gov/compound/9554); Lide (2007)
Color/Physical State	White powder (ammonium salt)	PubChem Identifier (CID 9554) (URL: https://pubchem.ncbi.nlm.nih.gov/compound/9554)
Boiling Point	192.4 ºC	HSDB (2012); Lide (2007); SRC (2016)
Melting Point	54.3 ºC	HSDB (2012); Lide (2007); SRC (2016)
Vapor Pressure	0.525 mm Hg at 25 °C (measured) 0.962 mm Hg at 59.25 °C (measured)	Hekster et al. (2003); HSDB (2012); SRC (2016) ATSDR (2015); Kaiser et al. (2005)
K_{AW}	0.00102 (experimentally determined; equivalent to Henry's Law Constant of 0.000028 Pa-m^3/mol at 25 °C)	Li et al. (2007)
K_{OW}	Not measurable	UNEP (2015)
Organic carbon water partitioning coefficient (K_{OC})	2.06	Higgins and Luthy (2006)
pKa	3.15 (mean measured)	Burns et al. (2008) and 3M Company (2003) as reported in EPA Chemistry Dashboard (URL: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID8031865#properties)
Solubility in Water	9,500 mg/L (estimated); 3,300 mg/L at 25 ºC (measured)	Hekster et al. (2003); Inoue et al. (2012)

Property	PFOA, acidic form[1]	Source
Half-Life in Water	Stable	UNEP (2015)
Half-Life in Air	Stable	UNEP (2015)

[1]: PFOA is most commonly produced as an ammonium salt (CASRN 3825-26-1). Properties specific to the salt are not included.

PFOA is water soluble, nonvolatile, and stable, with a low vapor pressure and is a solid at room temperature (UNEP 2015). The EPA's chemistry dashboard reported a mean experimental acid dissociation constant (pKa) for PFOA of 3.15 calculated from pKa values determined from Burns et al. (2008) and 3M Company (2003). Burns et al. (2008) measured an acid dissociation constant (pKa) for PFOA of 3.8 using a standard water-methanol mixed solvent approach, which indicates PFOA is a moderate acid, while 3M Company (2003) reported a measured PFOA pKa of 2.5.

Due to the surfactant properties of PFOA, it forms three layers when added to octanol and water in a standard test system used to measure a n-octanol-water partition co-efficient (K_{OW}), thus preventing direct measurement (EFSA 2008; Giesy et al. 2010). Although a K_{OW} cannot be directly measured, a K_{OW} for PFOA has been estimated from its individual water and octanol solubilities (estimated PFOA K_{OW} range = 2.69 – 6.3; UNEP 2015); however, the veracity of such estimates is uncertain (UNEP 2015). Lacking a reliable K_{OW} for PFOA 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 PFOA would bring into question the use of K_{OW} as a predictor of environmental behavior; for example, bioaccumulation of PFOA is thought to be mediated via binding to proteins rather than partitioning into lipids (EFSA 2008; Giesy et al. 2010), the latter being the theoretical basis for K_{OW}-based prediction of bioaccumulation.

2 PROBLEM FORMULATION

A problem formulation provides a strategic framework for water quality criteria 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 was consistent with the EPA's Guidelines for Ecological Risk Assessment (U.S. EPA 1998).

2.1 Overview of PFOA Sources

2.1.1 Manufacturing of PFOA

PFOA is primarily produced through Electrochemical Fluorination (ECF) in which an organic raw material, in the case of PFOA as octanoyl fluoride ($C_7H_{15}COF$), undergoes electrolysis in anhydrous hydrogen fluoride solution. This electrolysis leads to the replacement of all the hydrogen atoms by fluorine atoms and results in perfluorooctanoyl fluoride ($C_7F_{15}COF$), which is the major raw material used to manufacture PFOA and PFOA salts (Figure 2-1; Buck et al. 2011). Electrochemical Fluorination typically results in a mixture of branched and linear isomers.
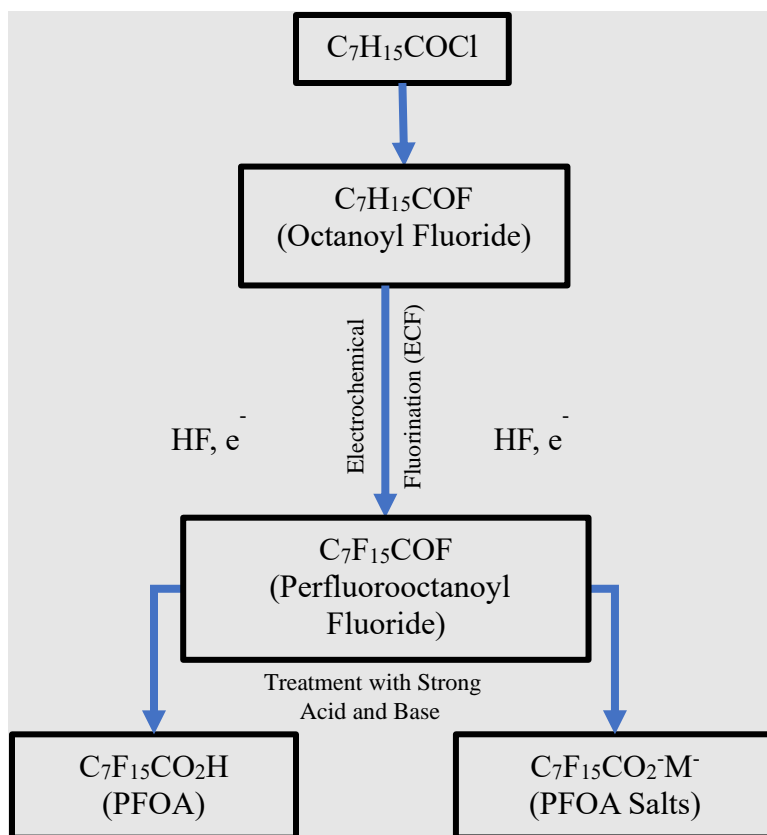
Figure 2-1. Synthesis of Perfluorooctanoic acid (PFOA) by Electrochemical Fluorination (ECF).
Modified from Buck et al. (2011).

Initial production of PFOA started in the 1940s and commercial production and use as protective coatings starting in the mid-1950s. From 1951 – 2004 the total global historic industry wide emission of PFCAs (including PFOA) from all sources (i.e., direct and indirect sources such as manufacture, use, consumer products, and PFCA precursors) ranged from 3,200 tons to 7,300 tons (Prevedouros et al. 2006). In 2006, the EPA initiated the 2010/2015 PFOA Stewardship Program, resulting in major PFOA producers committing to a 95% reduction in PFOA facility emissions and product contents across the globe by 2010. The 2010/2015 PFOA Stewardship Program further aimed to eliminate PFOA emissions and product content by 2015 (U.S. EPA 2006).

2.1.2   Sources of PFOA to Aquatic Environments

PFCAs, including PFOA are primarily released into water (95% of PFCAs are emitted to water; 3M Company 2000b) and can enter the aquatic environment from both industrial and consumer products during manufacturing, along the supply chains, during product use and/or disposal (Ahrens and Bundschuh 2014; Kannan 2011). Occurrence of PFOA in the aquatic environment arises from both direct and indirect sources (Ahrens et al. 2011a). However, the quantitative assessments of their production, direct and indirect emissions, and environmental measurements are lacking (Ahrens and Bundschuh 2014; Prevedouros et al. 2006).

The direct sources of PFOA to the aquatic environment include both municipal and industrial wastewater treatment plants (WWTPs), landfill leachate, and runoff from contaminated biosolids (Renner 2009). WWTPs in particular are an important source of PFOA to aquatic systems (Ahrens et al. 2009).

Indirect sources of PFOA to aquatic environments include dry and wet atmospheric deposition, runoff from contaminated soils, and consumer product use and disposal (Kannan 2011). Identification of indirect sources of PFOA and understanding their relative contribution to aquatic ecosystems is difficult. Overall, the presence of indirect sources of PFOA and their contributions are dependent on the system and the nearby land uses. Overall PFAS concentrations, including PFOA, in the environment are positively correlated with population density. Overall, PFOA occurrence in aquatic environments is driven by legacy PFOA sources because use of PFOA in the United States was largely phased out by 2010, and completely phased out by 2015 in accordance with the EPA's 2010/2015 PFOA Stewardship Program.

In addition to direct discharge, environmental breakdown of precursor compounds containing a seven-member perfluoro moiety can provide an additional source of PFOA. Metabolic transformation of PFAS precursors, such as PAPs, FTCAs, FTUCAs, FTSAs, and

FASAAs, and the degradation of volatile PFAS, such as FTOHs, FASAs, and FASEs, can be

potential sources of PFOA as these compounds can transform into more persistent PFAS,

including PFCAs and PFOA (Ahrens and Bundschuh 2014). For example, fluoroacrylate

polymers can breakdown in soil and release fluorotelomer alcohol (FTOH) which can further

degrade into PFOA (Russell et al. 2008). Similarly, polyfluoroalkyl phosphoric acid diesters

(diPAPs) are used in commercial applications, such as food packaging, and can be found in

WWTP sludge and contaminated biosolids. In environmental media, diPAPs can release FTOH

that further degrades into PFOA (Lee et al. 2010; Sinclair and Kannan 2006; Washington et al.

2009). Current understanding of these transformation processes remains limited, and additional

work is needed to fully understand these processes and their role in generation of sources of

PFOA to aquatic environments (Lau et al. 2007).

PFOA can also be re-emitted to the aquatic environment from ice melt and sediment

transport. Release of PFOA will continue into the future from the transformation of other PFAS

and the historical products still in use (e.g., consumer goods manufactured and/or obtained

before the PFOA discontinuation).

2.2   Environmental Fate and Transport of PFOA in the Aquatic Environment

2.2.1   Environmental Fate of PFOA in the Aquatic Environment

In natural waters near neutral pH, PFOA rapidly dissociates into ionic components. In

aquatic environments, PFOA has an affinity to remain in the water column rather than sediments,

but can also adsorb to sediments in the presence of organic carbon, with the partitioning

coefficients (K_d) increasing with salinity (Canada 2012; Hekster et al. 2003). Because of its

water solubility and preferential binding to proteins, once PFOA enters a waterbody it tends to

remain dissolved in the water column, where it is mobile, unless it adsorbs to organic particulate

matter or is assimilated by organisms.

PFOA has low volatility in the ionized form but can adsorb to particles in air where it can be transported globally, including remote locations (Del Vento et al. 2012; Shoeib et al. 2006). PFOA is water soluble and has been found in surface water, ground water, and drinking water. Because of the relatively low log K_{OC} of PFOA, it does not easily adsorb to sediments and tends to stay in the water column.

In the water column, and other environmental compartments, PFOA is stable and resistant to hydrolysis, photolysis, volatilization, and biodegradation (Higgins and Luthy 2006; UNEP 2015). The persistence of PFOA 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 PFOA are known. In aquatic environments, the only dissipation mechanisms for PFOA are physical mechanisms, such as environmental dilution and sorption.

2.2.2   Environmental Transport of PFOA in the Aquatic Environment

The physiochemical properties discussed in Section 1.2.1 above enable PFOA to be highly persistent in the aquatic environment. PFOA tends to be distributed in waters and in the atmosphere (Ahrens 2011; Yamashita et al. 2008). PFOA concentrations in seawater are typically greater than PFOS, which has been attributed to the relatively lower bioaccumulation potential, lower sorption to sediments, and greater water solubility (Ahrens 2011; Ahrens et al. 2009).

Numerous uncertainties exist in the understanding of environmental transport of PFOA in aquatic systems. Both point and non-point sources contribute PFOA to the aquatic environment. PFOA can be transported from these sources into rivers, streams, lakes, and marine environments. There is a general decrease in PFOA concentrations along a transport pathway resulting from dilution in the water column. For example, measured PFOA concentrations in

WWTP effluents were generally an order of magnitude greater than riverine concentrations, with the upper end of riverine concentrations being similar to WWTP effluents. Although minimum and maximum PFOA concentrations in coastal waters (ranging from hundreds of pg/L to several ng/L) were below corresponding measurements in riverine systems, coastal PFOA concentrations in general were largely similar to riverine concentrations. Open oceans contained the lowest PFOA concentrations resulting from immense dilution. Overall, open ocean concentrations of PFOA were roughly 2.5 orders of magnitude lower than those reported in WWTP effluents (Ahrens 2011).

PFOA has been found in a diversity of environments, including in the arctic and Antarctic, despite the limited number of manufacturing facilities and/or small population sizes typically found in these areas (Del Vento et al. 2012; Shoeib et al. 2006). Although PFOA has low volatility, particularly in the ionized form, it can absorb to air particles before being deposited via atmospheric deposition to these remote regions. For example, Kim and Kannan (2007) reported PFOA in snow in the United States ranging from below the limit of quantification to 20 ng/L. Similarly, Young et al. (2007) reported mean PFOA concentrations in snow in Canada ranging from 0.01 ng/L to 0.15 ng/L.

The continued presence of PFOA in open oceans and in remote polar areas may be due to multiple exposure pathways, including those caused by direct production, use, and discharge of PFOA itself, degradation and transformation of precursor compounds, and via long range aqueous and atmospheric transport (Armitage et al. 2009).

2.3 Transformation and Degradation of PFOA Precursors in the Aquatic Environment

Included among major sources of PFOA to the environment is from the abiotic and biotic transformation and degradation of polyfluoroalkyl precursor substances (see Section 2.2.2

above). Polyfluoroalkyl substances are one type of precursor substance that have the potential to be transformed abiotically or biotically into PFCAs such as PFOA (Buck et al. 2011). On a global scale, production volumes of polyfluoroalkyl substances, many of which are likely polyfluoroalkyl precursor substances that ultimately degrade or transform to PFOA, greatly exceed direct emissions of PFOA through its manufacture, use and disposal (Butt et al. 2014; Liu and Mejia Avendaño 2013). According to the OECD (2006), there were approximately one thousand polyfluorylalkyl chemicals commercially produced at the time that could conceivably degrade to PFCAs such as PFOA. For example, Buck et al. (2011) identified 42 families of compounds and numerous individual PFAS detected in environmental and human matrices, many of which have not been evaluated for their biodegradability (Liu and Mejia Avendaño 2013). Any or all members of these PFAS have the ability to be transformed or degraded to PFAAs such as PFCAs or perfluoroalkane sulfonic acids (PFSAs).

Critical reviews by Butt et al. (2014) and Liu and Mejia Avendaño (2013) provided a comprehensive summary of the qualitative and quantitative relationships between biodegradation and transformation of polyfluoroalkyl precursors and generation of PFOA and other PFCAs. The most well-studied polyfluoroalkyl precursor substances are fluorotelomer-based compounds, which are produced through telomerization technology and are associated with PFOA as the final product (Buck et al. 2011).

2.3.1 Biodegradation of fluorotelomer-based precursors

The aerobic biodegradation pathway of fluorotelomer alcohols (8:2 and 6:2 FTOH) have been thoroughly studied. Dinglasan et al. (2004) was among the first to investigate the biotransformation of 8:2 FTOH in a mixed microbial culture. Additional studies of the aerobic microbial degradation of 8:2 FTOH by Liu et al. (2010) and Wang et al. (2005, 2009, and 2012) have since confirmed the formation of PFOA via this pathway. The observed half-lives of 8:2

FTOH ranged from <2 days to 30 days in these laboratory studies. Molar yield of PFOA ranged anywhere from 0.5 to 40% depending on type of microbes or microcosm used in the study, with Wang et al. (2009) observing relatively higher PFOA yield in aerobic soils relative to PFOA yield in pure bacterial culture (Liu et al. 2010). Thus, aerobic microbial degradation of 8:2 FTOH can be a significant source of PFOA in some environmental compartments. Anaerobic microbial degradation of 8:2 FTOH, on the other hand, is inefficient and likely an insignificant source of PFOA to the environment (Zhang et al. 2013b). Additional studies are needed, however, to understand anaerobic biodegradability of FTOHs and related compounds in general (Liu and Mejia Avendaño 2013).

Aerobic biodegradation of several other types of fluorotelomer-based, polyfluoroalkyl precursor substances definitively linked to PFOA formation include: fluorotelomer stearate (8:2 FTS) with observed half-life in aerobic soils of 5-28 days and molar yield of about 01.7-4% (Dasu et al. 2012, 2013); fluorotelomer acrylate (8:2 FTAC) and fluorotelomer methacrylate (8:2 FTMAC) monomers with observed half-life in aerobic soils of 3-5 days and 15 days and molar yields of 7.8 and 10%, respectively (Royer 2011); fluorotelomer ethoxylates (FTEOs) with observed half-life in unfiltered WWTP effluent of approximately one day and molar yield of about 0.3% (Frömel and Knepper 2010); and fluorotelomer urethanes, specifically, aliphatic diurethane ester (8:2 HMU), with an half-life in aerobic soils of >180 days and molar yield of 0.9% (Dasu 2011).

2.3.2   Biodegradation of side-chain polymers

At present, a crucial need exists to understand the potential degradation of side-chain fluorinated polymers in natural environments because they currently represent a high percentage of all commercial and industrial PFAS sales products (Liu and Mejia Avendaño 2013). Side-chain polymers are those with polyfluoroalkyl or perfluoroalkyl chains attached to non-

fluorinated backbones (Buck et al. 2011). Russell et al. (2008) investigated the biodegradation of a high molecular weight (~40,000 amu, 100–300 nm in diameter) polyacrylate polymer aqueous dispersion product in four aerobic soils over two years. Two approaches (molar mass balance and kinetic modeling) gave conflicting results. The molar mass balance approach indicated no evidence of biodegradation because the PFOA generated was mostly accounted for by impurity (residual non-polymerized PFAS) degradation. Conversely, using the kinetic modeling approach half-lives of PFOA were estimated to be around 1,200-1,700 years among the four soils tested. Upon further investigation using a low molecular weight (~3,500 amu) polyurethane polymer product and a similar approach, Russell et al. (2010) clearly demonstrated biodegradability of the low molecular weight polyurethane polymer product compared to the polyacrylate polymer, as the levels of PFOA produced were several orders of magnitude greater than what the impurities could account for. Applying a similar kinetic modeling approach, the half-lives of the polyurethane polymer were estimated to range from 28 to 241 years among the four test soils. Given the large disparity in half-life prediction between the two studies, however, additional research is needed to clarify the contributions of polyfluoroalkyl polymers to PFOA formation due to the high percentage of side-chain fluorinated polymers that exist in commercial and industrial sales products.

2.3.3   Biodegradation of other polyfluoroalkyl substances

Recently, Mejia-Avendaño et al. (2016) examined the formation of PFOA from aerobic biotransformation of quaternary ammonium polyfluoroalkyl surfactants (QACs). Capitalizing on several recent studies focused on identifying specific PFAS in major PFAS-based aqueous film-forming foam (AFFF) formulations, all the newly identified PFAS were polyfluoroalkyl compounds. These compounds have perfluoroalkyl carbon chain lengths varying from four to 12 and possess functionalities such as sulfonyl, thioether, tertiary amine, quaternary ammonium,

carboxylate, sulfonate, amine oxide, and betaine, etc. (Mejia-Avendaño et al. 2016). Importantly, the identified cationic PFAS in these studies contain either tertiary amine or quaternary ammonium groups. In this first study of the fate of polyfluoroalkyl cationic surfactants used in aqueous AFFF formulations, the biotransformation of perfluorooctaneamido quaternary ammonium salt (PFOAAmS) was characterized by a DT50 value (time necessary to consume half of the initial mass) of 142 days and significant generation of perfluoroalkyl carboxylic acid (PFOA) at a yield of 30% (mol) by day 180. Three novel biotransformation intermediates were identified for PFOAAmS, and it was demonstrated that despite overall high stability of QACs and their biocide nature, the ones with perfluoroalkyl chains can be substantially biotransformed into perfluoroalkyl acids in aerobic soil.

The above microbial biotransformation and degradation pathways are all dependent on environmental conditions, degradation kinetics, and the chemical structures and properties of the individual polyfluoroalkyl precursors (Buck et al. 2011; Butt et al. 2014; Liu and Mejia Avendaño 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 Avendaño 2013). It is evident through these studies that the biotransformation and biodegradability of polyfluoroalkyl precursor substances is due to the breakdown of the non-fluorinated functionality of the precursor substances, which precedes the breakdown of the perfluorinated carbons. In contrast, perfluoroalkyl chemicals in general resist biotransformation and defluorination under natural conditions. Using ^{14}C-labeled PFOA to examine five different microbial communities, a range of electron donors for reductive defluorination processes, and the possibility of co-metabolism during reductive dechlorination of

trichloroethene, Liou et al. (2010) was able to confirm that PFOA is highly resistant to microbial degradation in natural environments.

2.3.4 Non-microbial biodegradation of other polyfluoroalkyl substances

Butt et al. (2014) reviewed the current state of knowledge regarding the biotransformation of fluorotelomer-based, polyfluoroalkyl precursor substances that degrade to form PFCAs (PFOA) in microbial systems, rats, mice, and fish. Consistent with information presented above, the majority of biotransformation studies thus far used 8:2 FTOH (a fluorotelomer alcohol) as the substrate; only a few studies of non-FTOH biotransformation exist. The biotransformation studies of 8:2 FTOH metabolism universally show the formation of PFOA. As above, the overall yield of PFOA is low, presumably because of the multiple branches in the biotransformation pathways, including conjugation reactions in animal systems which are capable of phase II metabolism and results in the formation of conjugated metabolites such as glucuronide, sulfate, and glutathione metabolites. Butt et al. (2014) also showed that fluorotelomer carboxylates (FTCAs) appear to be more stable in animal models, whereas they are relatively labile in microbial systems. In contrast, the unsaturated form of FTCAs – FTUCAs appear to be readily degraded in animal models.

2.4 Environmental Monitoring of PFOA in Abiotic Media

PFOA 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 media that PFOA is found (Lau et al. 2007). Occurrence and detection of PFOA in other aquatic abiotic media found in the aquatic environment are summarized below.

2.4.1 PFOA Occurrence and Detection in Ambient Surface Waters

PFOA is one of the dominant PFAS detected in ambient surface waters, along with PFOS (Ahrens 2011; Benskin et al. 2012; Dinglasan-Panlilio et al. 2014; Nakayama et al. 2007; Remucal 2019; Zareitalabad et al. 2013). Most of the current, published PFOA occurrence studies have focused on a handful of broad geographic regions, many times targeting sites with known manufacturing or industrial uses of PFAS, such as the Great Lakes, the Cape Fear River and waterbodies near Decatur, Alabama (Figure 2-2; Boulanger et al. 2004; Cochran 2015; Hansen et al. 2002; Konwick et al. 2008; Nakayama et al. 2007; 3M Company 2001).
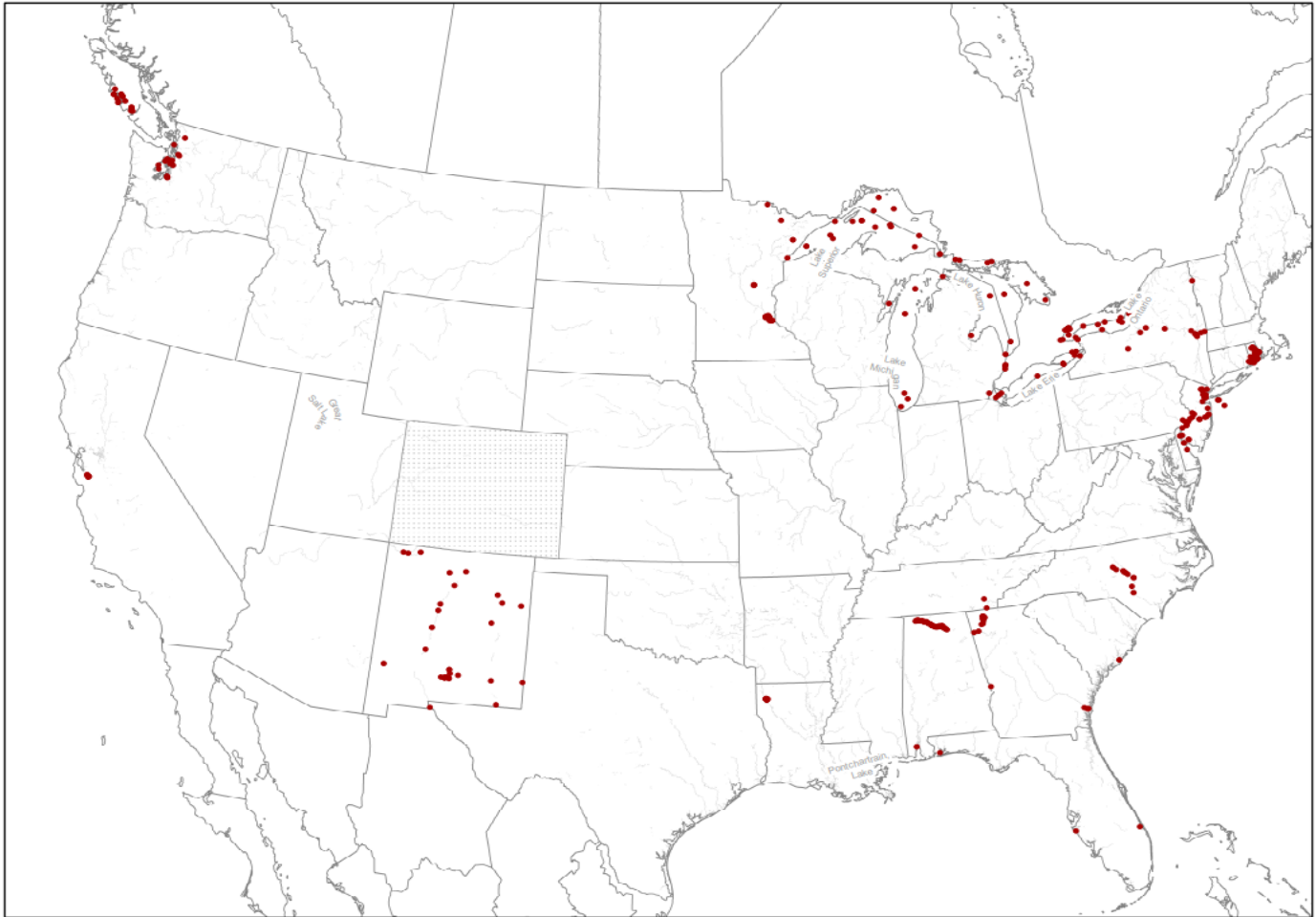
Figure 2-2. Map Indicating Sampling Locations for Perfluorooctanoic acid (PFOA) Measured in Surface Waters Across the United States (U.S.) Based on Data Reported in the Publicly Available Literature.
Colorado sampling coordinates were not available, these data are represented by the dash marks to indicate measured PFOA surface water concentrations are available in Colorado.

Concentrations of PFOA in surface waters vary widely (Figure 2-3), with observed concentrations ranging over seven orders of magnitude and detected generally between pg and ng per liter with some sites with reported concentrations in µg/L (Zareitalabad et al. 2013). For the purposes of this overview and comparison, all concentrations reported here are in ng per liter (ng/L). Unlike other contaminants commonly found in aquatic ecosystems, PFAS are synthetic compounds and therefore have no natural source. Thus, the occurrence of any PFAS in the environment is an indication of anthropogenic sources, including consumer and industrial use,

long-range transport, atmospheric deposition, surface water runoff, and general persistence in the environment.
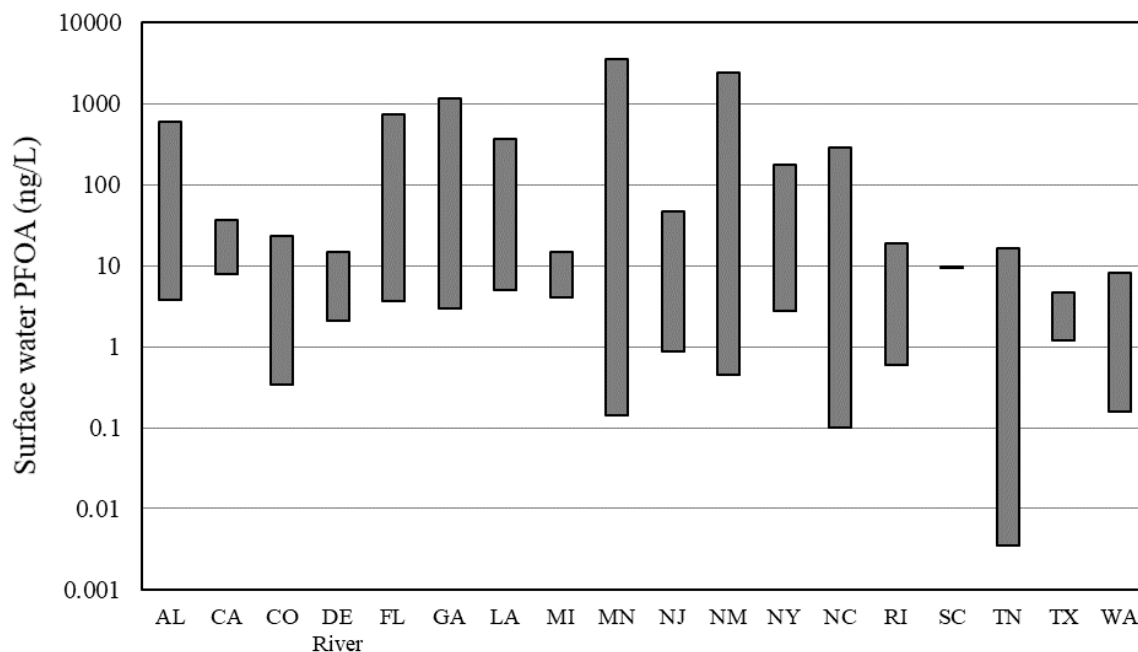
Figure 2-3. Distribution of the minimum and maximum concentrations (ng/L) of Perfluorooctanoic acid (PFOA) measured in surface waters for each state or waterbody (excluding the Great Lakes) with reported data in the publicly available literature. The distribution is arranged alphabetically by state and waterbody.

PFOA concentrations in surface water tend to increase with levels of urbanization. Across the Great Lakes region, PFOA was higher in the downstream lakes of Erie and Ontario and lower in the upstream lakes of Superior, Michigan, and Huron (Remucal 2019). Similarly, Zhang et al. (2016) observed measured PFOA concentrations in urban areas (urban average PFOA concentration = 10.17 ng/L; n = 20) to be more than three time greater than concentrations in rural areas (rural average PFOA concentration = 2.95 ng/L; n = 17) within New Jersey, New York, and Rhode Island. Temporal variation of PFOA in surface waters remains largely unknown due to data limitations. See Appendix M for further discussion of PFOA occurrence in surface waters and other abiotic media such as aquatic sediments, groundwater, air, and ice.

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2.5 Bioaccumulation and Biomagnification of PFOA in Aquatic Ecosystems

PFOA is 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, which facilitates their long-range transport to aquatic ecosystems in the remote arctic and mid-oceanic islands (Haukås et al. 2007; Houde et al. 2006a). Several physical-chemical properties of PFAS contribute to their bioaccumulation within aquatic and aquatic-dependent species once they have entered an aquatic ecosystem.

2.5.1 PFOA Bioaccumulation in Aquatic Life

In contrast to many persistent organic pollutants which tend to partition to fats, PFOA preferentially binds to proteins (Martin et al. 2003a, 2003b). Within the body, PFOA 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, 2003b). PFOA may also bind to ovalbumin, and the transfer of PFOA to such albumin in eggs can be an important mechanism for depuration in female oviparous species, as well as a mechanism for maternal transfer (Jones et al. 2003; Kannan et al. 2005).

The stability of PFOA contributes to its bioaccumulation potential, as PFOA has not been found to undergo biotransformation within the organism and is primarily depurated through excretion in urine or across gill surfaces (De Silva et al. 2009; Martin et al. 2003b). Within an organism, PFOA may undergo enterohepatic recirculation, in which PFOA is excreted from the liver in bile to the small intestine, then reabsorbed and transported back to the liver (Goecke-Flora and Reo 1996). Among PFAS, this process becomes increasingly more efficient the longer the perfluorinated chain length, resulting in longer half-lives for chemicals like PFOA with a relatively long chain length, as they are less readily excreted. PFAS with carboxylate head

groups, such as PFOA, are less efficiently resorbed by the small intestine and transported back to the liver than sulfonate PFAS, resulting in lower bioaccumulation levels (Hassell et al. 2020; Martin et al. 2003a).

Sex differences in the elimination rate of PFOA chemicals have been observed in some species. Lee and Schultz (2010) observed that the elimination rate of PFOA from blood plasma was ten times faster in female fathead minnows compared to males. The faster elimination rate may be related to sex hormones (i.e., androgen and estrogen) levels, as the elimination rate in females decreased four-fold following exposure to the androgen trenbolone (Lee and Schultz 2010). This pattern has also been observed in rats, where the elimination of PFOA was 70 times faster in females than males and was attributed to sex-related differences in the expression of organic anion transporters in kidneys resulting in higher excretion rates (Kudo et al. 2002). The mechanism for the higher elimination rate in female fathead minnows has not been determined, and the degree to which gender-related differences in elimination rate apply to other fish species, or other taxonomic groups, is unknown. However, it does suggest that the sex of the organism should be considered when assessing ecosystem level bioaccumulation, and that there may be another mechanism in addition to egg production that can result in lower concentration of PFAS in females.

The structure of PFOA also contributes to its bioaccumulation potential, with linear forms being more bioaccumulative than branched forms (De Silva et al. 2009; Hassell et al. 2020). The preferential accumulation of linear PFOA occurs because the elimination rate, of branched isomers of PFOA is higher, particularly across gill surfaces (De Silva et al. 2009). 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 Potential for PFOA Bioaccumulation and Biomagnification in Aquatic Ecosystems

PFOA 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 carboxylate head groups (Higgins and Luthy

2006). In a series of laboratory studies, Higgins and Luthy (2006) demonstrated that PFOA

sorption to sediments increased with increasing organic content, increasing calcium ions, and

decreasing pH. The strongest effect was observed in response to increasing organic content,

demonstrating the importance of hydrophobic effects, while the increased sorption in response to

calcium ions and decreasing pH demonstrated the role of electrostatic effects (Higgins and Luthy

2006). Across all PFAS, sorption to sediments increased with increasing perfluorinated chain

length, and for a given chain length, PFAS such as PFOS, had approximately 1.7 times the

sorption capacity as perfluoroalkyl carboxylic acids (PFCA) such as PFOA (Higgins and Luthy

2006). The capacity of PFOA to bind to particulate matter increases with increasing salinity.

Jeon et al. (2010) observed that water column PFOA partitioned more readily to particulate

organic matter as salinity increased from 10 to 34 ppt, resulting in increased uptake of PFOA in

Pacific oysters (*Crassostrea gigas*). In a recent review, Li et al. (2018a) found no single

parameter strongly predicted PFOA sorption to sediments and Li et al. (2019) reported that the

protein content of soil was a better predictor of sorption than organic carbon. Overall, these

results suggest that sorption to sediments should be an important mechanism for PFOA entry into

an aquatic ecosystem.

Evidence of the PFOA sediment pathway in aquatic ecosystems, although mixed, overall demonstrates the importance of bioaccumulation from sediments and biofilms via diet into aquatic invertebrates. In laboratory studies, PFOA concentrations in sediment were positively correlated to PFOA tissue concentrations in *Lumbriculus variegatus* (Lasier et al. 2011), but not for *Chironomus plumosus* (Wen et al. 2016) or the amphipods *Gammarus fossarum* and *G. pulex* (Bertin et al. 2016). In field studies PFOA concentrations were positively correlated between sediments and biofilms and benthic feeding organisms (Lescord et al. 2015; Loi et al. 2011; Martin et al. 2004; Penland et al. 2020). In addition, the distribution of PFAS in sediments was more similar to their distribution in the tissues of benthic invertebrates (Lescord et al. 2015) and benthic-feeding fish (Thompson et al. 2011) than they were to their distribution in pelagic organisms.

PFOA can also enter aquatic organisms directly from the water column through respiration. Because of its binding affinity to proteins, PFOA can enter the body of gill-breathing organisms by binding to proteins in the blood at gill surfaces (De Silva et al. 2009; Jones et al. 2003; Martin et al. 2003a, 2003b).

The relative distribution of PFOA in tissues is related to the primary route of exposure (dietary or respiratory). In rainbow trout, the rank order of PFOA 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 PFAS 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.

Data from multiple field studies suggest trophic biomagnification potential of PFOA is low, and is often not observed, particularly with respect to aquatic organisms. In a review of PFOA and PFOS concentration data across major taxonomic groups, Ahrens and Bundschuh (2014) found that maximum PFOA and PFOS concentrations were similar for invertebrates, but that maximum PFOS concentrations in fish were nearly an order of magnitude greater than PFOA, and several orders of magnitude greater for aquatic-dependent birds and mammals.

When individual aquatic species pairs were considered, biomagnification factors (BMF) greater than one, indicating biomagnification, have been observed for PFOA (e.g., Fang et al. 2014; Penland et al. 2020; Tomy et al. 2009), suggesting trophic biomagnification. However, when ecosystem-level biomagnification is assessed using trophic biomagnification factors (TMF), which measures the change in the concentration of a chemical per trophic level within a food web, PFOA is nearly always shown not to biomagnify (Loi et al. 2011; Martin et al. 2004; Tomy et al. 2004; Xu et al. 2014; Zhou et al. 2012), unless aquatic-dependent species, such as aquatic-dependent birds, are included in the food web model (Houde et al. 2006a; Kelly et al. 2009; Tomy et al. 2009). The overall lack of biomagnification in PFOA relative to PFOS is attributed to its physical-chemical properties, including a shorter perfluorinated chain length and the carboxylate head group, both of which are associated with less efficient assimilation into tissues and faster excretion rates (e.g., Martin et al. 2003a, 2003b).

2.5.3 Environmental Monitoring of PFOA in Biotic Media

Generally, PFOA is one of the dominant PFAS detected in aquatic ecosystems, along with PFOS (Ahrens 2011; Benskin et al. 2012; Dinglasan-Panlilio et al. 2014; Nakayama et al. 2007; Remucal 2019; Zareitalabad et al. 2013). PFAS were first detected in human serum samples in the late 1960s, and subsequent studies across several continents demonstrated the global distribution of PFAS in humans (Giesy and Kannan 2001; Houde et al. 2006b). Since

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then, the global distribution of PFAS in tissues of aquatic and aquatic-dependent species has been demonstrated in studies conducted in freshwater and marine environments across every continent, including remote regions far from direct sources, such as the high arctic, Antarctica, and oceanic islands (Giesy and Kannan 2001; Houde et al. 2006b).

In lentic surface waters of the United States, one of the most comprehensive studies of PFOA 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, PFOA was measured in fish collected at 12% of the sites, with a 90th centile concentration of 0.16 ng/g wet weight (ww), and a maximum concentration of 0.97 ng/g ww (Stahl et al. 2014). Lake trout (31% of samples), smallmouth bass (14%), and walleye (13%) were the most commonly sampled species from the Great Lakes samples.

Martin et al. (2004) measured PFOA in whole body samples of invertebrates and fish in Lake Ontario, near the town of Niagara-on-the-Lake. PFOA concentrations were much higher in the benthic amphipod *Diporeia hoya* (90 ng/g ww) than in the more pelagic *Mysis relicta* (2.5 ng/g ww), suggesting sediments are an important source of PFOA in this area (Martin et al. 2004). Among the four fish species sampled, PFOA concentrations were highest in the slimy sculpin (44 ng/g ww), which feeds on *M. relicta* and *D. hoya*. Although lake trout occupy the highest trophic level at this site, their PFOA concentrations were the lowest of all sampled fish species (1.0 ng/g ww) (Martin et al. 2004). PFOA concentrations were lower in lake trout than in alewife (1.6 ng/g ww), which comprise 90% of the lake trout diet, suggesting a lack of PFOA biomagnification in this system (Martin et al. 2004).

Guo et al. (2012) measured PFOA in lake trout muscle tissues in Canadian waters of Lakes Ontario, Erie, Huron, and Superior, as well as Lake Nipigon, Ontario. The average PFOA concentration across all sites was 0.045 ng/g ww and was not significantly different (P<0.1) across the different lakes (Guo et al. 2012). Finally, Delinsky et al. (2010) sampled bluegill, black crappie, and pumpkinseed muscle tissues in 59 lakes in Minnesota, including four lakes in the Minneapolis-St. Paul metropolitan area, and did not detect PFOA in any of the samples (limit of quantification = 0.77 ng/g ww; see Table 2 of Delinsky et al. 2009).

In flowing surface waters of the United States, one of the most comprehensive studies of PFOA concentrations included fish muscle tissue data from 164 urban river sites (5th order or higher) across the coterminous 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). Largemouth bass (34% of samples), smallmouth bass (25%), and channel catfish (11%) were the most commonly sampled species from the urban stream sites (Stahl et al. 2014). PFOA was not detected in any of the urban river sites (Stahl et al. 2014). The lack of detection may have been related to the method detection limit of 2.37 ng/g ww, which was higher than the highest PFOA concentration measured in the Great Lakes coastal survey described above, which also followed a probabilistic sampling design (Stahl et al. 2014).

In 2005, Ye et al. (2008) detected average PFOA concentrations of 0.17 ng/g ww and 0.2 ng/g ww from whole body composite samples of multiple fish species from the Ohio River and Mississippi River, respectively. PFOA was not detected (<1.0 ng/g ww) in whole body composite fish samples collected from the Missouri River (Ye et al. 2008). Delinsky et al. (2010) sampled PFOA in bluegill, black crappie, and pumpkinseed muscle tissues at eleven locations

along the upper Mississippi River in 2007, and did not detect it at any location, including the heavily impacted Pool Two site in the Minneapolis-St. Paul metropolitan area.

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. PFOA was detected in whole body tissues of unionid mussels (7.41 ng/g ww) and aquatic insects (10.68 ng/g ww), but was not detected in Asian clam, snails, or crayfish. PFOA was measured in muscle tissue of two of the 11 sampled fish species, the channel catfish (21.19 ng/g ww) and notchlip redhorse (45.66 ng/g ww). PFOA was not detected in the eggs of a robust redhorse sample, which had the highest measured PFOS concentration (482.9 ng/g ww) of any sample from the Penland et al. (2020) study.

Houde et al. (2006a) measured whole body PFOA in six fish species in Charleston Harbor, South Carolina, and whole body PFOA of zooplankton and five fish species in Sarasota Bay, Florida. Charleston Harbor was the more developed of the two sites and had higher overall PFOA concentrations. PFOA was detected in four of the six fish species in Charleston Harbor and ranged from 0.5 ng/g ww in spot to 1.8 ng/g ww in spotted seatrout. In Sarasota Bay, PFOA concentrations averaged 0.3 ng/g ww in zooplankton and was not detected in any of the fish species (Houde et al. 2006a).

Overall, these results illustrate the distribution of PFOA in biotic media collected from invertebrate and fish samples. In contrast to PFOS, PFOA concentrations in biotic media are often low, or below detection levels, highlighting the lower overall bioaccumulation potential for this chemical, based on its physical-chemical properties, including a shorter perfluorinated chain length, and a carboxylate head group. In addition, trophic biomagnification is rarely observed with PFOA, as concentrations in invertebrates are often similar to concentrations in fish.

2.6 Exposure Pathways of PFOA in Aquatic Environments

There are multiple potential exposure pathways of PFOA in the aquatic environment, including: (1) direct aqueous (dermal and respiratory) exposure, (2) direct exposure from contaminated sediment (for benthic organisms), (3) diet (e.g., bioaccumulation and biomagnification), and (4) maternal transfer (Ankley et al. 2020). Exposure of PFOA 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). Elevated PFOA concentrations in eggs of fish and piscivorous birds suggests that PFOA may maternally transfer to offspring. Given these exposure pathways, aquatic organisms, such as fish and aquatic invertebrates, are exposed to PFOA when it is present in the environment. This exposure occurs through multiple exposure routes including water, sediment, diet, and maternal transfer.

2.7 Effects of PFOA on Biota

Currently, PFOA aquatic ecotoxicity data are primarily available for freshwater fish, aquatic invertebrates, plants, and algae. Section 3 and Section 4 provide study summaries of individual publicly available ecotoxicity studies, Appendix A through Appendix F summarize the current quantitatively acceptable PFOA aquatic life ecotoxicity data, and Appendix G and Appendix H list current qualitatively acceptable PFOA aquatic life ecotoxicity data.

2.7.1 Mechanisms of PFOA Toxicity

The mechanisms underpinning the toxicity of PFOA to aquatic organisms, like other PFAS, is an active and on-going area of research. Much work is still needed from a mechanistic perspective to better understand how the different modes of action elicit specific biological responses. Molecular disturbance at the cellular- and organ-level resulting in effects on reproduction, growth and development at the individual-level are associated with the sex-related

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endocrine system, thyroid-related endocrine system, and neuronal-, lipid-, and carbohydrate-metabolic systems (see Ankley et al. 2020 and Lee et al. 2020 for the latest reviews on the subject). The underlying mechanisms of PFOA toxicity to aquatic animals, and fish in particular, appear to be related to oxidative stress, apoptosis, thyroid disruption, and development-related gene expression (Lee et al. 2020). The published research suggests that many of these molecular pathways interact with each other and could be linked. For example, for several PFAS including PFOA, oxidative stress appears 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 PFAS induce oxidative stress require additional study, but increased ß-oxidation of fatty acids and mitochondrial toxicity are proposed triggers (Ankley et al. 2020).

Of particular importance to this document is that PFOA exposure-related disruption of the sex-related endocrine system (e.g., androgen and estrogen) at the molecular, tissue and organ levels appears to have adverse reproductive outcomes in fish and invertebrates, and likely in both freshwater and saltwater and via multiple exposure routes, i.e., waterborne and dietary (Lee at al. 2020). The reproductive effects were observed in the F_0, F_1 and F_2 generations of zebrafish, *Danio rerio*, in the multi-generational PFOA exposure reported by Lee et al. (2017).

It is clear that PFOA, and many other PFAS, cause a wide range of adverse effects in aquatic organisms, including: reproductive failure, developmental toxicity; androgen, estrogen and thyroid hormone disruption; immune system disruption; and, neuronal and developmental damage. Study of the systematic interactions among the relevant biological pathways in fish is a research need, as well as a better understanding of several knowledge gaps in non-fish aquatic organisms where mechanistic-based investigations need to be prioritized.

2.7.2   Potential Interactions with Other PFAS

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

Findings of the PFAS-specific studies described below are as reported by the study authors without any additional interpretation or analysis of uncertainty. At both the organismal and cellular levels, studies on zebrafish (*Danio rerio*; Ding et al. 2013), a water flea (*Daphnia magna*; Yang et al. 2019), a bioluminescent cyanobacterium (*Anabaena sp.*; Rodea-Palomares et al. 2012), or with cultured hepatocytes of the cyprinid, *Gobiocypris rarus* (Wei et al. 2009), demonstrate that the effects observed from *in vivo* and *in vitro* tests on PFAS mixtures vary. PFAS mixture studies on zebrafish reported interactions for combinations of PFOA and PFOS, but departures from additive models were also minor (Ding et al. 2013). Menger et al. (2020)

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reported zebrafish behavioral effects from a PFAS mixture that were less than individual PFAS, however evaluation of chemical dose response and comparison to mixture models was not conducted. Yang et al. (2019) exposed the water flea, *Daphnia magna*, to single and binary mixtures of PFOA and PFOS. The authors reported synergism in acute and chronic toxic effects. Conversely, Rodea-Palomares et al. (2012) showed binary PFOA and PFOS mixture as having an antagonistic interaction at the whole range of effect levels tested using the bioluminescent cyanobacterium, *Anabaena.*

In tests with cultured hepatocytes of the cyprinid *G. rarus*, co-exposure of PFOA with a mixture of five other PFAS [PFNA, PFDA, PFDoA, PFOS, 8:2 FTOH] altered genes involved in multiple biological functions and processes, including fatty acid metabolism and transport, xenobiotic metabolism, immune response, and oxidative stress. Additionally, greater than 80% of the altered genes in both the PFOA- and PFOS-dominant mixture groups were of the same gene set (Wei et al. 2009). Finally, Conley et. al. (2022) observed PFOA and PFOS interacting in an additive manner to reduce pup body weight, pup liver weight, and maternal liver weight in the Sprague-Dawley rat.

2.8   Conceptual Model of PFOA 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 2-4 summarizes sources, potential pathways of PFOA exposure for aquatic life and aquatic-dependent wildlife and possible toxicological effects.

PFOA initially enters the aquatic environment through direct discharge from wastewater treatment facilities, atmospheric deposition, and runoff from contaminant surfaces such as PFAS disposal sites or contaminated biosolids. PFOA enters the aquatic environment primarily in the

dissolved form and to a lesser extent, particle-bound forms. Exposure pathways for the biological

receptors of concern (i.e., aquatic organisms) and potential effects (e.g., impaired survival,

growth, and reproduction) in those receptors are represented in the conceptual model (Figure

2-4). Both direct (i.e., exposure from the water column which is represented by *) and indirect

(i.e., bioconcentrated by producers and bioaccumulated by consumers in higher trophic levels

represented by **) pathways are represented in the conceptual model.

Cycling

PFOA Source
Point Sources
(from municipal and industrial dischargers from applications such as surfactants, textile stain and soil repellents)

PFOA in Water
Dissolved & Particle-Bound

PFOA Source
Nonpoint Sources
(from landfill leachate, land application of biosolids)

Settling/Resuspension

Absorption/Desorption

Degradation & Metabolism of Other PFASs

PFOA in Sediment

*

**

Receptors

Aquatic Life

Producers
1st Trophic Transfer
(from phytoplankton, periphyton, macrophytes; e.g., algae, cyanobacteria, waterweed/common eelgrass)

Consumers
2nd Trophic Transfer
(to zooplankton, macroinvertebrates; e.g., cladocerans/copepods & mayflies/ribbed mussels)

Consumers
3rd Trophic Transfer
(to predatory fish; e.g., longnose dace/ American shad)

Consumers
4th Trophic Transfer
(to predatory fish; e.g., largemouth bass/ striped bass)

Deformities, Reproductive and Growth Impairments, Mortality

Population Decline

Loss of species & community Structure/Function Change

Figure 2-4. Conceptual Model Diagram of Sources, Compartmental Partitioning, and Trophic Transfer Pathways of Perfluorooctanoic acid (PFOA) in the Aquatic Environment and its Bioaccumulation and Effects in Aquatic Life and Aquatic-dependent Wildlife.
PFOA sources represented in ovals, compartments within the aquatic ecosystem represented by rectangles, and effects (on trophic levels of aquatic-dependent wildlife, represented by shaded box) in pentagons. Examples of organisms in each trophic transfer provided as freshwater/marine. Movement of PFOA from water to receptors indicated by two separate pathways: direct exposure to all trophic levels within box (*) and bioconcentration by producers (**). Relative proportion of PFOA transferred between each trophic level is dependent on life history characteristics of each organism.

2.9 Assessment Endpoints

Assessment endpoints are defined as the explicit expressions of the environmental values to be protected and are comprised of both the ecological entity (e.g., a species, community, or other entity) and the attributes or characteristics of the entity to be protected (U.S. EPA 1998). Assessment endpoints may be identified at any level of organization (e.g., individual, population, community). In context of the CWA, aquatic life criteria for toxic substances are typically determined based on the results of toxicity tests with aquatic organisms, for which adverse effects on growth, reproduction, or survival are measured. 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 5th percentile of genera and are, thus intended to be protective of approximately 95 percent of aquatic genera to ensure aquatic communities are protected. Assessment endpoints consistent with the criteria developed in this document are summarized in Table 2-1.

The use of laboratory toxicity tests to protect bodies of water and resident aquatic species was based on the theory 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 diverse, the 1985 Guidelines recommend that acceptable data be available for at least eight genera with a specified taxonomic diversity (the standard eight minimum data requirements, or MDRs). The intent of the eight MDRs is to serve as a typical 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).

2.10 Measurement Endpoints

2.10.1 Overview of Toxicity Data Requirements

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

- Acute freshwater criteria require data from the following eight taxonomic groups:
  a) the family Salmonidae in the class Osteichthyes
  b) a second family 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 criteria 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 components of the aquatic ecosystem from long term exposures, chronic toxicity test data are recommended for the same minimum of eight diverse taxonomic groups that are recommended for freshwater acute criterion derivation. If the eight diverse taxonomic groups are not available to support chronic criterion derivation using a genus distribution approach, the chronic criterion may be derived using an acute-to-

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chronic ratio (ACR) approach. To apply an ACR approach to derive a chronic freshwater criterion a minimum of three taxa are recommended, with at least one chronic test being from an acutely sensitive species. Acute-to-chronic ratios (ACRs) can be calculated with data for aquatic organisms.

- Chronic aquatic life criteria require data from the following taxonomic groups:
  a) At least one is a fish
  b) At least one is an invertebrate
  c) At least one is an acutely sensitive freshwater species, for freshwater chronic criterion (the other two may be saltwater species)
  d) At least one is 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 are examined to determine whether aquatic plants are likely to be adversely affected by the concentration expected to be protective for other aquatic organisms.

2.10.2 Measure of PFOA Exposure Concentrations

These PFOA ambient water quality criteria are for the protection of aquatic life. This criteria document provides a critical review of all data identified in the EPA's literature search for PFOA, including:

- the anionic form (CAS No. 45285-51-6),
- the acid form (CAS No. 335-67-1), and;
- the ammonium salt (CAS No. 3825-26-1).

PFOA toxicity studies typically do not conduct an analysis or separation sufficient to determine if the test compound is purely linear or branched. Data for possible inclusion in the PFOA criteria were obtained from published literature reporting acute and chronic exposures of PFOA that were associated with mortality, growth, and reproduction. 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:

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 were subsequently

evaluated by the Office of Water. All studies were evaluated for data quality as described by

U.S. EPA (1985), the EPA's Office of Chemical Safety and Pollution Prevention (OPP)'s

Ecological Effects Test Guidelines (U.S. EPA 2016b), and the EPA OW's internal data quality standard operating procedure (SOP), which is consistent with OPP's data quality review approach (U.S. EPA 2016b). Office of Water completed a Data Evaluation Record (DER) for each species by chemical combination from the PFOA 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 P with the intent to convey the meticulous level of evaluation, review, and documentation each PFOA study identified by ECOTOX was subject to.

Studies that did not fully meet the data quality objectives outlined by the EPA SOP were not considered for inclusion in the criteria derivation, including some studies with other PFAS exposures, but were considered qualitatively as supporting information and are characterized in the Effects Characterization. These studies are listed in Appendix G and Appendix H. Furthermore, only single chemical toxicity tests with PFOA were considered for possible inclusion in criteria derivation, studies that tested chemical mixtures, including mixtures with PFAS were excluded from criteria derivation. Both controlled laboratory experiments and field observations/studies were included.

The 1985 Guidelines recommend only toxicity tests focused on North American resident species be considered. Due to the EPA's interest in using all available quality data, particularly for a data-sparse chemical like PFOA (relative to chemicals such as cadmium or ammonia), toxicity studies were considered for possible inclusion regardless of the test species residential status in North America. Use of non-North American residential species is also consistent with other published aquatic life criteria (U.S. EPA 2018). Non-North American resident species also 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 4.1). These analyses provided an additional line-of-evidence that supports inclusion of non-resident species in PFOA criteria derivation.

Toxicity tests used in many previous aquatic life criteria documents are typically based on measured chemical concentrations only. For PFOA, the EPA has examined the issue of whether nominal (unmeasured) and measured concentrations are in close agreement with each other (Jarvis et al. 2023). While measured PFOA toxicity tests are generally preferred, results of Jarvis et al. (2023) demonstrated that experimental conditions had little influence on observed discrepancies between nominal and measured concentrations for PFOA, with the exception of freshwater studies that contained substrate. Nominal and measured concentrations in the analysis generally displayed a high degree of linear correlation (>0.98 freshwater, >0.84 saltwater) and relatively low median percent differences (Jarvis et al. 2023). In freshwater tests, when tests with substrate were removed, 89% of the 527 PFOA and PFOS measured concentrations were within 20% of their nominal counterparts (Note: the EPA's OCSPP's Ecological Effects Test Guidelines (2016b) consider tests acceptable when measured concentrations are within 20% of nominal, and Rewerts et al. (2021) suggested that PFAS-specific toxicity tests may even be acceptable if measured and nominal concentrations do not differ by up to 30%.). Of the observed disparities between measured and nominal concentrations in the PFOA freshwater data sets, those with substrate (McCarthy et al. 2021; Oakes et al. 2004) were concluded to be the primary contributor to observed differences with measured PFOA concentrations systematically lower than corresponding nominal concentrations, indicating that added PFOA may have sorbed to

substrate, reducing PFOA in the water column (Jarvis et al. 2023). PFOA concentrations in test waters are expected to remain relatively constant over the course of acute and chronic exposures given its ability to resist breakdown and transformation (Ahrens 2011). Since toxicity tests with substrate and nominal concentrations only were not used quantitatively in PFOA criteria derivation, the EPA determined nominal test concentrations adequately represent actual PFOA exposures in standard acute and chronic laboratory-based toxicity tests. Consequently, PFOA toxicity tests were not excluded from quantitative use in criteria derivation on the basis of unmeasured test concentrations alone.

Typically, per the 1985 Guidelines acute toxicity data from all measured flow-through tests would be used to calculate species mean acute values (SMAVs), 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 the PFOA, static, renewal, and flow-through experiments were considered for possible inclusion for both species mean acute and chronic values regardless of whether PFOA concentrations were measured because PFOA is a highly stable compound, resistant to hydrolysis, photolysis, volatilization, and biodegradation (Section 1.2.1) and, therefore, expected to vary only minimally in the course of a toxicity test.

Additionally, chronic values were based on endpoints and exposure durations that were appropriate to the species. Thus, both life- and partial life-cycle tests were utilized for the derivation of the chronic criterion. However, it should be noted that the 1985 Guidelines specify life-cycle chronic tests are typically used for invertebrates. The chronic studies used in the

derivation of the chronic water column-based PFOA criterion followed taxon-specific exposure duration requirements from various test guidelines (i.e., the EPA's 1985 Guidelines and the EPA's OCSPP's Ecological Effects Test Guidelines) when available. For example, only chronic daphnid studies of 21 days were considered in the chronic criterion derivation because the EPA 1985 Guidelines states daphnid tests should begin with young < 24-hours old and last at least 21 days. When taxon-specific exposure duration requirements were not available for a particular test organism in the PFOA toxicity literature, both life- and partial life-cycle tests were considered in the derivation of the chronic criterion.

PFOA toxicity in aquatic life is manifested as effects on survival, growth, and reproduction. Measurements of fish tissue may be linked to the chronic adverse effects of PFOA, since PFOA is highly persistent and potentially bioaccumulative.

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 data were 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. LC_{50} is the concentration of a chemical that is estimated to kill 50 percent of the test organisms. EC_{50} is the concentration of a chemical that is estimated to produce a specific effect in 50 percent of the test organisms. And the IC_{50} is the concentration of a chemical that is estimated to inhibit some biological process (e.g., enzyme

inhibition associated with an apical endpoint such as mortality) in 50 percent of the test organisms.

2.10.3.2 Chronic Measures of Effect

The endpoint for chronic exposures is the effect concentration estimated to produce a chronic effect on survival, growth, or reproduction in 10 percent of the test organisms (EC_{10}). The EPA selected an EC_{10} to estimate a low level of effect that would be both different from controls and not expected to be severe enough to cause effects at the population level for a potentially bioaccumulative contaminant, such as PFOA. 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). Use of a 10% effect concentration for deriving chronic criteria magnitudes is also consistent with the harmonized guidelines from OECD and the generally preferred effect level for countries such as Canada, Australia, and New Zealand (CCME 2007; Warne et al. 2018).

Regression analysis was used preferentially to characterize a concentration-effect relationship and to estimate concentrations at which chronic effects are expected to occur (i.e., point estimate). Reported No Observed Effect Concentrations (NOECs) and Lowest Observed Effect Concentrations (LOECs) were only used for the derivation of a 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 were used, a Maximum Acceptable Toxicant Concentration (MATC) was calculated, which is the geometric mean of the NOEC and LOEC. For the calculation of a chronic criterion, 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

provide additional information that is difficult to determine with an MATC, such as a measure of effect level across a range of tested concentrations. A decision rule was also applied to the PFOA toxicity data when an author-reported NOEC or LOEC was used in conformity with the 2013 Ammonia Freshwater Aquatic Life Criteria (U.S. EPA 2013) such that "greater than" values for concentrations of a relatively low magnitude compared to the other available toxicity data, and "less than" values for concentrations of relatively high magnitude were considered to add little significant information to the analyses and were not used quantitatively. Conversely, if data from studies with relatively low "less than" values indicated a significant effect or studies with relatively high "greater than" values only found an incomplete response for a chronic endpoint (indicating low toxicity of the test material), those data significantly enhanced the understanding of PFOA toxicity. Thus, the decision rule was applied as follows: "greater than" (>) high toxicity values and "less than" (<) low toxicity values were used quantitatively to derive the chronic water column-based PFOA criterion (U.S. EPA 2013). Data that met the quality objectives and test requirements were utilized quantitatively in deriving freshwater 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 PFOA.

Assessment Endpoints for the Aquatic Community	Measures of Effect
Aquatic Life: Survival, growth, and reproduction of freshwater and estuarine/marine aquatic life (i.e., fish, amphibians, aquatic invertebrates)	For effects from acute exposure: 1. LC_{50}, EC_{50}, or IC_{50} concentrations in water For effects from chronic exposure: 1. EC_{10} concentrations in water 2. NOEC and LOEC concentrations in water; Only used when an EC_{10} could not be calculated for a genus.

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

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 when adequate concentrations-response data were published in the study or could be obtained from authors. When concentration-response data were not presented in toxicity studies, concentration-response data were requested from study authors to independently calculate toxicity values. In cases where study authors did not respond to the EPA's request for data or were unable to locate concentration-response data, the toxicity values were not independently calculated by the EPA, and the reported toxicity values were retained for criteria deviation. 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. The R drc package has various 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 PFOA aquatic life criteria were included in each study summary below and were used to derive criteria for aquatic life, where available. Details relating to the independent verification of toxicity values for each toxicity study used to derive the criteria were included in Appendix A.2 and Appendix C.2.

2.11 Analysis Plan

2.11.1 Derivation of Water Column Criteria

During CWA Section 304(a) criteria development, the EPA reviews and considers all relevant toxicity test data. Information available for all relevant species and genera were reviewed to identify: 1) data from acceptable tests that meet data quality standards, and 2)

whether the acceptable data meet the MDRs as outlined in the EPA's 1985 Guidelines (U.S. EPA 1985). The taxa represented by the different MDR groups represent taxa with different ecological, trophic, taxonomic and functional characteristics in aquatic ecosystems, and are intended to be a representative subset of the diversity within a typical aquatic community. MDRs for derivation of acute and chronic freshwater criteria were met for all the eight MDRs.

Acute and chronic MDRs for PFOA estuarine/marine criteria derivation were not met and, consequently, acute and chronic estuarine/marine criteria were not derived. The EPA is, however, including an acute aquatic life benchmark for estuarine/marine environments (see Appendix L), using available estuarine/marine species toxicity data and application of ORD's peer-reviewed web-ICE tool. A minimal number of tests from acceptable studies of aquatic algae and vascular plants were also available for possible derivation of a Final Plant Value. However, the relative sensitivity of freshwater plants to PFOA exposures indicated plants are less sensitive than aquatic vertebrates and invertebrates so plant criteria were not developed.

2.11.2 Derivation of Tissue-Based Criteria

Chronic toxicity studies (both laboratory and field studies) were further screened to ensure they contained the relevant chronic PFOA exposure conditions to aquatic organisms (i.e., dietary, or dietary and waterborne PFOA exposure), measurement of chronic effects, and measurement of PFOA in tissue(s). The EPA considered deriving tissue-based criteria using empirical toxicity tests with studies that exposed organisms to PFOA in water and/or diet and reported exposure concentrations based on measured tissue concentrations. This approach would also correspond with the 2016 Selenium Aquatic Life Freshwater Criterion, which is the only 304(a) aquatic life criterion with tissue-based criterion elements. However, the freshwater chronic PFOA toxicity data with measured tissue concentrations were limited, with no quantitatively acceptable tissue-based tests. Qualitatively acceptable tissue-based tests were

reported for four species (three fish species and one amphibian) across five publications.

Therefore, there were insufficient data to derive tissue-based criteria using a GSD approach from

empirical tissue data from toxicity studies. The EPA thus developed protective tissue-based

criteria through a bioaccumulation factor approach (Burkhard 2021).

2.11.3 Translation of Chronic Water Column Criterion to Tissue Criteria

Because there were insufficient chronic toxicity data with measured tissue concentrations

to derive chronic PFOA tissue criteria using a GSD approach, the EPA derived PFOA chronic

tissue-based criteria by translating the chronic freshwater water column criterion (see Section

3.2.1.3) into tissue-based criteria magnitudes using bioaccumulation factors and the following

equation:

$$Tissue\ Criteria = Chronic\ Water\ Column\ Criterion \times BAF \quad \textit{(Equation 1)}$$

The resulting tissue-based criteria magnitudes correspond to the tissue type from the BAF used

in the equation (see Section 2.11.3.1).

2.11.3.1 Aquatic Life Bioaccumulation Factors

A bioaccumulation factor (BAF) is determined from field measurements and is calculated

using the equation:

$$BAF = \frac{C_{biota}}{C_{water}} \qquad\qquad \textit{(Equation 2)}$$

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

The EPA considered BAF data from field measurements to capture all PFOA exposure

routes, *i.e.*, dietary, water, contact with sediments via dermal exposure and ingestion, and

maternal transfer. Depending on the tissue residue measurement, BAFs can be based upon residues in the whole organism, muscle, liver, or any other tissue.

Searching for literature reporting on PFOA 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), 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 the 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 PFOA 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 for freshwater and estuarine/marine species. BAFs from both freshwater and estuarine/marine species were considered because: (1) inclusion of estuarine/marine BAFs expanded the relatively limited PFOA BAF dataset, and (2) Burkhard (2021) did not specifically observe notable differences in PFAS BAFs between freshwater and estuarine/marine systems, instead stating additional research is needed to formulate conclusions.

Data from papers with appropriate BAF information were further 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. Table 2-2 below outlines the screening criteria for study evaluation and ranking. Only BAFs of high and medium quality were

used to derive the tissue criteria (Appendix O). For further details on BAFs compilation and

ranking, see Burkhard (2021).

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

Screening Factor	High Quality	Medium Quality	Low Quality
Number of Water Samples	>3	2 – 3	1
Number of Organism Samples	>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

Modified from Burkhard (2021).

PFOA bioaccumulation potential in aquatic life is expected to be relatively low compared

to PFOS bioaccumulation, or PFOA bioaccumulation in aquatic-dependent birds and mammals

The high water solubility of PFOA may allow aquatic organisms to excrete PFOA through the

gills. Conversely, aquatic dependent birds and mammals lack such an excretion mechanism, as

the low vapor pressure of PFOA limits its ability to be transferred across the alveolar membrane

from lungs to air (Canada 2012; Kelly et al. 2004). The tissue criteria for fish and invertebrates

presented here protect aquatic life populations from PFOA exposure accumulating in tissues and

provide U.S. states and Tribes greater context to their fish-tissue monitoring programs, which are

actively measuring PFAS, including PFOA.

3   EFFECTS ANALYSIS FOR AQUATIC LIFE

3.1   Toxicity to Aquatic Life

All available studies relating to the acute and chronic toxicological effects of PFOA on aquatic life were considered in the derivation of these national recommended PFOA criteria. Data for possible inclusion in these PFOA criteria were obtained from published literature reporting acute and chronic exposures of PFOA that were associated with mortality, survival, growth, and reproduction. The latest search was conducted through the March 2024 ECOTOX database update. Acute and chronic data meeting the quality objectives and test requirements were utilized quantitatively in deriving these criteria for aquatic life and are presented in Appendix A: Acceptable Freshwater Acute PFOA Toxicity Studies; Appendix B: Acceptable Estuarine/Marine Acute PFOA Toxicity Studies; Appendix C: Acceptable Freshwater Chronic PFOA Toxicity Studies, and; Appendix D: Acceptable Estuarine/Marine Chronic PFOA Toxicity Studies.

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

Quantitatively acceptable acute PFOA toxicity data were available for 27 freshwater species, representing 19 genera and 17 families in five phyla, and four estuarine/marine species, representing four genera and three families in three phyla (Table 3-1). Quantitatively acceptable chronic PFOA toxicity data were available for 13 freshwater species, representing 12 genera and ten families in three phyla and two estuarine/marine species, representing two genera and two families in two phyla. The following study summaries present the key acute and chronic freshwater toxicity data with effect values that were used quantitatively to derive the acute and chronic freshwater and estuarine/marine criteria to protect aquatic life. Study summaries for the most sensitive taxa are presented below and are grouped by acute or chronic exposure and sorted by sensitivity to PFOA.

Acute and chronic values were presented as reported by the study authors for each individual study, unless stated otherwise. The EPA independently calculated these toxicity values if sufficient raw data were available for the EPA to conduct statistical analyses. The EPA's independently-calculated toxicity values were used preferentially, where available. Author-reported toxicity values and the EPA's independently calculated values (where available) were included in each study summary and in appendices, as applicable. The results of all toxicity values, such as LC values, EC values, NOECs, LOECs, and species- and genus-mean values, are given to four significant figures to prevent round-off error in subsequent calculations, not to reflect the precision of the value. The specific toxicity value utilized in the derivation of the corresponding PFOA criteria is stated for each study at the end of the summaries below and in the respective appendices.

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 PFOA.

MDR[a, b]	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	3	3	2	2
Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)	5	10	2	2
Planktonic Crustacean	3	6	3	4
Benthic Crustacean	1	1	1	1
Insect	1	1	1	1
Family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, or Mollusca)	4	4	1	1
Family in any order of insect or any phylum not already represented	1	1	1	1
Total	**19**	**27**	**12**	**13**
MDR	Saltwater[a]			
	GMAV	SMAV	GMCV	SMCV
Family in the phylum Chordata	0	0	1	1
Family in the phylum Chordata	0	0	0	0
Either the Mysidae or Penaeidae family	2	2	0	0
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			0	0
Family in a phylum other than Chordata			0	0
Any other family			0	0
Total	**4**	**4**	**2**	**2**

[a] The 1985 Guidelines require that data from a minimum of eight families are needed to calculate an estuarine/marine criterion. Insufficient data exist to fulfill all eight of the taxonomic MDR groups. Consequently, the EPA cannot derive an estuarine/marine acute criterion, based on the 1985 Guidelines. However, the EPA has developed estuarine/marine benchmarks through use of surrogate data to fill in missing MDRs using the EPA's WebICE tool and other New Approach Methods. These benchmarks are provided in Appendix L.

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

The acute data set for PFOA contains 19 genera representing all eight taxonomic MDR groups (Table 3-3). Quantitatively-acceptable data for acute PFOA toxicity were available for four freshwater fish species, representing four genera and three families and fulfilled two of the eight MDRs. Quantitatively acceptable data for acute PFOA toxicity were also available for 13 freshwater invertebrate species, representing ten genera and nine families, and fulfilled five of the eight MDRs. Quantitatively acceptable acute data were available for 10 freshwater amphibian species, representing five genera and five families fulfilling one of the MDRs. Summaries of studies for the most sensitive acute genera are described below, with the four most sensitive genera provided in Table 3-2.

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

Rank	Genus	GMAV (mg/L)	Species
1	*Moina*	8.885	Cladoceran (*Moina macrocropa*)
			Cladoceran (*Moina micrura*)
2	*Neocloeon*	13.05	Mayfly (*Neocloeon triangulifer*)
3	*Chydorus*	93.17	Cladoceran (*Chydorus sphaericus*)
4	*Daphnia*	142.8	Cladoceran (*Daphnia carinata*)[a]
			Cladoceran (*Daphnia magna*)
			Cladoceran (*Daphnia pulicaria*)

[a] Not a North American resident but in same genus as resident species.

3.1.1.1.1   Most acutely sensitive genus: Moina (cladoceran)

Ji et al. (2008) performed a 48-hour static, unmeasured acute test of PFOA (CAS # 335-67-1, purity unreported) with *Moina macrocopa*. Testing method followed U.S. EPA/600/4-

90/027F (2002). The test involved four replicates of five daphnids (<24 hours old) each in five unmeasured test concentrations (62.5, 125, 250, 500 and 1,000 mg/L) plus a negative control. Dilution water was moderately hard reconstituted water. Survival of daphnids in the negative control was not reported, although EPA/600/4-90/027F requires at least 90% survival for test acceptability. The author-reported 48-hour EC_{50} for the study was 199.51 mg/L. The EPA performed C-R analysis for the test. The EPA-calculated EC_{50} (166.3 mg/L PFOA) was acceptable for quantitative use in deriving the acute freshwater PFOA criterion and served directly as the *Moina macrocopa* Species Mean Acute Value (SMAV).

Razak et al. (2023) tested the acute toxicity of perfluorooctanoic acid (PFOA, ≥98% purity) to *Moina micrura* in a 48-hour static measured experiment. Testing methods followed OECD 202 (OECD 2004) with nominal testing concentrations of 10, 25, 50, 75, 100, 250, 500, 750, 1,000, 2,500, 5,000, 7,500, and 10,000 µg/L, plus a control, with four replicates per treatment. Filtered surface lake water was used as test water. Measured concentrations were not reported, but the authors noted they were 94.3±6.1% of nominal on average. Each replicate consisted of 10 neonates (<48 hours old) in 50 mL of solution in a 100 mL beaker, and organisms were not fed during the study. Lethal effect concentrations (LC) were calculated using Probit analysis, and the 48-hour LC_{50} value of 474.7 µg/L, or 0.4747 mg/L was determined to be acceptable for quantitative use. The 48-hr LC_{50} was acceptable for quantitative use in deriving the acute freshwater PFOA criterion and served directly as the *Moina micrura* SMAV.

Moina macrocopa (SMAV of 166.3 mg/L) is much more tolerant to acute freshwater PFOA exposures than *M. micrura* (SMAV of 0.4747 mg/L) but both species were used to determine the *Moina* Genus Mean Acute Value (GMAV) of 8.885 mg/L. If the EPA were to exclude the *M. micrura* SMAV on the basis of it being an overly sensitive outlier (relative to *M.*

macrocopa and the overall quantitatively acceptable acute data), that would result in the final PFOA acute criterion potentially being underprotective of untested sensitive invertebrate species or other taxa, considering that the available data serve as surrogate information for the thousands of untested freshwater species. Conversely, excluding the *M. macrocopa* SMAV on the basis of it being a tolerant outlier (relative to *M. micrura*) would result in the final PFOA acute criterion being highly influenced by a single test/species with an LC_{50} that was uniquely sensitive (i.e., *M. micrura* SMAV = 0.4747 mg/L) compared to the overall acute data. Averaging the *M. micrura* and *M. moina* SMAVs resulted in a GMAV (8.885 mg/L) that was the most sensitive GMAV, yet still in the general range of the data overall.

3.1.1.1.2   Second most acutely sensitive genus: Neocloeon (mayfly)

Soucek et al. (2023) exposed the parthenogenetic mayfly, *Neocloeon triangulifer*, to PFOA (CAS # 335-67-1, 95% purity) in water in a 96-hour acute toxicity test. The test was performed under static, non-renewal conditions beginning with <24-hour old larvae. Mayflies were fed live diatom biofilm scraping beginning on day 0. Feeding only occurred on day 0, and the authors indicated test organisms required food to survive the entire 96-hour exposure, with previous studies demonstrating greater than 80% mortality at 48 hours with no food (Soucek and Dickinson 2015). Percent survival in the control treatment after 96 hours was 100%. The EPA-calculated acute LC_{50} (i.e., 13.045 mg/L) was similar to the author-reported LC_{50} of 13.451 mg/L. The EPA-calculated LC_{50} value (i.e., 13.045 mg/L) was acceptable for quantitative use in deriving the acute freshwater PFOA criterion and served directly as the *Neocloeon* GMAV.

3.1.1.1.3   Third most acutely sensitive genus: Chydorus (cladoceran)

Le and Peijnenburg (2013) performed a 48-hour static unmeasured acute PFOA toxicity test with the cladoceran, *Chydorus sphaericus*. The authors reported the 48-hour EC_{50} was 0.22 mM PFOA (91.10 mg/L). The EPA performed concentration-response (C-R) analysis for the test

and calculated a LC$_{50}$ of 93.17 mg/L PFOA that is acceptable for quantitative use. No other quantitatively acceptable acute toxicity data were available for *Chydorus sphaericus* or other members of the genus *Chydorus*. Therefore, the LC$_{50}$ (i.e., 93.17 mg/L) from this test served directly as the *Chydorus sphaericus* SMAV and the *Chydorus* GMAV.

3.1.1.1.4 Fourth most acutely sensitive genus: Daphnia (cladoceran)

Logeshwaran et al. (2021) conducted an acute PFOA test with the cladoceran, *Daphnia carinata*, and PFOA (95% purity, purchased from Sigma-Aldrich Australia) following OECD guidelines (2000) with slight modifications. Authors used nominal test concentrations (0, 0.5, 1, 2.5, 5, 10, 20, 30, 40, 50, 100, 150, 200 and 250 mg/L PFOA) with three replicates per treatment. No mortality occurred in the controls. The author-reported 48-hour EC$_{50}$ was 78.2 mg/L PFOA. The EPA-calculated 48-hour EC$_{50}$ value was 66.80 mg/L, which was acceptable for quantitative use. No other quantitatively acceptable acute tests were available for this species and the EC$_{50}$ of 66.80 mg/L from Logeshwaran et al. (2021) served directly as the *Daphnia carinata* SMAV.

Boudreau (2002) performed a 48-hour static PFOA (CAS # 335-67-1, ≥97% purity) acute test with *Daphnia pulicaria*, following ASTM E729-96 (1999). Five unmeasured test concentrations plus a negative control were used with 3-4 replicates per treatment and 10 daphnids per replicate. Nominal concentrations were 0 (negative control), 26.3, 52.6, 105, 210 and 420 mg/L. Mortality of daphnids in the negative control was not reported, but the protocol followed by the authors (i.e., ASTM E729-96) required ≥ 90% survival in negative controls. The 48-hour *D. pulicaria* EC$_{50}$ reported in the publication was 203.7 mg/L, which was acceptable for quantitative use. No other quantitatively acceptable acute tests were available for this species and the EC$_{50}$ of 203.7 mg/L from Boudreau (2002) served directly as the *D. pulicaria* SMAV.

Boudreau (2002) also performed a 48-hour static PFOA (CAS # 335-67-1, ≥97% purity) acute test with *Daphnia magna* following the same methods used in the *D. pulicaria* acute test.

The 48-hour *D. magna* EC$_{50}$ reported in the publication was 223.6 mg/L, which was acceptable for quantitative use.

Colombo et al. (2008) conducted a 48-hour static PFOA (ammonium salt, CAS # 3825-26-1, 99.7% purity) acute test on *Daphnia magna*. Authors stated the test followed OECD test guideline 202 (1992). The nominal test concentrations included control, 100, 178, 316, 562 and 1,000 mg/L, with four replicates/treatment and five animals/replicate. No mortality was observed in the controls, and the 48-hour EC$_{50}$ reported in the study was 480 mg/L, which was acceptable for quantitative use.

Ji et al. (2008) performed a 48-hour static acute test of PFOA (CAS # 335-67-1, purity unreported; obtained from Sigma Aldrich, St. Louis, MO) on *D. magna*. Authors stated the test followed U.S. EPA/600/4-90/027F (2002). The test involved four replicates of five daphnids each in five unmeasured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 62.5, 125, 250, 500 and 1,000 mg/L. Mortality of daphnids in the negative control was not reported, although EPA/600/4-90/027F requires at least 90% survival for test acceptability. The author-reported 48-hour EC$_{50}$ for the study was 476.52 mg/L (95% C.I. = 375.3 – 577.7 mg/L). The EPA performed C-R analysis for the test. The EPA-calculated EC$_{50}$ was 542.5 mg/L PFOA and was acceptable for quantitative use.

Li (2009) conducted a 48-hour static PFOA (ammonium salt, >98% purity) acute test with *Daphnia magna*. Authors stated the test generally followed OECD 202 (1984). The test employed five replicates of six daphnids each in five test concentrations (nominal range = 31 – 250 mg/L) plus a negative control. No control daphnids were immobile at the end of the test. The author-reported 48-hour EC$_{50}$ for the study was 181 mg/L (95% C.I.: 166-198 mg/L) which was averaged across three tests. The EPA performed C-R analysis for each individual test. All three

tests had acceptable curves with the EPA-calculated EC_{50} values of 220.8 mg/L, 157.9 mg/L, and 207.3 mg/L, which were acceptable for quantitative use.

Yang et al. (2014) conducted a 48-hour acute test of PFOA (CAS # 335-67-1, 99% purity) with *Daphnia magna*, following ASTM E729 (1993). The test employed three replicates of 10 daphnids each in six test concentrations plus a negative and solvent control. Nominal concentrations were 0 (negative and solvent controls), 50, 80, 128, 204.8, 327.68 and 524.29 mg/L. Test concentrations were measured in low and high treatments only. Negative control and solvent control mortality were 0% each. The author-reported 48-hour LC_{50} was 201.85 mg/L (95% C.I. = 134.68 – 302.5 mg/L). The EPA performed C-R analysis for the test and fit an acceptable curve with an EPA-calculated LC_{50} of 222.0 mg/L PFOA, which was acceptable for quantitative use.

Barmentlo et al. (2015) performed a 48-hour static, measured acute test of PFOA (CAS # 335-67-1, >96% purity) with *Daphnia magna* following OECD 202 (2004) test guidelines. The test involved four to six replicates of five daphnids each in five test concentrations plus a negative control. Nominal concentrations were not provided, but PFOA was measured in the control, lowest, and highest test concentrations. Based on these measurements, the authors interpolated all test concentrations to be: 0.053 (negative control), 81, 128, 202, 318 and 503 mg/L. The author-reported 48-hour EC_{50} was 239 mg/L (95% C.I. = 190-287 mg/L). The EPA performed C-R analysis for the test and fit an acceptable curve with an EPA-calculated EC_{50} of 215.6 mg/L PFOA, which was acceptable for quantitative use.

Ding et al. (2012a) conducted a 48-hour static, partially measured acute test on PFOA (CAS # 335-67-1; 96% purity from Sigma Aldrich) with *D. magna*. The test generally followed OECD test guideline 202 (2004). Authors employed four replicates of five daphnids each in six

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test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 144.9, 165.6, 186.3, 207.0, 227.7, and 248.4 mg/L. Concentrations of PFOA were confirmed in the highest and lowest concentrations, though only nominal concentrations were reported. It was stated that the verified concentrations were "well in line with nominal concentrations". The 48-hour EC_{50} was reported as 211.6 mg/L (95% C.I. = 184.7 – 255.5 mg/L). The EPA performed C-R analysis for the test. The EPA-calculated EC_{50} was 216.1 mg/L PFOA, which was acceptable for quantitative use.

Lu et al. (2016) evaluated the acute toxicity of PFOA (CAS# 335-67-1, 98% purity, purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) on *Daphnia magna* immobilization. The test was conducted following modified OECD standard test procedure 202, whereby five concentration treatments (3, 10, 30, 100 and 300 mg/L) plus a blank control were employed with three replicates per treatment. Authors reported immobility/survival to be a more sensitive endpoint than survival alone. The author-reported 48-hour EC_{50} for immobility/survival was 110.7 mg/L and the EPA-calculated 48-hour EC_{50} was 114.6 mg/L, which was acceptable for quantitative use.

Yang et al. (2019) evaluated the acute effects of PFOA (CAS# 335-67-1, purchased from Sigma-Aldrich in St. Louis, MO) on *Daphnia magna* in a 48-hour unmeasured static exposure. Authors stated the protocol for all testing followed OECD Guideline 202. Nominal acute test concentrations included 0 (control), 66.67, 79.92, 96.06, 115.1, 138.3, and 166.0 mg/L PFOA, with four replicates per treatment. Authors reported an LC_{50} of 120.9 mg/L PFOA. The EPA-calculated 48-hour LC_{50} was 117.2 mg/L, which was acceptable for quantitative use.

Chen et al. (2022) tested the acute toxicity of ammonium perfluorooctanoic acid (APFO, $\geq$98% purity) to *Daphnia magna* in a 48-hour unmeasured, static experiment. The acute APFO

exposure generally followed test method OECD 202 (OECD 2004). For each replicate, 10 neonates were exposed to 50 mL of test solutions with nominal concentrations of 0, 10, 50, 100, 200, and 500 mg/L APFO, made by diluting the stock solution with culture medium. There were three replicates per treatment and test organisms were not fed during the experiment. Control mortality was less than 10% in the AFPO and reference toxicity tests. The 48-hour EC_{50} for immobility was 156.9 mg/L, which was acceptable for quantitative use.

Quantitatively acceptable *D. magna* acute values from Boudreau (2002; $EC_{50} = 223.6$ mg/L), Colombo et al. (2008; $EC_{50} = 480$ mg/L), Ji et al. (2008; $EC_{50} = 542.5$ mg/L), Li (2009; $EC_{50} = 220.8$, 157.9, and 207.3 mg/L), Yang et al. (2014; $LC_{50} = 222.0$ mg/L), Barmentlo et al. (2015; $EC_{50} = 215.6$ mg/L), Ding et al. (2012a; $EC_{50} = 216.1$ mg/L), Lu et al. (2016; $EC_{50} = 114.6$ mg/L), Yang et al. (2019; $LC_{50} = 117.2$ mg/L), and Chen et al. (2022; $EC_{50} = 156.9$ mg/L) were taken together as a geometric mean value to calculate the *D. magna* SMAV of 213.9 mg/L. The *D. carinata* SMAV (i.e., 66.80 mg/L), *D. pulicaria* SMAV (i.e., 203.7 mg/L), and *D. magna* SMAV (i.e., 213.9 mg/L) were used to determine the *Daphnia* GMAV of 142.8 mg/L.

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

Rank[a]	GMAV (mg/L PFOA)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOA)
1	8.885	D	*Moina*	Cladoceran, *Moina macrocopa*	166.3
				Cladoceran, *Moina micrura*	0.4747
2	13.05	F	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	13.05
3	93.17	D	*Chydorus*	Cladoceran, *Chydorus sphaericus*	93.17
4	142.8	D	*Daphnia*	Cladoceran, *Daphnia carinata*	66.80
				Cladoceran, *Daphnia magna*	213.9
				Cladoceran, *Daphnia pulicaria*	203.7
5	150.0	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	150.0
6	161.0	G	*Ligumia*	Black sandshell, *Ligumia recta*	161.0
7	164.4	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	164.4
8	377.0	C	*Xenopus*	Frog, *Xenopus sp.*	377.0
9	383.6	H	*Dugesia*	Planaria, *Dugesia japonica*	383.6
10	431.5	E	*Neocaridina*	Green neon shrimp, *Neocaridina denticulata*	431.5
11	450.4	B	*Danio*	Zebrafish, *Danio rerio*	450.4
12	593.6	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	593.6
13	646.2	C	*Hyla*	Gray treefrog, *Hyla versicolor*	646.2
14	664.0	B	*Lepomis*	Bluegill, *Lepomis macrochirus*	664.0
15	681.1	G	*Physella*	Bladder snail, *Physella acuta*	681.1
16	689.4	C	*Ambystoma*	Jefferson salamander, *Ambystoma jeffersonianum*	1,070
				Small-mouthed salamander, *Ambystoma texanum*	407.3

Rank[a]	GMAV (mg/L PFOA)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOA)
				Eastern tiger salamander, *Ambystoma tigrinum*	752.0
17	793.9	C	*Anaxyrus*	American toad, *Anaxyrus americanus*	793.9
18	951.5	C	*Lithobates*	American bullfrog, *Lithobates catesbeiana*	1,020
				Green frog, *Lithobates clamitans*	1,070
				Northern leopard frog, *Lithobates pipiens*	751.7
				Wood frog, *Lithobates sylvatica*	999
19	4,001	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	4,001

a Ranked from the most sensitive to the most resistant based on Genus Mean Acute Value.

b From Appendix A: Acceptable Freshwater Acute PFOA Toxicity Studies.

c MDR Groups – Freshwater:

    A. the family Salmonidae in the class Osteichthyes

    B. a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)

    C. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)

    D. a planktonic crustacean (e.g., cladoceran, copepod, etc.)

    E. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)

    F. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)

    G. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)

    H. a family in any order of insect or any phylum not already represented.
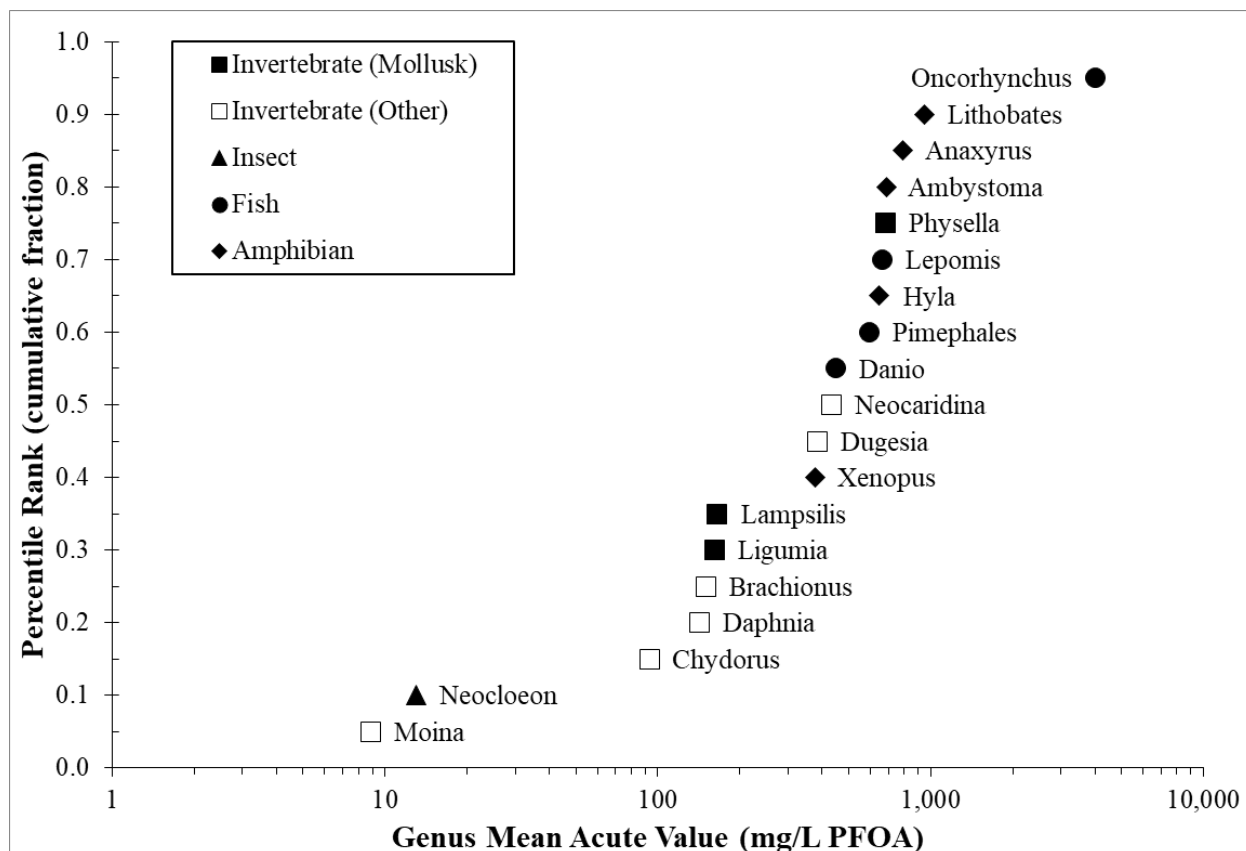
Figure 3-1. Ranked Freshwater Acute PFOA GMAVs Fulfilling the Acute Family MDR.

3.1.1.2   Summary of Quantitatively Acceptable Acute PFOA Toxicity Studies for Estuarine/Marine Species

Quantitatively-acceptable empirical data for acute PFOA toxicity were available for four estuarine/marine species, representing four genera and three families in three phyla. The data available for estuarine/marine invertebrates fulfilled three of the eight MDRs. In the interest of providing information to states/authorized Tribes on protective values, the EPA developed an estuarine/marine acute benchmark using the available empirical data supplemented with toxicity values generated through the use of 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 (web-ICE) tool (Raimondo et al. 2010). These benchmarks are provided in Appendix L.

The following section provides information and summaries of studies for the sensitive estuarine/marine taxa, based on the limited available data (Table 3-4). Study summaries for the 4 most sensitive genera are provided below.

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

Rank	Genus	GMAV (mg/L)	Species
1	*Siriella*	15.5	Mysid (*Siriella armata*)[a]
2	*Mytilus*	17.58	Mediterranean mussel (*Mytilus galloprovincialis*)
3	*Strongylocentrotus*	20.63	Purple sea urchin (*Strongylocentrotus purpuratus*)
4	*Americamysis*	24	Mysid (*Americamysis bahia*)

[a] Not a North American resident species, but a member of the *Siriella* genus and serves as a surrogate for untested *Siriella* species residing in North America (Heard et al. 2006).

3.1.1.2.1 Most sensitive estuarine/marine genus: Siriella (mysid)

Mhadhbi et al. (2012) performed a 96-hour static, unmeasured acute test with PFOA (96% purity) on the mysid, *Siriella armata*. Mysids were exposed to one of ten nominal PFOA treatments (0.1, 0.5, 1, 2, 5, 10, 20, 30, 40 and 80 mg/L). Neonates were fed 10-15 *Artemia salina* nauplii daily and mortality was recorded after 96 hours. The 96-hour LC_{50} reported in the study was 15.5 mg/L PFOA and was acceptable for quantitative use. No other quantitatively acceptable acute toxicity data were available for *Siriella armata* or other members of the genus *Siriella*. Therefore, the LC_{50} (i.e., 15.5 mg/L) from this test served directly as the *Siriella* GMAV. Although *S. armata* is not a North American resident species, it is a member of the Mysidae Family and serves as a surrogate for untested mysid species, including members of the genus *Siriella*, residing in North America (Heard et al. 2006).

3.1.1.2.2 Second most sensitive estuarine/marine genus: Mytilus (mussel)

The acute toxicity of PFOA (purity not provided) on the Mediterranean mussel, *Mytilus galloprovincialis,* which occurs in California and other parts of the Pacific Northwest (Green 2014), was evaluated by **Fabbri et al. (2014)**. The endpoint was the percent reduction of normal D-larvae in each well. Authors noted that controls had ≥80% normal D-larvae across all tests, meeting the >75% acceptability threshold outlined by ASTM (2004). PFOA was only measured once in one treatment which was similar to the nominal concentration. 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. Although authors report ~27% effect at the LOEC (i.e., 0.0001 mg/L), the test concentrations failed to elicit 50% 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 quantitatively acceptable.

Hayman et al. (2021) reported the results of a 48-hour static, measured acute PFOA (CAS # 335-67-1, 95% purity, purchased from Sigma-Aldrich, St. Louis, MO) test on the Mediterranean mussel, *Mytilus galloprovincialis*. Authors note that tests followed U.S. EPA (1995b) and ASTM (2004) protocols. Six test solutions were made in 0.45 µm filtered seawater (North San Diego Bay, CA) with PFOA dissolved in methanol. The highest concentration of methanol was 0.02% (v/v) and each treatment solution contained five replicates. 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-hour EC_{50}, based on normal larvae survival was 9.98 mg/L PFOA. The EPA-calculated 48-hour EC_{50} value was 17.58 mg/L, which was acceptable for quantitative use.

Although the 48-hour EC_{50} based on malformation of >1 mg/L from Fabbri et el. (2014) met the EPA's data quantitatively objectives, it was not used directly in the calculation of the *M. galloprovincialis* SMAV because it was a "greater than LC_{50}" value and a definitive LC_{50} was available for the same species as reported by Hayman et al. (2021). The definitive LC_{50} value from Hayman et al. (2021) of 17.58 mg/L served directly as the *Mytilus galloprovincialis* SMAV and as the *Mytilus* GMAV.

3.1.1.2.3 *Third most sensitive estuarine/marine genus: Strongylocentrotus (urchin)*

Hayman et al. (2021) reported the results of a 96-hour static, measured PFOA (CAS # 335-67-1, 95% purity, purchased from Sigma-Aldrich, St. Louis, MO) test with the purple sea urchin, *Strongylocentrotus purpuratus*. Authors note that tests followed U.S. EPA (1995b) and ASTM (2004) protocols. Six test solutions were made in 0.45 µm filtered seawater (North San Diego Bay, CA) with PFOA dissolved in methanol and each treatment was replicated five times. The highest concentration of methanol was 0.02% (v/v). At test termination (96 hours), the first 100 larvae were enumerated and observed for normal development (organisms distinguished as being in the four 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, was 19 mg/L PFOA. The EPA-calculated 96-hour EC_{50} value was 20.63 mg/L, which was acceptable for quantitative use. The EC_{50} value of 20.63 mg/L was the only acceptable acute value for *Strongylocentrotus purpuratus* or any members of the genus *Strongylocentrotus*. Therefore, it served directly as the *Strongylocentrotus purpuratus* SMAV and the *Strongylocentrotus* GMAV.

3.1.1.2.4 Fourth most sensitive estuarine/marine genus: Americamysis (mysid)

Hayman et al. (2021) conducted a 96-hour static, measured test to assess effects of

PFOA (CAS # 335-67-1, 95% purity, purchased from Sigma-Aldrich, St. Louis, MO) on the

mysid, *Americamysis bahia*. Authors note that tests followed U.S. EPA (2002) protocols. Six test

solutions were made in 0.45 µm filtered seawater (North San Diego Bay, CA) with PFOA

dissolved in methanol. The highest concentration of methanol was 0.02% (v/v) and each test

solution was replicated six times with five mysids per replicate. There were no significant

differences between solvent control and filtered seawater, suggesting no adverse effects of

methanol. No organisms were found dead in the controls at test termination. The EPA was

unable to fit a concentration-response model with significant parameters and relied on the author-

reported 96-hour LC_{50} of 24 mg/L PFOA as the quantitatively acceptable acute value. The LC_{50}

value of 24 mg/L was the only acceptable acute value for *Americamysis bahia* or any members

of the genus *Americamysis.* Therefore, it served directly as the *Americamysis bahia* SMAV and

the *Americamysis* GMAV.

The estuarine/marine acute data set for PFOA contained four genera (Figure 3-2)

representing only three of the eight taxonomic MDR groups. The missing MDR groups included

two families in the phylum Chordata, two families in a phylum other than Chordata, and any

other family not already represented (Table 3-5). As noted above, the EPA used the available

acute toxicity data and ORD's peer-reviewed web-ICE tool to develop aquatic life benchmarks

for consideration by states and Tribes (see Appendix L).

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

Rank[a]	GMAV (mg/L PFOA)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOA)
1	15.5	C	*Siriella*	Mysid, *Siriella armata*	15.5
2	17.58	D	*Mytilus*	Mediterranean mussel, *Mytilus galloprovincialis*	17.58
3	20.63	F	*Strongylocentrotus*	Purple sea urchin, *Strongylocentrotus purpuratus*	20.63
4	24	C	*Americamysis*	Mysid, *Americamysis bahia*	24

a Ranked from the most sensitive to the most resistant based on Genus Mean Acute Value.
B From Appendix B: Acceptable Estuarine/Marine Acute PFOA Toxicity Studies
c MDR Groups identified in Footnote C of Table 3-1.
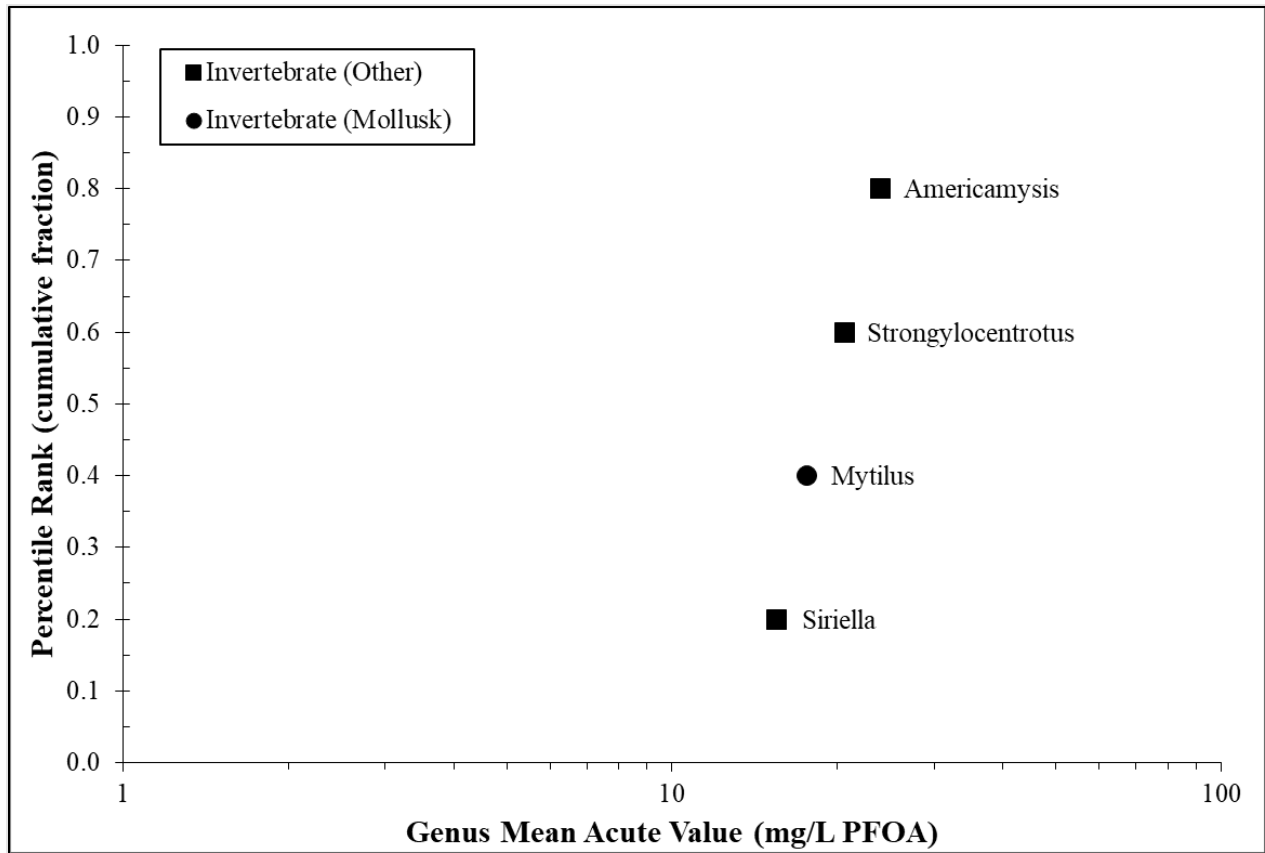
Figure 3-2. Acceptable Estuarine/Marine GMAVs.

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

Acceptable chronic PFOA toxicity data in freshwater were available for a total of 13 species representing 12 genera and ten families in three phyla. All eight of the required MDRs were fulfilled. The following section provides information and summaries of studies for the sensitive taxa with effect values used in the quantitative calculation of the chronic freshwater PFOA criterion (Table 3-6). The chronic data set for PFOA based on quantitatively acceptable data contains 12 genera representing all eight taxonomic MDR groups (Table 3-7).

Table 3-6. The Most Sensitive Genera Used in Calculating the Chronic Freshwater Water Column Criterion (Sensitivity Rank 1-4).
Ranked Below from Most to Least Sensitive

Rank	Genus	GMCV (mg/L)	Species
1	*Hyalella*	0.147	Amphipod (*Hyalella azteca*)
2	*Lithobates*	0.288	American bullfrog (*Lithobates catesbeiana*)
3	*Daphnia*	0.3695	Cladoceran (*Daphnia carinata*)[a]
			Cladoceran (*Daphnia magna*)
4	*Brachionus*	0.7647	Rotifer (*Brachionus calyciflorus*)

[a] Not a North American resident but in same genus as resident species.

3.1.1.3.1 Most chronically sensitive genus: Hyalella (amphipod)

Bartlett et al. (2021) evaluated the chronic effects of PFOA (CAS# 335-67-1, 96% purity, solubility in water at 20,000 mg/L, purchased from Sigma-Aldrich) on *Hyalella azteca* via a 42-day static-renewal, measured study. Methods for this study were adapted from Borgmann et al. (2007), and organisms were two to nine days old at the test initiation. A 100 mg/L stock solution was prepared to yield measured test concentrations of 0 (control), 0.84, 3.3,

8.9, 29 and 97 mg/L PFOA. Two separate tests were performed with five replicates per concentration and 20 amphipods per replicate. At test termination (day 42), adults were sexed and weighed, as well as their young counted. The 42-day author-reported LC_{10} value for survival was 23.2 mg/L PFOA. The author-reported EC_{10} values for growth and reproduction were 0.160 mg/L and 0.0265 mg/L, respectively. The EPA only performed C-R analysis for the growth and reproduction-based endpoints for this test, given the apparent tolerance of the survival-based endpoint. The EPA calculated EC_{10} values for the 42-day growth endpoint (i.e., control normalized wet weight/amphipod) and the 42-day reproduction endpoint (i.e., number of juveniles per female). The 42-day growth-based EC_{10} of 0.488 mg/L was not selected as the primary endpoint from this test because it was more tolerant than the reproduction-based EC_{10} of 0.147 mg/L, which was acceptable for qualitative use. The EC_{10} value of 0.147 mg/L was the only acceptable chronic value for *Hyalella azteca* or any members of the genus *Hyalella*. Therefore, it served directly as the *Hyalella azteca* Species Mean Chronic Value (SMCV) and the *Hyalella* Genus Mean Chronic Value (GMCV).

3.1.1.3.2 Second most chronically sensitive genus: Lithobates (frog)

Flynn et al. (2019) evaluated the chronic effects of PFOA (CAS# 335-67-1, purchased from Sigma-Aldrich) on the American bullfrog (*Lithobates catesbeiana*, formerly, *Rana catesbeiana*) during a 72-day static-renewal unmeasured exposure. The authors tested a negative control and two treatment concentrations (i.e., 0.144 and 0.288 mg/L), which were the only three PFOA-only treatments within the larger factorially-designed experiment. Each treatment contained 10 tadpoles (Gosner stage 25) and treatments were replicated four times. On day 72 of the experiment, all tadpoles were euthanized and measured (snout vent length and mass). The most sensitive chronic endpoint was growth (snout-vent length), with a 72-day NOEC and LOEC of 0.144 mg/L and 0.288 mg/L, respectively. The EPA could not independently calculate an EC_{10}

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value because there were minimal effects observed across the limited number of treatment concentrations tested. Consequently, the EPA used the LOEC of 0.288 mg/L as the chronic value from this chronic test. The LOEC was used preferentially to the MATC from this test because a ~7% reduction in snout-vent length relative to control responses was observed at the LOEC (i.e., 0.288 mg/L), which is a similar effect level to the chronic 10% effect level (i.e., EC_{10}) used preferentially to derive the chronic criterion. The LOEC value of 0.288 mg/L was the only acceptable chronic value for *Lithobates catesbeiana* or any members of the genus *Lithobates.* Therefore, it served directly as the *Lithobates catesbeiana* SMCV and the *Lithobates* GMCV.

3.1.1.3.3   *Third most chronically sensitive genus: Daphnia (cladoceran)*

Logeshwaran et al. (2021) conducted a static-renewal unmeasured PFOA (95% purity, purchased from Sigma-Aldrich Australia) chronic toxicity test with the cladoceran, *Daphnia carinata*. Authors stated the chronic test protocol followed OECD guidelines (2012). Authors tested a negative control and five PFOA concentrations (i.e., 0.001, 0.01, 0.1, 1.0 and 10 mg/L PFOA). Each test treatment was replicated 10 times with one daphnid (six to12 hours old) per treatment. At test termination (21 days) test endpoints included survival, days to first brood, average offspring in each brood and total live offspring. No mortality occurred in the controls or lowest test concentration. Of the three endpoints measured, average offspring in each brood and total live offspring were the more sensitive endpoints with 21-day NOEC and LOEC values of 0.01 and 0.1 mg/L PFOA, respectively. The EPA was unable to calculate statistically robust EC_{10} estimates from C-R models for these endpoints, largely because of the 10X dilution series across five orders of magnitude. The LOECs for these endpoints were not selected as the chronic value because the LOECs produced a 29.23% reduction in the average number of offspring per brood relative to controls and a 39.89% reduction in the total living offspring relative to controls. Therefore, the MATC (i.e., 0.03162 mg/L) was selected as the quantitatively acceptable chronic

value from this test. The MATC value of 0.03162 mg/L was the only acceptable chronic value for *Daphnia carinata,* and it served directly as the *Daphnia carinata* SMCV.

Centre International de Toxicologie (2003) and **Colombo et al. (2008)** conducted a 21-day renewal measured chronic test on PFOA with the daphnid, *Daphnia magna*. Authors stated that the toxicity test conducted followed OECD test guideline 211. Average number of live young was the most sensitive endpoint reported by Colombo et al. (2008), with a NOEC of 20 mg/L. The author-reported NOEC for the average number of live young was 20 mg/L, the LOEC was 44.2 mg/L and the MATC was 29.73 mg/L. The EPA performed C-R analysis for each reported endpoint. The most sensitive endpoint with an acceptable C-R curve was the average number of live young per starting adult, with an EPA-calculated EC_{10} of 20.26 mg/L PFOA and was acceptable for quantitative use.

Ji et al. (2008) conducted a chronic life-cycle test on the effects of PFOA with *Daphnia magna*. Authors stated that the *D. magna* test followed OECD 211 (1998). The most sensitive endpoint for *D. magna* reported in the publication was days to first brood with a 21-day NOEC of 6.25 mg/L (LOEC = 12.5 mg/L; MATC = 8.839 mg/L); however, number of young per starting female (an endpoint not reported in the publication, which only assessed number of young per surviving female) was calculated by the EPA and considered to be a more sensitive endpoint with an EPA-calculated EC_{10} of 7.853 mg/L. Therefore, the EPA-calculated EC_{10} of 7.853 mg/L PFOA for *D. magna* (number of young per starting female) was considered quantitatively acceptable.

Li (2010) conducted an unmeasured chronic life cycle 21-day test on the effects of PFOA on *Daphnia magna*. Authors stated the test followed OECD 211 (1998). The *D. magna* 21-day NOEC (reproduction as number of young per female, broods per female, and mean brood size)

was 10 mg/L (LOEC = 32 mg/L; calculated MATC = 17.89 mg/L). The EPA performed C-R

analysis for each reported endpoint. The EPA also revaluated all endpoints that were based on

number of surviving females to be based on the number of starting females. This recalculation

was done with the intent to account for starting females that were unable to contribute to the

population as reproduction/female due to mortality. The most sensitive endpoint with an

acceptable C-R curve was the number of young per starting female with an EPA-calculated EC_{10}

of 12.89 mg/L PFOA and was acceptable for quantitative use.

Yang et al. (2014) evaluated the chronic 21-day renewal, measured test of PFOA with

Daphnia magna, following ASTM E729 (1993). The author-reported *D. magna* 21-day EC_{10} for

reproduction (total number of spawning) was 7.02 mg/L. The EPA performed C-R analysis for

each reported endpoint. Both chronic survival and reproduction endpoints resulted in acceptable

C-R curves. The EPA-calculated EC_{10} for reproduction as total number of spawning events was

6.922 mg/L, similar to the EC_{10} reported by the authors (i.e., 7.02 mg/L). Chronic survival was

more sensitive than reproduction, with an EPA-calculated EC_{10} of 5.458 mg/L PFOA. Therefore,

the survival based EC_{10} calculated by the EPA (i.e., 5.458 mg/L) was acceptable for quantitative

use.

Lu et al. (2016) evaluated the chronic toxicity of PFOA (CAS# 335-67-1, 98% purity,

purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) on *Daphnia magna*

immobilization, growth and reproduction in a 21-day semi-static test with unmeasured treatment

solutions. Authors stated the test protocol followed OECD Test Method 211. Neonates (<24 h

old) were exposed to PFOA in one of six PFOA treatments (i.e., 0 [control] 0.032, 0.16, 0.8, 4

and 20 mg/L), with 20 replicates for each treatment. The 21-day growth and reproductive NOEC

and LOEC values were 0.032 and 0.16 mg/L PFOA, respectively. The EPA was unable to fit a

C-R model with significant parameters to the chronic data associated with reproduction from this test. The EPA-calculated EC_{10} values for mean intrinsic rate of increase (r; population-level endpoint that accounts for births and deaths over time) and growth (as length) were 0.0173 mg/L and 0.0124 mg/L, respectively. Both EC_{10} values were nearly two times lower than the NOEC for both endpoints (i.e., 0.032 mg/L) and four times lower than the LOEC values (i.e., 0.16 mg/L) for both endpoints, where a 15.2% reduction in intrinsic rate of natural increase ® and an 11.9% reduction in length were observed. As a result, the MATC of 0.0716 mg/L for growth and reproduction was selected as the most appropriate chronic value for quantitative use to in deriving the chronic water column-based criterion.

Yang et al. (2019) evaluated the chronic effects of PFOA (CAS# 335-67-1, purchased from Sigma-Aldrich in St. Louis, MO) on *Daphnia magna* via a 21-day unmeasured, static-renewal test that assessed reproductive effects. The protocol for testing followed OECD Guideline 211. Authors tested a negative control and four PFOA treatment concentrations (6.708, 10.10, 15.11, and 22.61 mg/L), with each treatment replicated 10 times and each replicate containing one neonate (12–24 hours old) in a 100 mL glass beaker. The reproductive NOEC and LOEC values were 6.708 and 10.10 mg/L PFOA, respectively. The EPA performed C-R analysis for the test. The EPA-calculated EC_{10} based on mean offspring at 21-days as a proportion of the control response was 8.084 mg/L and was used quantitatively to derive the chronic water column criterion.

The chronic values from Centre International de Toxicologie/Colombo et al. (2003/2008; EC_{10} = 20.26 mg/L; endpoint = average number of live young), Ji et al. (2008; EC_{10} = 7.853 mg/L; endpoint = number of live young per starting female), Li (2010; EC_{10} =12.89 mg/L; endpoint = number of young per starting female), Yang et al. (2014; EC_{10} = 5.458 mg/L;

endpoint = survival), Lu et al. (2016; MATC = 0.0716 mg/L; endpoint = length and rate of natural increase), and Yang et al. (2019; EC_{10} = 8.084 mg/L; endpoint = mean offspring as a proportion of control response) were taken together as a geometric mean to serve as the *Daphnia magna* SMCV (i.e., 4.317 mg/L).

The *D. carinata* SMCV (i.e., 0.03162 mg/L) and the *D. magna* SMCV (i.e., 4.317 mg/L) were used to calculate the *Daphnia* GMCV of 0.3695 mg/L. Exclusion of the *D. carinata* SMCV under the basis of it being an overly sensitive outlier (relative to *D. magna* and the overall quantitatively acceptable chronic data) would have resulted in the *Daphnia* GMCV being potentially underprotective. Conversely, excluding the *D. magna* SMCV under the basis of being a tolerant outlier (relative to *D. carinata*) would result in the *Daphnia* GMCV being highly influenced by a single test/species with a relatively sensitive chronic value. The *D. carinata* chronic value was also an MATC, calculated as the geometric mean of the NOEC (0.01 mg/L) and LOEC (0.1 mg/L) from a 10X dilution series, meaning the MATC was influenced by a relatively low NOEC and broadly spaced dosing. However, the corresponding LOEC (i.e., 0.1 mg/L) remains relatively sensitive and produced effects of 29.23% to 39.89%, depending on the specific offspring-based endpoint. Using both the *D. magna* and *D. carinata* SMAVs resulted in a *Daphnia* GMCV (0.3695 mg/L) that was among the four most sensitive genera and in the general range of the data overall.

3.1.1.3.4 *Fourth most chronically sensitive genus: Brachionus (rotifer)*

Zhang et al. (2013a) conducted a chronic life-cycle renewal test of PFOA (CAS # 335-67-1, 96% purity) with *Brachionus calyciflorus*. The test consisted of a negative control and four PFOA concentrations (0.25, 0.5, 1.0, 2.0 mg/L PFOA). For each treatment level, fifteen amictic rotifers were placed individually into culture plate wells containing two mL of test solution that was renewed daily. Numbers of eggs produced and starting rotifer lifetimes were recorded for

every individual, and the test was conducted until every starting rotifer from every treatment level died, which occurred around 200 hours after test initiation. Data from this test were used to construct survivorship and fertility tables using conventional life-history techniques, which were used to calculate net reproductive rate, generation time, and intrinsic rate of natural increase. The EPA calculated EC_{10} values from C-R data reported in the publication, and the most sensitive endpoint with an acceptable C-R curve was the intrinsic rate of natural increase, with an EC_{10} of 0.5015 mg/L PFOA.

The intrinsic rate of natural increase (d^{-1}) is a population-level endpoint that accounts for births and deaths over time. In Zhang et al. (2013a), the intrinsic rate of natural increase was calculated as the natural log of the lifetime net reproductive rate for all individuals within a population (defined here as a PFOA treatment level) divided by the average generation time of those individuals.

The EC_{10} calculated for the intrinsic rate of natural increase was similar to the EC_{10} value for average net reproductive rate (0.514 mg/L). Zhang et al. (2013a) also reported significant reductions in egg size, with an EPA-calculated $EC_{10} = 0.193$ mg/L. However, this endpoint displayed a relatively poor concentration response relationship and may not be relevant for assessing population level effects. For these reasons, it was not selected as the primary effect concentration from this study. Zhang et al. (2013a) also reported effects to average juvenile period, which was a relatively tolerant endpoint. Juvenile period decreased with increasing exposure concentration, with the average juvenile period being about 16% faster than the control responses in the highest treatment concentration (2.0 mg/L). Effects to chronic apical endpoints in this publication and Zhang et al. (2014b) generally appear as a threshold effect from 0.25 mg/L to 1.0 mg/L, providing further support for selection of the EC_{10} value (i.e., 0.5015 mg/L)

based on rate of natural increase as the primary chronic value for quantitative use from Zhang et al. (2013a).

In addition to the life cycle exposure, Zhang et al. (2013a) also conducted a second multi-generational 28-day study to measure effects of PFOA on growth patterns, population density, and population dynamics. The 28-day test consisted of a negative control and two PFOA concentrations (0.25, and 2.0 mg/L PFOA). Population densities were lower than controls at both PFOA treatment levels; however, because this study was limited to two treatment levels, it was considered to be of secondary importance compared to the life-cycle test.

Zhang et al. (2014b) describes the results of three experiments involving *Brachionus calyciflorus* exposures to PFOA (CAS # 335-67-1, 96% purity). The effects of PFOA concentration on mictic ratios of *B. calyciflorus* was examined by placing individual neonates in culture plate wells with two mL of medium containing of two PFOA concentrations (0.25 mg/L and 2.0 mg/L) plus a control. Each treatment level, as well as the control, was replicated three times. All eggs produced by these exposed individuals were individually incubated in culture wells with one mL control medium. The mature F_1 offspring were subsequently identified as producing mictic or amictic eggs, and these data were used to calculate mictic ratios. The proportions of mictic eggs increased with increasing PFOA concentration (0.56 – control, 0.72 – 0.25 mg/L, 0.75 – 2.0 mg/L), and the results were statistically significant ($p<0.05$). In contrast, mictic ratios were not affected by PFOA concentrations in Zhang et al. (2013a). Because of the inconsistent result in the mictic ratio endpoint between Zhang et al. (2013a) and Zhang et al. (2014b), it was not selected as the representative endpoint from either publication.

The effects of PFOA concentration on resting egg production of *B. Calyciflorus* was examined by exposing rotifers to one of five PFOA concentrations (plus control) in the dark for

six days. Resting eggs were collected on the sixth day and then hatched in control medium 30 days later. Resting egg production decreased with increasing PFOA concentration. The EPA-calculated EC_{10} calculated from C-R data reported in Figure 1 of Zhang et al. (2014b) was 0.076 mg/L. Because there was only one replicate (as implied by lack of error bars in Figure 1 of the publication, no clear description of replicates in the methods section, and no author-reported statistical analysis of this endpoint), resting egg production from this study was not considered quantitatively acceptable but was retained for qualitative use. In a second resting egg exposure study, resting eggs were produced under control conditions, then allowed to hatch while exposed to one of five PFOA concentrations plus a control. In this study, the effects of PFOA exposure on resting egg hatching rate were not statistically significant.

Finally, the effects of PFOA concentration on *B. calyciflorus* population growth was examined during a four-day study in which 10 neonates were placed into chambers with 10 mL of medium containing one of eight PFOA concentrations, plus a control. Each treatment level, as well as the control, was replicated at least six times. After four days, the total numbers of rotifers in each chamber were counted, and these data were used to calculate the intrinsic rate of natural increase (d^{-1}), the most sensitive acceptable endpoint from this study, with an EPA-calculated EC_{10} of 1.166 mg/L. Beyond Zhang et al. (2013a) and Zhang et al. (2014b), no other quantitatively acceptable chronic tests were available for *Brachionus calyciflorus*. The EPA-calculated EC_{10} values from Zhang et al. (2013a) (i.e., 0.5015 mg/L; endpoint = rate of natural increase) and Zhang et al. (2014b) (i.e., 1.166 mg/L; endpoint = rate of natural increase) were taken together as a geometric mean to serve as the *Brachionus calyciflorus* SMCV (i.e., 0.7647 mg/L). No other quantitatively acceptable chronic toxicity data were available for other members

of the genus *Brachionus* and the *Brachionus calyciflorus* SMCV (i.e., 0.7647 mg/L) served

directly as the *Brachionus* GMCV.

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

Rank[a]	GMCV[b] (mg/L PFOA)	MDR Group[c]	Genus	Species	SMCV[b] (mg/L PFOA)
1	0.147	E	*Hyalella*	Amphipod, *Hyalella azteca*	0.147
2	0.288	C	*Lithobates*	American bullfrog, *Lithobates catesbeiana*	0.288
3	0.3695	D	*Daphnia*	Cladoceran, *Daphnia carinata*	0.03162
				Cladoceran, *Daphnia magna*	4.317
4	0.7647	G	*Brachionus*	Rotifer, *Brachionus calyciflorus*	0.7647
5	2.194	D	*Moina*	Cladoceran, *Moina macrocopa*	2.194
6	>3.085	H	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	>3.085
7	9.487	C	*Oryzias*	Medaka, *Oryzias latipes*	9.487
8	23.56	D	*Ceriodaphnia*	Cladoceran, *Ceriodaphnia dubia*	23.56
9	>30	B	*Gobiocypris*	Rare minnow, *Gobiocypris rarus*	>30
10	>40	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	>40
11	>76	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	>76
12	88.32	F	*Chironomus*	Midge, *Chironomus dilutus*	88.32

a Ranked from the most sensitive to the most resistant based on Genus Mean Chronic Value.
b From Appendix C: Acceptable Freshwater Chronic PFOA Toxicity Studies.
c MDR Groups identified in Footnote C of Table 3-3.
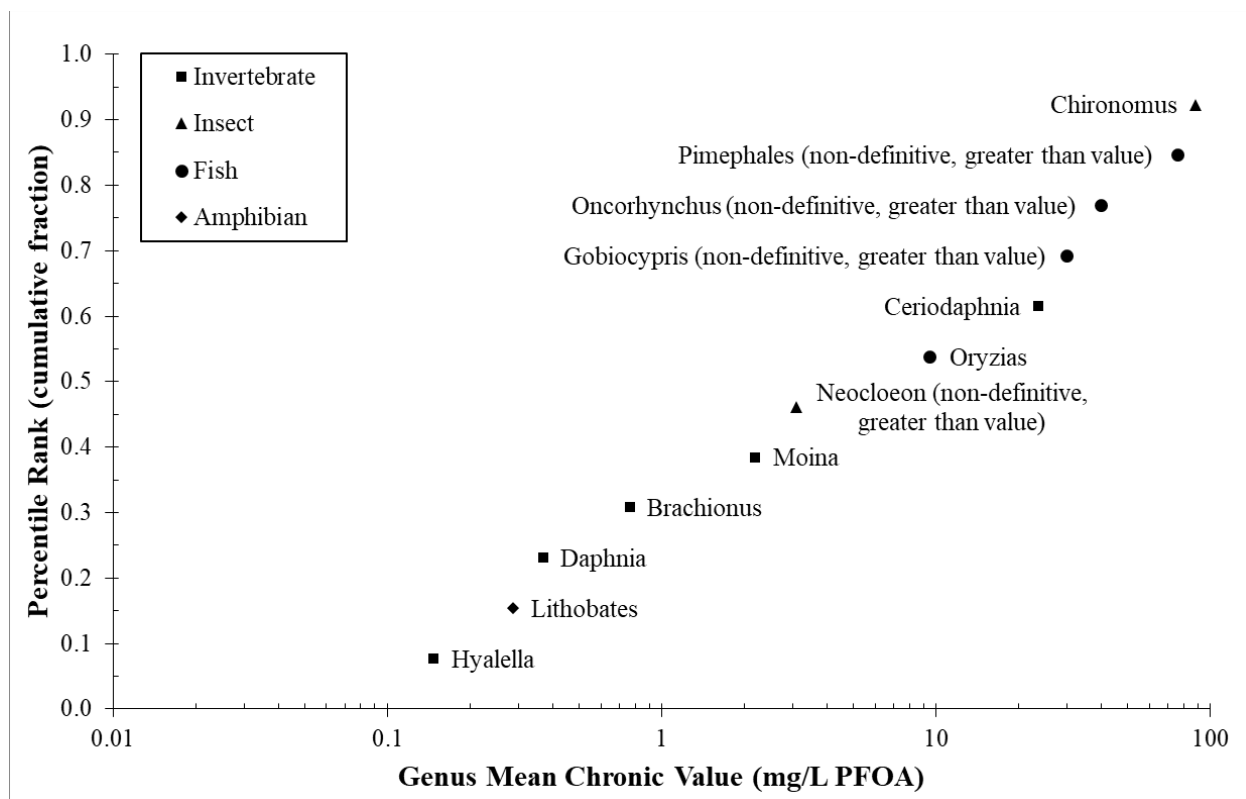
Figure 3-3. Freshwater Genus Mean Chronic Values for PFOA.

3.1.1.4   Summary of Chronic PFOA Toxicity Studies Used to Derive the Estuarine/Marine Aquatic Life Criterion

Quantitatively-acceptable empirical data for chronic PFOA toxicity were available for two estuarine/marine species, fulfilling two of the eight MDRs (Table 3-8).

3.1.1.4.1 Most chronically sensitive estuarine/marine species: Oryzias (fish)

Oh et al. (2013) evaluated the chronic toxicity of PFOA on the Japanese medaka, *Oryzias latipes*, in a 30-day static exposure. Prior to test initiation, the fish were acclimated to a seawater environment. Fish were exposed for 30 days to one PFOA concentration (1.0 mg/L), a 0.22 µm filtered seawater control, and a DMSO carrier solvent control. The 30-day condition factor NOEC of 1.0 mg/L PFOA was selected as the primary endpoint from this study. The methods section of Oh. et al. (2013) also stated, "In our preliminary study, fish mortality was altered 30 days after perfluorinated compound exposure, suggesting that repeated exposure to

PFCs for 30 days at 1 µg/mL [1 mg/L] causes adverse effect on *O. latipes*." The statement about

mortality-based effects in their preliminary test is in direct conflict with the condition-based

results from the primary test that was the focus of the publication. Few details are provided about

the preliminary test. Results of the final test (i.e., condition factor NOEC of 1.0 mg/L reported in

Table 1 of Oh et al. 2013) were retained as quantitatively acceptable because they provide

chronic estuarine/marine data that were otherwise limited.

3.1.1.4.2 *Second most chronically sensitive estuarine/marine species: Paracentrotus (sea urchin)*

Savoca et al. (2022) tested chronic toxicity of perfluorooctanoic acid (PFOA, >98%

purity) to sea urchins (*Paracentrotus lividus*) in a 28-day measured, static experiment. Four sea

urchins were placed into one of nine aquariums containing 15 L of seawater at nominal

concentrations (0 [control], 10, and 100 mg/L), with three replicates per concentration treatment.

The average recovery of PFOA was stable and close to nominal across all treatments throughout

the experiment, and PFOA in control water was less than 10 ng/L. Coelomic fluid was sampled

from all individuals weekly to measure PFOA uptake. During the exposure period, sea urchins at

the highest test concentration showed sublethal signs of toxicity, including reduced spine

mobility, spine loss, and reduced ability to remain anchored to the bottom of the test vessels.

Mortality was only observed at the highest test concentration. Adult mortality, adult PFOA

accumulation in coelomic fluid, percentage of normal and abnormal embryos, and gene

expression in embryos were measured. The NOEC and LOEC for adult mortality were 10 mg/L

and 100 mg/L, respectively, and the MATC of 31.62 mg/L was determined to be acceptable for

quantitative use.

Table 3-8. Ranked Estuarine/Marine Genus Mean Chronic Values.

Rank[a]	GMCV[b] (mg/L PFOA)	MDR Group[c]	Genus	Species	SMCV[b] (mg/L PFOA)
1	>10	A	*Oryzias*	Japanese medaka, *Oryzias latipes*	>10
2	31.62	E	*Paracentrotus*	Purple sea urchin, *Paracentrotus lividus*	31.62

a Ranked from the most sensitive to the most resistant based on Genus Mean Chronic Value.
b From Appendix D: Acceptable Estuarine/Marine Chronic PFOA Toxicity Studies.
c MDR Groups identified in Footnote C of Table 3-3.
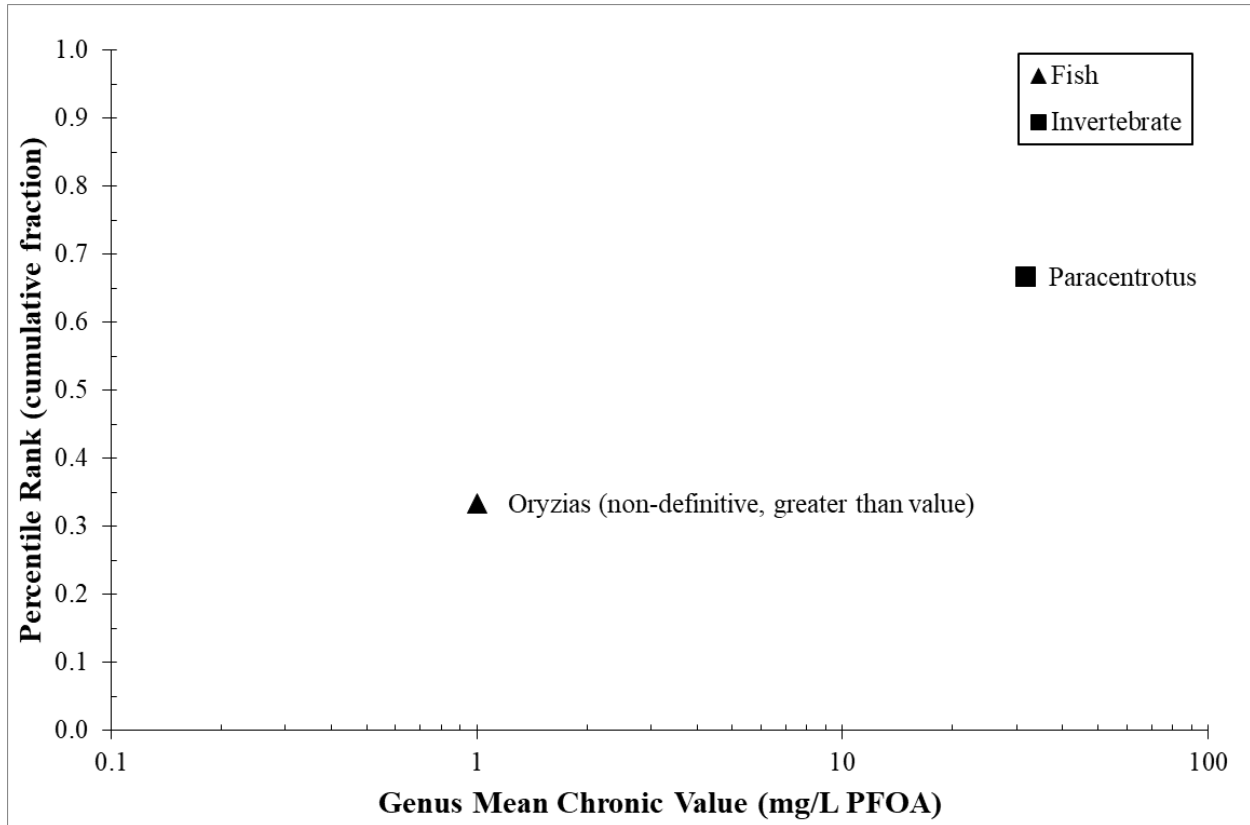
Figure 3-4. Estuarine/Marine Genus Mean Chronic Values for PFOA.

3.2 Derivation of the PFOA Aquatic Life Criteria

3.2.1 Derivation of Water Column-based Criteria

3.2.1.1 Derivation of Acute Water Criterion for Freshwater

The acute data set for PFOA contains 19 genera representing all eight taxonomic MDR groups. GMAVs for the four most sensitive genera were within a factor of 16.1 of each other.

The freshwater Final Acute Value (FAV) (i.e., the 5th percentile of the genus sensitivity distribution, intended to address 95 percent of the genera) for PFOA is 6.237 mg/L, calculated using the procedures described in the 1985 Guidelines (Table 3-9). The FAV is lower than all the GMAVs for the tested species. The FAV was then divided by two to obtain a concentration yielding a minimal effect acute criterion value. Based on the above, the FAV/2, which is the freshwater acute criterion water column magnitude (criterion maximum concentration, CMC), is 3.1 mg/L PFOA (rounded to two significant figures) and is expected to be protective of 95% of freshwater genera exposed to PFOA 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 3-5).

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

Calculated Freshwater FAV based on 4 lowest values: Total Number of GMAVs in Data Set = 19						
Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Moina*	8.885	2.18	4.77	0.050	0.224
2	*Neocloeon*	13.05	2.57	6.60	0.100	0.316
3	*Chydorus*	93.17	4.53	20.56	0.150	0.387
4	*Daphnia*	142.8	4.96	24.61	0.200	0.447
		Σ (Sum):	14.25	56.54	0.50	1.37

$S^2 =$ 208.28   S = slope
L = -1.396   L = X-axis intercept
A = 1.831   A = lnFAV
FAV = 6.237   P = cumulative probability
CMC = **3.1 mg/L PFOA** (rounded to two significant figures)

Figure 3-5. Ranked Freshwater Acute PFOA GMAVs and CMC.

3.2.1.2 Derivation of Acute Water Criterion for Estuarine/Marine Water

The 1985 Guidelines state that data from a minimum of eight families are needed to calculate an estuarine/marine FAV. Insufficient data exist to fulfill all eight of the taxonomic MDR groups. Notably, no acceptable test data on fish species were available. Since data were available for only three families, an estuarine/marine FAV could not be derived, and consequently, the EPA cannot derive an estuarine/marine acute criterion. The EPA has, however, developed an estuarine/marine acute benchmark value using available empirical data and the EPA/ORD's web-ICE tool to estimate missing data. The acute estuarine/marine benchmark is provided in Appendix L.

3.2.1.3   Derivation of Chronic Water Criterion for Freshwater

The freshwater Final Chronic Value (FCV) (i.e., the 5th percentile of the genus sensitivity distribution, intended to address 95 percent of the genera) for PFOA is 0.1041 mg/L, calculated using the procedures described in the 1985 Guidelines (Table 3-10). The freshwater chronic criterion water column magnitude (CCC) is the FCV rounded to two significant figures, or 0.10 mg/L PFOA, and is expected to be protective of 95% of freshwater genera potentially exposed to PFOA if the four-day average magnitude is not exceeded more than once in three years.

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

Calculated Freshwater FCV based on 4 lowest values: Total Number of GMCVs in Data Set = 12						
Rank	**Genus**	**GMCV (mg/L)**	**ln(GMCV)**	**ln(GMCV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Hyalella*	0.147	-1.917	3.676	0.077	0.277
2	*Lithobates*	0.288	-1.245	1.550	0.154	0.392
3	*Daphnia*	0.3695	-0.996	0.991	0.231	0.480
4	*Brachionus*	0.7647	-0.268	0.072	0.308	0.555
		Σ (Sum):	**-4.43**	**6.29**	**0.77**	**1.70**

$S^2 =$ 32.54   S = slope
L =   -3.538   L = X-axis intercept
A =   -2.262   A = lnFCV
FCV = 0.1041   P = cumulative probability
CCC = **0.10 mg/L PFOA** (rounded to two significant figures)

Figure 3-6. Freshwater Quantitative GMCVs and CCC.

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

The EPA 1985 Guidelines set the standard chronic duration at four days for two primary reasons. The 1985 Guidelines state, "*An averaging period of four days seems appropriate for use with the CCC for two reasons. First, it is substantially shorter than the 20 to 30 days that is obviously unacceptable. Second, 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.*"

Among tests with chronically sensitive genera, Bartlett et al. (2021) measured effects of PFOA on *H. azteca* (the most chronically sensitive GMCV) survival at 7, 14, 21, 28, 35, and 42 days and concluded, "Toxicity increased approximately two-fold over the duration of exposure, with LC_{50}s of 110 mg/L after seven days and 51 mg/L after 42 days." Based on Table S6 of Bartlett et al. (2021), LC_{50} values decreased from seven to 21-days and remained generally stable from 28 to 42 days, suggesting the chronic PFOA LC_{50} value became time-independent between 21 and 28 days. Although *H. azteca* survival was tolerant at seven days, clear time-dependent toxicity may not occur for more sensitive endpoints such as reproduction.

Bartlett et al. (2021) determined effects to reproduction to be more sensitive than long-term survival, but only measured effects to reproduction after 42 days of exposure, which is substantially longer than the four-day chronic duration. Bartlett et al. (2021) noted, effects to amphipod reproduction are typically the result of effects to growth under the premise, "larger amphipods have a greater reproductive output" and "reduced growth delays sexual maturity." However, results observed in Bartlett et al. (2021), suggested "the effects on reproduction may also have occurred independently of growth." Therefore, the reproductive-specific effects observed by Bartlett et al. (2021) may not have been caused by the long-term effects of reduced growth but were possibly the result of a sexually-developing and uniquely-sensitive life stage

that existed during a relatively brief duration within the longer 42-day test. Such instances are among the two primary reasons why the 1985 Guidelines prescribed the standard four-day chronic duration.

PFOA effects observed for other chronically sensitive and short-lived species further suggests a four-day chronic duration was appropriate. For example, the SMCV for *Brachionus calyciflorus* and the *Brachionus* GMCV (fourth most sensitive genus) are both the geometric mean of a full life-cycle test (i.e., up to 200 hours) test by Zhang et al. (2013a) and a four-day test by Zhang et al. (2014b). Chronic values for both studies correspond to the effect on population intrinsic growth rate. The full life-cycle test lasted up to 200 hours and yielded an EPA-calculated EC_{10} of 0.5015 mg/L. The four-day test yielded an EPA-calculated EC_{10} of 1.166 mg/L, suggesting chronic PFOA effects to short-lived species may occur after four days and increase with exposure duration.

Similarly, the SMCV for *Moina macrocopa* and the *Moina* GMCV (fifth most sensitive genus) are based on a seven-day life-cycle test by Ji et al. (2008), which also suggested reproductive effects (endpoint of number of young per starting female) occur in as little as seven days.

Overall, no chronic PFOA toxicity tests systematically 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 PFOA exposures, or assessed the potential for latent toxicity. However, chronic tests, including life cycle tests with relatively short-lived species suggest chronic effects may occur at durations shorter than those of standard toxicity tests (e.g., 28 days) and a chronic four-day duration component of the water column criterion was considered protective for these species/genera. Therefore, the EPA has set the duration component of the PFOA chronic water

column criterion at four days to reflect the chronic criterion duration recommended in the 1985 Guidelines. This four-day duration component of the chronic water column criterion is also consistent with U.S. EPA (1991), which considered the default four-day chronic averaging period as "the shortest duration in which chronic effects are sometimes observed for certain species and toxicants," and concludes that four-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 1985 Guidelines recommend that data from a minimum of eight families are needed to calculate an estuarine/marine FCV or sufficient data to transform an estuarine/marine FAV to a CCC through the use of an ACR. Insufficient data exist to fulfill all eight of the taxonomic MDR groups. There are only two quantitatively acceptable GMCVs for estuarine/marine genera and no estuarine/marine ACRs. Consequently, the EPA could not derive an estuarine/marine chronic criterion (see Appendix L for derivation of acute estuarine/marine benchmarks).

3.2.2   Derivation of Tissue-Based Criteria

Chronic PFOA toxicity data with measured tissue concentrations were limited. There were no aquatic life tissue-based toxicity studies considered acceptable for quantitative use. Therefore, there were not sufficient data to derive chronic tissue criteria using a sensitivity distribution approach. Instead, the water column chronic criterion was transformed into corresponding tissue-based criteria through a BAF approach, as outlined in Section 2.11.3. The chronic PFOA tissue-based criteria were derived by translating the chronic freshwater column criterion (i.e., 0.10 mg/L; see equation *1* in Section 3.2.1.3) into corresponding tissue-based criteria. The resulting tissue criterion corresponded to the tissue type from the 20th percentile BAF used in the equation (see Section 2.11.3). 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 PFOA and resultant tissue-based exposures is relatively low as well as those conditions with the bioaccumulation potential of PFOA is relatively high.

3.2.2.1 PFOA Bioaccumulation Factors (BAFs)

Section 2.11.3.1 above summarizes the literature search, calculation, and evaluation of the PFOA BAFs for aquatic life. These BAFs were compiled by and can be found in Burkhard (2021). BAFs used in the derivation of the PFOA tissue-based 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 across and within water bodies, 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 PFOA BAFs used in derivation of the tissue-based criteria are presented in Table 3-11 and individual BAFs are provided in Appendix O.

Table 3-11. Summary Statistics for PFOA BAFs in Invertebrate Tissues and Various 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)
Invertebrates	21	105.3	84.8	11.76	0.985	9,680
Fish Muscle	17	7.152	7.94	1.331	0.292	656
Fish Whole Body	25	198.6	219	64.93	1	16,273

[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 to represent that site).

3.2.2.2 Deriving Tissue-Based Criteria from the Chronic Water Column Criterion

Invertebrate whole-body, fish whole-body, and fish muscle tissue criteria were derived separately by multiplying the freshwater chronic water column criterion by the respective 20th centile of the distribution of BAFs described in Section 3.2.2.1, using Equation *1* from Section 2.11.3. The use of a 20th centile BAF results in more protective tissue criteria than those derived from a BAF based on a central tendency measure (e.g., geometric mean or median), which would only be protective on average or approximately 50% of the time.

The invertebrate whole-body tissue chronic criterion was calculated by multiplying the 20th centile BAF of 11.76 L/kg wet weight and the PFOA freshwater chronic water criterion of 0.10 mg/L, which resulted in a chronic invertebrate whole-body tissue criterion of 1.18 mg/kg wet weight. The fish muscle tissue chronic criterion was calculated by multiplying the 20th centile BAF of 1.331 L/kg wet weight and the PFOA freshwater chronic water criterion of 0.10 mg/L, which resulted in a chronic fish muscle-based chronic criterion of 0.133 mg/kg wet weight. The fish whole-body tissue chronic criterion was calculated by multiplying the 20th centile BAF of 64.93 L/kg wet weight and the PFOA freshwater chronic water criterion of 0.10 mg/L, which resulted in a chronic fish whole-body tissue criterion of 6.49 mg/kg wet weight. The chronic tissue-based criteria are expected to be protective of 95% of freshwater genera potentially exposed to PFOA under long-term exposures if the tissue-based criteria magnitudes are not exceeded.

The EPA acknowledges that there is uncertainty in deriving protective tissue criteria magnitudes by transforming the chronic water column criterion (which was based on tests that only added PFOA 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, PFOA 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-based and dietary-based PFOA 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. No available quantitatively acceptable data on the effects of dietary exposures to aquatic species were available, thus the EPA elected to develop protective values for aquatic organism tissues based on the observed relationship between water column concentrations and tissue concentrations and observed PFOA toxicity in chronic tests where PFOA was only added directly to the water column.

3.2.2.3   Deriving Protective Duration and Exceedance Frequencies for the Tissue-based Chronic Criteria

3.2.2.3.1   Duration: Chronic Tissue-Based Criteria

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

3.2.2.3.2   Frequency: Chronic Tissue-Based Criteria

The PFOA tissue-based criteria frequencies are set as "not to be exceeded" to ensure protection of aquatic life populations. This frequency accounts for the many variables influencing ecological recovery and the uncertainty in PFAS-specific ecological recovery time due to the lack of case studies on recovery rates following elevated PFOA concentrations in

aquatic biota. Ecological recovery times following chemical disturbances are context-dependent, 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.

PFOA-specific case studies are unavailable to directly inform rates of ecological recovery following elevated concentrations in fish and aquatic invertebrates. Metals and other chemical pollutants may be retained in the sediment and biota, where they can result in residual effects over time that further delay recovery. Few studies are available concerning PFOA 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 PFOA in adult rainbow trout exposed to PFOS for 28 days via the diet followed by 28 days depuration was estimated to be seven days in muscle tissue (Falk et al. 2015), while the terminal half-life in rainbow trout receiving a one-time intra-arterial injection of PFOA was 12.6 days (Consoer et al. 2014). Additionally, the depuration half-life in northern leopard frog tadpoles via 40-day aqueous exposure to 0.10 mg/L PFOA was estimated to be only 2.6 days (Hoover et al. 2017). It's unclear whether PFOA half-life in aquatic organism tissues is the mechanistic result of rapid depuration via gills or an artifact of these measurements that are taken during relatively short testing times (e.g., 28 days) where a steady state condition between PFOA and water and tissues has not occurred. Long-term uptake and subsequent excretion rates of PFOA have been extensively studied in humans relative to aquatic life. For example, Li et al. (2017) reported a median PFOA half-life of 2.7 years in human serum

following exposure to PFOA in drinking water, which authors stated was in the range of previously published estimates.

Due to chemical retention in both the environment and 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, 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 effects of selenium that led to severe reproductive failure and deformities in fish, were still measurable (as fish deformities) in 1992 (seven years later) and in 1996 (ten years later). Lemly (1997, 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 PFOA has been attributed to the strong C-F bond, with no known biodegradation or abiotic degradation processes for PFOA (see Section 2.3). Similarly, 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 = one year; 75th centile ~ three years; n = 20). Unlike metals, however, PFOA is not naturally-occurring and aquatic organisms possess no evolved detoxification mechanisms to aid in recovery at the individual level. Furthermore, the degradation of other

PFAS into PFOA may represent a continued source of PFOA in aquatic systems that further delays recovery.

The persistence and bioaccumulative/human-made nature of PFOA in aquatic systems, in combination with the documented recovery times of pollutants with similar chemical attributes (Lemly 1997; Gergs et al. 2016; Mebane 2022), suggests aquatic systems may recover from PFOA tissue criteria exceedances on the order of five to 10 years. However, recovery times could be longer if the source of PFOA and other PFAS that degrade into PFOA is not removed. There is a large amount of uncertainty in specifying a time interval associated with ecological recovery from PFOA tissue criteria exceedances given the lack of PFOA-specific examples of ecological recovery and the many situational-specific factors influencing recovery (Mebane et al. 2020). For example, the lack of PFOA degradation in the environment, and the fact that other PFAS in the environment can degrade into PFOA could act as ongoing PFOA sources that further delay recovery. Given these uncertainties, the PFOA tissue-based criteria frequencies are set as "not to be exceeded" to ensure protection of aquatic life populations. Moreover, if tissue-based criteria were exceeded, then PFOA has likely built up through the food web and PFOA source reservoirs are likely to exist, representing a broad level of PFOA contamination throughout the aquatic ecosystem.

The "not to exceed" frequency components of the tissue-based criteria do not suggest aquatic ecosystems could never recover from PFOA tissue-based criteria exceedance, under the right conditions. For example, ecological recovery from such an exceedance could begin once PFOA sources are decreased or eliminated, PFOA source reservoirs existing within the ecosystem (including other PFAS that degrade into PFOA), became permanently isolated from possible uptake by the ecosystem (e.g., long-term burial with no benthic disturbances), and

unaffected organisms were able to repopulate the system through immigration and/or reproductive events producing generations that are no longer exposed to PFOA.

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

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

This Aquatic Life Ambient Water Quality Criteria for PFOA document includes water column based acute and chronic criteria and tissue-based criteria for fresh waters. Acute and chronic water column criteria magnitudes for estuarine/marine waters could not be derived at this time due to data limitations; however, an acute estuarine/marine benchmark is provided for states/authorized tribal consideration (see Appendix L). The freshwater acute water column-based criterion magnitude is 3.1 mg/L, and the chronic water column-based chronic criterion magnitude is 0.10 mg/L. The fish whole-body tissue criterion magnitude is 6.49 mg/kg wet weight, the fish muscle tissue criterion magnitude is 0.133 mg/kg wet weight and the invertebrate whole-body tissue criterion magnitude is 1.18 mg/kg wet weight (Table 3-12). The assessment of the available data for fish, invertebrates, amphibians, and plants indicates these criteria will protect the freshwater aquatic community.

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

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

Table 3-12. Recommended Perfluorooctanoic acid (PFOA) Ambient Water Quality Criteria for the Protection of Aquatic Life in Freshwater.

Type/Media	Acute Water Column (CMC)[1,4]	Chronic Water Column (CCC)[1,5]	Invertebrate Whole-Body[1,2]	Fish Whole-Body[1,2]	Fish Muscle[1,2]
Magnitude	3.1 mg/L	0.10 mg/L	1.18 mg/kg ww	6.49 mg/kg ww	0.133 mg/kg ww
Duration	One-hour average	Four-day average	Instantaneous[3]		
Frequency	Not to be exceeded more than once in three years on average	Not to be exceeded more than once in three years on average	Not to be exceeded[6]		

[1] All five of these water column and tissue criteria are intended to be independently applicable and no one criterion takes primacy. All of the above recommended criteria (acute and chronic water column and tissue criteria) are intended to be protective of aquatic life. These criteria are applicable throughout the year.
[2] Tissue criteria 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 PFOA over time and space in aquatic life population(s) at a given site.
[4] Criterion Maximum Concentration; applicable throughout the water column.
[5] Criterion Continuous Concentration; applicable throughout the water column.
[6] PFOA chronic freshwater tissue-based criteria should not be exceeded, based on measured tissue concentrations representing the central tendency of samples collected at a given site and time.

This Aquatic Life Ambient Water Quality Criteria and Acute Saltwater Benchmark for PFOA document includes a water column based acute benchmark for estuarine/marine waters. The derivation of this benchmark is described in detail in Appendix L. The saltwater acute benchmark 7.0 mg/L (magnitude component), expressed as a one-hour average (duration component), that is not to be exceeded more than once in three years on average (Table 3-13).

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

methods (NAMs), such as computer-based toxicity estimation tools (e.g., the EPA's Web-ICE)

or other new approach methods intended to reduce reliance on additional animal testing

(https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-vertebrate-animals-chemical), including the use of read-across estimates based on other

chemicals with similar structures. The EPA's aquatic life benchmark values are not regulatory,

nor do they automatically become part of a state's water quality standards.

Table 3-13. Acute Perfluorooctanoic Acid (PFOA) Benchmark for the Protection of Aquatic Life in Estuarine/Marine Waters.

Type/Media	Acute Water Column Benchmark
Magnitude	7.0 mg/L
Duration	One hour average
Frequency	Not to be exceeded more than once in three years on average

4 EFFECTS CHARACTERIZATION FOR AQUATIC LIFE

This section describes supporting information for the derivation of these PFOA aquatic life criteria. Specifically, this chapter: (1) assesses the influence of including non-North American resident species in criteria derivation (Section 4.1), (2) considers relatively sensitive toxicity data from qualitatively acceptable studies that were used as supporting information (Section 4.2), (3) describes the available PFOA ACRs (Section 4.3), (4) compares the tissue-based criteria magnitudes to the empirical tissue-based effect concentrations available (Section 4.4), (5) evaluates aquatic plant tolerance to PFOA exposures (Section 4.5), and (6) evaluates the tolerance of threatened and endangered species to PFOA exposures (Section 4.6).

4.1 Influence of Using Non-North American Resident Species on PFOA Criteria

The EPA conducted an additional analysis of the water column-based criteria by limiting the toxicity datasets to organisms that are residents to the conterminous United States that have established populations to evaluate the influence of including non-North American resident species in criteria derivation.

4.1.1 Freshwater Acute Water Criterion with Resident Organisms

Three species, the green neon shrimp (*Neocaridina denticulata*), the cladoceran (*Daphnia carinata*) and the planarian (*Dugesia japonica*), are not resident or reproducing in the conterminous United States, while it remains uncertain if there are established resident zebrafish (*Danio rerio*) populations in the conterminous United States (U.S. FWS 2018). Nevertheless, zebrafish are common ecotoxicity test organisms that serve as taxonomic surrogates for untested fish species and are also considered in effects assessments conducted under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Removal of the green neon shrimp, *Daphnia carinata*, and *Dugesia japonica*, while retaining

zebrafish truncated the freshwater acute dataset to 24 species representing 17 genera (Table 4-1). The freshwater acute dataset truncated to North American resident species only was missing one MDR group (a benthic crustacean). The *Daphnia carinata* SMAV was the third most sensitive; however, its removal along with the removal of the other non-North American resident species had limited impact on the exploratory FAV and subsequent acute water column concentration (Table 4-2). The acute water column concentration based on North American resident species only, including zebrafish, was 2.6 mg/L PFOA, which was slightly lower than the chronic criteria CMC (CMC of 3.1 mg/L) based on both North American and non-North American species. Had zebrafish also been removed from the exploratory FAV and acute water column concentration based on North American resident species only, the four most sensitive genera would have remained the same, but the number of genera in the dataset would have decreased by one, which causes the resulting exploratory FAV (i.e., 4.793 mg/L) and acute water column concentration (i.e., 2.4 mg/L) to decrease slightly. The exploratory FAV and CMC based on North American resident species only with zebrafish excluded were both similar to the FAV and CMC described in Section 3.2.1.1.

Table 4-1. Ranked Freshwater Genus Mean Acute Values with North American Resident Organisms.

Rank[a]	GMAV (mg/L PFOA)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOA)
1	8.885	D	*Moina*	Cladoceran, *Moina macrocopa*	166.3
				Cladoceran, *Moina micrura*	0.4747
2	13.05	F	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	13.05
3	93.17	D	*Chydorus*	Cladoceran, *Chydorus sphaericus*	93.17
4	150.0	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	150.0

Rank[a]	GMAV (mg/L PFOA)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOA)
5	161.0	G	*Ligumia*	Black sandshell, *Ligumia recta*	161.0
6	164.4	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	164.4
7	208.7	D	*Daphnia*	Cladoceran, *Daphnia magna*	213.9
				Cladoceran, *Daphnia pulicaria*	203.7
8	377.0	C	*Xenopus*	Frog, *Xenopus sp.*	377.0
9	450.4	B	*Danio*	Zebrafish, *Danio rerio*	450.4
10	593.6	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	593.6
11	646.2	C	*Hyla*	Gray treefrog, *Hyla versicolor*	646.2
12	664.0	B	*Lepomis*	Bluegill, *Lepomis macrochirus*	664.0
13	681.1	G	*Physella*	Bladder snail, *Physella acuta*	681.1
14	689.4	C	*Ambystoma*	Jefferson salamander, *Ambystoma jeffersonianum*	1,070
				Small-mouthed salamander, *Ambystoma texanum*	407.3
				Eastern tiger salamander, *Ambystoma tigrinum*	752.0
15	793.9	C	*Anaxyrus*	American toad, *Anaxyrus americanus*	793.9
16	951.5	C	*Lithobates*	American bullfrog, *Lithobates catesbeiana*	1,020
				Green frog, *Lithobates clamitans*	1,070
				Northern leopard frog, *Lithobates pipiens*	751.7
				Wood frog, *Lithobates sylvatica*	999
17	4,001	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	4,001

a Ranked from the most sensitive to the most tolerant based on Genus Mean Acute Value.
b From Appendix A: Acceptable Freshwater Acute PFOA Toxicity Studies.
c MDR Groups identified in Footnote C of Table 3-3.

Table 4-2. Freshwater Exploratory Final Acute Value and Acute Water Column Concentration with North American Resident Organisms (zebrafish included).

Calculated Freshwater FAV based on 4 lowest values: Total Number of GMAVs in Data Set = 17					
Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Moina*	8.885	2.18	4.77	0.059	0.243
2	*Neocloeon*	13.05	2.57	6.60	0.118	0.343
3	*Chydorus*	93.17	4.53	20.56	0.176	0.420
4	*Brachionus*	150.0	5.01	25.11	0.235	0.485
		Σ (Sum):	**14.30**	**57.04**	**0.59**	**1.49**

S^2 = 181.32   S = slope
L = -1.444   L = X-axis intercept
A = 1.567   A = lnFAV
FAV = 4.793   P = cumulative probability
CMC = **2.4 mg/L PFOA** (rounded to two significant figures)

4.1.2 Freshwater Chronic Water Criterion with North American Resident Organisms

Three species, the cladoceran (*Daphnia carinata*), the rare minnow (*Gobiocypris rarus*) and the medaka (*Oryzias latipes*), are not resident or reproducing in the conterminous United States. Removal of these species truncated the freshwater chronic dataset to ten species representing ten genera (Table 4-3). The freshwater chronic dataset truncated to North American resident species fulfilled all MDR groups. Calculating the FCV based on the chronic GSD comprised of North American resident species only resulted in an exploratory FCV of 0.05857 mg/L and a chronic water column criterion of 0.059 mg/L (rounded to two significant figures; Table 4-4). The exploratory chronic water column concentration CCC (0.059 mg/L PFOA) based on North American species only was about half of the CCC based on North American and non-North American species (0.10 mg/L PFOA) and is lower than all of the quantitatively-acceptable GMCVs (Table 3-7). The reduction in the exploratory FCV based on North American resident species only was primarily an artifact of the FCV calculation procedure rather than inclusion of more sensitive toxicity data. That is, the reduced "*n*" used in the exploratory criterion calculation

and the increase in the *Daphnid* GMCV (which was the result of excluding *D. carinata* SMCV), increased the slope of the GSD which decreased the extrapolated FCV. The EPA retained the chronic water column criterion which includes North American and non-North American species, with a magnitude of 0.10 mg/L, to ensure the fullest, high-quality dataset available is used to represent the thousands of untested aquatic taxa present in U.S. ecosystems when deriving the chronic criterion for PFOA.

Table 4-3. Ranked Freshwater Genus Mean Chronic Values with Resident Organisms.

Rank[a]	GMCV[b] (mg/L PFOA)	MDR Group[c,d]	Genus	Species	SMCV[b] (mg/L PFOA)
1	0.147	E	*Hyalella*	Amphipod, *Hyalella azteca*	0.147
2	0.288	C	*Lithobates*	American bullfrog, *Lithobates catesbeiana*	0.288
3	0.7647	G	*Brachionus*	Rotifer, *Brachionus calyciflorus*	0.7647
4	2.194	D	*Moina*	Cladoceran, *Moina macrocopa*	2.194
5	>3.085	H	*Neocloeon*	Mayfly, *Neocloeon triangulifer*	>3.085
6	4.317	D	*Daphnia*	Cladoceran, *Daphnia magna*	4.317
7	23.56	D	*Ceriodaphnia*	Cladoceran, *Ceriodaphnia dubia*	23.56
8	>40	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	>40
9	>76	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	>76
10	88.32	F	*Chironomus*	Midge, *Chironomus dilutus*	88.32

a Ranked from the most sensitive to the most resistant based on Genus Mean Chronic Value.
b From Appendix C: Acceptable Freshwater Chronic PFOA Toxicity Studies.
c MDR Groups identified in Footnote C of Table 3-3.

Table 4-4. Freshwater Exploratory Final Chronic Value and Chronic Water Column Concentration with North American Resident Organisms.

Calculated Freshwater FCV based on 4 lowest values: Total Number of GMCVs in Data Set = 10						
Rank	Genus	GMCV (mg/L)	ln(GMCV)	ln(GMCV)^2	P=R/(N+1)	sqrt(P)
1	*Hyalella*	0.147	-1.917	3.676	0.091	0.302
2	*Lithobates*	0.288	-1.245	1.550	0.182	0.426
3	*Brachionus*	0.7647	-0.268	0.072	0.273	0.522
4	*Moina*	2.194	0.786	0.617	0.364	0.603
		Σ (Sum):	**-2.64**	**5.91**	**0.91**	**1.85**

$S^2 =$ 82.45   S = slope
L = -4.868   L = X-axis intercept
A = -2.838   A = lnFCV
FCV = 0.05857   P = cumulative probability
CCC = **0.059 mg/L PFOA** (rounded to two significant figures)

4.2 Consideration of Relatively Sensitive Qualitatively Acceptable Water Column-Based Toxicity Data

A multitude of studies were identified as not meeting the EPA's data quality guidelines for inclusion in the criteria derivation; however, these studies were used qualitatively as supporting information to the PFOA criteria derived to protect aquatic life and provide additional evidence of the observed toxicity and effects of PFOA, including the relative sensitivities. Most of these studies produced relatively tolerant effect concentrations relative to the criteria and are listed in Appendix G. The key studies used qualitatively in the derivation of the PFOA criteria were identified as being from either relatively sensitive genera or relatively sensitive tests and are described below. That is, qualitatively acceptable data for tests with species among the four most sensitive genera that were used to derive the criteria magnitudes are discussed. Qualitatively acceptable tests with relevant exposure durations and apical effects that also observed effect concentrations less than or similar to (e.g., factor of two) the corresponding criteria magnitude are discussed. Qualitatively acceptable studies described below were separated by acute (Section 4.2.1) and chronic (Section 4.2.2) data and only included those

studies that reported apical endpoints. The toxicity values summarized as part of this Effects

Characterization were not used in any quantitative analyses or in the numerical derivation of the

PFOA aquatic life criteria.

4.2.1 Consideration of Qualitatively Acceptable Acute Data

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

4.2.1.1.1 *Most acutely sensitive genus, Moina*

There were no qualitatively acceptable acute tests for species within the genus, *Moina*.

4.2.1.1.2 *Second most acutely sensitive genus, Neocloeon*

There were no qualitatively acceptable acute tests for species within the genus,

Neocloeon.

4.2.1.1.3 *Third most acutely sensitive genus, Chydorus*

There were no qualitatively acceptable acute tests for species within the genus, *Chydorus*.

4.2.1.1.4 *Fourth most acutely sensitive genus, Daphnia*

3M Co. (2000a) exposed *D. magna* to PFOA (CAS # 335-67-1) in a 48-hour static,

unmeasured acute toxicity test that followed USEPA-TSCA Guideline 797.1300. The toxicant

was part of the 3M production lot number 269 and was characterized as mixture of PFOA (96.5-

100% of the compound) and C_6, C_7 and C_9 perfluoro homologue compounds (0-3.5% of the

compound). The 48-hour reported EC_{50}, based on death/immobility, was 360 mg/L PFOA. This

test was not acceptable for quantitative use because of possible mixture effects from other

perfluoro homologue compounds in the test substance, the amount of isopropanol and missing

exposure details, but was retained for qualitative use.

3M Co. (2000a) summarized four 48-hour static, unmeasured APFO (CAS # 3825-26-1)

acute toxicity tests with the cladoceran, *Daphnia magna* and APFO. The toxicant was part of the

3M production lot number 37 and was characterized as a mixture of APFO (96.5-100% of the

compound) and C_6, C_7 and C_9 perfluoro analogue compounds (0-3.5% of the compound). The

48-hour EC_{50} determined from tests conducted in May 1982, based on death/immobility, was >1,000 mg/L APFO, while the EC_{50} for a subsequent test in June 1982 was reported to be 126 mg/L APFO. Possible mixture effects of other perfluoro analogue compounds, and the authors indication that the tests may have included food, did not make these tests acceptable for quantitative use and they were retained for qualitative use.

3M Co. (2000a) summarized a 48-hour static, unmeasured acute toxicity test with the cladoceran, *Daphnia magna* and APFO (CAS # 3825-26-1). The toxicant was part of the 3M production lot number 390 and was characterized as a mixture of APFO (78-93% of the compound) and C_5, C_6 and C_7 perfluoro analogue compounds (7-22% of the compound). The author-reported 48-hour EC_{50}, based on mortality, was 221 mg/L APFO. The possible mixture effects of APFO with other perfluoro analogue compounds in the test material and possible low (<80%) test substance purity did not make this test acceptable for quantitative use. This test was retained for qualitative use only.

3M Co. (2000a) summarized a 48-hour static, unmeasured acute toxicity test with the cladoceran, *Daphnia magna* and APFO (CAS # 3825-26-1). The acute test followed USEPA-TSCA Guideline 797.1300 protocol. The toxicant was part of the 3M production lot number HOGE 205 and was not sufficiently characterized but was considered a mixture of APFO (30% of the compound) and water (80% of the compound). The author-reported 48-hour EC_{50}, based on mortality, was 1,200 mg/L test substance. The authors reported that the test substance was considered a mixture of APFO (with unreported purity) and other impurities, so the EC_{50} does not accurately reflect the toxicity of APFO and therefore the value was not acceptable for quantitative use but was retained for qualitative use.

3M Co. (2000a) summarized a 48-hour static, unmeasured acute toxicity test with the cladoceran, *Daphnia magna* and APFO (CAS # 3825-26-1). The acute test followed test guidance from OECD 202. The toxicant was part of the 3M production lot number 2327 and was characterized as a mixture of APFO (<45% of the compound), water (50% of the compound), inert perfluorinated compound (<3% of test substance), and C_5 and C_7 perfluoro analogue compounds (1-2% of the compound). The author-reported 48-hour EC_{50}, based on death/immobility, was 584 mg/L test substance. Because of the possible mixture effects of the inert perfluorinated compounds, other perfluoro analogue compounds and unreported test substance purity, the test was not acceptable for quantitative use but was retained for qualitative use.

3M Co. (2000a) summarized a 21-day static-renewal, unmeasured chronic toxicity test with the cladoceran, *Daphnia magna,* and APFO (CAS # 3825-26-1) and also briefly described a corresponding acute test with a reported 48-hour EC_{50} of 266 mg/L APFO. Very few details were provided about the acute test methodology. The test compound was assumed to be that of the chronic test, where the toxicant was part of the 3M production lot number 37 and was characterized as a mixture of APFO (96.5-100% of the compound) and C_6, C_7 and C_9 perfluoro analogue compounds (0-3.5% of the compound). The 48-hour EC_{50} from this test was not used quantitatively because of missing study details and the possible presence of additional PFAS, but the study was retained for qualitative use.

Overall, these *D. magna* acute effect concentrations were all greater than the FAV (i.e., 6.237 mg/L) and the acute criterion magnitude (i.e., 3.1 mg/L). These additional data suggest the *Daphnia* GMAV (i.e., 142.8 mg/L) used to derive the acute criterion was sufficiently protective.

4.2.1.2   Consideration of Relatively Sensitive Freshwater Tests based on Qualitatively
Acceptable Data

This section focuses on qualitatively acceptable tests that were most relevant to informing

the appropriateness of the acute freshwater criterion. Specifically, those tests used to

qualitatively inform the acute freshwater criterion magnitude were identified as most relevant if

they met all parameters listed below:

1. reported effect concentrations that were less than or similar to (e.g., factor of two) the
   acute criterion magnitude;
2. evaluated an animal species;
3. conducted the test for a relevant acute exposure duration (e.g., ~48 hours to ~96 hours);
4. evaluated apical effects (i.e., acute mortality/inhibition), and;
5. not already discussed in the previous section (i.e., not a species discussed among the four
   most sensitive genera).

The toxicity values summarized below were not used quantitatively to derive the acute PFOA

criterion. Results of each individual study (as well as the rationale why a study was not

quantitatively acceptable) were considered relative to the acute criterion magnitude to ensure the

acute PFOA criterion was not underprotective and to provide additional supporting evidence of

the potential toxicity of PFOA to aquatic organisms.

4.2.1.2.1 Genus: Danio (zebrafish)

Truong et al. (2014) evaluated the sub-chronic effects of 1,060 compounds (U.S. EPA

ToxCast phase 1 and 2) on zebrafish, *Danio rerio*, through the use of high-throughput

characterization of multidimensional *in vivo* effects. The effects of APFO and PFOA on

mortality, growth, behavior, morphology, histology and physiology were observed until 120

hours post fertilization (hpf) (114-hour exposure duration) with the water quality conditions not

reported. The most sensitive endpoint was mortality with a reported LOEC of 0.02759 mg/L

APFO. There were no effects of PFOA on mortality for zebrafish embryos with a reported

NOEC of 26.50 mg/L PFOA. This test was not used quantitatively and retained for qualitative

use only because the exposure durations were too long for an acute test and too short for a chronic test.

Han et al. (2021) evaluated the bioconcentration factors and phenotypic toxicities of 74 PFAS in zebrafish after five days of exposure. Toxicity tests followed OECD test guidance with minor modifications. Embryos (64-cell stage) were exposed to one measured PFAS concentration or DMSO solvent controls with solutions renewed daily. At test termination no mortality effects were observed at 3.94 µM PFOA (1.631 mg/L) and 3.67 µM APFO (1.582 mg/L). This test was not used quantitatively and retained for qualitative use only because the exposure durations were too long for an acute test and too short for a chronic test with no effects observed.

Rericha et al. (2021) conducted a 114-hour static unmeasured PFOA toxicity test with the zebrafish, *Danio rerio.* The authors reported a 114-hour mortality NOEC of 0.60 µM, or 0.2484 mg/L PFOA, based on a molecular weight of 414.07 g/mol. This test was not acceptable for quantitative use as the exposure duration was too long to be an acute test and too short to be a chronic test. Additionally, the authors only tested one exposure concentration that produced a relatively low NOEC. Although relatively low, the NOEC of 0.2484 mg/L does not suggest *D. rerio* early life stages are sensitive to sub-chronic PFOA exposures.

Haimbaugh et al. (2022) evaluated the acute toxic effects of low-level (≤700 ng/L) PFOA on zebrafish from zero to five days post fertilization. Unmeasured test concentrations (7, 70, or 700 ng/L PFOA) were renewed daily. At test termination, the highest test concentration (700 ng/L or 0.0007 mg/L) had no effects on mortality or abnormal development. This test was not used quantitatively and retained for qualitative use only because the exposure durations were too long for an acute test and too short for a chronic test with no effects observed.

Yu et al. (2022) evaluated the sub-chronic effects of PFOA on embryonic *Danio rerio*. Exposure to PFOA concentrations up to 0.100 mg/L had no effect on growth/length, mortality/hatch, or survival of larvae. The 70-hour growth, mortality, and hatch NOECs of 0.100 mg/L PFOA were not acceptable for quantitative use because of the short exposure duration, especially in the context of other available tests for this species that were of a sufficient exposure duration. Yu et al. (2022) also reported that heartbeat (at 48 hpf) and locomotor behavior (at 120 hpf) were significantly decreased by PFOA at 0.025 mg/L; however, these effects did not translate to effects on growth, mortality, or hatch at nominal test concentrations that were four times greater.

Liu et al. (2023b) evaluated the acute effects of PFOA on zebrafish embryos (5 hpf) exposed to one of five test concentrations (1, 2, 5, 10, and 20 mg/L). Test concentrations were renewed daily and test concentrations were measured with hatching rate, mortality and deformity recorded at test termination (96 hpf). The author reported that the 91-hour LC_{50} was approximately 2 mg/L PFOA. This test was not acceptable for quantitative use because the exposure duration was not a 96-hour exposure duration and other quantitatively acceptable data suggests the relatively-sensitive LC_{50} reported here (~2 mg/L) is an outlier, with the *Danio* SMAV being 450.4 mg/L.

Wang et al. (2023) evaluated the acute effects of PFOA on zebrafish embryos (2 hpf) exposed to 5 or 500 µg/L up to 120 hours post fertilization. Test concentrations were renewed daily and PFOA concentrations were measured using high-performance liquid chromatography-tandem mass spectrometry. At 96 hpf, there were no effects observed on mortality or abnormal development at 491.5 µg/L (0.4915 mg/L PFOA). Since this exposure was shorter than the

recommended 96 hour exposure (only 94 hours) and no effects were observed, the test was only

used qualitatively.

Quantitatively acceptable acute tests used to calculate the SMAV (Ye et al. 2007;

Hagenaars et al. 2011; Zhao et al. 2016; Godfrey et al. 2017a; Stengel et al. 2017, 2018)

suggested *D. rerio* is relatively tolerant to acute PFOA exposures with a *D. rerio* SMAV of

450.4 mg/L. Another quantitatively acceptable acute test with the zebrafish was conducted by

Corrales et al. (2017). However, the LC_{50} value from this test was excluded from the SMAV

calculation because a comparative assessment between this LC_{50} value and the other seven

quantitatively-acceptable values available indicated the LC_{50} was an outlier, about 5 times lower

than the other lowest LC_{50} value. It is expected that *D. rerio* will be tolerant to acute PFOA

exposures.

4.2.2   Consideration of Qualitatively Acceptable Chronic Data

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

4.2.2.1.1 *Most chronically sensitive genus, Hyalella*

Kadlec et al. (2024) tested the sub-chronic toxicity of PFOA (96-99% purity) to *Hyalella*

azteca for seven days in a measured, static-renewal experiment. Testing protocols followed

ASTM methodologies (ASTM 2002) with a shortened exposure period. The test was conducted

with a 0.5x dilution series of measured PFOA concentrations, with four replicates of each

concentration, and ~10 organisms per replicate (note: some replicates were overstocked and

included 11 test organisms). Mean test concentrations were 0.53 (control), 5.3, 11, 21, 41 and 83

mg/L. Amphipods were placed in 100 mL glass beakers with two holes (covered by stainless-

steel mesh) on opposite sides and filed with 60 mL of test solution. Beakers also included 2.5 mL

of silica sand substrate to fully cover the bottoms of each chamber and each test chamber was

renewed daily. Little to no mortality was observed in any treatment with survival ranging from

98-100% at test termination. The author reported EC_{20} for biomass was 5.0 mg/L. The EPA did not recalculate an EC_{10} from this study since the exposure duration was too short for a chronic exposure. However, this value is much larger than the 42-day exposure (Bartlett et al. 2021) with an EPA-calculated EC_{10}, based on reproduction, of 0.147 mg/L PFOA.

4.2.2.1.2 Second most chronically sensitive genus, Lithobates

Hoover et al. (2017) tested PFOA (96% purity) toxicity on the northern leopard frog, *Lithobates pipiens* (formerly, *Rana pipiens*) in a chronic renewal toxicity test using measured PFOA treatment concentrations. The 40-day NOEC was ≥1.0 mg/L PFOA based on Gosner stage reached at test termination and snout-vent length. The test used water renewals rather than the required flow-through design for chronic ALC development; however, leopard frogs commonly do not tolerate flow-through test systems and the use of renewal system was appropriate for this study organism. Also, PFOA was detected in the control organisms at concentrations three orders of magnitude lower than any PFOA treatment groups, indicating the trace contamination in controls may not be considered a significant issue. The 40-day NOEC of ≥1.0 mg/L was classified as acceptable for quantitative use based on meeting data quality objectives; however, it was not used to derive the chronic criterion because the study showed no adverse effects at the highest treatment concentrations (i.e., 1.0 mg/L). Because the highest treatment group that showed no effects was a relatively low treatment concentration, including this NOEC value in the criterion calculation would have resulted in the criterion magnitude being influenced by the relatively low-test concentration selected by study investigators (that did not produce an adverse response), rather than a concentration-response relationship. Therefore, this test was not used quantitatively and was considered qualitatively acceptable for use in criterion derivation.

Flynn et al. (2021) evaluated the chronic effects of PFOA (CAS # 335-67-1, ≥96% purity, purchased from Sigma-Aldrich) on northern leopard frogs, *Lithobates pipiens* (formerly *Rana pipiens*), via a 30-day sediment-spiked, static outdoor mesocosm study. At test termination (30 days) there was no effect on survival or growth (snout-vent length and weight). The 30-day NOEC, based on survival and growth, was 0.066 mg/L. However, on test-day five and at test termination all frogs in the spiked sediment mesocosm were less developed, based on Gosner stage, than the control mesocosms. The study was not acceptable for quantitative use because the test design was an outdoor spiked sediment mesocosm exposure with algal and zooplankton communities present and because of the relatively low NOEC value that did not quantitatively inform criteria derivation based on an exposure-response effect.

Flynn et al. (2022) evaluated the chronic effects of PFOA on the northern leopard frog [*Lithobates* (formerly *Rana*) *pipiens*] during a 30-day measured renewal exposure. Animals were monitored daily for mortality and abnormalities and sampled to quantify snout–vent length (SVL), body mass, and developmental stage. The SVL and body mass were used to calculate the scaled mass index (SMI). The NOEC for SVL was >1.376 mg/L and not used quantitatively because of a lack of a clear dose-response relationship and high variability in organismal responses despite a 10X dilution series. The NOEC and LOEC for growth (as mass) were 0.1251 and 1.376 mg/L resulting in a MATC of 0.4149 mg/L, which was also not used quantitatively for the same reasons. The effects of PFOA on SMI and Gosner stage were also not quantitatively acceptable because, while the highest test concentration was over 100X greater than the lowest, the response in the highest test concentration did not differ from control responses. Results of this test were retained for qualitative use.

Overall, these three studies showed minimal effects to the northern leopard frog at the concentrations tested, while the indoor laboratory test by Flynn et al. (2019; used to derive the *Lithobates catesbeiana* SMCV) showed a ~7% reduction in SVL after 72-day exposures at 0.288 mg/L. Although Hoover et al. (2017) reported a NOEC of 1.0 mg/L, the tests only consisted of a 40-day exposure, which may not have been long enough to elicit the chronic effects to SVL observed by Flynn et al. (2019) after 72 days. For example, Flynn et al. (2019) reported effects of PFOS on *Lithobates catesbeiana* tadpole mass after 21, 42, 56, 63, 70, and 72 days, with PFOS dose-dependent effects only becoming apparent at 56 days. Results of Flynn et al. (2021) could not meaningfully inform the appropriateness of the chronic criterion or the *Lithobates catesbeiana* SMCV (0.288 mg/L) because Flynn et al. (2021) did not observe effects of PFOA on *Lithobates pipiens*, with a relatively low NOEC of 0.066 mg/L. Similarly, results of Flynn et al. (2022) did not suggest the *Lithobates* GMAV value was underprotective, as Flynn et al. (2022) did not observe obvious PFOA effects with increasing exposure concentrations.

4.2.2.1.3 *Third most chronically sensitive genus, Daphnia*

3M Co. (2000a) summarized a 21-day static-renewal, unmeasured chronic toxicity test with the cladoceran, *Daphnia magna,* and APFO (CAS # 3825-26-1). The toxicant was part of the 3M production lot number 37 and was characterized as a mixture of APFO (96.5-100% of the compound) and C_6, C_7 and C_9 perfluoro analogue compounds (0-3.5% of the compound). The test followed U.S. EPA (1982) and OECD (1997) test protocols. The 21-day NOEC and LOEC, based on reproduction and survival were 22 and 36 mg/L APFO, respectively with a corresponding MATC of 28.14 mg/L. This test was acceptable for qualitative use only because of the possible mixture effects of other perfluoro analogue compounds and missing exposure details but does not suggest *D. magna* will be chronically sensitive relative to the chronic freshwater criterion.

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Seyoum et al. (2020) evaluated the chronic effects of PFOA (CAS# 335-67-1, >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. The 21-day reproductive (fecundity) LOEC of 0.4141 mg/L PFOA was reported by the study authors, where a ~38.25% reduction in mean number of daphnids relative to the control was observed. The EPA was unable to fit a model with significant parameters to the reproduction-based concentration-response data due to a lack of clear concentration-dependent effects beyond the LOEC. The reproduction-based LOEC (i.e., 0.4141 mg/L) was selected as the chronic value from this test; however, it was not considered acceptable for quantitative use because chronic responses in this test did not display concentration-dependent effects beyond the LOEC despite a 25X increase in treatment concentrations. Moreover, additional EC_{10} values from other, quantitatively acceptable tests, were available to inform the chronic sensitivity of *Daphnia magna* in criteria derivation.

4.2.2.1.4 Fourth most chronically sensitive genus, Brachionus

The qualitatively acceptable chronic value for *Brachionus* from **Zhang et al. (2014b)** was discussed in greater detail in Section 3.1.1.3.4. Briefly, Zhang et al. (2014b) conducted a full life-cycle test using renewal conditions for approximately four days on the rotifer, *Brachionus calyciflorus*. Zhang et al. (2014b) reported several endpoints, including intrinsic rate of natural increase, which was selected as the primary endpoint for criterion derivation. Zhang et al. (2014b) also observed effects to resting egg production. Resting egg production is an ecologically important endpoint for this species because it represents the final result of sexual reproduction. NOEC and LOEC values were not reported for resting egg production, but 0.25 mg/L PFOA produced more than a 50% reduction in resting egg production. Based on the authors description of results in the text, it was assumed the *B. calyciflorus* four-day NOEC for resting egg production was 0.125 mg/L and the LOEC was 0.25 mg/L and the calculated MATC

was 0.1768 mg/L, suggesting resting egg production may be a relatively sensitive endpoint. Because there was only one replicate (as implied by lack of error bars in Figure 1 of the publication, no clear description of replicates in the methods section, and no author-reported statistical analysis of this endpoint), resting egg production from this study was not considered quantitatively acceptable and was instead considered in a qualitative manner. Overall, effects to chronic apical endpoints for this genus, reported in this publication and Zhang et al. (2013a), generally appear as a threshold effect from 0.25 mg/L to 1.0 mg/L, providing support for the endpoint and effect level selected for quantitative use in criterion derivation (i.e., intrinsic rate of natural increase), and further suggests the chronic criterion magnitude is adequately protective of the genus, *Brachionus*.

4.2.2.2 Consideration of Relatively Sensitive Freshwater Tests based on Qualitatively Acceptable Data

This section focuses on qualitatively acceptable chronic tests that were most relevant to informing the appropriateness of the chronic freshwater criterion. Specifically, those tests used to qualitatively inform the chronic freshwater criterion magnitude were identified as most relevant if they met all parameters listed below:

1. reported effect concentrations that were less than or similar to (e.g., factor of two) the chronic freshwater criterion magnitude;
2. evaluated an animal species;
3. conducted the test for a non-acute exposure duration (e.g., greater than seven days);
4. evaluated apical effects (i.e., long-term survival, growth, and/or reproduction), and;
5. not already discussed in the previous section (i.e., not a species discussed among the four most sensitive genera).

The toxicity values summarized below were not used quantitatively to derive the chronic PFOA freshwater criterion. Results of each individual study (as well as the rationale why a study was not quantitatively acceptable) were considered relative to the chronic criterion magnitude to

ensure the chronic PFOA criterion was not underprotective and to provide additional supporting evidence of the potential toxicity of PFOA to aquatic organisms.

4.2.2.2.1 Genus: Chironomus (midge)

Stefani et al. (2014) conducted a chronic (10 generation) test of PFOA with a midge, *Chironomus riparius*. The 10 generations (each approximately 20 to 28 days) were tested under static conditions. The NOEC for the study, based on effect on emergence, reproduction or sex ratio at the only concentration tested, was 0.0089 mg/L PFOA. **Marziali et al. (2019)** provides further analysis of the same chronic test conducted by Stefani et al. (2014; reported in Table G.1) by reporting measurements of alterative endpoints/responses. The LOEC based on developmental time, adult weight was 0.0098 mg/L (time-weighted average; NOEC <0.0098 mg/L). While Stefani et al. (2014) reported no effects across the chronic test, Marziali et al. (2019) reported effects to select generations. Overall, however, effects were sporadic with reductions in growth observed in several generations. There were no effects on "survival, development, or reproduction" and Marziali et al. (2019) concluded "no effects at population level (population growth rate) were proved, thus a toxicity risk in real ecosystems at the tested concentrations seems unlikely." The results from these studies were deemed not acceptable for quantitative use because of limited test concentrations assessed, and uncertainty pertaining to sediment characteristics, as well as poor control survival in four of the 10 generations.

Zhai et al. (2016) exposed *Chironomus plumosus* larvae to PFOA (perfluorooctanoic acid, obtained from Acros Organics, Morris Plains, NJ, 96% purity) spiked in sediment for 10.3 days. The sediment was collected from the upstream region of the Yongding River in Beijing, China. Sediments were physically mixed with a steel blender at 60 rpm for 24 hours in the darkness at room temperature. Then, 1 mL of PFOA methanol solution (20 mg/L) was added to obtain a concentration of 100 ng/g PFOA, and the sample was thoroughly mixed in a fume hood.

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When the methanol had evaporated, 300 mL of distilled water was added to each beaker. Finally, the beakers were sealed, kept in the darkness at 25°C, and aged for 60 days before test initiation. The midge larvae used in this study were collected from the uncontaminated upstream area of the Yangliuqing River in the outer suburbs of Tianjin, China. Male larvae in the third or fourth instars of similar size were chosen and placed in pure water to purify their guts for more than 48 hours in the laboratory. There were 20 larvae in each 500 mL beaker containing sediment and 300 mL of solution and three replicates per concentration (9.8 ng PFOA/g sediment) and control (pure water). The static measured exposure lasted 10 days without food supply and involved a 16:8 hour (light:dark) photoperiod at $25 \pm 1°C$. At the end of the experiment, the surviving larvae were counted. The 10-day mortality NOEC was 9.8 ng/g, the only concentration tested. The test was not acceptable for quantitative use because: (1) exposures were spiked sediments rather than water only, (2) test duration (~10 days) was sub-chronic, too long for an acute test and too short for a chronic test, and (3) no effects were observed at the highest concentration tested which produced a relatively low "> NOEC" value that did not provide information on the sensitivity of this species to PFOA at concentrations consistent with criteria magnitudes. Results of this test were retained for qualitative use.

Quantitatively acceptable midge data (McCarthy et al. 2021) were available to derive the chronic PFOA freshwater criterion and these chronic data further suggest *Chironomus* is relatively tolerant to chronic PFOA exposures.

4.2.2.2.2 Genus: Salmo (Atlantic salmon)

Spachmo and Arukwe (2012) exposed Atlantic salmon (*Salmo salar*) to PFOA (95% purity) in a 52-day flow-through test with unmeasured treatment concentrations. The 52-day growth NOEC and LOEC were 0.10 and >0.10 mg/L PFOA, respectively. These data were acceptable for quantitative use based on meeting data quality objectives; however, were not used

in deriving the chronic criterion because the study only included one treatment group that showed no adverse effects. Because the one treatment group that showed no effects was a relatively low treatment concentration, including this NOEC value in the criterion calculation would have resulted in the criterion magnitude being strongly influenced by the low test concentration selected by study investigators (that did not produce an adverse response), rather than a concentration-response relationship.

Arukwe et al. (2013) exposed Atlantic salmon (*Salmo salar*) embryos to PFOA at 100 µg/L for 49 days. This test was not used quantitatively because of the lack of effects in the one treatment group tested. The 49-day growth-based NOEC of 0.1 mg/L PFOS was selected as the most sensitive and relevant endpoint. However, given there was only one treatment group and that the test concentration was a relatively low concentration (at 0.1 mg/L) compared to the other PFOA data, resulting in a > low value, this test was not informative enough for quantitative use in the derivation of the PFOA aquatic life criteria. Results of this test were retained for qualitative consideration.

4.2.2.2.3 Genus: Cyprinus (carp)

Petre et al. (2023) evaluated the bioconcentration and biotransformation of PFOA in common carp (*Cyprinus carpio*) through a 14-week exposure and 3-week depuration period. Carp (30-40 g) were exposed to one of two PFOA treatments (10 and 100 µg/L) in a static renewal measured experiment with solutions renewed every 48 hours. After 14 weeks, the fish were visually analyzed, measured, and weighed. No mortalities or visible physiological were observed in any treatment. Therefore, the 14-week NOEC, based on mortality, weight and length, was >92.1 µg/L (>0.0921 mg/L). The test not acceptable for quantitative use because the chronic value was a "greater than" NOEC which would have influenced the criterion magnitude despite no effects observed at the chronic value.

4.2.2.2.4 Genus: Danio (zebrafish)

Jantzen et al. (2017a) evaluated the effects of PFOA on the morphometric, behavioral

and gene expression in *D. rerio* exposed via five-day static unmeasured exposures. Zebrafish

embryos were exposed at 3-hpf to PFOA for 120 hours in what is equivalent to a rapid early-life

stage test. The observation period in clean water was extended beyond the exposure time points

from 120 hpf to 14 days post fertilization (dpf) to assess possible latent effects. The five-day

(plus nine days for observation) chronic value for growth-based endpoints, including body

length, was an MATC of 0.6325 mg/L (NOEC = 0.2 mg/L; LOEC = 2.0 mg/L), but the MATC

for swimming activity, a non-apical endpoint, was reported as 0.06325 mg/L (NOEC = 0.02

mg/L; LOEC = 0.2 mg/L). The reported chronic values based on growth and swimming activity

were not considered quantitatively acceptable because of the relatively brief chronic (i.e., 5-day)

exposure duration compared to other acceptable acute exposures that indicated *D. rerio* was

tolerant to brief (i.e., 96-hours) PFOA exposures.

4.2.2.2.5 Genus: Oryzias (medaka)

Ji et al. (2008) evaluated the chronic toxicity of PFOA (CAS # 335-67-1, purity not

provided) to the Japanese medaka, *Oryzias latipes*, via renewal unmeasured exposures. For the

F0 fish exposure study, breeding medaka pairs were exposed to PFOA for 14 days. Eggs were

counted every day, and the eggs spawned on the seventh day were saved for the F1 generation

exposure study. For the F1 fish exposure study, fertilized eggs collected from F0 fish were

exposed until all living embryos had hatched. 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 from each treatment group were transferred to beakers without PFOA for observation

through 100 days post hatch. The F0 (parental generation) adult survival, condition factor and

adult male and female GSI and HSI 14-day LOECs were all >10 mg/L PFOA. For the F1 (progeny generation), the LOEC for larval survival was 0.1 mg/L, while the corresponding NOEC was considered <0.1 because effects were observed in the lowest concentration tested. This test was not used quantitatively because uncertainties associated with the responses across the range of concentrations tested. In many instances, the authors did not report increasing chronic effects with increasing concentrations that differed by an order of magnitude. Additionally, endpoints associated with longer term effects to juveniles were also rejected because of pseudoreplication resulting from a lack of replicates in the hatching stage. Since this test is a static-renewal unmeasured test, the EPA chose to rely exclusively on the test by Lee et al. (2017) to derive the SMCV for this species since Lee et al. (2017) was a flow-through measured test with fewer concerns pertaining to test design (i.e., no pseudoreplication) and results (lack of increasing effects despite a 10-fold increase in exposure concentrations).

4.3 Acute to Chronic Ratios

When sufficient empirical data are not available, the 1985 Guidelines allow the use of a Final Acute-to-Chronic Ratio (FACR) to convert the FAV to a FCV as an alternative approach to derive the chronic criterion (U.S.EPA 1985). An ACR approach was not used for the derivation of the chronic freshwater PFOA criterion, which was derived from empirical chronic data with all of the eight MDRs met. For illustrative purposes only, 11 individual ACRs for five invertebrate species, and one amphibian could be calculated from the quantitatively acceptable acute and chronic toxicity data (Appendix A and Appendix C). Appendix I includes the ACRs for freshwater aquatic species with quantitatively acceptable acute values for which comparable quantitatively acceptable chronic 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.

Individual ACRs ranged from <4.229 to 3,493 across all species and SMACRs ranged from <4.299 to 3,493. Except for *D. magna*, all SMACRs consisted of a single ACR. *Lithobates catesbeiana* had the largest ACR (i.e., 3,493). The denominator of the *L. catesbeiana* ACR was a LOEC where authors reported a significant effect to snout vent length (SVL) despite a reduction of only ~7% relative to control responses (Flynn et al. 2019). The ~7% decrease to SVL observed at the LOEC was a relatively mild effect level compared to the denominator (i.e., chronic value) of most other PFOA ACRs, with chronic effect levels typically being EC_{10} values or MATCs that had corresponding LOECs that produced a >10% effect. Consequently, the relatively mild effect to SVL observed by Flynn et al. (2019) may have contributed to an artificially high ACR relative to the other PFOA ACRs available.

Daphnia carinata had the second highest ACR (2,113) and the denominator was based on a MATC, with the corresponding NOEC and LOEC (which reduced reproduction by ~40%) that differed by a factor of 10 (Logeshwaren et al. 2021). The 10X difference between the *D. carinata* NOEC and LOEC published by Logeshwaran et al. (2021) likely contributed to an artificially low MATC that, in turn, produced an artificially high ACR.

The mayfly, *Neocloeon triangulifer*, had the lowest non-definitive ACR (<4.229). The 96-hour LC_{50} of 13.05 mg/L was divided by the highest chronic test concentration from Soucek et al. (2023) that did not result in any adverse survival, weight or emergence effects at test termination (i.e., NOEC > 3.085 mg/L). The relatively high acute sensitivity of this species produced a low ACR compared to other test organisms.

Four out of the five *D. magna* ACRs ranged from 14.50 to 69.08, with the remaining ACR from Lu et al. (2016) being 1,602. The *D. magna* ACR from Lu et al. (2016) was removed from the SMACR calculation because it was an order of magnitude greater than other ACRs for the species. Overall, the range of SMACRs was greater than a factor of 100 and there was not an apparent relationship between SMACRs and SMAVs. The 1985 Guidelines do not provide for calculation of a FACR under these circumstances.

4.4 Tissue-based Toxicity Studies Compared to the Chronic Tissue-based Criteria

Tissue-based PFOA toxicity data were reported for four species (three fish and one frog species) across five publications, all of which were classified as qualitatively acceptable. Feng et al. (2015) conducted a 96-hour study with juvenile goldfish (*Carassius auratus*) and observed no effects of PFOA on mortality or antioxidant enzyme activity in the highest aqueous PFOA treatment concentration (4.931 mg/L, measured), which corresponded to liver, gill, and muscle PFOA concentrations of 17.11, 35.13, and 6.07 mg/kg dry weight, respectively.

Giari et al. (2016) measured PFOA in several tissues of two-year-old common carp (*Cyprinus carpio*) exposed to nominal PFOA water concentrations of 0.0002 mg/L and 2 mg/L, plus a control, for 56 days. Corresponding tissue PFOA average concentrations in blood, liver, and muscle in fish from the 2 mg/L treatment after 56 days were 0.0649, 0.0281, and 0.0075 mg/kg wet weight, respectively. No effects of mortality, condition factor, hepatic somatic index (HSI) or gonadal somatic index (GSI) were observed. Manera et al. (2017) performed a separate study that replicated the study design of Giari et al. (2016), in which two-year-old common carp (*Cyprinus carpio*) were exposed to nominal PFOA water concentrations of 0.0002 mg/L and 2 mg/L, plus a control, for 56 days. PFOA was measured in liver, and average concentrations in fish exposed to 2 mg/L for 56 days was 0.0284 mg/kg wet weight, similar to Giari et al. (2016).

PFOA in the livers of fish exposed to 0.0002 mg/L and the control were below detection (<0.0002 mg/kg ww). No apical endpoints were reported; however, evidence of degenerative liver morphology in PFOA exposed fish was observed.

Hagenaars et al. (2013) exposed adult zebrafish (*D. rerio*) to aqueous PFOA for 28 days. Several reproductive and biochemical endpoints were measured. Whole-body PFOA concentrations in the highest concentration (1 mg/L PFOA, nominal) after 28 days averaged 0.550 mg/kg wet weight in males and 0.301 mg/kg wet weight in females. No statistically significant differences were observed in reproductive endpoints (total egg production, fertilized egg production, and hatching rate) for any treatment levels compared to controls. Statistically significant effects were observed among non-apical endpoints. Decreased whole body glycogen content was lower in male and female fish across all exposure treatments, and liver mitochondrial electron transport activity was lower in males exposed to the highest PFOA concentration. Differences in several liver proteins of PFOA exposed males and females were also observed.

Hoover et al. (2017) exposed juvenile (Gosner stage 26) northern leopard frogs (*Lithobates pipiens*, formerly, *Rana pipiens*) to three PFOA concentrations (0.011, 0.109, and 1.11 mg/L PFOA, respectively) for 40 days. Survival, growth (snout-vent length), and developmental time were measured. Whole body PFOA concentrations in frogs exposed to the highest aqueous treatment level averaged 3.61 mg/kg dry weight after 40 days. 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 criterion, 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). The resulting whole-body concentration at the highest treatment level after 40 days was 1.01 mg/kg wet weight. No effects of PFOA on mortality, growth, or development time were reported.

Tissue PFOA concentrations reported in all but one of the qualitative studies were lower than the chronic freshwater tissue-based criteria (i.e., invertebrate whole-body criterion = 1.18 mg/kg ww; fish whole-body criterion = 6.49 mg/kg ww; fish muscle criterion = 0.133 mg/kg ww). The exception was reported by Feng et al. (2015), where the 96-hour muscle-based NOEC (i.e., 6.07 mg/kg dw) was greater than the muscle tissue criterion magnitude of 0.133 mg/kg. However, the liver- and muscle-based NOECs reported by Feng et al. (2015) were from an acute duration (96-hour exposure), whereas the tissue-based values were derived to protect species from longer-term chronic exposures, where effects to sensitive species at concentrations lower than the whole body-based NOEC reported by Feng et al. (2015) may occur. In addition, the tissue concentrations were reported by Feng et al. (2015) as dry weight, as tissue specific percent moisture contents were not available. Had the values in Feng et al. (2015) been expressed as wet weight, the magnitude of the differences would have been smaller.

Although all other tissue-based concentrations were lower than the corresponding tissue-based criteria, no statistically significant effects of apical endpoints were observed in any of these studies. Results of these studies do not provide any evidence that the aquatic community will experience unacceptable chronic effects at tissue-based criteria magnitudes.

4.5 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 PFOA and if they were likely to be more sensitive to PFOA than aquatic animals (see Section 4 and Appendix E: Acceptable Freshwater Plant PFOA

Toxicity Studies). Toxicity values for freshwater plants reported in Appendix E were all greater than the chronic freshwater criterion (i.e., 0.10 mg/L PFOA). The lowest effect concentrations reported were all NOECs for the green alga (*Chlorella pyrenoidosa*). All of these were relatively low NOEC values, which do not provide meaningful information about the sensitivity of this alga relative to the chronic water column criterion. However, a definitive EC_{50} for the same species of 190.99 mg/L (Xu et al. 2013) suggests the PFOA criteria magnitudes are protective of *C. pyrenoidosa*. Excluding the relatively low NOECs, effect concentrations for freshwater plants and algae ranged from 5 to 745.7 mg/L relative to animal chronic values of 0.03162 to 88.32 mg/L (Appendix C). Therefore, it was not necessary to develop a criterion based on the toxicity of PFOA to aquatic plants and the PFOA freshwater criteria are expected to be protective of freshwater plants.

4.6 Protection of Threatened and Endangered Species

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

4.6.1 Quantitatively Acceptable Acute Toxicity Data for Listed Species

Quantitatively acceptable acute toxicity test data evaluating the effects of PFOA on threatened and endangered freshwater species were available for rainbow trout (*Oncorhynchus mykiss*) with a SMAV of 4,001 mg/L PFOA (DuPont Haskill Laboratory 2000). The rainbow trout SMAV is more than 1,290 times greater than the recommended acute criterion (CMC) of 3.1 mg/L, indicating the acute criterion is protective of rainbow trout and expected to be

protective of other listed salmonid species. There were no acceptable acute toxicity data for endangered or threatened estuarine/marine aquatic species.

4.6.2 Quantitatively Acceptable Chronic Toxicity Data for Listed Species

Quantitatively acceptable chronic toxicity test data evaluating the effects of PFOA on threatened and endangered freshwater species were available for rainbow trout (*Oncorhynchus mykiss*) with a SMCV of >40 mg/L PFOA (Centre International de Toxicologie 2004; Colombo et al. 2008). The rainbow trout SMCV is 400 times greater than the recommended chronic criterion (CCC) of 0.10 mg/L, indicating the chronic criteria are protective of rainbow trout and other listed salmonid species. There are no acceptable chronic toxicity data for endangered or threatened estuarine/marine aquatic species.

4.6.3 Qualitatively Acceptable Toxicity Data for Listed Species

Focusing on qualitatively acceptable tests with apical endpoints and water column exposures, there available data for the listed Atlantic salmon (*Salmo salar*). Spachmo and Arukwe (2012) and Arukwe et al. (2013) observed no adverse effects for growth at the highest treatment concentration (0.1 mg/L PFOA) following 49-day and 53-day exposures, respectively. For both exposures, the qualitatively-acceptable NOECs were greater than the recommended acute criterion (CCC) of 0.10 mg/L, which further indicates that the chronic criteria are protective of listed salmonid species. There were no qualitative acute or chronic toxicity data for endangered or threatened estuarine/marine aquatic species.

4.7 Summary of the PFOA Aquatic Life Criteria and the Supporting Information

The PFOA 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 PFOA. This Aquatic Life Ambient Water Quality Criteria for

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Perfluorooctanoic acid (PFOA) document includes water column based acute and chronic criteria and tissue-based criteria for fresh waters. Acute and chronic water column criteria magnitudes for estuarine/marine waters could not be derived at this time due to data limitations; however acute estuarine/marine benchmarks are provided in Appendix L. The freshwater acute water column-based criterion magnitude is 3.1 mg/L, and the chronic water column-based chronic criterion magnitude is 0.10 mg/L. The fish whole-body tissue criterion magnitude is 6.49 mg/kg wet weight, the fish muscle tissue criterion magnitude is 0.133 mg/kg wet weight and the invertebrate whole-body tissue criterion magnitude is 1.18 mg/kg wet weight (Table 3-12). Although empirical PFOA toxicity data for estuarine/marine species were not available to fulfill the eight MDRs directly, the EPA included an acute aquatic life benchmark for estuarine/marine environments 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 7.0 mg/L; this value provides information on a concentration that should be protective of aquatic estuarine/marine life from acute PFOA exposures. As noted earlier, the benchmark value has greater uncertainty than the freshwater PFOA criteria, due to the paucity of empirical data of PFOA effects on estuarine/marine organisms.

The EPA evaluated the influence of including non-North American resident species on the acute and chronic criteria magnitudes and concluded their inclusion did not substantiality affect the criteria magnitudes. These PFOA aquatic life criteria are expected to be protective of aquatic life, such as fish and aquatic invertebrates, on a national basis.

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Appendix A   Acceptable Freshwater Acute PFOA Toxicity Studies

A.1   Summary Table of Acceptable Quantitative Freshwater Acute PFOA 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 hours	PFOA >98%	-	25	LC$_{50}$	458	-	**458**	-	Li 2008
Planaria (0.9 cm), *Dugesia japonica*	S, U	96 hours	PFOA >98%	-	25	LC$_{50}$	337[h]	321.8	**321.8**	-	Li 2009
Planaria (0.9 cm), *Dugesia japonica*	S, U	96 hours	PFOA >98%	-	25	LC$_{50}$	337[h]	383.0	**383.0**	383.6	Li 2009
Fatmucket (glochidia, <24 hours), *Lampsilis siliquoidea*	S, M	24 hours	PFOA 96%	8.46	20	EC$_{50}$ (viability)	164.4	-	**164.4**	-	Hazelton et al. 2012, Hazelton 2013
Fatmucket (juvenile, 4-6 weeks), *Lampsilis siliquoidea*	R, M	96 hours	PFOA 96%	8.46	20	LC50	>500	-	>500[f]	164.4	Hazelton et al. 2012, Hazelton 2013
Black sandshell (glochidia, <24 hours), *Ligumia recta*	S, M	24 hours	PFOA 96%	8.46	20	EC$_{50}$ (viability)	161.0	-	**161.0**	-	Hazelton et al. 2012, Hazelton 2013
Black sandshell (juvenile, 4-6 weeks), *Ligumia recta*	R, M	96 hours	PFOA 96%	8.46	20	LC$_{50}$	>500	-	>500[f]	161.0	Hazelton et al. 2012, Hazelton 2013
Pewter Physa (mixed age), *Physella acuta* (formerly, *Physa acuta*)	S, U	96 hours	PFOA >98%	-	25	LC$_{50}$	672[h]	762.0	**762.0**	-	Li 2009
Pewter Physa (mixed age), *Physella acuta*	S, U	96 hours	PFOA >98%	-	25	LC$_{50}$	672[h]	659.9	**659.9**	-	Li 2009
Pewter Physa (mixed age), *Physella acuta*	S, U	96 hours	PFOA >98%	-	25	LC$_{50}$	672[h]	628.3	**628.3**	681.1	Li 2009
Rotifer (<2-hour old neonates), *Brachionus calyciflorus*	S, U[b]	24 hours	PFOA 96%	-	20	LC$_{50}$	150.0	-	**150.0**	150.0	Zhang et al. 2013a
Cladoceran (<24 hours), *Chydorus sphaericus*	S, U	48 hours	PFOA Unreported	-	20	EC$_{50}$ (death/immobility)	91.10[c]	93.17[c]	**93.17[c]**	93.17	Le and Peijnenburg 2013

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 (6-12 hours), *Daphnia carinata*	S, U	48 hours	PFOA 95%	-	21	EC_{50} (death/immobility)	78.2	66.80	**66.80**	66.80	Logeshwaran et al. 2021
Cladoceran (<24 hours), *Daphnia magna*	S, U	48 hours	PFOA ≥97%	-	21	EC_{50} (immobility)	223.6	-	**223.6**	-	Boudreau 2002
Cladoceran (STRAUS-clone 5; 6-24 hours), *Daphnia magna*	S, U	48 hours	APFO 99.7%	-	18-22	EC_{50}	480[d]	-	**480[d]**	-	Colombo et al. 2008
Cladoceran (<24 hours), *Daphnia magna*	S, U	48 hours	PFOA Unreported	-	21	EC_{50} (immobility)	476.52	542.5	**542.5**	-	Ji et al. 2008
Cladoceran (<24 hours), *Daphnia magna*	S, U	48 hours	PFOA >98%	7.82-7.91	25	LC_{50}	181[h]	220.8	**220.8**	-	Li 2009
Cladoceran (<24 hours), *Daphnia magna*	S, U	48 hours	PFOA >98%	7.82-7.91	25	LC_{50}	181[h]	157.9	**157.9**	-	Li 2009
Cladoceran (<24 hours), *Daphnia magna*	S, U	48 hours	PFOA >98%	7.82-7.91	25	LC_{50}	181[h]	207.3	**207.3**	-	Li 2009
Cladoceran (<24 hours), *Daphnia magna*	S, M	48 hours	PFOA 96%	-	20	EC_{50} (death/immobility)	211.6[c]	216.1[c]	**216.1[c]**	-	Ding et al. 2012a
Cladoceran (<24 hours), *Daphnia magna*	S, M	48 hours	PFOA 99%	7	22	LC_{50}	201.85	222.0	**222.0**	-	Yang et al. 2014
Cladoceran (<24 hours), *Daphnia magna*	S, M	48 hours	PFOA >96%	7.0-7.82	20	EC_{50} (immobility)	239	215.6	**215.6**	-	Barmentlo et al. 2015
Cladoceran (<24 hours), *Daphnia magna*	S, U	48 hours	PFOA 98%	-	20	EC50 (death/immobility)	110.7	114.6	**114.6**	-	Lu et al. 2016
Cladoceran (12-24 hours), *Daphnia magna*	S, U	48 hours	PFOA Unreported	6-8.5	20	LC_{50}	120.9[c]	117.2[c]	**117.2[c]**	-	Yang et al. 2019
Cladoceran (<24 hours), *Daphnia magna*	S, U	48 hours	APFO ≥98%	-	20	EC_{50} (death/immobility)	156.9	-	**156.9**	213.9	Chen et al. 2022
Cladoceran (<24 hours), *Daphnia pulicaria*	S, U	48 hours	PFOA ≥97%	-	21	EC_{50} (immobility)	203.7	-	**203.7**	203.7	Boudreau 2002
Cladoceran (<24 hours), *Moina macrocopa*	S, U	48 hours	PFOA Unreported	-	25	EC_{50} (immobility)	199.51	166.3	**166.3**	166.3	Ji et al. 2008
Cladoceran (<48 hours), *Moina micrura*	S, U	48 hours	PFOA ≥98%	-	27	LC_{50}	0.4747	-	**0.4747**	0.4747	Razak et al. 2023

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
Green neon shrimp, *Neocaridina denticulata*	S, U	96 hours	PFOA >98%	-	25	LC_{50}	454[h]	499.7	**499.7**	-	Li 2009
Green neon shrimp, *Neocaridina denticulata*	S, U	96 hours	PFOA >98%	-	25	LC_{50}	454[h]	428.1	**428.1**	-	Li 2009
Green neon shrimp, *Neocaridina denticulata*	S, U	96 hours	PFOA >98%	-	25	LC_{50}	454[h]	375.5	**375.5**	431.5	Li 2009
Mayfly (<24 hr larva), *Neocloeon triangulifer*	S, M	96 hours	PFOA 95%	-	23	LC_{50}	13.45	13.05	**13.05**	13.05	Soucek et al. 2023
Rainbow trout (2.8 cm, 0.21 g), *Oncorhynchus mykiss*	S, M	96 hours	APFO 99.4%	7.1-7.2	11.8	LC_{50}	4,001	-	**4,001**	4,001	DuPont Haskell Laboratory 2000
Zebrafish (embryo), *Danio rerio*	R, U	96 hours	PFOA Unreported	7-8.5	26	LC_{50}	499	-	**499**	-	Ye et al. 2007
Zebrafish (embryo), *Danio rerio*	S, U	96 hours	PFOA ≥97%	7.2-7.5	26	LC_{50}	>500	-	**>500**	-	Hagenaars et al. 2011
Zebrafish (3 mo), *Danio rerio*	S, M	96 hours	PFOA 98%	-	23	LC_{50}	118.82	122.5	**122.5**	-	Zhao et al. 2016
Zebrafish (embryo), *Danio rerio*	S, U	96 hours	PFOA Unreported	7.5	26-28	LC_{50}	24.6	22.77	22.77[g]	-	Corrales et al. 2017
Zebrafish (embryo, 4 hpf), *Danio rerio*	R, U	96 hours	PFOA Unreported	7-7.5	28	LC_{50}	473[h]	548.00	**548.0**	-	Godfrey et al. 2017a
Zebrafish (embryo, 4 hpf), *Danio rerio*	R, U	96 hours	PFOA Unreported	7-7.5	28	LC_{50}	473[h]	508.5	**508.5**	-	Godfrey et al. 2017a
Zebrafish (embryo, 4 hpf), *Danio rerio*	R, U	96 hours	PFOA Unreported	7-7.5	28	LC_{50}	473[h]	547.0	**547.0**	-	Godfrey et al. 2017a
Zebrafish (embryo), *Danio rerio*	R, U	96 hours	PFOA Unreported	-	26	LC_{50}	759	806.6	**806.6**	450.4	Stengel et al. 2017, 2018
Fathead minnow, *Pimephales promelas*	S, U	96 hours	PFOA 95-98%	7.5-7.7	19-20	LC_{50}	843	852.7	**852.7**	-	3M Co. 2000a
Fathead minnow (larva), *Pimephales promelas*	S, U	96 hours	PFOA Unreported	7.5	25	LC_{50}	413.2	-	**413.2**	593.6	Corrales et al. 2017
Bluegill (2.1 cm, 0.228 g), *Lepomis macrochirus*	S, U	96 hours	APFO 99%	6.9-7.4	21.4–22.1	LC_{50}	634	664.0	**664.0**	664.0	DuPont Haskell Laboratory 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
American toad (larva, Gosner stage 26), *Anaxyrus americanus*	S, U	96 hours	PFOA Unreported	-	21	LC_{50}	711[h]	781.4	**781.4**	-	Tornabene et al. 2021
American toad (larva, Gosner stage 41), *Anaxyrus americanus*	S, U	96 hours	PFOA Unreported	-	21	LC_{50}	711[h]	806.6	**806.6**	793.9	Tornabene et al. 2021
Gray treefrog (larva, Gosner stage 26), *Hyla versicolor*	S, U	96 hours	PFOA Unreported	-	21	LC_{50}	557	646.2	**646.2**	646.2	Tornabene et al. 2021
American bullfrog (tadpole, Gosner stage 25), *Lithobates catesbeiana* (formerly, *Rana catesbeiana*)	S, U	96 hours	PFOA Unreported	-	21	LC_{50}	1,004	1,006	**1,006**	-	Flynn et al. 2019
American bullfrog (larva, Gosner stage 26), *Lithobates catesbeiana*	S, U	96 hours	PFOA Unreported	-	21	LC_{50}	1,060	1,035	**1,035**	1,020	Tornabene et al. 2021
Green frog (larva, Gosner stage 26), *Lithobates clamitans* (formerly, *Rana clamitans*)	S, U	96 hours	PFOA Unreported	-	21	LC_{50}	1,070	-	**1,070**	1,070	Tornabene et al. 2021
Northern leopard frog (larva, Gosner stage 26), *Lithobates pipiens* (formerly, *Rana pipiens*)	S, U	96 hours	PFOA Unreported	-	21	LC_{50}	752	751.7	**751.7**	751.7	Tornabene et al. 2021
Wood frog (larva, Gosner stage 26), *Lithobates sylvatica* (formerly, *Rana sylvatica*)	S, U	96 hours	PFOA Unreported	-	21	LC_{50}	999	-	**999**	999	Tornabene et al. 2021
Frog (embryo stage 8.5), *Xenopus sp.*	R, U	96 hours	PFOA Unreported	-	23	LC_{50}	377[c]	**-**	**377[c]**	377	Kim et al. 2013

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 hours	PFOA Unreported	-	21	LC$_{50}$	1,070	-	**1,070**	1,070	Tornabene et al. 2021
Small-mouthed salamander (larva, Harrison stage 40), *Ambystoma texanum*	S, U	96 hours	PFOA Unreported	-	21	LC$_{50}$	474	407.3	**407.3**	-	Tornabene et al. 2021
Small-mouthed salamander (larva, Harrison stage 45), *Ambystoma texanum*	S, U	96 hours	PFOA Unreported	-	21	LC$_{50}$	1,000	-	1,000[f]	407.3	Tornabene et al. 2021
Eastern tiger salamander (larva, Harrison stage 40), *Ambystoma tigrinum*	S, U	96 hours	PFOA Unreported	-	21	LC$_{50}$	752	-	**752**	752	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 PFOA 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 414.07 mg/mmol.

d Concentration of APFO in publication indirectly determined through quantification of the anion (PFO-).

e Values in bold used in the SMAV calculation.

f Only the most sensitive life-stage used in the SMAV calculation.

g Value is considered an outlier and not used in SMAV calculation.

h Author pooled test of life stages.

A.2 Detailed PFOA Acute Toxicity Study Summaries and Corresponding Concentration-Response Curves (when calculated)

The purpose of this section is to present detailed study summaries for tests that were considered quantitatively acceptable for criterion derivation, with summaries grouped and ordered by genus sensitivity. C-R models developed by the EPA that were used to determine acute toxicity values used for criterion derivation are also presented. C-R models included here with study summaries were those for the four most sensitive genera. In many cases, authors did not report concentration-response data in the publication/supplemental materials and/or did not provide concentration-response data upon request by the EPA. In such cases, the EPA did not independently calculate toxicity values and the author-reported effect concentrations were used to derive the criterion.

A.2.1 Most acutely sensitive genus – *Moina*

Ji et al. (2008) performed a 48-hour static, unmeasured acute test of PFOA (CAS # 335-67-1, purity unreported; obtained from Sigma Aldrich, St. Louis, MO) with *Moina macrocopa*. Authors stated the test followed U.S. EPA/600/4-90/027F (2002). *M. macrocopa* used for testing were obtained from brood stock cultured at the Environmental Toxicology Laboratory at Seoul National University, 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 as 16-hour:8-hour, light:dark, the same conditions as the daphnid cultures. Preparation of test solutions was not described. The test involved four replicates of five daphnids each in five unmeasured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 62.5, 125, 250, 500 and 1,000 mg/L. Test temperature was maintained at $25 \pm 1°C$. Authors noted water quality parameters (pH, temperature, conductivity, and dissolved oxygen)

were measured 48-hours after exposure, but the information was not reported. Survival of daphnids in the negative control was not reported, although EPA/600/4-90/027F requires at least 90% survival for test acceptability. The author-reported 48-hour EC_{50} for the study was 199.51 mg/L (95% C.I. = 163.9 – 245.1). The EPA performed C-R analysis for the test. The EPA-calculated EC_{50} was 166.3 mg/L PFOA (95% C.I. = 138.6 – 194.1 mg/L) and was acceptable for quantitative use.

Razak et al. (2023) tested the acute toxicity of perfluorooctanoic acid (PFOA) to *Moina micrura* in a 48 hour static measured experiment. In addition to acute toxicity, the effects of PFOA on heart rate, heart size, and individual size were also examined. PFOA (≥98% purity) analytical standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and PFOA and solvents for making test solutions were purchased from Fisher Scientific (New Jersey, USA). Organisms were obtained from the Aquatic Animal Health and Therapeutics Laboratory (Aquahealth) at the Institute of Bioscience, Universiti Putra Malaysia. Culturing procedures followed International Organisation for Standardization (ISO) procedure 6341:2012. Cultures were kept under a 12:12 light:dark cycle at 27±1°C. Culture water was renewed every two weeks, and culture organisms were fed green algae (*Chlorella vulgaris*) three times weekly. Both culture and test water was filtered (0.2 µm) surface lake water. A stock solution of 100 mg/L PFOA with filtered surface lake water was made just before testing began. Testing methods followed OECD 202 (OECD 2004) with nominal testing concentrations of 10, 25, 50, 75, 100, 250, 500, 750, 1,000, 2,500, 5,000, 7,500, and 10,000 µg/L, plus a control, with four replicates per treatment. Measured concentrations were not reported, but the authors noted they were 94.3±6.1% of nominal on average. Each replicate consisted of 10 neonates (<48 hours old) in 50 mL of solution in a 100 mL beaker, and organisms were not fed during the study. Nonparametric

Kruskal-Wallace tests followed by post-hoc tests were used to calculate significant (P<0.05) differences between controls and treatment concentrations for all endpoints. The lethal effect concentrations (LC_{10}, LC_{50}, LC_{75}, LC_{90}) were calculated using Probit analysis, and the 48-hour LC_{50} value of 474.7 µg/L, or 0.4747 mg/L was determined to be acceptable for quantitative use. C-R data could not be obtained for this test (beyond the visual presentation in the Razak et al. (2023), so the EPA was unable to perform independent C-R analysis.

A.2.2 Second most acutely sensitive genus – *Neocloeon*

Soucek et al. (2023) exposed the parthenogenetic mayfly, *Neocloeon triangulifer*, to PFOA (CAS # 335-67-1, 95% purity) in a 96-hour acute toxicity test. The test was performed under static, non-renewal conditions beginning with <24-hour old larvae. Exposures consisted of five mayfly nymphs per 30 mL polypropylene cup filled with 20 mL test solution. The control and each of six PFOA test concentrations were replicated five times for a total of 25 test organisms per treatment. Nominal test concentrations were 0.0 (control), 1.25, 2.5, 5.0, 10, and 20 mg/L PFOA. Mean measured PFOA concentrations (EPA Analytical Method 1633; LC-MC/MS) were 0.0003 (control), 1.765, 3.896, 8.137, 13.39 and 30.40 mg/L PFOA, respectively. Mayflies were exposed at 23 ± 1°C under a 16:8-hour light:dark cycle and fed live diatom biofilm scraping beginning on day 0. Feeding only occurred on day 0, and the authors indicated that test organisms required food to survive the entire 96-hour exposure, with previous studies demonstrating greater than 80% mortality at 48 hours with no food (Soucek and Dickinson 2015). Percent survival in the control treatment after 96 hours was 100%. A steep concentration-response relationship between percent survival and increasing PFOA test concentration was observed. Survival decreased from 100% at 8.137 mg/L PFOA to 38.5% at 13.39 mg/L, and to 0% at 30.40 mg/L. The EPA-calculated acute LC_{50} (i.e., 13.045 mg/L) was similar to the author-

reported LC_{50} of 13.451 mg/L. The EPA-calculated LC_{50} value (i.e., 13.045 mg/L) was acceptable for quantitative use in deriving the acute freshwater PFOA criterion.

Recent research has led to the development of successful culturing methods for mayflies that are used in laboratory-based toxicity studies (Soucek and Dickinson 2015; Soucek et al. 2023). These resulting toxicity studies have shown that mayflies (including *Neocloeon triangulifer*) are commonly among the most sensitive taxa to different chemical exposures, including PFOA, PFOS, major geochemical ions, pesticides, and heavy metals (Johnson et al. 2015; Kim et al. 2012; Raby et al. 2018; Soucek and Dickinson 2015; Soucek et al. 2020; Soucek et al. 2023; Wesner et al. 2014). The high sensitivity of mayflies to contaminant exposures has also been observed In mesocosm-based experiments (Mebane et al. 2020) and field-based surveys (U.S. EPA 2011). Many of these laboratory-based toxicity tests used mayflies capable of adapting to laboratory settings. It is hypothesized that mayfly species unable to survive in laboratory settings may also be more sensitive to contaminant exposures than the relatively hardy mayfly species (e.g., *N. triangulifer*) commonly used for toxicity testing.

Publication: Soucek et al. (2023)
Species: Mayfly (*Neocloeon triangulifer*)
Genus: *Neocloeon*
EPA-Calculated LC_{50}: 13.045 mg/L (95% C.I. = 12.46 – 13.63 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	10.8557	6.1850	1.7552	0.0792
e	13.4934	0.3271	41.248	$<2.0e^{-16}$

Concentration-Response Model Fit:

Soucek et al. 2023
Neocleon triangulifer
Weibull type 1, 2 para
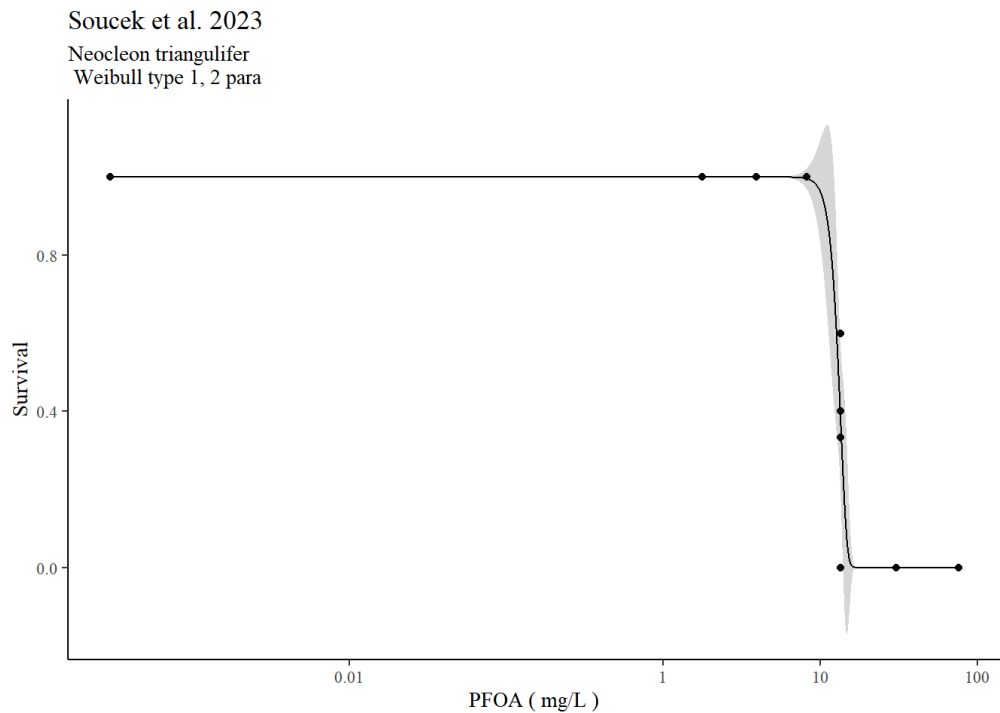
A.2.3 <u>Third most acutely sensitive genus – *Chydorus*</u>

   Le and Peijnenburg (2013) performed a 48-hour static unmeasured test on PFOA

(unreported purity) with the cladoceran, *Chydorus sphaericus*. Authors stated the test followed

the protocol of the "Chydotox toxicity test" developed by the National Institute for Public Health

and the Environment, The Netherlands. In-house cultures of neonates (<24 hours) were exposed

to 250 ML of test solutions in 2 ML vials of unreported material. Each vial contained five

neonates and each test concentration was replicated four times. No solvent was used in the test

solutions with 18-20 test concentrations. *C. sphaericus* was cultured at $20 \pm 1°C$ and a cycle of

16-hour:8-hour light:dark without the addition of food. At test termination vials were shaken

slightly and the mobility of the neonates was determined. The author-reported 48-hour EC_{50} was

0.22 MM PFOA (91.10 mg/L). The EPA performed concentration-response (C-R) analysis for

the test and calculated a LC$_{50}$ of 93.17 mg/L PFOA (95% C.I. = 82.52 – 103.8 mg/L) that was

acceptable for quantitative use.

Publication: Le and Peijnenburg (2013)
Species: Cladoceran (*Chydorus sphaericus*)
Genus: *Chydorus*
EPA-Calculated LC$_{50}$: 93.17 mg/L (95% C.I. = 82.52 – 103.8 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	3.007	0.4165	7.2181	5.27e^{-13}
d	0.9560	0.0248	38.5566	<2.2e^{-16}
e	5.9057	5.9057	15.7767	<2.2e^{-16}

Concentration-Response Model Fit:
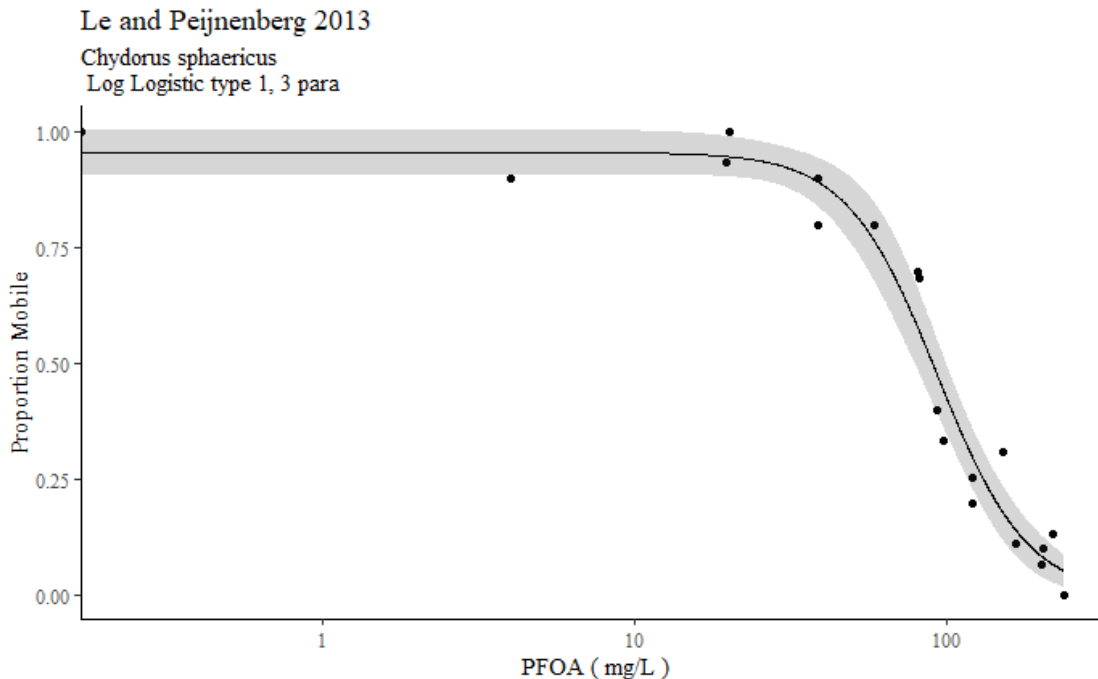
A.2.4 <u>Fourth most acutely sensitive genus – *Daphnia*</u>

Logeshwaran et al. (2021) conducted acute and chronic toxicity tests with the

cladoceran, *Daphnia carinata*, and PFOA (95% 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-hour:8-hour light:dark photoperiod. The acute

test protocol followed OECD guidelines (2000) with slight modifications. A PFOA stock

solution (100 mg/L) was prepared in deionized water. Cladoceran culture medium was used to

prepare the PFOA stock and test solutions. Ten daphnids (six to 12 hours old) were transferred to

polypropylene containers containing one of 14 nominal test concentrations (0, 0.5, 1, 2.5, 5, 10,

20, 30, 40, 50, 100, 150, 200 and 250 mg/L PFOA). Each test treatment was replicated three

times and held under the same conditions as culturing. At test termination (48 hours) immobility

was determined after 15 seconds of gentle stirring. No mortality occurred in the controls. The

author-reported 48-hour EC_{50} was 78.2 mg/L PFOA. The EPA-calculated 48-hour EC_{50} value

was 66.80 mg/L (95% C.I. = 57.10 – 76.50 mg/L), which was acceptable for quantitative use.

Publication: Logeshwaran et al. (2021)
Species: Cladoceran (*Daphnia carinata*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 66.80 mg/L (95% C.I. = 57.10 – 76.50 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.6249	0.1565	10.3860	<2.2e^{-16}
e	83.6974	5.9263	14.1230	<2.2e^{-16}

Concentration-Response Model Fit:

Logeshwaran et al. 2021
Daphnia carinata
Weibull type 1, 2 para
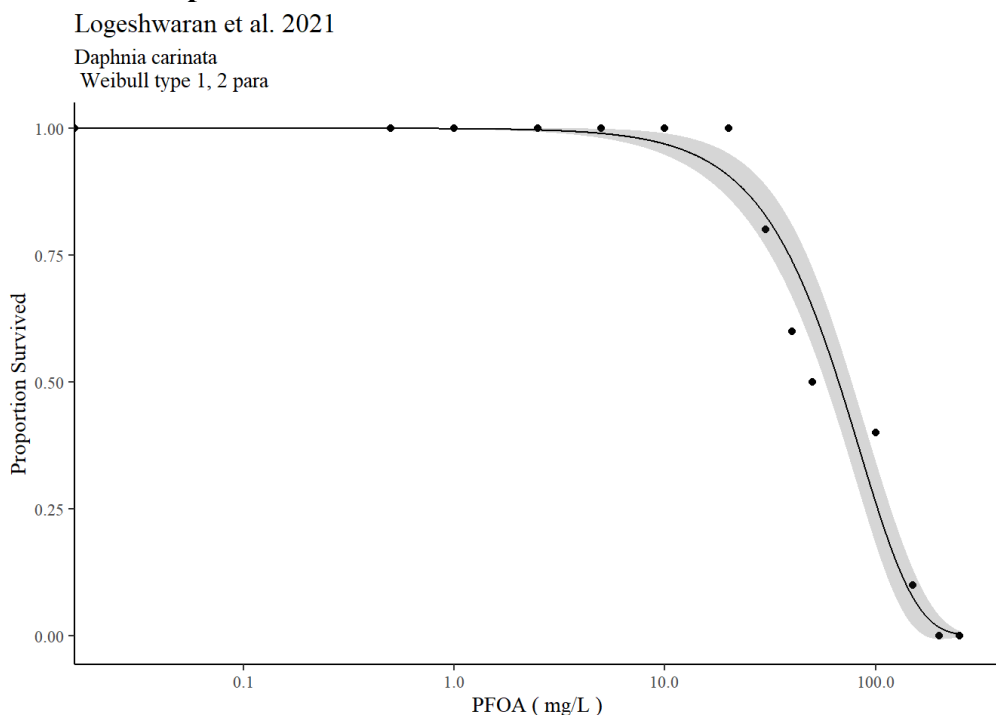
Boudreau (2002) performed a 48-hour static unmeasured test on PFOA (CAS # 335-67-1, ≥97% purity) with *Daphnia magna* and *Daphnia pulicaria* as part of a Master's thesis at the University of Guelph, Ontario, Canada. The results were subsequently published in the open literature (Boudreau et al. 2003). Authors stated 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. Total hardness was softened by addition of distilled deionized water to achieve a range of 200-225 mg/L of $CaCO_3$. Photoperiod was 16-hours of illumination 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 150 mL of test solution. All toxicity testing involved three to four replicates of 10 daphnids each in five unmeasured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 26.3, 52.6, 105, 210 and 420 mg/L. Experiments were conducted in environmental chambers at a test temperature of $21 \pm 1°C$. Authors note that temperature and pH were measured at the beginning and end of the study, but this information is not reported. Mortality of daphnids in the negative control was also not reported, although ASTM E729-96 requires at least 90% survival for test acceptability. The 48-hour *D. magna* EC_{50} reported in the publication was 223.6 mg/L. The 48-hour *D. pulicaria* EC_{50} reported in the publication was 203.7 mg/L.

Publication: Boudreau (2002)
Species: Cladoceran (*Daphnia pulicaria*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: Not calculable, concentration-response data not available
Concentration-Response Model Fit: Not Applicable

Publication: Boudreau (2002)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: Not calculable, concentration-response data not available
Concentration-Response Model Fit: Not Applicable

Colombo et al. (2008) conducted a 48-hour static unmeasured acute test on ammonium

perfluorooctanoate (APFO, CAS # 3825-26-1, 99.7% purity) with the daphnid, *Daphnia magna*.

The authors stated that the toxicity test was conducted following OECD test guideline 202

(1992). Neonates, six to 24-hours old, were acclimated to test conditions for six-hours before test

initiation with test solutions made in reconstituted M4 media. There were four replicates for each

test treatment containing five animals each. Exposure vessel material and size were not reported.

Based on loading, exposure vessels contained at least 100 mL test solution. Nominal test

concentrations were used based on the known stability of the test substance in water. The

nominal test concentrations included control, 100, 178, 316, 562 and 1,000 mg/L. Dissolved

oxygen was >60% saturation and temperature were maintained between 18-22°C. Illumination

involved 16-hours of light with an unreported intensity. No mortality was observed in the

controls. C-R data were available for this acute test; however, the EPA was unable to fit a model

with significant parameters and relied on the 48-hour EC$_{50}$ reported in the study of 480 mg/L,

which was acceptable for quantitative use. The authors note the contribution of ammonia from

APFO exposure indicates that un-ionized ammonia could be a potential contributor to the

observed acute toxicity of APFO. The EPA believes ammonia does not contribute substantively

to the acute toxicity to *D. magna* in this test based on the following rationale. U.S. EPA (2013)

derived a SMAV of 157.7 mg N/L at pH=7 and temperature of 20°C for *D. magna*. Using the

EPA equations, this translates to approximately 30.36 mg N/L at the authors' assumed acute test

pH=8.1 (from their Table 7) and their midrange test temperature of 20°C, which in turn translates

to an un-ionized ammonia concentration of approximately 1.76 mg un-ionized ammonia/L at this

pH and temperature. The authors' Table 7 lists the un-ionized ammonia concentration at their

APFO EC_{50} as 0.73 mg un-ionized ammonia/L, which is 2.4-fold lower than the EPA's SMAV

for D. *magna* re-expressed as an un-ionized ammonia concentration for the test condition.

Therefore, it is unlikely that ammonia contributes substantively to the acute toxicity of APFO to

D. magna in this test, and therefore, the 48-hour EC_{50} is used in the calculation of the SMAV for

this species.

> **Publication:** Colombo et al. (2008)
> **Species**: Cladoceran (*Daphnia magna*)
> **Genus**: *Daphnia*
> **EPA-Calculated LC_{50}**: Not calculable, unable to fit a model with significant parameters
> **Concentration-Response Model Fit**: Not Applicable

Ji et al. (2008) also performed a 48-hour static, unmeasured acute test of PFOA (CAS #

335-67-1, purity unreported; obtained from Sigma Aldrich, St. Louis, MO) with *D. magna*.

Authors stated that the test followed U.S. EPA/600/4-90/027F (2002). *D. magna* used for testing

were obtained from brood stock cultured at the Environmental Toxicology Laboratory at Seoul

National University, 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 as 16-hour:8-hour, light:dark, the same conditions as the daphnid cultures. Preparation

of test solutions was not described. The test involved four replicates of five daphnids each in five

unmeasured test concentrations plus a negative control. Nominal concentrations were 0 (negative

control), 62.5, 125, 250, 500 and 1,000 mg/L. Test temperature was maintained at $21 \pm 1°C$. The authors noted that water quality parameters (Ph, temperature, conductivity, and dissolved oxygen) were measured 48-hours after exposure, but the information was not reported. Mortality of daphnids in the negative control was not reported, although EPA/600/4-90/027F requires at least 90% survival for test acceptability. The author-reported 48-hour EC_{50} for the study was 476.52 mg/L (95% C.I. = 375.3 – 577.7 mg/L). The EPA performed C-R analysis for the test. The EPA-calculated EC_{50} was 542.5 mg/L PFOA (95% C.I. = 461.1 – 623.8 mg/L), which was acceptable for quantitative use.

Publication: Ji et al. (2008)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 542.5 mg/L (95% C.I. = 461.1 – 623.8 mg/L),
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	3.7248	1.8230	2.0432	0.0410
d	0.8985	0.0393	22.8879	$< 2.0\ e^{-16}$
e	598.5588	66.9972	8.9341	$< 2.0\ e^{-16}$

Concentration-Response Model Fit:
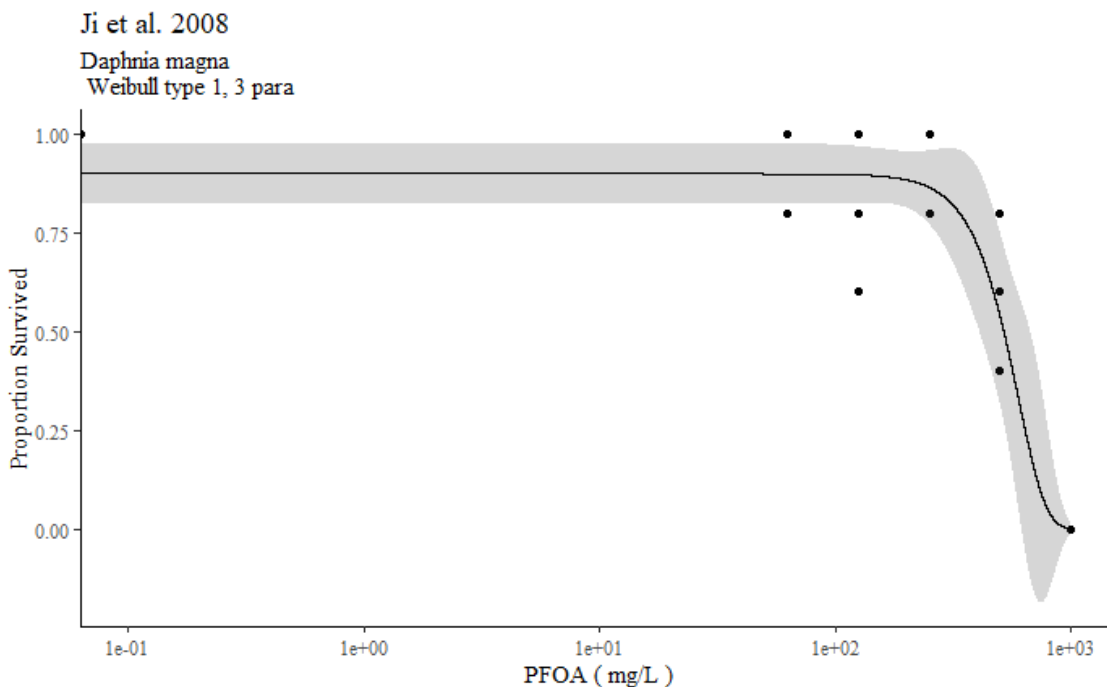
Li (2009) conducted a 48-hour static unmeasured acute test on PFOA (ammonium salt,

>98% purity) with *Daphnia magna*. The authors stated that the test followed OECD 202 (1984)

with slight modifications. *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 five

test concentrations plus a negative control. Based on water solubility of test chemicals and preliminary toxicity results, nominal test concentrations were in the range of 31-250 mg/L for PFOA. The test was conducted in a temperature incubator at $25 \pm 2°C$. Water quality parameters including water Ph, 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 $67.7 \pm 6.8\%$ saturation and $101.8 \pm 6.8 \mu S/cm$, respectively. After the 48-hour testing period, dissolved oxygen and specific conductivity were $55.6 \pm 1.26\%$ saturation (implying 4.56 mg/L) and $109.1 \pm 3.5 \mu S/cm$, respectively. None of the control animals became immobile at the end of the test. The author-reported 48-hour EC_{50} for the study was 181 mg/L (95% C.I.: 166-198 mg/L) which was averaged across three tests. The EPA performed C-R analysis for each individual test. All three tests had acceptable curves with the EPA-calculated EC_{50} values of 220.8 mg/L (95% C.I. = 191.8 – 250.0 mg/L), 157.9 mg/L (95% C.I. = 135.9 – 180.0 mg/L), and 207.3 mg/L (95% C.I. = 176.1 – 238.5 mg/L), which were acceptable for quantitative use.

Publication: Li (2009)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 220.8 mg/L (95% C.I. = 191.8 – 250.0 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	3.2035	0.6834	4.6875	2.766 e^{-6}
e	247.6075	17.6535	14.0260	< 2.2 e^{-16}

Concentration-Response Model Fit:
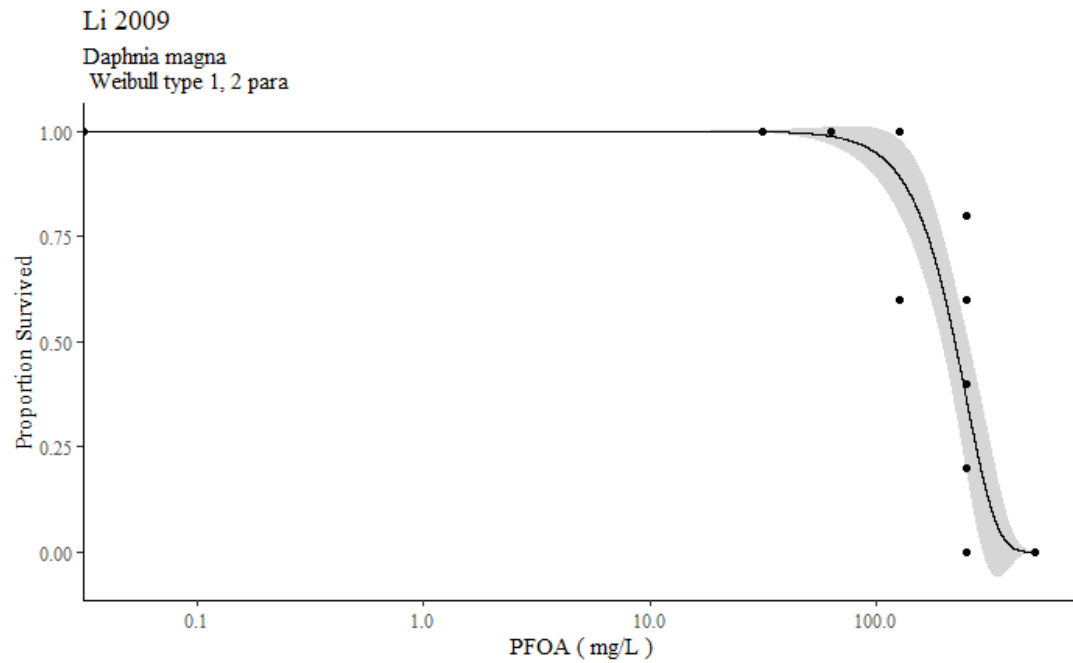
Publication: Li (2009)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 157.9 mg/L (95% C.I. = 135.9 – 180.0 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	2.8137	0.4368	6.4423	1.177 e^{-10}
e	179.9061	12.3471	14.5707	< 2.2 e^{-16}

Concentration-Response Model Fit:
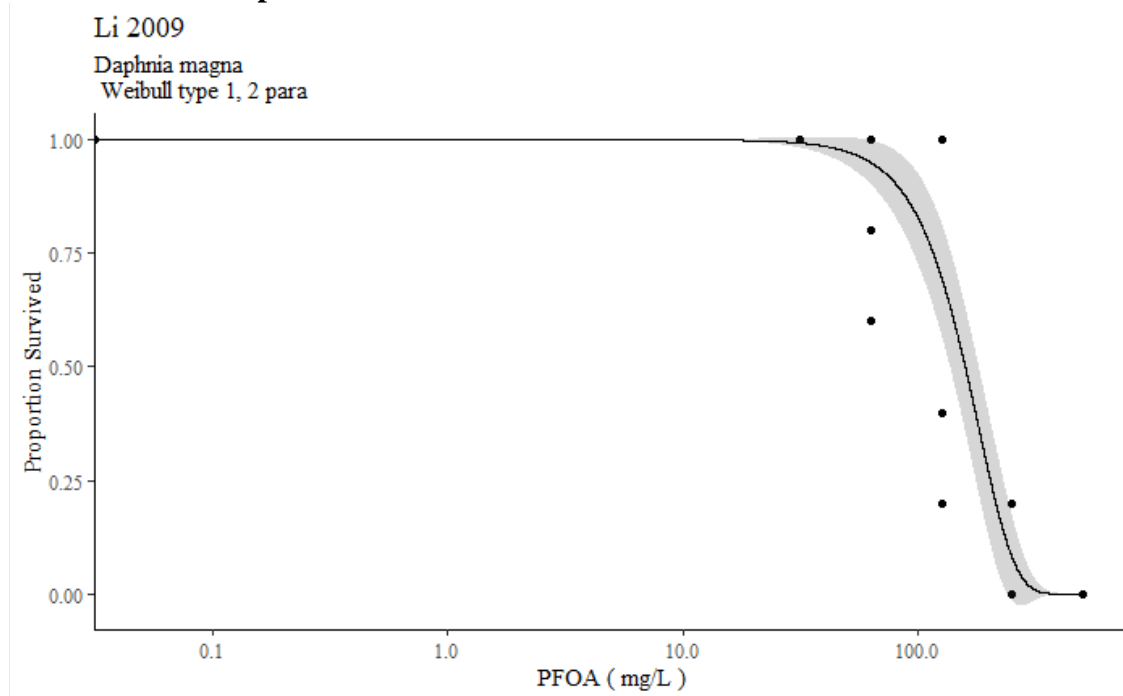
Publication: Li (2009)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 207.3 mg/L (95% C.I. = 176.1 – 238.5 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	2.5732	0.4477	5.7479	9.036 e^{-9}
e	239.0336	19.0886	12.5223	< 2.2 e^{-16}

Concentration-Response Model Fit:

Li 2009

Daphnia magna
Weibull type 1, 2 para
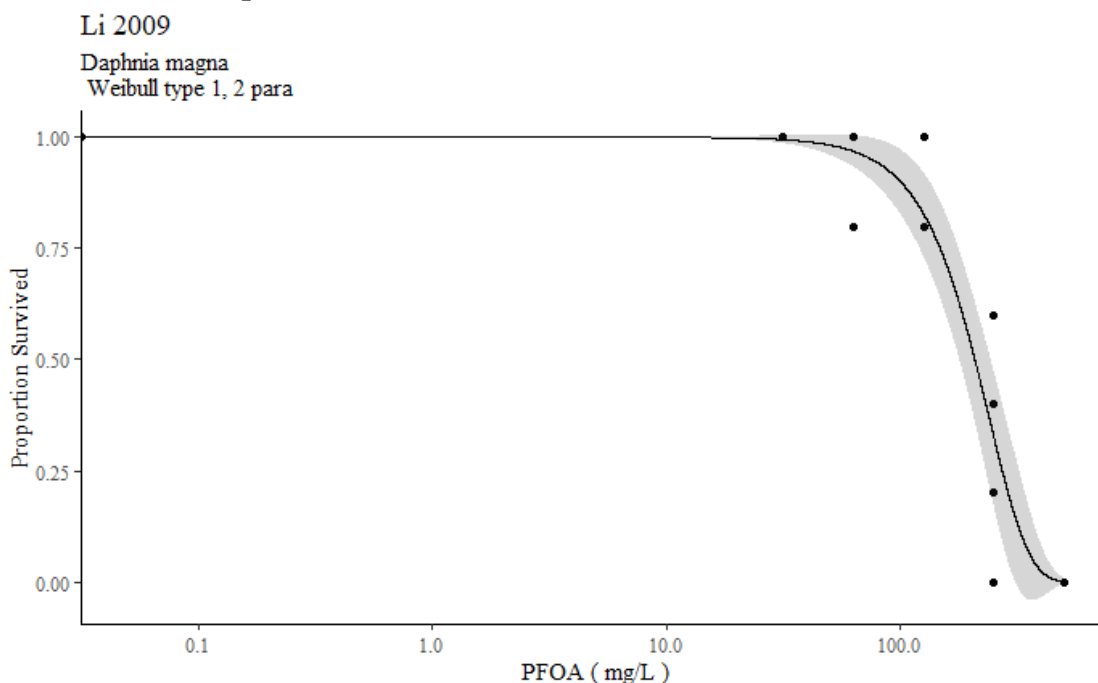
Yang et al. (2014) conducted a 48-hour measured acute test of PFOA (CAS # 335-67-1,

99% purity) with *Daphnia magna*, following ASTM E729 (1993). Although the authors termed

the test conditions "static", they also mentioned PFOA measurements before and after renewal;

based on this distinction the test was assumed to be renewed at least once. Daphnids 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 total hardness,

190.0 ± 0.1 mg/L as CaCO$_3$). The photoperiod consisted of 12-hours of illumination at an

unreported intensity. A primary stock solution was prepared by dissolving PFOA in deionized water and solvent, DMSO, and 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 daphnids 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), 50, 80, 128, 204.8, 327.68 and 524.29 mg/L. The authors provided mean measured concentrations before and after renewal: 49.62 and 43.93 mg/L (lowest concentration) and 526.9 and 476.41 mg/L (highest concentration). Analyses of test solutions were performed using HPLC/MS and negative electrospray ionization. The concentration of PFOA 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 99%. 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 and solvent control mortality were 0% each. The author-reported 48-hour LC_{50} for the study was 201.85 mg/L (95% C.I. = 134.7 - 302.5 mg/L). The EPA performed C-R analysis for the test and had an acceptable curve with an EPA-calculated LC_{50} of 222.0 mg/L PFOA (95% C.I. = 190.5 – 253.5 mg/L). The acute value was acceptable for quantitative use.

Publication: Yang et al. (2014)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 222.0 mg/L (95% C.I. = 190.5 – 253.5 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.1031	0.1773	6.2226	4.89 e^{-10}
e	309.4319	36.3820	8.5051	< 2.2 e^{-16}

Concentration-Response Model Fit:
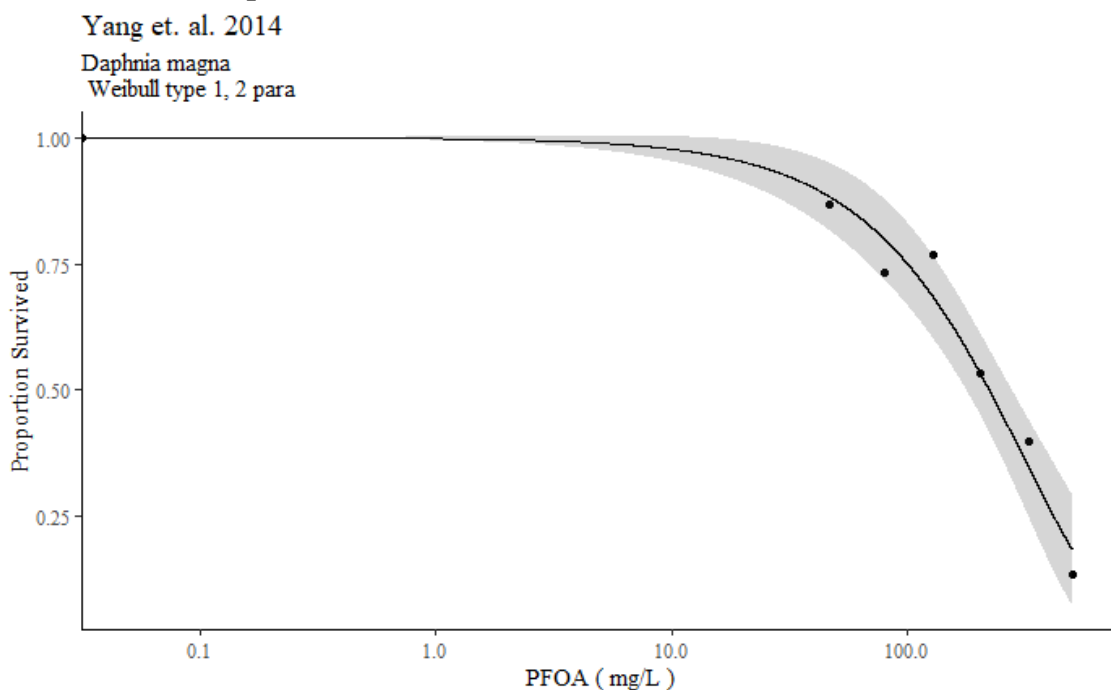
Barmentlo et al. (2015) performed a 48-hour static, measured acute test of PFOA (CAS # 335-67-1, >96%) with *Daphnia magna*. The authors stated the test followed OECD 202 (2004) guidelines for testing. *D. magna* used for testing were obtained from Grontmij, Amsterdam, and cultured in M4 media according to OECD 211 (2012). Test organisms were less than 24-hours old at test initiation. Dilution water was ISO medium. Experiments were conducted in 50 Ml polypropylene tubes with 20 Ml of test solution. The photoperiod consisted of 16-hours of illumination at an unreported intensity. PFOA stock was made with demineralized water. The test involved four to six replicates of five daphnids each in five test concentrations plus a

negative control. Nominal concentrations were not provided, but PFOA was measured in the control, lowest and highest test concentrations. Based on these measurements, the authors interpolated all test concentrations, 0.053 (negative control), 81, 128, 202, 318 and 503 mg/L. Test temperature was maintained at $20 \pm 1°C$, Ph ranged from 7.00-7.82, and D.O. ranged from 8.54-9.42 mg/L. Mortality of daphnids in the negative control was not reported. The author-reported 48-hour EC_{50} for the study was 239 mg/L (95% C.I.: 190 – 287 mg/L). The EPA performed C-R analysis for the test and had an acceptable curve with an EPA-calculated EC_{50} of 215.6 mg/L PFOA (95% C.I. = 181.7 – 249.5 mg/L). The acute value was acceptable for quantitative use.

Publication: Barmentlo et al. (2015)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 215.6 mg/L PFOA (95% C.I. = 181.7 – 249.5 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	2.3893	0.4288	5.5728	2.507 e^{-8}
e	251.3332	19.6475	12.7921	< 2.2 e^{-16}

Concentration-Response Model Fit:
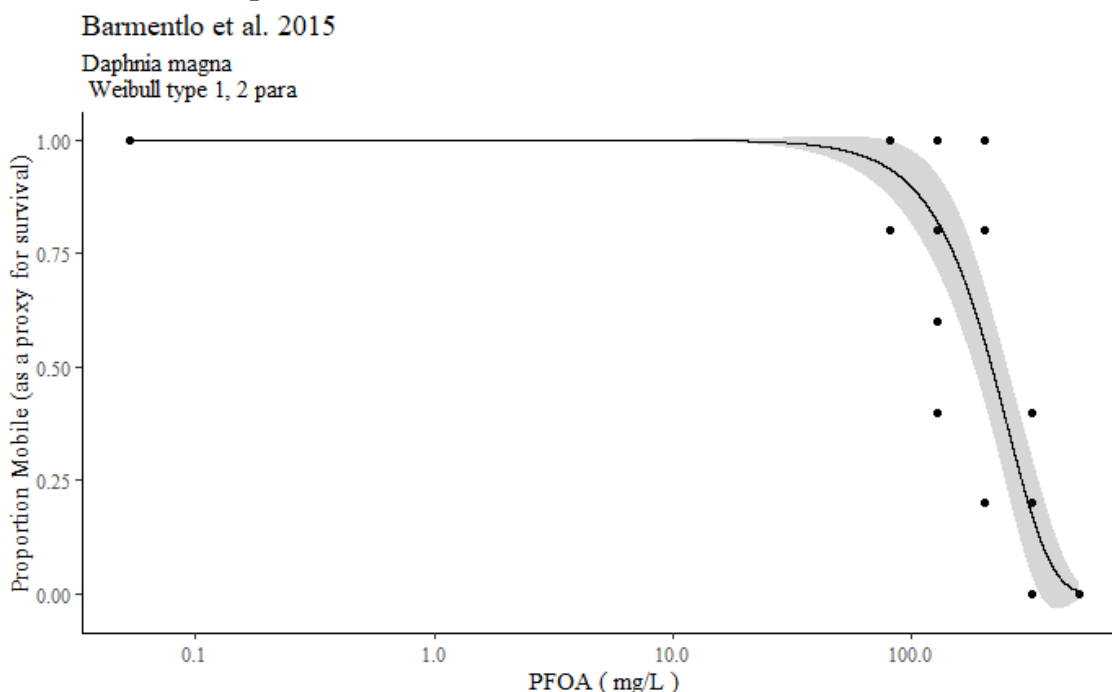
Ding et al. (2012a) conducted a 48-hour static, partially measured acute test on PFOA (CAS # 335-67-1; 96% purity from Sigma Aldrich) with *D. magna*. The test was performed following OECD test guideline 202 (2004) with slight modifications. *D. magna* used for testing were purchased from local suppliers and cultured for two months prior to use. Test organisms were less than 24-hours old at test initiation. Dilution water was M4 solution prepared following the OECD test guideline. The photoperiod consisted of a 16 hour:8-hour light:dark cycle at an unreported light intensity. A primary stock solution was prepared in dilution (reconstituted M4)

water. Exposure vessels were 50 Ml polypropylene disposable tubes containing 20 Ml of test solution. The test involved four replicates of five daphnids each in six test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 0.35, 0.4, 0.45, 0.5, 0.55 and 0.6 Mm PFOA, or 0, 144.9, 165.6, 186.3, 207.0, 227.7 and 248.4 mg/L, respectively, after conversion by multiplying the Mm concentration by a molecular weight of 414.07 g/mol for PFOA. The subsequent concentrations are reported in the converted units of mg/L. Concentrations of PFOA were confirmed in the highest and lowest concentrations, though only nominal concentrations were reported. It was stated that the verified concentration was "well in line with nominal concentrations". Test temperature was maintained at 20 ± 1°C. Observations were made at 24-hours and 48-hours after test initiation. EC_{50} values were reported for both observational time periods. The 48-hour EC_{50} was reported as 211.6 mg/L with the 95% confidence levels of 184.7 - 255.5 mg/L and a NOEC of 207.0 mg/L. The EPA performed C-R analysis for the test. The EPA-calculated EC_{50} was 216.1 mg/L PFOA (95% C.I. = 206.1 – 225.9 mg/L) for *D. magna*, which was acceptable for quantitative use.

(It should be noted that these authors also conducted and reported on a similar acute toxicity test with *Chydorus sphaericus*. However, this test was not used qualitatively or quantitatively because personal correspondence with the authors indicated high control mortality. Raw data provided by the authors did not clearly show 24- and 48-hour EC_{50} data reported in the publication. Data provided by the authors contained many PFOA C-R datasets for *C. sphaericus*. Of these, the C-R dataset with nominal concentrations and 24- and 48-hour EC_{50} values that best matched those in the publication indicated high mortality in control responses. Therefore, the results of this test were not included in the PFOA aquatic life criteria document.)

Publication: Ding et al. (2012a)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 216.1 mg/L PFOA (95% C.I. = 206.1 – 225.9 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	7.5008	1.3478	5.5650	2.621 e^{-8}
e	0.5478	0.0128	42.6800	< 2.2 e^{-16}

Concentration-Response Model Fit:
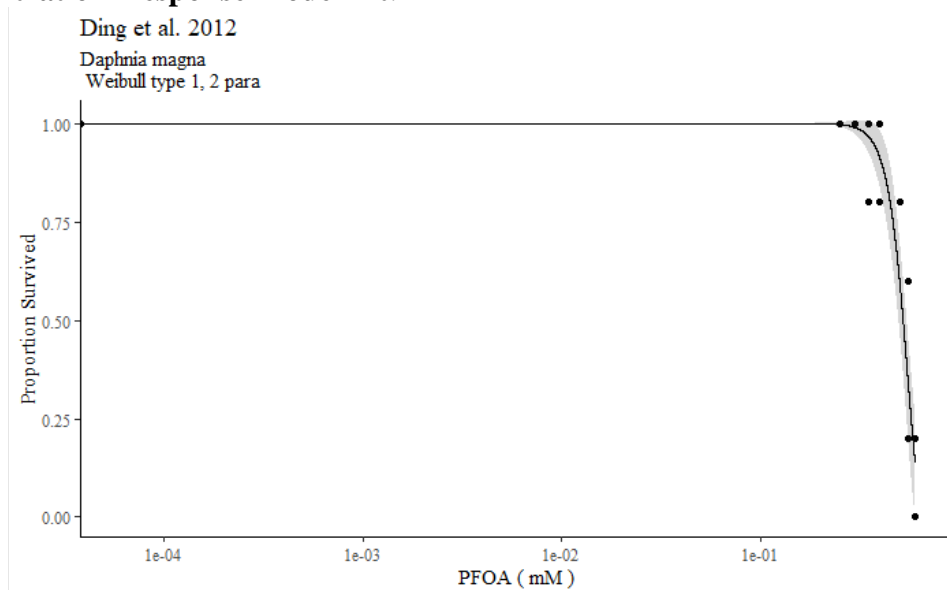
Ding et al. 2012
Daphnia magna
Weibull type 1, 2 para

Lu et al. (2016) evaluated the acute toxicity of PFOA (CAS# 335-67-1, 98% purity, purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) on *Daphnia magna* immobilization. Reconstituted daphnia culture media was used for both culturing and test solution preparation as described in OECD Test Guideline 202. *D. magna* cultures (originally obtained from the Chinese Center for Disease Control and Prevention (Beijing, China) were fed with the green algae *Scenedesmus obliquus* daily, maintained at 20°C and a light/dark photoperiod of 16-hour/8-hour and the medium renewed three times weekly. The 48-hour static unmeasured acute test was conducted via a modified OECD standard test procedure 202, whereby five concentration treatments (3, 10, 30, 100 and 300 mg/L) plus a blank control were

employed. Ten neonates (<24-hours old) from a designated brood were placed in a 100 Ml glass beaker containing 45 Ml test solution for each test concentration and control. Test daphnids were not fed during the testing period and each treatment was replicated three times. The status of immobilization and mortality was checked at 48 hours (daphnids unable to swim within 15 seconds after gentle agitation of the test container are considered to be immobile and those animals whose heartbeats have stopped are considered dead). The authors reported immobility/ survival to be a more sensitive endpoint than survival alone. The author-reported 48-hour EC_{50} for immobility/survival was 110.7 mg/L and the EPA-calculated 48-hour EC_{50} was 114.595 mg/L (95% C.I. = 93.71 – 135.5 mg/L).

Publication: Lu et al. (2016)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 114.595 mg/L (95% C.I. = 93.71 – 135.5 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	0.5649	0.1050	5.3783	7.517 e^{-8}
e	219.2463	68.2427	3.2127	0.0013

Concentration-Response Model Fit:

Lu et al. 2016
Daphnia magna
Weibull type 1, 2 para
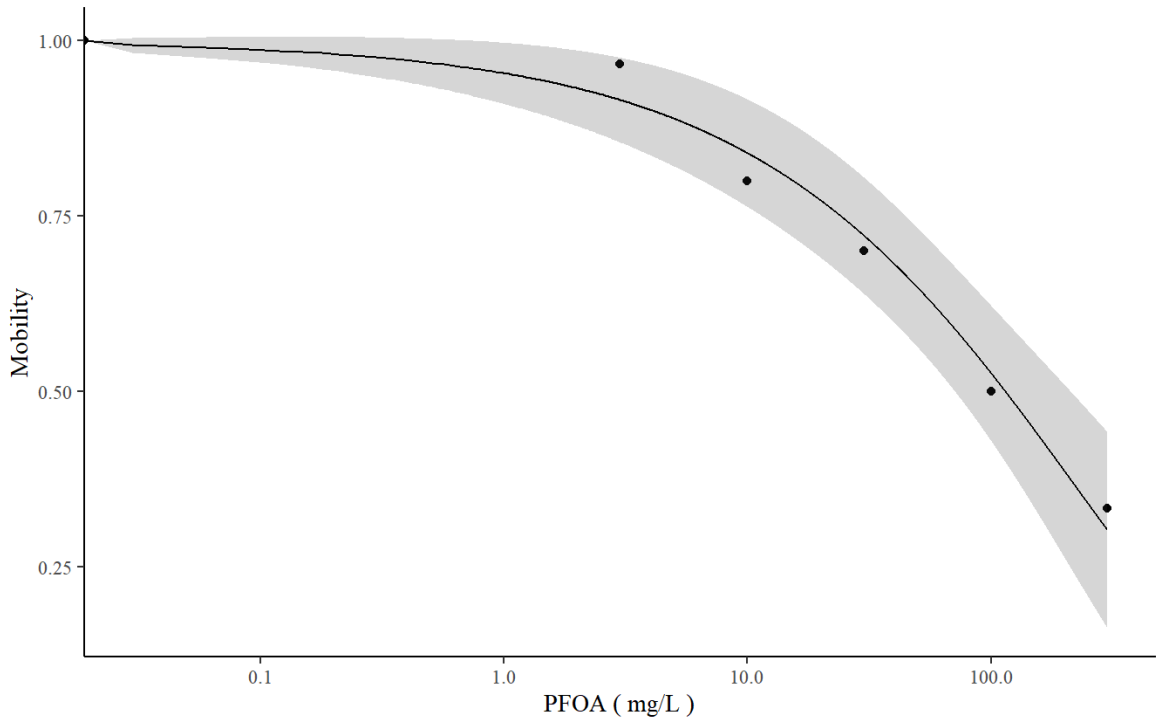
Yang et al. (2019) evaluated the acute effects of PFOA (CAS# 335-67-1, purchased from

Sigma-Aldrich in St. Louis, MO) on *Daphnia magna* via a 48-hour unmeasured static exposure.

D. magna cultures were originally 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 specified in OECD Guideline 202. Protocol for all testing followed

OECD Guideline 202. Cladocerans were cultured in artificial freshwater maintained at 20 ± 1°C

under a 16-hour:8-hour light:dark photoperiod and a light intensity of 1,000-1,500 lux at the surface of the water. Cultures were fed *Scenedesmus obliquus* daily and the water was changed twice weekly. Reported water quality parameters include total hardness of 140-250 mg/L as $CaCO_3$ and Ph of 6-8.5. Acute test concentrations included 0 (control), 0.000161, 0.000193, 0.000232, 0.000278, 0.000334 and 0.000401 mol/L (or 0 (control), 66.67, 79.92, 96.06, 115.1, 138.3 and 166.0 mg/L, respectively, given the molecular weight of the form of PFOA used in the study, CAS # 335-67-1, of 414.07 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 0.000292 mol/L, or 120.9 mg/L PFOA. The EPA-calculated 48-hour LC_{50} was 117.192 mg/L (95% C.I. = 112.2 – 122.2 mg/L).

Publication: Yang et al. (2019)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC$_{50}$: 117.192 mg/L (95% C.I. = 112.2 – 122.2 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	6.0229	1.0808	5.5724	2.512 e^{-8}
d	0.9998	0.0103	96.6600	< 2.2 e^{-16}
e	117.1921	4.8691	24.0685	< 2.2 e^{-16}

Concentration-Response Model Fit:

Yang et al. 2019
Daphnia magna
Log Logistic type 1, 3 para
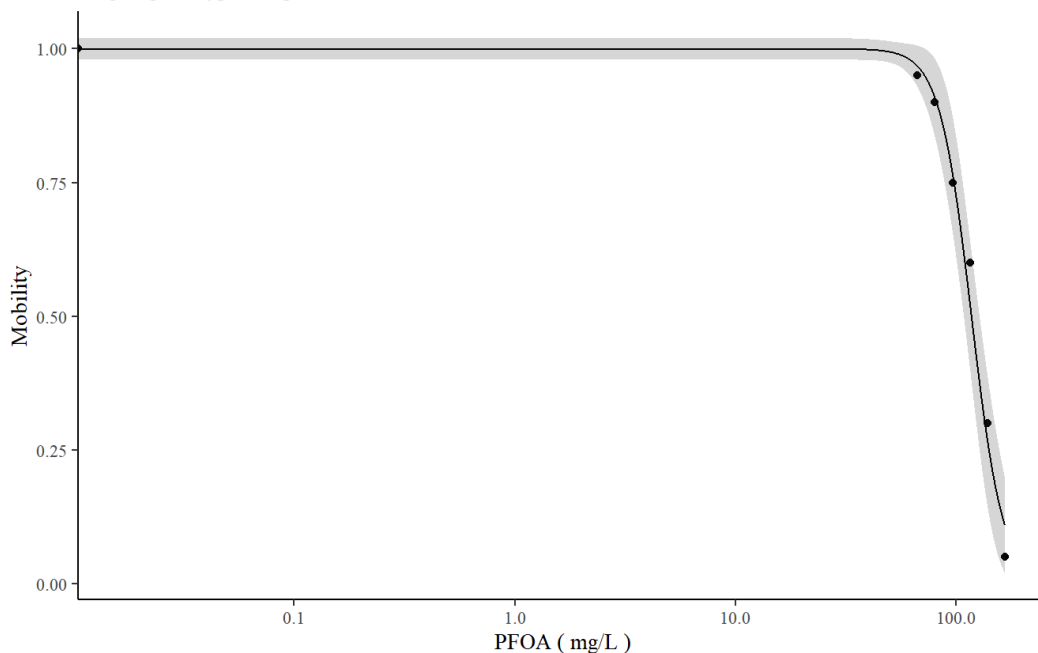
Chen et al. (2022) tested the acute toxicity of ammonium perfluorooctanoic acid (APFO) to *Daphnia magna* in a 48-hour unmeasured, static experiment. In addition, the authors tested the acute effects of pristine and aged polystyrene particles, and the combined effects of these microplastics and AFPO, but only the results of the AFPO exposure are summarized here. AFPO (≥98% purity) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., China. Organisms were purchased from Everbright Algae Species Co., China. They were housed in

culture medium described in method OECD 202 (OECD 2004) at 20±1°C under a 12:12

light:dark cycle at 2000±200 lux. Culture medium was renewed twice a week, and organisms

were fed green algae daily. The acute APFO exposure generally followed test method OECD

202 (OECD 2004). Stock solution of 50 g/L APFO was prepared by mixing the chemical with

ultrapure laboratory water. For each replicate, 10 neonates were exposed to 50 Ml of test

solutions with nominal concentrations of 0, 10, 50, 100, 200, and 500 mg/L APFO, made by

diluting the stock solution with culture medium. There were three replicates per treatment, and

test vessels were housed on a bath oscillator rotating at 150 rpm. Test organisms were not fed

during the experiment. A reference toxicity test was performed with K_2CrO_7. Control mortality

was less than 10% in the AFPO and reference toxicity tests. The LC_{50} and EC_{50} values were

calculated using a logistic model in GraphPad Prism, version 9.2.0, and the 48-hour EC_{50} for

immobility was 156.9 mg/L, which was determined to be acceptable for quantitative use.

A.2.5   Fifth most acutely sensitive genus – *Brachionus*

   Zhang et al. (2013a) performed a 24-hour static test of PFOA (CAS # 335-67-1, 96%

purity) with *Brachionus calyciflorus*. Organisms were neonates 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-hour:8-hour, 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 PFOA (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, six-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), 60, 80, 100, 120, 140, 160,

and 180 mg/L. PFOA 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 PFOA

measured every eight-hours over a 24-hour period in rotifer medium with green algae incurs

minimal change in the concentration range from 0.25 to 2.0 mg/L. The acute test was conducted

without green algae added to the exposure medium. Although this rotifer species has a short life

span, a 24-hour unfed test is not expected to cause starvation and 0% mortality was observed at

test termination in the negative control. The study reported 24-hour LC_{50} was 150.0 mg/L. The

acute value was acceptable for quantitative use.

>
> **Publication**: Zhang et al. (2013a)
> **Species**: Rotifer (*Brachionus calyciflorus*)
> **Genus**: *Brachionus*
> **EPA-Calculated LC$_{50}$**: Not calculable, concentration-response data not available
> **Concentration-Response Model Fit**: Not Applicable

A.2.6   <u>Sixth and seventh most acutely sensitive genera – *Ligumia* and *Lampsilis* (mussels)</u>
Hazelton (2013) and Hazelton et al. (2012) evaluated the acute effects of PFOA (96%

purity) on two freshwater mussels: *Lampsilis siliquoidea* and *Ligumia recta*. Acute toxicity was

observed under static conditions over a 24-hour period (<24-hour old glochidia) or renewal

conditions over a 96-hour period (four to six-week-old juveniles). The authors stated that the

tests followed ASTM E2455-06 (2006). Dilution water was hard reconstituted water.

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, while corresponding mean measured concentrations were less than the limit of

quantification (LOQ, specifics not provided), 0.0051, 0.0484, 0.490, 4.8, 51 and 476 mg/L

PFOA, respectively. Analyses of test solutions were performed at the U.S. EPA National

Exposure Research Laboratory in Research Triangle Park, NC using HPLC/MS. Measured test

concentrations of PFOA were within 10% of target in water from acute tests. Recovery of PFOA

standards ranged from 91.2-108%. For all acute tests, alkalinity ranged from 97 to 110 mg/L as

$CaCO_3$ with a mean of 104.4 mg/L; total hardness ranged from 132 to 162 mg/L as $CaCO_3$ with

a mean of 149.6 mg/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 at 20°C. Mortality of mussels in the negative control

was <10% in all exposures. The 24-hour EC_{50} reported for glochidia of *L. siliquoidea* was 164.4

mg/L (95% C.I.: 116.0 – 232.8 mg/L) and for *L. recta*, 161.0 mg/L (95% C.I.: 135.0 – 192.7

mg/L). The 96-hour LC_{50} values for the juvenile *L. siliquoidea* and *L. recta* were greater than the

highest test concentration (500 mg/L). The study reported 24-hour EC_{50}s for *L. siliquoidea* and

for *L. recta* represent acute values acceptable for quantitative use for the two mussel species. The

juvenile life stage is less sensitive, such that its LC_{50}s were not used quantitatively in SMAVs.

A.2.7   Eighth most acutely sensitive genus – *Xenopus*

Kim et al. (2013) conducted a 96-hour renewal unmeasured assay with perfluorooctanoic

acid (PFOA) using the frog embryo teratogenesis assay – *Xenopus* (FETAX). PFOA stock

solutions were prepared by dissolving PFOA in dimethyl sulfoxide (DMSO), and then diluting in

FETAX medium for exposure solutions (DMSO did not exceed 0.15%). Adult *Xenopus* were

purchased from Nasco (Fort Atkinson, WI) and housed in clear plastic aquariums with

dechlorinated tap water at $18 \pm 2°C$ with a 12-hour light cycle and fed three times a week. Ovulation was induced by injecting 1,000 IU of human chorionic gonadotropin just under the skin of a female in the evening. The next day, females laid eggs in 60 mm plastic dishes. The eggs were immediately fertilized in 0.1X modified Barth solution (MBS) (*Xenopus* testes were obtained from sacrificed males). Following successful fertilization, the jelly coat was removed by swirling the embryos in a 2% L-cysteine solution. The embryos were then transferred to 1X MBS containing 3% Ficoll 400. Unfertilized eggs and dead embryos were removed and maintained at $22 \pm 0.5°C$. Finely cleaved embryos in the blastula stage (stage 8.5) were selected, with 20-25 embryos used per concentration (nominal concentrations of 100, 500, 750, 1000 and 1,250 µM PFOA, or 41.4, 207.0, 310.6, 414.1 and 517.6 mg/L PFOA, respectively). DMSO alone (0.1%) and FETAX medium alone were used as controls. Embryos were incubated at 23°C until the end of the assay. The media were changed every day, and dead embryos were removed. At the end of the experiments, embryo mortality was recorded and surviving embryos were fixed in 4% formaldehyde to check for malformation. Head-tail lengths and malformations were analyzed to measure growth inhibition. The authors reported a 96-hour LC_{50} of 377 mg/L PFOA and the value was acceptable for quantitative use.

A.2.8   <u>Ninth most acutely sensitive genus – *Dugesia*</u>

Li (2008) conducted a 96-hour static, unmeasured acute toxicity test on PFOA (CAS # 3825-26-1, >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. The dilution water was dechlorinated tap water and a primary stock solution of PFOA was prepared in the same dilution water. The photoperiod consisted of 12-hours of illumination at an unreported intensity.

Exposure vessels were polypropylene beakers of unreported dimensions with a 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 100-750 mg/L PFOA. The test temperature was maintained at $25 \pm 1°C$. No other water quality parameters were reported for test solutions. Survival of negative control animals was not reported. The study reported a 96-hour LC_{50} was 458 mg/L (95% C.I. = 427 – 491 mg/L). The acute value was acceptable for quantitative use.

      Li (2009) conducted a second 96-hour static, unmeasured acute test of PFOA (ammonium salt, >98% purity) with *Dugesia japonica.* Again, 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. The dilution water was dechlorinated tap water and a primary stock solution of PFOA was prepared in the same dilution water. The photoperiod consisted of 12-hours of illumination at an unreported intensity. Exposure vessels were made of polyethylene with unreported dimensions and 50 Ml fill volume. The test employed three replicates of 10 planarians each in at least five test concentrations plus a negative control. The test was repeated three times with different test concentrations. Nominal test concentrations were in the range of 150-750 mg/L PFOA. The test temperature was maintained at $25 \pm 2°C$. 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 provided. Organisms were not fed, and no mortality was observed in the control groups in any of the three tests. The author-reported 96-hour LC_{50} was 337 mg/L (95% C.I. = 318-357 mg/L) which was averaged across the three tests. The EPA performed C-R analysis for each individual test. Two of the tests had acceptable

curves with the EPA-calculated LC_{50} values of 321.8 mg/L PFOA (95% C.I. = 290.6 – 353.1 mg/L) and 383.0 mg/L PFOA (95% C.I. = 347.8 – 418.2 mg/L) which were acceptable for quantitative use. The third curve had a poor concentration response and the LC_{50} (427.7 mg/L; 95% C.I. = 251.4 – 604.1 mg/L) was, therefore, not used quantitatively but considered for qualitative use only.

A.2.9 <u>Tenth most acutely sensitive genus – *Neocaridina*</u>

Li (2009) conducted a 96-hour acute test on PFOA (ammonium salt, >98% purity) with the freshwater shrimp species, *Neocaridina denticulata* (a non-North American species). Test conditions were static (no solution renewal), and test concentrations were unmeasured. 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 and were reported to be 1.3 ± 0.2 cm long. 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 organisms each in at least five test concentrations plus a negative control. Each treatment was tested three different times. Nominal test concentrations were in the range of 50-1,000 mg/L PFOA. The test temperature was maintained at $25 \pm 2°C$. 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 is not reported. Mortality of negative control animals was 10% for one treatment, but 0% in others. The author-reported 96-hour LC_{50} reported in the study was 454 mg/L (95% C.I.: 418-494 mg/L) which was averaged across three tests. The EPA performed C-R analysis for each individual test. All three tests had acceptable curves with the EPA-calculated LC_{50}s of 499.7

mg/L (95% C.I. = 457.4 – 542.1 mg/L), 428.1 mg/L (95% C.I. = 396.3 – 459.9 mg/L), and 375.5

mg/L (95% C.I. = 296.5 – 454.4 mg/L), which were acceptable for quantitative use.

A.2.10 <u>Eleventh most acutely sensitive genus – *Danio*</u>

The acute effects of PFOA on the zebrafish, *Danio rerio*, have been reported by

numerous researchers. **Ye et al. (2007)** evaluated the acute effects of perfluorooctanoic acid

(PFOA, purity not reported) on *D. rerio* in a 96-hour static-renewal test with unmeasured

treatment concentrations. The PFOA stock solution of 1,500 mg/L was maintained at a Ph of 8.2

with phosphate buffer and test substances were agitated in the reconstituted water by

ultrasonification. The solutions were stored at 4 ± 1°C. No added solvents were used to

formulate test concentrations from the stock solution. Test fish (AB strain) were obtained from

the School of Life Sciences at Fandan University, Shanghai, China. Breeding fish (1.5 years old)

were fed live brine shrimp twice daily and kept with a light:dark period of 14:10 in aquaria

containing aerated natural water. The Ph ranged from 7 to 8.5 and water temperature was

maintained at 26 ±1°C in test solutions. Embryos were obtained from spawning adults, usually

five male and three female. For each toxicant concentration, 48 embryos were randomly

distributed into each well of 24-well polystyrene multi-well plates, with four eggs per well. Each

well was filled with 2.5 mL of test solution, which was totally renewed daily by transferring

embryos in newly cleaned wells. Nominal exposure concentrations were 0 (control), 1.5, 45, 100,

400, 800 and 1,500 mg/L PFOA. The multi-well plates were kept at 26 ± 1°C, with a

photoperiod of 16:8-hour light:dark. The observations of embryos were made at distinct stages,

which represent important steps of zebrafish development. The author reported 96-hour LC_{50}

was 499 mg/L PFOA. The EPA could not calculate an LC_{50} value because sufficient C-R data

were not reported by Ye et al. (2007). The author-reported LC_{50} value (i.e., 499 mg/L) was

acceptable for quantitative use in deriving the freshwater acute PFOA criterion.

Hagenaars et al. (2011) exposed *D. rerio* embryos to PFOA (CAS #335-67-1, purity ≥97%) under static unmeasured conditions for 120 hours. The PFOA was dissolved in medium-hard reconstituted laboratory water, which was aerated and kept at 26°C until use (no solvent). Adult wildtype 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, with fertilized eggs collected in egg traps within 30 minutes of spawning. Eggs were transferred to the test solutions 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 embryo 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, Ph 7.2-7.5 and subjected to a 14-hour:10-hour, light:dark cycle. A test was considered valid if more than 90% of the controls successfully hatched and showed neither sublethal nor lethal effects. The authors reported a 96-hour LC_{50} of >500 mg/L PFOA and was classified as quantitative.

Zhao et al. (2016) performed a 96-hour static acute PFOA (98% purity; obtained from J&K Scientific) test on zebrafish, *D. rerio*. Authors stated that the test followed OECD Guideline 203. Zebrafish were obtained from Wuhan Institute of Hydrobiology at the Chinese Academy of Science. Test organisms were three months old and 2.5-3.0 cm in length at test initiation. The dilution water used for testing was not provided. Experiments were conducted in 5-liter glass fish

tanks filled with three liters of solution. The photoperiod and preparation of test solutions was not described. The test involved 10 fish in five unmeasured test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 72.3, 86.8, 104.2, 125.0 and 150.0 mg/L. However, nominal concentrations of 50, 100, 200 mg/L PFOA were confirmed by HPLC in a side study without organisms. Measured concentrations in the side study were 80-120 percent of nominal. Test temperature was maintained at $23 \pm 2°C$, but other water quality parameters were not reported. There was no mortality in the negative control at the end of the exposure period. The author-reported 96-hour LC_{50} was 118.82 mg/L (95% C.I. = 107.46 – 134.33 mg/L). The EPA performed C-R analysis for the test and calculated an LC_{50} of 122.5 mg/L PFOA (95% C.I. = 119.1 – 125.8 mg/L), which was acceptable for quantitative use in deriving the acute PFOA freshwater criterion. *D. rerio* embryos (4 hours post-fertilization; hpf) were also subjected to PFOA by **Godfrey et al. (2017a)** in a 96-hour acute toxicity test using static renewal exposures that were not analytically confirmed. Stock solutions were prepared by dissolving PFOA in 1 L of reverse osmosis (RO) water containing 12.5 µL Replenish (Seachem Laboratories Inc.) and then adjusted to neutral Ph (7-7.5). Adult zebrafish, AB wild-type, were maintained at a water temperature of $28 \pm 1°C$ and a photoperiod of 14-h L:10-h D. Fish were fed twice daily, *Artemia* nauplii in the morning and Tetramin in the afternoon, and genders were kept separate overnight at a ratio of 2 males:1 female. Randomly collected embryos (20 per concentration, gastrula stage, 4-hpf) were placed in plastic petri dishes containing 25 Ml of exposure solution for 96-hours at 28°C. Each test consisted of a minimum of two replicates per dose and test solutions were renewed daily (nominal exposure solutions ranged from 250-1,000 mg/L PFOA). The author-reported 96-hour LC_{50} was 473.0 mg/L PFOA which was averaged across four tests. The EPA performed C-R analysis for each individual test. Three tests had

acceptable curves with the EPA-calculated LC_{50}s of 548.0 mg/L (95% C.I. = 530.6 – 565.5 mg/L), 508.5 mg/L (95% C.I. = 471.4 – 545.6 mg/L), and 547.0 mg/L (95% C.I. = 516.0 – 578.0 mg/L), which were acceptable for quantitative use. The fourth test had an unacceptable curve and therefore the EPA-calculated LC_{50} of 560.1 mg/L PFOA (95% C.I. = 556.4 – 563.8 mg/L) was not used.

Stengel et al. (2017, 2018) exposed *D. rerio* embryos to PFOA for 96-hours using renewal unmeasured procedures as specified in OECD (2013) guidelines. PFOA 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 are provided as described previously (Kimmel et al. 1995, 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 were exposed at the latest from 1 hpf in glass vessels, which had been preincubated (saturated) for at least 24 hours, to a series of nominal PFOA dilutions (0, 400, 512, 640, 800 and 1,000 mg/L). After verifying fertilization success, embryos were individually transferred to the wells of 24-well plates, which had been pre-incubated with 2 mL of the test solution per well for 24 hours prior to the test start and kept in an incubator at 26.0 ± 1.0°C under a 14-hour:10-hour light:dark regime. In order to prevent evaporation or cross-contamination between the wells, the plates were sealed with self-adhesive foil. Embryo tests were classified as valid if the mortality in the negative control was ≤10%, and if the positive control (3,4-dichloroaniline) showed mortalities between 20% and 80%. All fish embryo tests were run in three independent replicates. The author-reported 96-hour LC_{50} was 759 mg/L PFOA. The EPA performed C-R analysis for the

test and had an acceptable curve with an EPA-calculated LC_{50} of 806.6 mg/L (95% C.I. = 773.6 – 839.6 mg/L) and was determined to be quantitatively acceptable for criterion derivation.

Corrales et al. (2017) exposed *D. rerio* embryos to PFOA for 96-hours employing static unmeasured procedures (U.S. EPA 2002, OECD 2013). Tropical 5D wild type adult zebrafish were kept at a density of less than four fish per liter in a z-mod recirculating system with water (Ph 7.0, 260 ppm Instant Ocean) maintained at 26-28°C and a 16-hour:8-hour light/dark cycle. Zebrafish were fed twice daily with brine shrimp (*Artemia sp*. Nauplii) and once per day with TetraMin Tropical Flakes. Sexually mature fish were bred to produce embryos for toxicity studies. Glass beakers were used as experimental units; 15 zebrafish embryos in 100 Ml beakers containing 30 ml test solution. Before the start of each experiment, all solutions were titrated to Ph 7.5 following standard methods. General water chemistry measures (e.g., alkalinity, total hardness, dissolved oxygen, and temperature) were also routinely monitored (assume same culture and test physico-chemical test conditions). The author-reported 96-hour LC_{50} was 24.6 mg/L PFOA. The EPA-calculated LC_{50} was 22.77 mg/L PFOA (95% C.I. = 13.30 – 32.20 mg/L), which was acceptable for quantitative use. This LC_{50} value, however, was excluded from derivation of the acute criterion because a comparative assessment between this LC_{50} value and the other seven quantitatively-acceptable zebrafish LC_{50} values available (Ye et al. 2007; Zhao et al. 2016; Godfrey et al. 2017a; Hagenaars et al. 2011; Stengel et al. 2017, 2018), indicated that the LC_{50} reported by Corrales et al. (2017) was an outlier, falling out more than five times lower than the next lowest *D. rerio* LC_{50} value (Zhao et al. 2016) and nearly 20 times lower than the final *D. rerio* SMAV.

A.2.11 Twelfth most acutely sensitive genus – *Pimephales*

The **3M Company (2000a)** conducted a 96-hour static, unmeasured acute toxicity test with the fathead minnow, *Pimephales promelas*, and PFOA (CAS # 335-67-1). The toxicant was

part of the 3M production and was characterized as a mixture of PFOA (95-98% of the compound) and perfluorochemical inert compounds (1-5% of the compound). No specific test protocol was identified. A stock of PFOA was made by dissolving the test substance with NaOH and diluting the stock with carbon-filtered well water to make five test concentrations (690, 750, 810, 870 and 930 mg/L) plus a control (well water only). Exposures were conducted in glass beakers with 5 L of test solution and five fish per beaker (0.5 g/L fish loading). There were two replicates for each treatment. Test conditions throughout the experiment varied little (D.O.: 6.1-7.7 mg/L; Ph: 7.5-7.7; 19-20°C). No mortality occurred in the control and PFOA treatments $\leq$750 mg/L. The author-reported 96-hour LC_{50} was 843 mg/L. The EPA performed C-R analysis for the test. The EPA-calculated LC_{50} was 852.7 mg/L PFOA (95% C.I. = 834.4 – 870.9 mg/L), which was acceptable for quantitative use and was used for criterion derivation.

Corrales et al. (2017) also evaluated the acute toxicity of PFOA to the fathead minnow (*Pimephales promelas*). Embryos were exposed to PFOA for 96-hours employing static unmeasured procedures (U.S. EPA 2002, OECD 2013). Fish were housed in a flow-through system supplied with aged, dechlorinated tap water at a constant temperature of 25 ±1°C under a 16-hour:8-hour light/dark photoperiod. They were fed twice daily with brine shrimp (*Artemia sp*. Nauplii) and TetraMin Tropical Flakes. Individuals were aged to at least 120 days before breeding at which time they were placed in tanks in a 1:4-5 male to female ratio. Embryos were collected, and within 24 hours post hatched larvae were used for toxicity studies. Glass beakers were used as experimental units; 10 fathead minnow larvae were placed in each 500 Ml beaker containing 200 ml test solution. Before the start of each experiment, all solutions were titrated to Ph 7.5 following standard methods. General water chemistry measures (e.g., alkalinity, total hardness, dissolved oxygen, and temperature) were also routinely monitored (assume same

culture and test physico-chemical test conditions). The reported 96-hour LC_{50} was 413.2 mg/L PFOA and was determined to be quantitatively acceptable for criterion derivation.

A.2.12 Thirteenth most acutely sensitive genus – *Hyla*

Tornabene et al. (2021) conducted an acute PFOA (purchased from Sigma Aldrich, Catalog # 171468-25G; purity not provided) toxicity test with the gray treefrog, *Hyla versicolor*. The acute test followed standard 96-hour acute toxicity test guidance (U.S. EPA 2002; ASTM 2017). Frog egg masses were 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. Before test initiation larvae were acclimated to test conditions (21°C and 12-hour:12-hour light:dark photoperiod) for 24 hours. A stock solution of PFOA (2,000 mg/L) was made in UV-filtered well water and diluted with well water to reach test concentrations (ranged from 0-2,000 mg/L PFOA). 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 solution and were not fed during the exposure period. There were nine to 10 replicates for each treatment and no mortality occurred in the controls. The author reported 96-hour LC_{50} was 557 mg/L and the EPA-calculated 96-hour LC_{50} values was 646.2 mg/L (95% C.I. = 588.0 – 704.4 mg/L), which was acceptable for quantitative use. Note, the authors also reported a qualitatively acceptable test for the same species (Gosner stage 40) that is described in Table G.1.

A.2.13 Fourteenth most acutely sensitive genus – *Lepomis*

The **DuPont Haskell Laboratory (2000)** evaluated the acute toxicity of ammonium perfluorooctanoate (APFO, 99% purity) to the bluegill sunfish, *Lepomis macrochirus*. The static unmeasured GLP study exposed 2.1 cm fish for 96 hours (dilution water not identified). Fish

used in this study were not fed approximately 24-hours prior to and during the test. Bluegill sunfish were assigned to the test chambers using random numbers. Nominal APFO concentrations were 262, 328, 410, 512, 640, 800 and 1,000 mg/L. Glass aquaria (20L) containing 10 L of test solution were employed. Positions of test chambers in the water bath used to maintain constant temperature were assigned using random numbers. Ten fish were added to each replicate using random numbers (2 replicates per concentration; total 20 fish per concentration). A photoperiod of 16 hours light (312-344 lux) versus eight hours darkness was employed with 25 minutes of transitional light (<2.15 lux) preceding and following the 16-hour light interval. Observations for mortality and behavioral effects were made daily. All chemical and physical parameters were within expected ranges. Total alkalinity and EDTA total hardness of the dilution water control were 79 mg/L $CaCO_3$ and 76 mg/L $CaCO_3$, respectively. During the test, dissolved oxygen concentrations ranged from 6.7-8.5 mg/L, Ph ranged from 6.9-7.4, and temperature ranged from 21.4-22.1°C. No fish died in the controls. The authors reported a 96-hour LC_{50} of 634 mg/L APFO. The EPA performed C-R analysis for the test and had an acceptable curve with an EPA-calculated LC_{50} of 664.0 mg/L (95% C.I. = 631.4 – 696.7 mg/L), which was determined to be quantitatively acceptable for criterion derivation.

A.2.14 Fifteenth most acutely sensitive genus – *Physella*

Li (2009) conducted a 96-hour static unmeasured acute test on PFOA (ammonium salt, >98% purity) with the snail species, *Physella acuta* (Note: formerly called *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. Snails of mixed ages (shell length 0.6 ± 0.2 cm) were used at test initiation. The dilution water was dechlorinated tap water, and a primary stock solution of PFOA was prepared in the same dilution water. The photoperiod

consisted of 12-hours of illumination at an unreported intensity. Exposure vessels were made of polyethylene with unreported dimensions and 1 L fill volume. The test employed five replicates of six snails each in at least five test concentrations plus a negative control. Nominal test concentrations were in the range of 100-1,000 mg/L PFOA. The test temperature was maintained at $25 \pm 2°C$. 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 is not reported. Organisms were not fed, and no animals died in the control groups. The author-reported 96-hour LC$_{50}$ was 672 mg/L (95% C.I.: 635-711 mg/L) which was averaged across three tests. The EPA performed C-R analysis for each individual test. All three tests had acceptable curves with the EPA-calculated LC$_{50}$s of 762.0 mg/L (95% C.I. = 706.1 – 817.9 mg/L), 659.9 mg/L (95% C.I. = 607.9 – 711.8 mg/L), and 628.3 mg/L (95% C.I. = 582.9 – 673.7 mg/L), which were acceptable for quantitative use.

A.2.15 <u>Sixteenth most acutely sensitive genus – *Ambystoma*</u>

Tornabene et al. (2021) conducted acute toxicity tests with three species of salamanders in the genus *Ambystoma* and PFOA (purchased from Sigma Aldrich, Catalog # 171468-25G; 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 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 salamanders reached Harrison stage 40, defined as when larvae are free swimming and feeding. Before test initiation larvae were acclimated to test conditions (21°C and 12-hour:12-hour light:dark photoperiod) for 24 hours. An additional acute test with Harrison stage 45 small-mouthed salamanders was run to determine if toxicity varied between life stages.

A stock solution of PFOA (2,000 mg/L) was made in UV-filtered well water and diluted with well water to reach test concentrations (ranged from 0-2,000 mg/L PFOA). 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 solution 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 45 small-mouthed salamander, five to 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. Only one salamander larva died in the controls across all tests (eastern tiger salamander test). acute values from the four tests include:

- **Jefferson salamander**: The author-reported 96-hour LC_{50} was 1,070 mg/L. The EPA was unable to fit a C-R model with significant parameters and relied on the author reported value as quantitatively acceptable.

- **Harrison stage 40 small-mouthed salamander**: The author-reported 96-hour LC_{50} was 474 mg/L. The EPA-calculated LC_{50} was 407.3 mg/L (95% C.I. = 303.7 – 0.510.9 mg/L), which was acceptable for quantitative use.

- **Harrison stage 45 small-mouthed salamander**: The author-reported 96-hour LC_{50} was 1,000 mg/L. The EPA was unable to fit a C-R model with significant parameters and relied on the author-reported value as quantitatively acceptable; however, the LC_{50} from this test was more than two times greater than the Harrison stage 40 small-mouthed salamander indicating the Harrison stage 45 was a relatively tolerant life stage. As a result, the LC_{50} from this test was not used in the SMAV calculation for *A. texanum*.

- **Eastern tiger salamander**: The author-reported 96-hour LC_{50} was 752 mg/L. Concentration-response data from this test lacked partial effects and the EPA was

unable to fit a C-R model with significant parameters and relied on the author reported

value as quantitatively acceptable.

A.2.16 <u>Seventeenth most acutely sensitive genus – *Anaxyrus*</u>

Tornabene et al. (2021) conducted acute PFOA (purchased from Sigma Aldrich, Catalog

171468-25G; purity not provided) toxicity tests with the American toad, *Anaxyrus americanus*.

The acute tests followed standard 96-hour acute toxicity test guidance (U.S. EPA 2002; ASTM

2017). The toad egg masses were 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 41 was

conducted to determine if toxicity varied between life stages. Before test initiation larvae were

acclimated to test conditions (21°C and 12-hour:12-hour light:dark photoperiod) for 24 hours. A

stock solution of PFOA (2,000 mg/L) was made in UV-filtered well water and diluted with well

water to reach test concentrations (ranged from 0-2,000 mg/L PFOA). 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 solution and were not fed during the exposure period. The number of replicates varied by

treatment for both tests; 10 replicates for each treatment except the 1,750 mg/L PFOA exposure

which had nine replicates. No mortality occurred in any of the control groups. The authors did

not find a significant difference between the life stages of the American toad, so results of the

two tests were pooled to determine the 96-hour author-reported LC_{50} of 711 mg/L. The EPA-

calculated 96-hour LC_{50} value was 781.4 mg/L (95% C.I. = 748.3 – 814.4 mg/L) for the Gosner

stage 26 test and was 806.6 (95% C.I. = 760.6 – 852.6 mg/L) mg/L for the Gosner stage 41 test,

both of which were quantitatively acceptable for use.

A.2.17 Eighteenth most acutely sensitive genus – *Lithobates*

Flynn et al. (2019) evaluated the acute effects of PFOA (CAS# 335-67-1, purchased from Sigma-Aldrich) on the American bullfrog (*Lithobates catesbeiana*, formerly, *Rana catesbeiana*) during a 96-hour static unmeasured 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 (21ºC and a 12-hour:12-hour light:dark photoperiod). A 2,000 mg/L PFOA stock solution was prepared with reverse osmosis water to produce 12 nominal test concentrations of PFOA [0 (control), 10, 100, 250, 500, 750, 1,000, 1,250, 1,500, 1,750, 2,000 and 2,500 mg/L]. Each test treatment contained 10 replicates with one Gosner Stage 25 tadpole in each 250 Ml plastic tub. Mortality was monitored twice daily. The author reported a 96-hour LC_{50} value of 1,004 mg/L PFOA. The EPA performed C-R analysis for the test and the EPA-calculated 96-hour LC_{50} was 1,006 mg/L (95% C.I. = 992.8 – 1,018 mg/L).

Tornabene et al. (2021) conducted acute PFOA (purchased from Sigma Aldrich, Catalog # 171468-25G; purity not provided) toxicity tests with four species of frogs in the genus *Lithobates* (formerly, *Rana*). Acute tests followed standard 96-hour guidance (U.S. EPA 2002; 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°C and 12-hour:12-hour light:dark photoperiod) for 24 hours. A stock solution of PFOA (2,000 mg/L) was made in UV-filtered well water and diluted with well water to reach test concentrations (ranged from 0-2,000 mg/L PFOA). 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 solution and were not fed during the exposure period. The number of replicates varied by species and treatment; 30 replicates in the control and five to 20 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. Acute values from the four tests include:

- **American bullfrog**: The author-reported 96-hour LC_{50} was 1,060 mg/L. The EPA-calculated LC_{50} was 1,035 mg/L (95% C.I. = 1,020 – 1,049 mg/L), which was acceptable for quantitative use.
- **Green frog**: The author-reported 96-hour LC_{50} was 1,070 mg/L. The EPA was unable to fit a C-R model with significant parameters and relied on the author reported value as quantitatively acceptable.
- **Northern leopard frog**: The author-reported 96-hour LC_{50} was 752 mg/L. The EPA-calculated LC_{50} was 751.7 mg/L (95% C.I. = 713.0 – 790.5 mg/L), which was acceptable for quantitative use.
- **Wood frog**: The author-reported 96-hour LC_{50} was 999 mg/L. The EPA was unable to fit a C-R model with significant parameters and relied on the author reported value as quantitatively acceptable.

A.2.18 <u>Nineteenth most acutely sensitive genus – *Oncorhynchus*</u>

The acute effects of ammonium perfluorooctanoate (APFO, 99.4% purity) to *Oncorhynchus mykiss* were investigated by researchers at the **DuPont Haskell Laboratory (2000)**. The static measured GLP study exposed 2.8 cm fish for 96-hours (dilution water not

identified). Rainbow trout used in this study were not fed approximately 29-hours prior to and during the test. Rainbow trout were assigned to the test chambers using random numbers. The addition of fish to the test solutions was initiated approximately 41 minutes after test solution mixing was completed. Mean measured concentrations of ammonium perfluorooctanoate were 554, 1,090, 2,280, 4,560 and 9,360 for the 625, 1,250, 2,500, 5,000 and 10,000 mg/L nominal dose levels, respectively (measured directly by high performance liquid chromatography/tandem mass spectrometry). Control solutions showed no detectable concentrations of ammonium perfluorooctanoate. All test substance solutions were clear and colorless with no insoluble test substance present during the test. Test chambers were stainless steel aquaria that held approximately 9 L of test solution. Two replicate test chambers were used per test concentration with 10 fish in each chamber (total of 20 fish per concentration). Each chamber was covered with a glass plate to prevent fish from escaping. Mortality and behavioral observations were made at test start, every 24-hours thereafter, and at test end. All chemical and physical parameters for the definitive test were within expected ranges. Total alkalinity and EDTA total hardness of the dilution water control were 49 mg/L $CaCO_3$ and 122 mg/L $CaCO_3$, respectively. During the test, dissolved oxygen concentrations ranged from 7.5-11.2 mg/L, pH ranged from 7.1-7.2, and mean temperature was 11.8°C (range 11.6-12.1°C). A photoperiod of 16 hours light (approximately 199-450 lux) and eight hours darkness was employed, which included 30 minutes of transitional light (11-157 lux) preceding and following the 16-hour light interval. The authors reported a 96-hour LC_{50} of 4,001 mg/L APFO and no mortality or sublethal effects were observed at concentrations ≤2,500 mg/L APFO. Unlike Colombo et al. (2008), DuPont Haskell Laboratory (2000) did not observe any ammonia toxicity resulting from the APFO test salt, perhaps a result of the relatively lower pH and temperature used by DuPont Haskell Laboratory

(2000), which decreased the proportion of un-ionized ammonia. Results of this study were considered quantitatively acceptable for use in criterion derivation.

Appendix B   Acceptable Estuarine/Marine Acute PFOA Toxicity Studies

B.1   Summary Table of Acceptable Quantitative Estuarine/Marine Acute PFOA 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)[e]	Species Mean Acute Value (mg/L)	Reference
Purple sea urchin (embryo), *Strongylocentrotus purpuratus*	S, M	96 hours	PFOA 95%	-	15	30	EC50 (normal development)	19	20.63	**20.63**	20.63	Hayman et al. 2021
Mediterranean mussel (larva), *Mytilus galloprovincialis*	S, U	48 hours	PFOA Unreported	7.9-8.1	16	36	EC50 (malformation)	>1	-	>1[c]	-	Fabbri et al. 2014
Mediterranean mussel (embryo), *Mytilus galloprovincialis*	S, M	48 hours	PFOA 95%	-	15	30	EC50 (normal and surviving)	9.98	17.58	**17.58**	17.58	Hayman et al. 2021
Mysid (3-days old), *Americamysis bahia*	S, M	96 hours	PFOA 95%	-	20	30	LC50	24	-	**24**	24	Hayman et al. 2021
Mysid (neonate, <24 hours), *Siriella armata*	S, U	96 hours	PFOA 96%	-	20	-	LC50	15.5	-	**15.5**	15.5	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] Values in bold used in the SMAV calculation

B.2 Detailed Study Summaries of Acute Saltwater PFOA Toxicity Studies Considered for Use in Saltwater Criterion Derivation

The purpose of this section is to present detailed study summaries for acute estuarine/marine tests that were considered quantitatively acceptable for criterion derivation, with summaries grouped and ordered by genus sensitivity. Unlike Appendix A.2 and Appendix C.2, the EPA-calculated C-R models were not presented below for the four most sensitive estuarine/marine genera because an estuarine/marine criterion was not developed exclusively based on these empirical data. Rather, an estuarine/marine benchmark was derived using a NAM, which is further described in Appendix L.

B.2.1 Most acutely sensitive estuarine/marine genera – *Siriella* (mysid)

Mhadhbi et al. (2012) performed a 96-hour static, unmeasured acute test with PFOA (96% purity) on the mysid, *Siriella armata*. A stock solution of PFOA was made either with filtered sea water from the Ria of Vigo (Iberian Peninsula) for low exposure concentrations, or with DMSO for high PFOA concentrations (a final maximum DMSO concentration of 0.01% (v/v) in the test medium). However, the authors do not indicate what is considered a high-test concentration, so it is unclear which test concentrations actually used DMSO as a solvent. If DMSO was used, a solvent control was also included. Mysids were exposed to one of ten nominal PFOA treatments (0.1, 0.5, 1, 2, 5, 10, 20, 30, 40 and 80 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$_{50}$ reported in the study was 15.5 mg/L

PFOA and was acceptable for quantitative use.

B.2.2   <u>Second most acutely sensitive estuarine/marine genus – *Mytilus* (mussel)</u>

The acute toxicity of perfluorooctanoic acid (PFOA, purity not provided) on the

Mediterranean mussel, *Mytilus galloprovincialis,* which occurs in California and other parts of

the Pacific Northwest (Green 2014), was evaluated by **Fabbri et al. (2014)**. 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 $16 \pm 1°C$. It is 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-hour:8-

hour light:dark photoperiod and exposed to one of six nominal PFOA concentrations (0.00001,

0.0001, 0.001, 0.01, 0.1 and 1 mg/L) or controls. The PFOA stock was made with ethanol, and

ASW control samples run in parallel. This 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 of >75% (ASTM 2004). Authors noted that controls had ≥80% normal D-larvae

across all tests. PFOA was only measured once in one treatment which was similar to the

nominal concentration, 0.000081 mg/L versus the nominal concentration of 0.0001 mg/L. PFOA

was below the limit of detection in the control ASW (0.04 ng/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

50% 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 PFOA (CAS # 335-67-1, 95% purity, purchased from Sigma-Aldrich, St. Louis, MO) on the Mediterranean mussel, *Mytilus galloprovincialis*. The authors note that the tests followed U.S. EPA (1995b) and ASTM (2004) protocols. Mussels were collected in the field (Sand Diego Bay, CA) and conditioned in a flow-through system at 15°C. Mussels were induced to spawn by heat-shock and approximately 250 embryos (2-cell stage) were added to 20 Ml borosilicate glass scintillation vials with 10 Ml of test solution. There were five replicates per test concentration. Test conditions were 30 ppt, 15°C and a 16-hour:8-hour light:dark photoperiod. Six test solutions were made in 0.45 µm filtered seawater (North San Diego Bay, CA) with PFOA dissolved in methanol. The highest concentration of methanol was 0.02% (v/v). Measured test concentrations ranged from 1.5-52 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-hour EC_{50}, based on normal survival larvae, was 9.98 mg/L PFOA. The EPA-calculated 48-hour EC_{50} value was 17.58 mg/L (95% C.I. = 13.73 – 21.43 mg/L), which was acceptable for quantitative use.

B.2.3 <u>Third most acutely sensitive estuarine/marine genus – *Strongylocentrotus* (sea urchin)</u>
Hayman et al. (2021) report the results of a 96-hour static, measured test on the effects of PFOA (CAS # 335-67-1, 95% purity, purchased from Sigma-Aldrich, St. Louis, MO) on the

purple sea urchin, *Strongylocentrotus purpuratus*. The authors noted that tests followed U.S. EPA (1995b) and ASTM (2004) protocols. Sea urchins were collected in the field (Sand Diego Bay, CA) and conditioned in a 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-hour:8-hour light:dark photoperiod. Six test solutions were made in 0.45 µm filtered seawater (North San Diego Bay, CA) with PFOA dissolved in methanol. The highest concentration of methanol was 0.02% (v/v). Measured test concentrations ranged from 1.5-52 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 (four-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, was 19 mg/L PFOA. The EPA-calculated 96-hour EC_{50} value was 20.63 mg/L (95% C.I. = 19.74 – 21.52 mg/L), which was acceptable for quantitative use.

B.2.4   Fourth most acutely sensitive estuarine/marine genus – *Americamysis* (mysid)
         Hayman et al. (2021) conducted a 96-hour static, measured test to assess effects of PFOA (CAS # 335-67-1, 95% purity, purchased from Sigma-Aldrich, St. Louis, MO) on the mysid, *Americamysis bahia*. The authors noted that tests followed U.S. EPA (2002) 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-hour:8-hour light:dark photoperiod). Six test solutions were made in 0.45 µm filtered seawater (North San Diego Bay, CA) with PFOA dissolved in methanol. The highest concentration of methanol was 0.02% (v/v). Measured test concentrations ranged from 1.1-29 mg/L. The highest test concentration (61.7

mg/L) was reported as nominal only because the sample was mistakenly not sent to the lab for verification. Controls were made in the same seawater and the acute test also included a solvent control. Five mysids (three-days old) were added to 120 Ml polypropylene cups and 100 Ml of test solution 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. No organisms were found dead in the controls at test termination. The EPA was unable to fit a concentration-response model with significant parameters and relied on the author-reported 96-hour LC_{50} of 24 mg/L PFOA as the quantitatively acceptable acute value.

Appendix C   Acceptable Freshwater Chronic PFOA Toxicity Studies

C.1   Summary Table of Acceptable Quantitative Freshwater Chronic PFOA 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
Rotifer (<2-hours old neonates), *Brachionus calyciflorus*	R, U[b]	Up to 200 hours	PFOA 96%	-	20	EC$_{10}$ (intrinsic rate of natural increase)	0.3536	0.5015	**0.5015**	-	Zhang et al. 2013a
Rotifer (<2-hours old neonates), *Brachionus calyciflorus*	R, U[b]	4 days	PFOA 96%	-	20	EC$_{10}$ (intrinsic rate of natural increase)	2.828	1.166	**1.166**	0.7647	Zhang et al. 2014b
Cladoceran (<8 hours), *Ceriodaphnia dubia*	R, M	7 days	PFOA 99.5%	7.72 (7.6-7.78)	24.7 (24.0-25.2)	EC$_{10}$ (neonates/female)	26.2	20.42	**20.42**	-	Kadlec et al. 2024
Cladoceran (<8 hours), *Ceriodaphnia dubia*	R, M	7 days	PFOA 99.5%	7.58 (7.47-7.67)	24.5 (24.0-25.2)	EC$_{10}$ (neonates/female)	25.1	21.69	**21.69**	-	Kadlec et al. 2024
Cladoceran (<8 hours), *Ceriodaphnia dubia*	R, M	7 days	PFOA 99.5%	7.64 (7.59-7.69)	24.9 (23.9-25.8)	EC$_{10}$ (neonates/female)	33.8	29.54	**29.54**	23.56	Kadlec et al. 2024
Cladoceran (6-12 hours old), *Daphnia carinata*	R, U	21 days	PFOA 95%	-	21	MATC (average # of offspring per brood and total # of living offspring)	0.03162	-	**0.03162**	0.03162	Logeshwaran et al. 2021
Cladoceran (STRAUS-clone 5; 6-24 hours old), *Daphnia magna*	R, M	21 days	APFO 99.7%	7.56-8.26	18-22	EC$_{10}$ (average # offspring per starting adult)	29.73	20.26	**20.26**[d]	-	Centre International de Toxicologie 2003; Colombo et al. 2008
Cladoceran, *Daphnia magna*	R, U	21 days	PFOA Unreported	-	21	EC$_{10}$ (# young/starting female)	17.68	7.853	**7.853**	-	Ji et al. 2008
Cladoceran (<24 hours old), *Daphnia magna*	R, U	21 days	APFO >98%	-	20	EC$_{10}$ (# young/starting female)	17.89	12.89	**12.89**	-	Li 2010
Cladoceran (<24 hours old), *Daphnia magna*	R, M	21 days	PFOA 99%	7	22	EC$_{10}$ (survival)	7.02[g]	5.458	**5.458**	-	Yang et al. 2014
Cladoceran (<24 hours old), *Daphnia magna*	S, U	21 days	PFOA 98%	-	20	MATC (growth and reproduction)	0.07155	-	**0.07155**	-	Lu et al. 2016

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 (12-24 hours old), *Daphnia magna*	R, U	21 days	PFOA Unreported	6-8.5	20	EC_{10} (# of offspring)	8.231[f]	8.084[f]	**8.084[f]**	4.317	Yang et al. 2019
Cladoceran (<24 hours old), *Moina macrocopa*	R, U	7 days	PFOA Unreported	-	25	EC_{10} (# young/starting female)	4.419	2.194	**2.194**	2.194	Ji et al. 2008
Amphipod (2-9 days old), *Hyalella azteca*	R, M	42 days	PFOA 96%	8.1	25	EC_{10} (# of juveniles/female)	0.0265	0.147	**0.147**	0.147	Bartlett et al. 2021
Midge (2-day old larvae), *Chironomus dilutus*	R, M	19 days	PFOA 97%	6.8-8.7	20.0-24.0	EC_{10} (survival)	89.8	88.32	**88.32**	88.32	McCarthy et al. 2021
Mayfly (<24 hr larva), *Neocloeon triangulifer*	R, M	23 days	PFOA 95%	-	23	NOEC (survival, weight, emergence)	>3.085	-	**>3.085**	>3.085	Soucek et al. 2023
Rainbow trout (embryo-larval-juvenile), *Oncorhynchus mykiss*	F, M	85 days (ELS)	APFO 99.7%	6.0-8.5	11.1-14.4	LOEC (growth and mortality)	>40	-	**>40[d]**	>40	Centre International de Toxicologie 2004; Colombo et al. 2008
Rare minnow (adult), *Gobiocypris rarus*	F, U	28 days	PFOA 98%	-	25	LOEC (survival)	>30	-	**>30**	>30	Wei et al. 2007
Fathead minnow (<18 hpf), *Pimephales promelas*	R, M	21 days	PFOA 96%	7.4-7.8	25	LOEC (mortality and growth)	>76	-	**>76**	>76	Bartlett et al. 2021
Medaka (adult-F0, embryo-F1, F2), *Oryzias latipes*	F, M	259 days[e]	PFOA Unreported	7.5	25	MATC (F2: sac-fry survival; F0, F1, F2: fecundity)	9.487	-	**9.487**	9.487	Lee et al. 2017
American bullfrog (tadpole, Gosner stage 25), *Lithobates catesbeiana* (formerly, *Rana catesbeiana*)	R, U	72 days	PFOA Unreported	-	21	LOEC (snout vent length)	0.288	-	**0.288**	0.288	Flynn 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 PFOA in the range of concentrations tested under similar conditions. Daily renewal of test solutions.

c Values in bold used in SMCV calculation.

d Concentration of APFO in publication indirectly determined through quantification of the anion (PFO-).

e Total exposure period across F0, F1, and F2 generations.

f Reported in moles, converted to grams based on a molecular weight of 414.07 g/mol.

g Value represents an EC_{10} based on reproduction.

C.2 Detailed PFOA Chronic Toxicity Study Summaries and Corresponding Concentration-Response Curves (when calculated)

The purpose of this section is 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 the EPA that were used to determine chronic toxicity values used for criterion derivation are also presented. C-R models included here with study summaries were those for the four most sensitive genera. In many cases, authors did not report concentration-response data in the publication/supplemental materials and/or did not provide concentration-response data upon request by the EPA. In such cases, the EPA did not independently calculate toxicity values and the author-reported effect concentrations were used to derive the criterion.

C.2.1 <u>Most chronically sensitive genus – *Hyalella*</u>

Bartlett et al. (2021) evaluated the chronic effects of PFOA (CAS# 335-67-1, 96% purity, solubility in water at 20,000 mg/L, purchased from Sigma-Aldrich) on *Hyalella azteca* via a 42-day static-renewal, measured study. Methods for this study were adapted from Borgmann et al. (2007), and organisms were two to nine days old at the test initiation. Experiments were conducted in standard artificial media with water quality characteristics of 52 to 60 mg/L alkalinity as $CaCO_3$, average specific conductivity of 0.41 Ms/cm, dissolved oxygen of 5.2 to 8.8 mg/L, total hardness of 120 to 140 mg/L $CaCO_3$, average Ph of 8.1 and average temperature of 25ºC. A 100 mg/L stock solution was prepared to yield measured test concentrations of 0 (control), 0.84, 3.3, 8.9, 29 and 97 mg/L PFOA. Two separate tests were performed with five replicates per concentration and 20 amphipods per replicate in 2-L HDPE containers filled with 1 L of testing solution, 2.5 mg of ground TetraMin and one piece of 5x5 cm cotton gauze. Test organisms were fed 2.5 mg TetraMin three times a week during weeks one

and two, 5 mg TetraMin three times a week during weeks three and four, and 5 mg TetraMin five times a week during weeks five and six. At test termination (day 42), adults were sexed and weighed, as well as their young counted. The 42-day author-reported LC_{10} value for survival was 23.2 mg/L PFOA. The author-reported EC_{10} values for growth and reproduction were 0.160 mg/L and 0.0265 mg/L, respectively. The EPA only performed C-R analysis for the growth and reproduction-based endpoints for this test, given the apparent tolerance of the survival-based endpoint. The EPA calculated EC_{10} values for the 42-day growth endpoint (i.e., control normalized wet weight/amphipod) and the 42-day reproduction endpoint (i.e., number of juveniles per female). The 42-day growth-based EC_{10} of 0.488 mg/L (95% C.I. $= 0.319 - 0.657$ mg/L) was not selected as the primary endpoint from this test because it was more tolerant than the reproduction-based EC_{10} of 0.147 mg/L (95% C.I. $= 0.147 - 0.147$ mg/L). The EPA-calculated EC_{10} was 0.147 mg/L with a corresponding EC_{50} of 0.911 mg/L. While the EC_{10} was relatively uncertain due to a lack of partial effects around the 10% effect level, the EC_{50} estimate remained relatively certain given the 47% effect observed in the lowest treatment concentration. EC_{50} to EC_{10} ratios from all quantitatively acceptable chronic concentration-response curves with similar species (i.e., small members of the subphylum Crustacea) and endpoints (i.e., offspring/female) were evaluated to understand the variability in the EC_{50}:EC_{10} ratios and provide further context to the reasonableness of the *H. azteca* EC_{10} estimate. Overall, three quantitatively acceptable chronic concentration-response curves with similar species/endpoints were available. See Table C-1 below for a description of the individual C-R curves and resultant EC_{50}:EC_{10} ratios from each C-R curve.

Table C-1. EC$_{50}$ to EC$_{10}$ ratios from all quantitatively acceptable chronic concentration-response curves with species similar to *H. azteca* (i.e., small members of the subphylum Crustacea) and with endpoints that were based on reproduction per female.

Citation	Species	Endpoint	EPA-Calculated EC$_{50}$ (mg/L)	EPA-Calculated EC$_{10}$ (mg/L)	EC$_{50}$:EC$_{10}$ Ratio
Ji et al. 2008	*Daphnia magna*	(# young/starting female)	61.67	7.853	7.853
Li 2008	*Daphnia magna*	(# young/starting female)	40.75	12.89	3.161
Ji et al. 2008	*Moina macrocopa*	(# young/starting female)	12.77	2.194	5.819

EC$_{50}$:EC$_{10}$ ratios from the three tests with similar species/endpoints ranged from 3.161 to 7.852 with a geometric mean ration of 5.247. Dividing the *H. azteca* reproduction-based EC$_{50}$ (i.e., 0.911 mg/L) by the geometric mean EC$_{50}$:EC$_{10}$ ratio (i.e., 5.247) produced an estimated *H. azteca* EC$_{10}$ of 0.174 mg/L, which was similar to EC$_{10}$ value calculated directly from the *H. azteca* C-R curve (i.e., 0.147 mg/L). The EC$_{10}$ value calculated directly from the *H. azteca* C-R dataset was, therefore, hypothesized to provide a robust estimate of a 10% reproductive-based effect concentration despite the lack of partial low-level effects observed along the C-R curve. The 42-day average number of young per female EC$_{10}$ value of 0.147 mg/L (95% C.I. = 0.147 – 0.147 mg/L) calculated from C-R data reported by Bartlett et al. 2021 was retained for quantitative use.

Publication: Bartlett et al. (2021)
Species: *Hyalella azteca*
Genus: *Hyalella*
EPA-Calculated EC$_{10}$: 0.147 mg/L (95% C.I. = 0.147 – 0.147 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.0325	2.1536 e^{-6}	479432	1.328 e^{-6}
d	1.7400	1.3029 e^{-7}	1335511	4.767 e^{-7}
e	1.2996	2.5393 e^{-6}	511775	1.244 e^{-6}

Concentration-Response Model Fit:

Bartlett et al. 2021

Hyalella azteca
Weibull type 1, 3 para
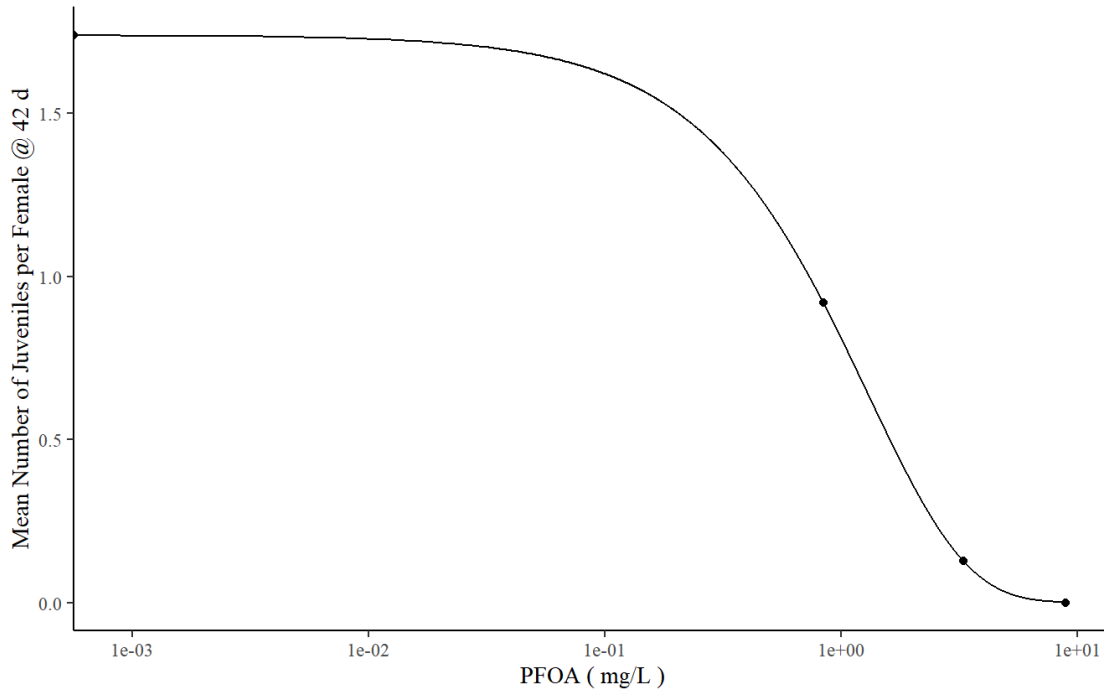
C.2.2 <u>Second most chronically sensitive genus – *Lithobates*</u>

Flynn et al. (2019) evaluated the chronic effects of PFOA (CAS# 335-67-1, purchased

from Sigma-Aldrich) on the American bullfrog (*Lithobates catesbeiana*, formerly, *Rana*

catesbeiana) during a 72-day static-renewal unmeasured exposure. 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 (21ºC and a 12-hour:12-hour light:dark photoperiod). A 2,000 mg/L PFOA stock solution was prepared with RO water to produce three concentrations for the chronic test (0, 0.144 and 0.288 mg/L). Each chronic test treatment contained 10 tadpoles (Gosner stage 25), replicated four times, in 15-L plastic tubs filled with 10 L of aged UV-irradiated, filtered well water. Complete water changes were performed every three to four days, at which time chemical treatments were reapplied. Each experimental unit was fed daily at a constant rate (10% per capita) based on tadpole wet biomass in the control treatment to assure that food was not limiting. On day 72 of the experiment, all tadpoles were euthanized, measured (snout vent length and mass) and staged. The most sensitive chronic endpoint was growth (snout-vent length), with a 72-day NOEC and LOEC of 0.144 mg/L and 0.288 mg/L, respectively. The EPA could not independently calculate an EC_{10} value because there were minimal effects observed across the limited number of treatment concentrations tested. Consequently, the EPA used the LOEC of 0.288 mg/L as the chronic value from this chronic test. The LOEC was used preferentially to the MATC from this test because a ~7% reduction in snout-vent length relative to control responses was observed at the LOEC (i.e., 0.288 mg/L).

Publication: Flynn et al. (2019)
Species: American bullfrog (*Lithobates catesbeiana*)
Genus: *Lithobates*
EPA-Calculated EC_{10}: Not calculable, unable to fit a model with significant parameters
Concentration-Response Model Fit: Not Applicable

C.2.3 Third most chronically sensitive genus – *Daphnia*

Logeshwaran et al. (2021) conducted a PFOA (95% purity, purchased from Sigma-Aldrich Australia) chronic toxicity test with the cladoceran, *Daphnia carinata*. 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-hour:8-hour light:dark photoperiod. The chronic test protocol followed OECD guidelines (2012). A PFOA stock solution (100 mg/L) was prepared in deionized water. Cladoceran culture medium was used to prepare the PFOA stock and test solutions. One daphnid (6-12 hours old) was transferred to each 100 Ml polypropylene container containing 50 Ml of the nominal test solution (0, 0.001, 0.01, 0.1, 1.0 and 10 mg/L PFOA). Each test treatment was replicated 10 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. No mortality occurred in the controls or lowest test concentration. Of the three endpoints measured, average offspring in each brood and total live offspring were the more sensitive endpoints with 21-day NOEC and LOEC values of 0.01 and 0.1 mg/L PFOA, respectively. The EPA was unable to calculate statistically robust EC_{10} estimates from C-R models for these endpoints, largely because of the 10X dilution series across five orders of magnitude. The LOECs for these endpoints were not selected as the chronic value because the LOECs produced a 29.23% reduction in the average number of offspring per brood relative to controls and a 39.89% reduction in the total living offspring relative to controls. Therefore, the MATC (i.e., 0.03162 mg/L) was selected as the quantitatively acceptable chronic value form this test.

Publication: Logeshwaran et al. (2021)
Species: Cladoceran (*Daphnia carinata*)
Genus: *Daphnia*
EPA-Calculated EC$_{10}$: Not used, unable to fit a statistically robust model
Concentration-Response Model Fit: Not Applicable

Ji et al. (2008) conducted a chronic life-cycle test on the effects of PFOA (CAS # 335-67-1, purity was not reported; obtained from Sigma Aldrich, St. Louis, MO, USA) with *Daphnia magna*. The test was done under renewal conditions over a 21-day period and test solutions were not analytically confirmed. Authors stated that the *D. magna* 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, 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 to be 16-hours of illumination, the same conditions as the daphnid cultures used as the source of the experimental organisms. 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), 3.125, 6.25, 12.5, 25 and 50 mg/L and test solutions were renewed three times per week. Test temperature was $21 \pm 1°C$ for the *D. magna* test. Authors note that the water quality parameters (pH, temperature, conductivity, and dissolved oxygen) were measured after changing the medium, but the information is not reported. Survival of daphnids in the negative control was 100%. The most sensitive endpoint for *D. magna* reported in the publication was days to first brood with a 21-day NOEC of 6.25 mg/L (LOEC = 12.5 mg/L; MATC = 8.839 mg/L); however, number of young per starting female (an endpoint not reported in the publication, which only assessed number of young per surviving female) was calculated by the EPA and considered to be a more sensitive endpoint with an EPA-calculated EC_{10} of 7.853 mg/L

(95% C.I. = 4.253 – 11.45 mg/L). Therefore, the EPA-calculated EC_{10} of 7.853 mg/L PFOA for

D. *magna* (number of young per starting female) was considered quantitatively acceptable.

Publication: Ji et al. (2008)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated EC_{10}: 7.853 mg/L (95% C.I. = 4.253 – 11.45 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	-0.4609	0.0633	-7.2762	0.0054
d	83.0500	3.3807	24.5662	0.0001
e	37.5761	6.5642	5.7244	0.0106

Concentration-Response Model Fit:

Ji et al. 2008
Daphnia magna
Weibull type 2, 3 para
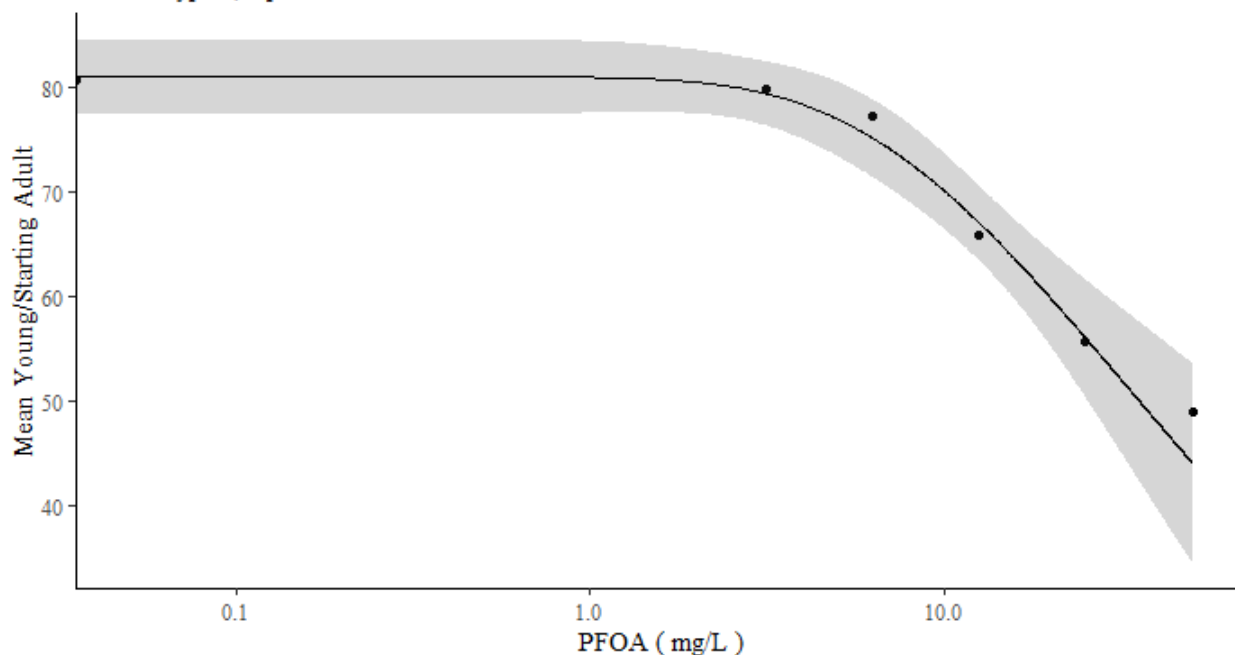
Li (2010) conducted an unmeasured chronic life cycle 21-day test on the effects of PFOA

(ammonium salt, >98% purity) on *Daphnia magna*. The authors stated that 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 salts added (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). The calculated total hardness was 169 mg/L as $CaCO_3$. The photoperiod had 16-hours of illumination with an unreported light intensity. A primary stock solution (1,000 mg/L) was prepared in ASTM medium. 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 and each test was repeated three times. Nominal concentrations were 0 (negative control), 1, 3.2, 10, 32 and 100 mg/L. Test temperature was maintained at $20 \pm 1°C$. Water quality parameters measured in test solutions were not reported. Survival of daphnids in the negative control was 96.7% across all three tests. The *D. magna* 21-day NOEC (reproduction as number of young per female, broods per female, and mean brood size) was 10 mg/L (LOEC = 32 mg/L; calculated MATC = 17.89 mg/L). The EPA performed C-R analysis for each reported endpoint. The EPA also revaluated all endpoints that were based on number of surviving females to be based on the number of starting females. This recalculation was done with the intent to account for starting females that were unable to contribute to the population as reproduction/female due to mortality. The most sensitive endpoint with an acceptable C-R curve was the number of young per starting female with an EPA-calculated EC_{10} of 12.89 mg/L PFOA (95% C.I. = 8.292 – 17.49 mg/L) and was acceptable for quantitative use.

Publication: Li (2010)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated EC$_{10}$: 12.89 mg/L (95% C.I. = 8.292 – 17.49 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.2765	0.2577	4.9540	0.0158
d	145.8089	3.0147	48.3655	$1.946\ e^{-5}$
e	57.4122	8.7260	6.5795	0.0071

Concentration-Response Model Fit:

Li 2010

Daphnia magna
Weibull type 1, 3 para
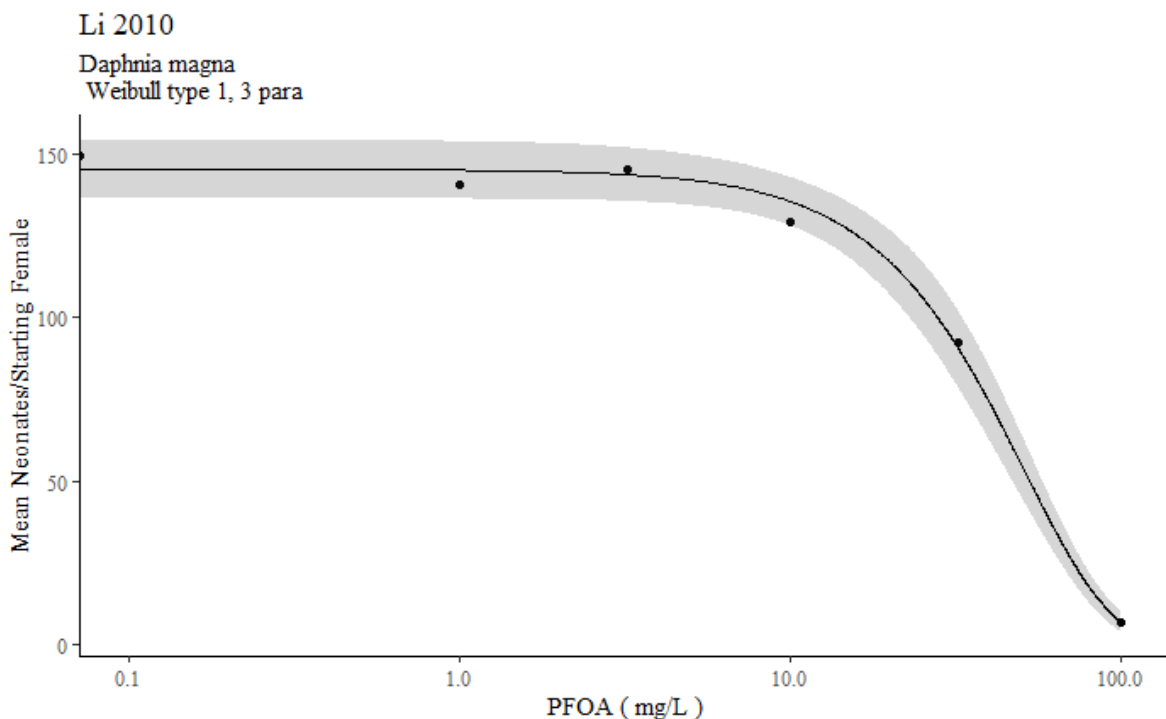
Yang et al. (2014) evaluated the chronic 21-day renewal, measured test of PFOA (CAS # 335-67-1, 99% purity) with *Daphnia magna*, following ASTM E729 (1993). Daphnids used for the test were donated by the Chinese Research Academy of Environmental Sciences. The daphnids were less than 24-hours old at 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 total hardness, 190.0 ± 0.1 mg/L as $CaCO_3$). The photoperiod consisted of 12-hours of illumination at an unreported intensity. A primary stock solution was prepared by dissolving

PFOA in deionized water and DMSO solvent, and 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 ten replicates of one daphnid each in six test concentrations plus a negative and solvent control. Nominal concentrations were 0 (negative and solvent controls), 5, 7.5, 11.25, 16.88, 25.31 and 37.97 mg/L and were renewed at 48-hour intervals. Test concentrations were measured in low and high treatments only. The authors provided mean measured concentrations before and after renewal: 4.96 and 4.49 mg/L (lowest concentration) and 37.66 and 32.88 mg/L (highest concentration). Analyses of test solutions were performed using HPLC/MS and negative electrospray ionization. The concentration of PFOA 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 99%. The temperature, DO, and Ph were reported as having been measured every day during the test, but results are not provided. Negative control and solvent control survival were 90% and 100%, respectively. The author-reported *D. magna* 21-day EC_{10} for reproduction (total number of spawning) was 7.02 mg/L. The EPA performed C-R analysis for each reported endpoint. Both chronic survival and reproduction endpoints resulted in acceptable C-R curves. The EPA-calculated EC_{10} for reproduction as total number of spawning events was 6.922 mg/L (95% C.I. = 4.865 – 8.979 mg/L), similar to the EC_{10} reported by the authors (i.e., 7.02 mg/L). Chronic survival was more sensitive than reproduction, with an EPA-calculated EC_{10} of 5.458 mg/L PFOA (95% C.I. = 3.172 – 7.743 mg/L). Therefore, the survival based EC_{10} calculated by the EPA (i.e., 5.458 mg/L) was acceptable for quantitative use.

Publication: Yang et al. (2014)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated EC$_{10}$: 5.458 mg/L (95% C.I. = 3.172 – 7.743 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.2765	0.2577	4.9540	0.0158
d	145.8089	3.0147	48.3655	1.946 e^{-5}
0.0158	57.4122	8.7260	6.5795	0.0071
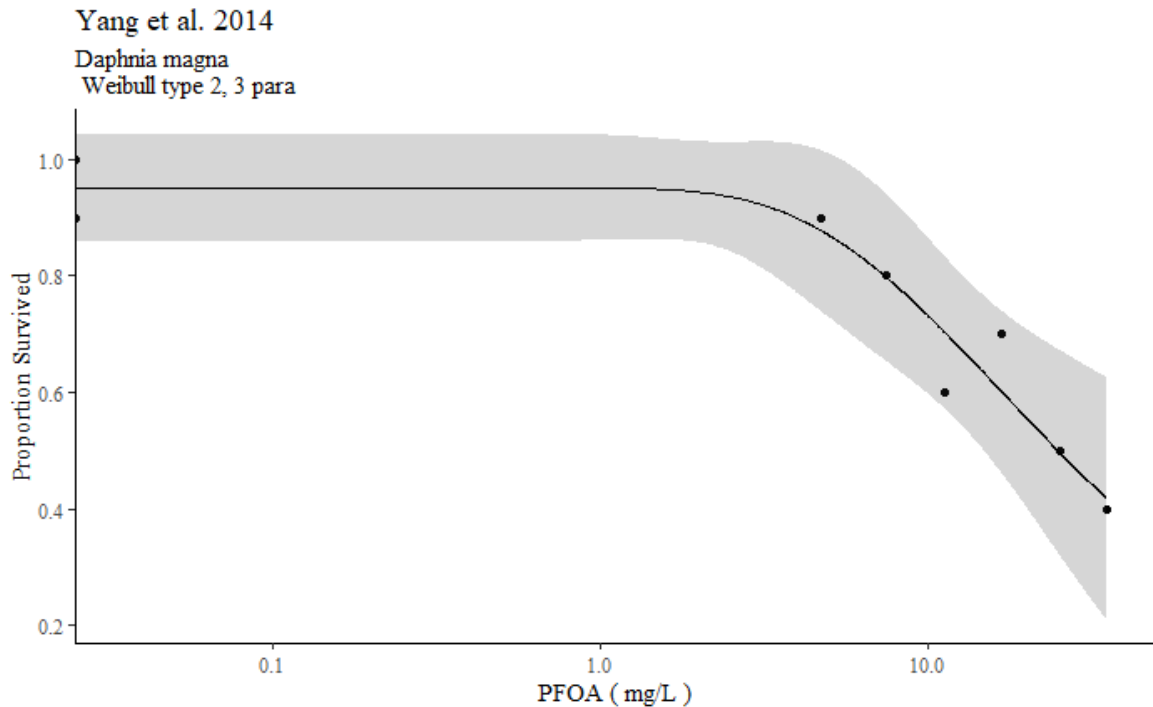
Concentration-Response Model Fit:

Yang et al. 2014
Daphnia magna
Weibull type 2, 3 para

Centre International de Toxicologie (2003) and **Colombo et al. (2008)** conducted a 21-day renewal measured chronic test on ammonium perfluorooctanoate (APFO CAS # 3825-26-1, 99.7% purity) with the daphnid, *Daphnia magna*. The authors stated that the toxicity test was conducted followed OECD test guideline 211. There were 10 replicates for each test treatment containing one neonate, six to 24-hours old, each. Exposure vessel material and size were not reported but filled with 50 Ml of test solution. Stock solutions of APFO were prepared by dissolving the test substance directly in M4 media. The stock was diluted with M4 media to

make the nominal test concentrations: control, 6.25, 12.5, 25, 50 and 100 mg/L. Test solutions

were analyzed by ion chromatography with electrochemical detection. Measured concentrations

were <LOQ, 4.31, 9.16, 20, 44.2 and 88.6 mg/L. Dissolved oxygen was >60% saturation and

temperature were maintained between 18-22°C. Illumination included 16-hours of light with an

unreported intensity. Test solutions were typically renewed every three days and daphnids were

fed daily. Control survival met the minimum survival guidance (80%). Average number of live

young was the most sensitive endpoint reported by Colombo et al. (2008), with a NOEC of 20

mg/L. Based on the author-reported EC_{50} for the average number of live young, the LOEC was

44.2 mg/L and the MATC was 29.73 mg/L. The EPA performed C-R analysis for each reported

endpoint. The most sensitive endpoint with an acceptable C-R curve was the average number of

live young, with an EPA-calculated EC_{10} of 20.26 mg/L PFOA (95% C.I. = -75.48 – 116.0

mg/L), which was acceptable for quantitative use. Although the *D. magna* C-R curve from

Colombo et al. (2008) displayed relatively wide 95% confidence bands, the C-R curve was

retained for use because the EC_{10} is just beyond the NOEC, where effects quickly increase from

0% to nearly 100%. Although there is a lack of partial effects, there appears to be a threshold

effect that occurs above the NOEC. Note, Colombo et al. (2008) reported treatment-mean C-R

data for the number of offspring per starting adult. In contrast, Centre International de

Toxicologie (2004) reported replicate-level C-R data for number of offspring per surviving adult

(producing an EPA-calculated EC_{10} of 21.25 mg/L; Model = Weibull Type 1, 3 parameters).

Basing the number of offspring on starting adult (as reported by Colombo et al. [2008]) rather

than per surviving adult (as reported by Centre International de Toxicologie [2003]), produces a

more sensitive endpoint that accounts for those parents that could not contribute to the overall

offspring count as a result of their own mortality. Because Colombo et al. 2008 and Centre

International de Toxicologie (2003) both reported only treatment-mean C-R survivorship data, the number of offspring per starting adult was based on treatment mean data rather than replicate-level data.

The authors note that the contribution of ammonia from APFO exposure in this test indicates that un-ionized ammonia could be a potential contributor to the observed chronic toxicity of APFO. The EPA believes ammonia does not contribute substantively to the chronic toxicity to *D. magna* in this test based on the following rationale. US EPA (2013) derived a GMCV of 41.46 mg N/L at pH=7 and temperature of 20°C for *D. magna*. Using the EPA equations, this translates to 12.6 mg N/L at the authors' assumed acute test pH=8.2 (from their Table 7) and the midrange reported test temperature (20°C). This in turn translates to an un-ionized ammonia concentration of approximately 0.91 mg un-ionized ammonia/L at this pH and temperature. Table 7 of Colombo et al. (2008) lists un-ionized ammonia concentration at the APFO 21-day reproductive NOEC as 0.048 mg un-ionized ammonia/L, which is almost twenty times lower than the EPA's SMAV for D. *magna* re-expressed as an un-ionized ammonia concentration for the test condition. Therefore, it is highly unlikely that ammonia contributed to the chronic toxicity of APFO to *D. Magna* in this test, and therefore, the EPA-calculated EC_{10} of 20.26 mg/L APFO is used in the calculation of the GMCV for this species.

Publication: Colombo et al. (2008)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated EC_{10}: 20.26 mg/L (95% C.I. = -75.48 – 116.0 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	2.79764	0.39868	7.0172	0.005944
d	65.85398	1.79847	36.6167	4.48 e^{-5}
e	45.28323	4.01408	11.2811	0.001494

Concentration-Response Model Fit:

Colombo et al. 2008

Daphnia magna
Weibull type 1, 3 para
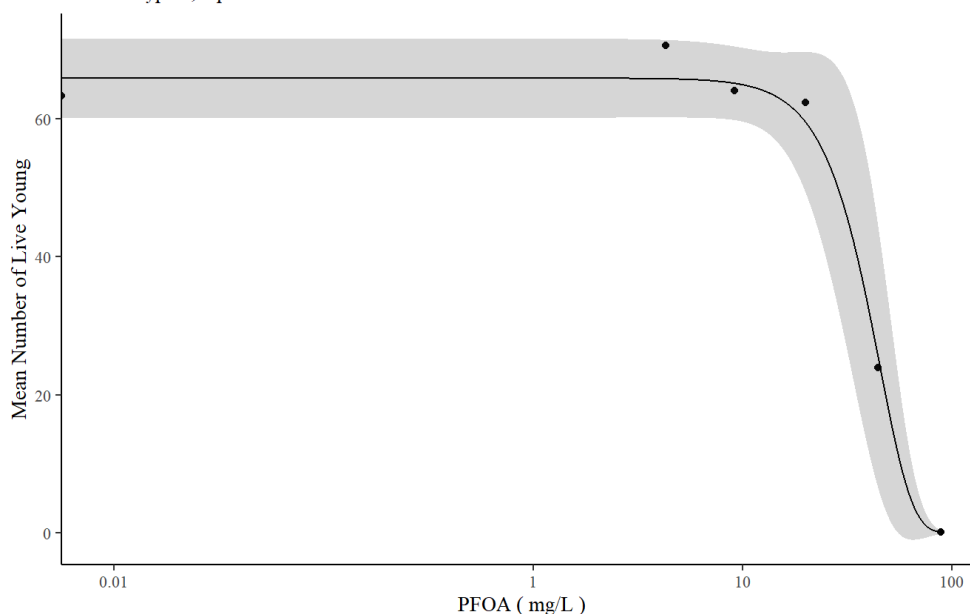
Lu et al. (2016) evaluated the chronic toxicity of PFOA (CAS# 335-67-1, 98% purity, purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) on *Daphnia magna* immobilization, growth and reproduction. Reconstituted daphnia culture media was used for both culturing and test solution preparation as described in OECD Test Guideline 202. *D. magna* cultures (originally obtained from the Chinese Center for Disease Control and Prevention (Beijing, China) were fed with the green algae *Scenedesmus obliquus* daily, maintained at 20°C and a light/dark photoperiod of 16-hours/8-hours and the medium renewed three times weekly. The 21-day chronic test endpoints were assessed by a semi-static unmeasured test according to OECD Test Method 211. Neonates (<24-hours old) were exposed to six concentrations of PFOA [0 (control), 0.032, 0.16, 0.8, 4 and 20 mg/L] maintained at 20 ± 1°C. One test organism was exposed in a 100 mL glass beaker filled with 45 mL of test solution, and there were 20 replicates for each exposure concentration. The daphnids were fed 1×10^6 cells of *Scenedesmus obliquus* per animal per day, and the test solution was renewed every other day. Survival, growth and

reproduction (fecundity) was determined during the 21-day exposure. The 21-day growth and reproductive NOEC and LOEC were 0.032 and 0.16 mg/L PFOA, respectively. The EPA was unable to fit a C-R model with significant parameters to the chronic data associated with reproduction from this test. The EPA-calculated EC_{10} values for mean intrinsic rate of increase ® and growth (as length) were 0.0173 mg/L (95% C.I. = 0.0170 – 0.0177 mg/L) and 0.0124 mg/L (95% C.I. = 0.0048 – 0.0200 mg/L), respectively. Both EC_{10} values were nearly two times lower than the NOEC of 0.032 mg/L and four times lower than the LOEC value (i.e., 0.16 mg/L) where only 15.2% and 11.9% reductions in intrinsic rate of natural increase (r) and length were observed, respectively. As a result, the MATC of 0.07155 mg/L for growth and reproduction was selected as the most appropriate chronic value for quantitative use to in deriving the chronic water column-based criterion.

Publication: Lu et al. (2016)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated EC_{10}: Not used, unable to fit a statistically robust model
Concentration-Response Model Fit: Not Applicable

Yang et al. (2019) evaluated the chronic effects of PFOA (CAS# 335-67-1, purchased from Sigma-Aldrich in St. Louis, MO) on *Daphnia magna* via a 21-day unmeasured, static-renewal test that assessed reproductive effects. *D. magna* cultures were originally 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 specified in OECD Guideline 202. Protocol for all testing followed OECD Guideline 211. Cladocerans were cultured in artificial freshwater maintained at 20 ± 1°C under a 16-hour:8-hour light:dark photoperiod and a light intensity of 1,000-1,500 lux at the surface of the water. Cultures were fed *Scenedesmus obliquus* daily and the water was changed twice weekly. Reported water quality parameters

include total hardness of 140-250 mg/L as $CaCO_3$ and pH of 6-8.5. The 21-day chronic study had nominal concentrations of 0 (control), 0.0000162, 0.0000244, 0.0000365 and 0.0000546 mol/L (or 0 (control), 6.708, 10.10, 15.11 and 22.61 mg/L, respectively, given the molecular weight of the form of PFOA used in the study, CAS # 335-67-1, of 414.07 g/mol). One neonate (12–24 hours old) was placed in A 100 mL glass beaker, replicated 10 times, and each container filled wiTH 80 mL of test solution maintained at $20 \pm 1°C$ and a 16-hour:8-hour light:dark photoperiod with a light intensity of 1,000-1,500 lux. *D. magna* were fed *S. obliquus* and test solutions were renewed every 72 hours. Test organisms were counted daily, with any young removed. The reproductive NOEC and LOEC were 0.0000162 and 0.0000244 mol/L, or 6.708 and 10.10 mg/L PFOA, respectively. The EPA performed C-R analysis for the test. The EPA-calculated EC_{10} based on mean offspring at 21-days as a proportion of the control response was 8.084 mg/L (95% C.I. = 7.830 – 8.334 mg/L) and was used quantitatively to derive the chronic water column criterion.

Publication: Yang et al. (2019)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated EC$_{10}$: 8.084 mg/L (95% C.I. = 7.830 – 8.334 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	-0.9632	0.1065	-9.0420	0.0029
e	19.2161	0.9269	20.7320	0.0002

Concentration-Response Model Fit:
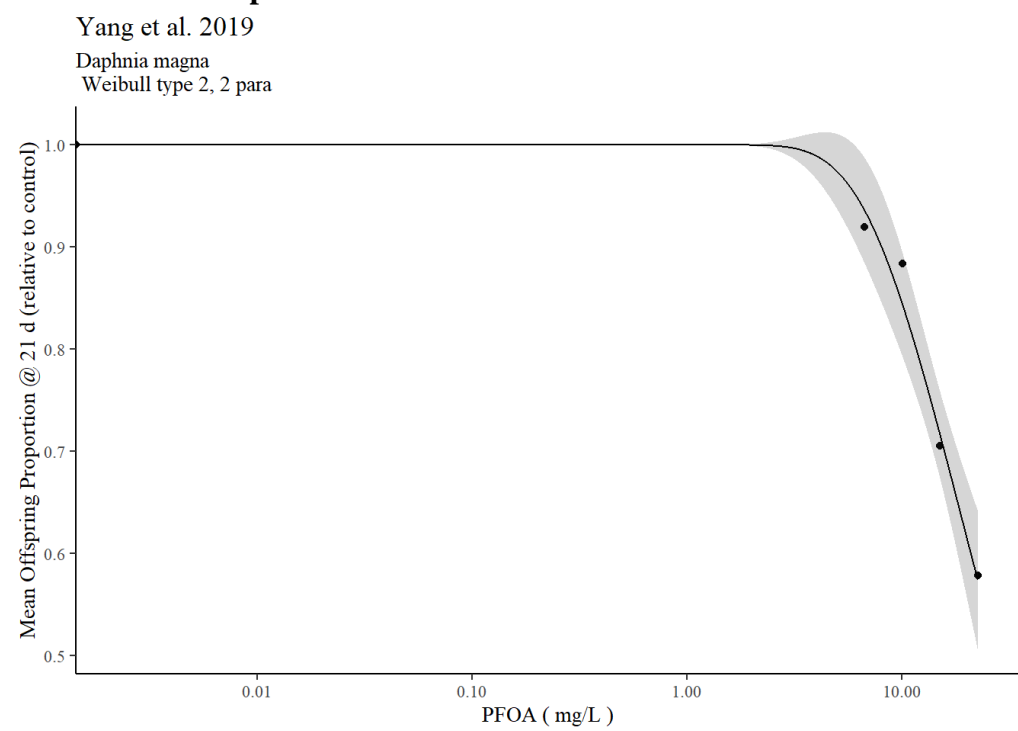
C.2.4 <u>Fourth most chronically sensitive genus – *Brachionus*</u>

Zhang et al. (2013a) conducted a chronic life-cycle renewal test of PFOA (CAS # 335-

67-1, 96% purity) with *Brachionus calyciflorus*. The test duration was up to 200-hours in a full-

life cycle test (primary emphasis), and 28 days in a population growth test (secondary emphasis:

only two 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-hours:8-hours, light:dark; 3,000 lux)

for more than six months before toxicity testing to acclimate to the experimental conditions.

Culture medium was an artificial inorganic medium and all toxicity tests were carried out in the

same culture medium and under the same conditions as during culture (i.e., pH, temperature,

illumination). Solvent-free stock solutions of PFOA (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 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, with one rotifer per cell. Treatments were repeated six times.

Nominal concentrations were 0 (negative control), 0.25, 0.5, 1.0 and 2.0 mg/L. PFOA

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 PFOA measured every 8 hours

over a 24-hour period in rotifer medium with green algae incurs minimal change in the

concentration range from 0.25 to 2.0 mg/L. One hundred percent survival was observed at 24

hours in the negative control in the corresponding acute test, but survival information is not

provided for the life-cycle test. Zhang et al. (2013a) demonstrated rotifer body size and mictic

ratio after 28-days were relatively tolerant endpoints with reported NOECs of ≥ 1.0 mg/L and 2.0

mg/L, respectively. The EPA performed C-R analysis for the remaining reported endpoints from

C-R data reported in the publication. The most sensitive endpoint with an acceptable C-R curve

was the intrinsic rate of natural increase with an EPA-calculated EC_{10} of 0.5015 mg/L PFOA

(95% C.I. = 0.1458 – 0.8572 mg/L), which was acceptable for quantitative use. The intrinsic rate

of natural increase (d^{-1}) is a population level endpoint that accounts for births and deaths over

time. In this study, the intrinsic rate of natural increase was defined as the natural log of the lifetime net reproductive rate for all individuals within a population (defined here as a PFOA treatment level) divided by the average generation time of those individuals. The effect associated with intrinsic rate of natural increase is similar to other chronic apical effects reported by Zhang et al. (2013a). For example, Zhang et al. (2013a) also reported net reproductive rate and juvenile period which produced an EPA-calculated EC_{10} value of 0.514 mg/L (95% C.I. = 0.1958 – 0.8329 mg/L). Zhang et al. (2013a) also reported effects to average juvenile period, which was a relatively tolerant endpoint. Juvenile period decreased with increasing exposure concentration, with the average juvenile period being about 16% faster than the control responses in the highest treatment concentration (2.0 mg/L; the EPA was unable to fit a statistically-robust C-R model for this endpoint). Zhang et al. (2013a) reported significant reductions in egg size with an EPA-calculated EC_{10} = 0.193 (95% C.I. = -0.1606 – 0.5466 mg/L); however, this endpoint displayed a relatively poor concentration response relationship and may not be relevant for assessing population level effects and was, therefore, not selected as the primary effect concentration from this test. Effects to chronic apical endpoints in this publication and Zhang et al. (2014b) generally appear as a threshold effect from 0.25 mg/L to 1.0 mg/L, providing further support for the endpoint and effect level selected for quantitative use from Zhang et al. (2013a).

Publication: Zhang et al. (2013a)
Species: Rotifer (*Brachionus calyciflorus*)
Genus: *Brachionus*
EPA-Calculated EC$_{10}$: 0.5015 mg/L (95% C.I. = 0.1458 – 0.8572 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	-0.6515	0.1231	-5.2930	0.0339
d	0.5058	0.0144	35.0478	0.0008
e	1.8042	0.2240	8.0546	0.0151

Concentration-Response Model Fit:

Zhang et al. 2013
Brachionus calyciflorus
Weibull type 2, 3 para
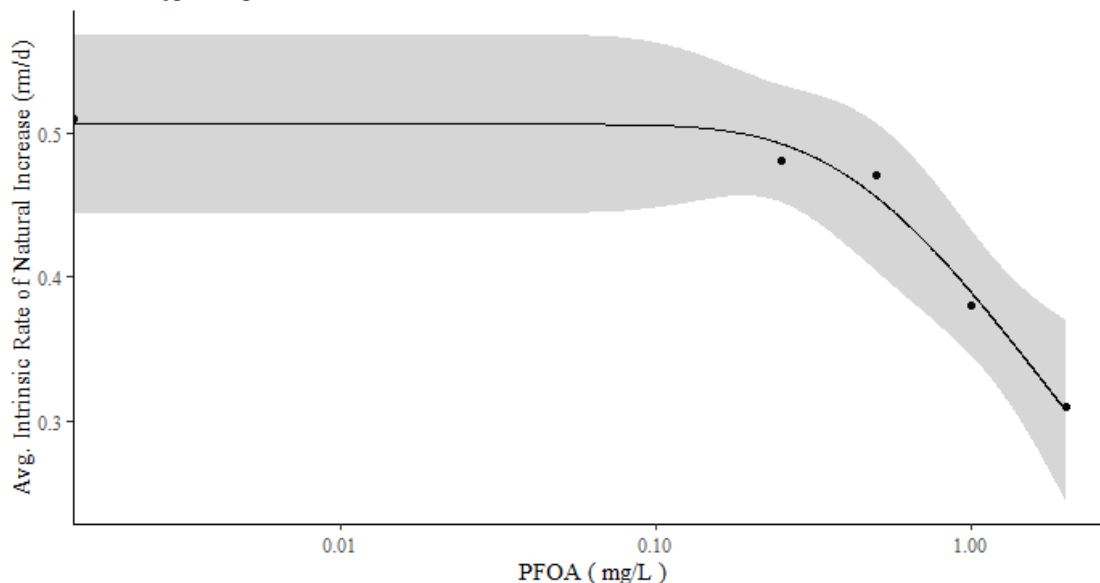
Zhang et al. (2014b) reports the results of a similar chronic life-cycle test of PFOA

(CAS # 335-67-1, 96% 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-hours:8-hours, 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 PFOA (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 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. The numbers of living rotifers were counted after four days for each treatment level. 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. PFOA 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 PFOA measured every eight-hours over a 24-hour period in rotifer medium with green algae incurs minimal change in the concentration, ranging from 0.25 to 2.0 mg/L. Negative control survival was not provided for the life-cycle test.

Resting egg production is an ecologically important endpoint for this species because it represents the final result of sexual reproduction. Based on authors description of results in the text, "PFOA exposure significantly reduced resting egg production of *B. calyciflorus* females during the three-day period." NOEC and LOEC values were not reported, but 0.25 mg/L PFOA produced more than a 50% reduction in resting egg production. Therefore, it is assumed The *B. calyciflorus* four-day NOEC for resting egg production was 0.125 mg/L and the LOEC was 0.25 mg/L, with a calculated MATC is 0.1768 mg/L. Concentration response data from Figure 1 of

Zhang et al. (2014b) were estimated (WebPlotDigitizer[1]) and used to derive an EPA-calculated EC_{10} of 0.076 mg/L (95% C.I. = 0.054 – 0.098 mg/L), further suggesting resting egg production may be a relatively sensitive endpoint. Because there was only one replicate (as implied by lack of error bars in Figure 1 of the publication, no clear description of replicates in the methods section, and no author-reported statistical analysis of this endpoint), resting egg production from this study was not considered quantitatively acceptable but was retained for qualitative use. Beyond resting egg production, PFOA did not clearly affect hatching rate of resting eggs when exposed to PFOA during the formation or hatching period, enhanced hatching rate relative to controls in most treatments (nominal test concentration range = 0 – 2.0 mg/L; see figures 3 and 4 of Zhang et al. 2014b) and displayed no clear concentration-response relationship, suggesting rotifer hatching rate was a relatively tolerant endpoint from this publication. In contrast to Zhang et al. (2013a), which observed no effect of PFOA on mictic ratio after 28 days at a nominal concentration as high as 2.0 mg/L PFOA, Zhang et al. (2014b) stated PFOA significantly increased the F1 mictic ratio from 0.56 in the control treatment to 0.75 and 0.72 in nominal PFOA test concentrations of 0.25 mg/L and 2.0 mg/L, respectively. Given conflicting results of PFOA on rotifer mictic ratio, it was not selected as the primary endpoint from Zhang et al. (2014b). The most sensitive quantitatively acceptable endpoint was the intrinsic rate of natural increase. The intrinsic rate of natural increase (d^{-1}) is a population level endpoint that accounts for births and deaths over time. In this study, the intrinsic rate of natural increase was defined as the natural log of the net increase in the number of rotifers (surviving parents and offspring) for each PFOA treatment level over a four-day exposure period. This endpoint was conceptually

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[1] WebPlotDigitizer is an online application used to convert values shown in figures to numerical values. This application was used to obtain numerical concentration-response data when they were only reported in figures. The application is free and available online (WebPlotDigitizer - Extract data from plots, images, and maps (automeris.io)).

equivalent to the intrinsic rate of natural increase endpoint calculated by Zhang et al. (2013a) but

was a simplification of the calculations performed in Zhang et al. (2013b), in that it only applied

to the four-day observational period, whereas the intrinsic rate of natural increase calculated in

Zhang et al. (2013a) represented the full lifetimes of all individuals within each population (i.e.,

exposure concentration). The EPA-calculated EC_{10} for this endpoint was 1.166 mg/L (95% C.I. =

0.7720 – 1.559 mg/L).

Publication: Zhang et al. (2014b)
Species: Rotifer (*Brachionus calyciflorus*)
Genus: *Brachionus*
EPA-Calculated EC_{10}: 1.166 mg/L (95% C.I. = 0.7720 – 1.559 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	1.1913	0.2118	5.6236	0.0014
d	0.2253	0.0081	27.9366	1.392 e^{-7}
e	7.7080	0.8191	9.4102	8.183 e^{-5}

Concentration-Response Model Fit:
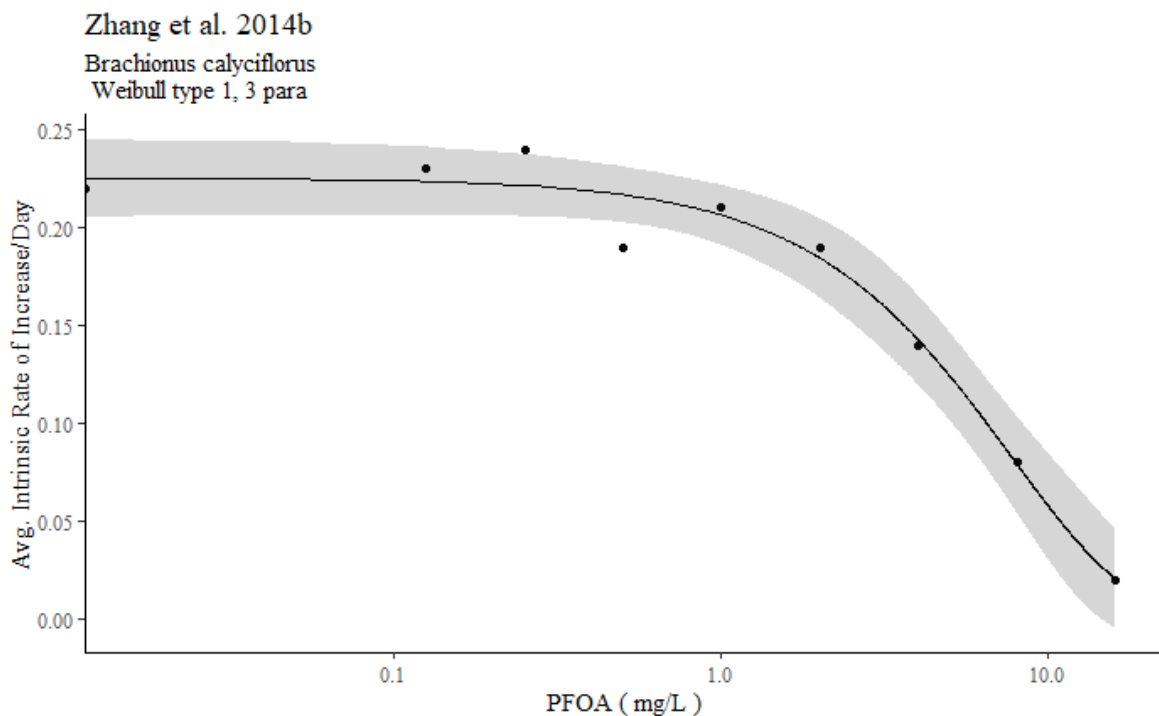
C.2.5   Fifth most chronically sensitive genus – *Moina*

Ji et al. (2008) conducted a chronic life-cycle test on the effects of PFOA (CAS # 335-67-1, purity unreported; obtained from Sigma Aldrich, St. Louis, MO, USA) with *Moina macrocopa*. Tests were done under renewal conditions over a seven-day period and test solutions were not analytically confirmed. Authors stated that the *M. macrocopa* test followed a protocol developed and reported by S.R. Oh (2007) (Master's thesis, Seoul National University, Seoul, Korea), which is similar to OECD 211 (1998), but with slight modification (i.e., shorter test duration, exposure temperature and different feeding regime: 100 µL yeast:cerophyll:Tetramin mixture and 200 µL algae suspension per day). *M. macrocopa* used for testing were obtained from brood stock cultured at the Environmental Toxicology Laboratory at Seoul National University, 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 for the test was not reported but was assumed to be 16-hours of illumination, the same conditions as the daphnid cultures reported in this same publication. Preparation of test solutions was not described. The test involved ten replicates of one individual each in five nominal test concentrations plus a negative control. Nominal concentrations were 0 (negative control), 3.125, 6.25, 12.5, 25 and 50 mg/L and test solutions were renewed three times per week. Test temperature was $25 \pm 1°C$ for *M. macrocopa*. Authors note that the 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 *M. macrocopa* seven-day NOEC (reproduction: number of young per adult) was 3.125 mg/L, the LOEC was 6.25 mg/L and the MATC is 4.419 mg/L. The EPA performed C-R analysis for this study. The most sensitive endpoint with an acceptable C-R curve was the number of young per

starting female. The EPA-calculated EC_{10} was 2.194 mg/L PFOA (95% C.I. = -0.7120 – 5.010

mg/L) for *M. macrocopa*. The lowest treatment concentration produced a greater than a 10%

effect which forced the EC_{10} calculation to extrapolate beyond the lowest treatment

concentration (i.e., not the control, but the nominal treatment of 3.25 mg/L). However, the

resultant EC_{10} value (i.e., 2.194 mg/L) was considered acceptable for quantitative use because it

was largely in agreement with the 14.3% effect observed at the test concentration of 3.125 mg/L.

C.2.6   Sixth most chronically sensitive genus – *Neocloeon*

Soucek et al. (2023) conducted a chronic life-cycle test to determine the effects of PFOA

(CAS # 335-67-1, 95% purity) on the parthenogenetic mayfly, *Neocloeon triangulifer*. The test

was performed under renewal conditions over 28 days beginning with <24-hour old nymphs.

Single mayfly exposures were static without renewal for the first four days due to the small size

of starting organisms and renewed three times per week thereafter by transferring organisms to

new exposure chambers. From day 0 to 14, mayflies were exposed in 30 Ml polypropylene cups

with 20 Ml exposure water. Organisms were transferred after 14 days into 250 Ml glass beakers

with 100 or 150 Ml of test water (or control) and to 300 Ml tall form beakers for emergence. At

test initiation, there were 16 replicates per test concentration and control. Nominal test

concentrations were 0.0 (control), 0.047, 0.094, 0.375, 0.750, 1.500 and 3.000 mg/L PFOA.

Mean measured PFOA concentrations (EPA Analytical Method 1633; LC-MC/MS) were

0.000176 (control), 0.043, 0.078, 0.419, 0.672, 1.739 and 3.085 mg/L PFOA, respectively.

Mayflies were exposed at 23 ± 1°C under a 16:8-hour light:dark cycle and fed 0.2 Ml diatom

slurry plus small scraping on test days 0 and 4 followed by live diatom biofilm scraping after day

4 on solution renewal days. Percent survival in the control after 14 days was 94%. Percent

survival of mayflies after 14 days in the remaining seven test concentrations ranged from 75 to

100%. On test day 14, replicates one through eight were destructively sampled to evaluate 14-

day weight. Replicates 9-18 were maintained under test conditions until test day 23. No clear effects of PFOA on 14-day weight or survival were observed in any of the treatments. Similarly, after 28-days of exposure, no clear effects of PFOA were observed on survival to pre-emergent nymph (PEN) stage, survival to emergence, number of days to emergence, imago live weight, or survival to subimago. The relatively high >NOEC (i.e., >3.085 mg/L) suggests *N. triangulifer* is relatively tolerant to chronic PFOA exposures and does not occur among the four most sensitive genera. The >NOEC (i.e., >3.085 mg/L) was acceptable for quantitative use in deriving the chronic freshwater PFOA criterion.

C.2.7   Seventh most chronically sensitive genus – *Oryzias*

Lee et al. (2017) conducted a multiple generation exposure to determine the effects of PFOA (CAS # 335-67-1, purity was not reported) on the reproductive toxicity and metabolic disturbances to *Oryzias latipes*. Fish were originally received from the Department of Risk Assessment of the National Institute of Environmental Research (NIER; South Korea) and maintained according to the following conditions: dissolved oxygen 7-8 mg/L, Ph 7.5 ± 0.2, water temperature 25 ± 1°C, 16-hour light, 8-hour dark photoperiod and total hardness 55-57 mg/L (as $CaCO_3$). *O. latipes* were fed *Artemia salina* once daily, based on the OECD test guideline 240 feeding schedule. Adults (about 13 weeks old, when genders could be visually differentiated) through the F2 generation were exposed to three nominal concentrations of PFOA (0.3, 3 and 30 mg/L PFOA) for a total exposure period of 259 days. At test initiation, four pairs of both genders were introduced into the test chambers (8 L glass tank) of a flow-through exposure system. PFOA solutions were replenished five times daily to keep the same water quality as fish maintaining condition. PFOA exposure continued for three weeks, during which eggs produced by mating of F0 fish were removed from test chamber and counted daily for fecundity. During test week four, (spawning period), the F1 generation eggs (n = 192) were

obtained per concentration. Right after the spawning period was finished, F0 fish were used to

evaluate metabolism disturbance. The F1 eggs were pooled and redistributed into an incubation

chamber containing PFOA solution. After the hatching was completed, the test organisms were

returned into test chambers and raised under flow-through PFOA exposure conditions until they

reached adult stage (at about 13 weeks old), during which sac-fry survival rate, hatching rate, and

abnormality of F1 were analyzed. When F1 fish reached the adult stage, sex ratio of total F1 fish

was determined and 32 individuals of F1 fish were used to analyze gonadosomatic (GSI),

hepatosomatic (HSI), condition factor (K), VTG expression, and histological alterations. In

addition, other F1 fish (32 male and female fish) were used for obtaining the F2 generation eggs

same as the F0 generation. The exposure conditions to F2 fish were carried out in the same

manner as F1 fish. Consequently, F0 fish were exposed to PFOA for four weeks and F1 and F2

fish were exposed to PFOA across all life cycle stages without exposure pause. The exposure

regime was applied equally in all test groups. The 259-day MATC of 9.487 mg/L PFOA was

reported for F2 sac-fry survival and fecundity for the F0, F1 and F2 generations and represented

the most sensitive endpoints from the study. Reproductive responses reported by Lee et al.

(2017) appear to be control normalized; however, use of control normalized data in this study

does not alter conclusions from hypothesis-based testing (i.e., use of a NOEC, LOEC, or

MATC). Beyond F2 survival (which had control mortality), the EPA attempted C-R analysis for

all endpoints reported by Lee et al. (2017). Given the large dilution factor between PFOA

treatments, C-R models could either not be fit, or when models could be fit, they performed

poorly on statistical metrics and were not used. Therefore, the 259-day MATC of 9.487 mg/L

PFOA was considered to be quantitatively acceptable for criterion derivation. The large dilution

factor from this test does not support concentration response modeling, consequently EPA relied on an MATC (i.e., 9.487 mg/L) as the chronic effect level from this test.

C.2.8 Eighth most chronically sensitive genus – *Ceriodaphnia*

Kadlec et al. (2024) tested the chronic toxicity of pefluorooctanoic acid (PFOA) to *Ceriodaphnia dubia* for seven days in three measured, renewal experiments. Similar chronic tests were also performed with *Chironomus dilutus* and *Hyallela azteca*, but this summary is limited to the results of the *C. dubia* tests. Test chemicals were obtained from Sigma, Alfa Aesar, Synquest, and Toronto Research Chemical (purity 96-99%). Test organisms were obtained from in-house cultures maintained following ASTM and EPA protocols. Test water was UV-treated and sand-filtered Lake Superior water supplemented with Na_2SO_4, NaCl, KCl, $CaCl_2$ x $2H_2O$, and $MgCl_2$ x $6H_2O$. Testing protocols followed species-specific ASTM methodologies (ASTM 2002). Three separate tests were conducted, each with a 0.5x dilution series of measured PFOA concentrations, with ten replicates of each concentration, and one organism per replicate. Test 1 mean concentrations were 0.019 (control), 0.19, 0.31, 0.62, 1.3, 2.7, 5.5, 11, 22, 44 and 91 mg/L. Test 2 mean concentrations were 0.67 (control), 6.7, 14, 28, 55, and 109 mg/L. Test 3 mean concentrations were 0.35 (control), 3.5, 6.8, 14, 30 and 57 mg/L. *C. dubia* neonates (<8-hour) were placed in one ounce polystyrene cups filled with 15 mL of test solution. Dissolved oxygen and pH were measured twice in each exposure per treatment and twice in the stock solutions. Test chambers were placed in a water bath to maintain a steady temperature under a 16:8 light:dark cycle. Study authors reported average water quality measurements of 24.7°C, 8.6 mg/L DO, 7.8 pH, 52 mg/L as $CaCO_3$ hardness, 41 mg/L as $CaCO_3$ alkalinity, and 145 µmhos/cm conductivity. Testing solutions were renewed daily, and organisms were fed daily with 100 µg/L YCT and algae. Control survival was 96.8% with a mean reproduction of 26.8 offspring per surviving female. EC_{20}s and EC_{50}s for survival and young per surviving female were calculated

following methods described in Mount et al. (2016), using custom software written with Intel Visual Fortran Compiler Xe (Intel Corporation) and Winteracter 13.0 (Interactive Software Services). The author reported EC_{20}s for young per surviving female for tests 1, 2, and 3 were 26.2, 25.1, and 33.8 mg/L, respectively. Concentration-response data were reported for these tests, allowing the EPA to independently model concentration-response curves using the dose-response curve package in R. The EPA-calculated EC_{10} values for tests 1, 2, and 3 were 20.42, 21.69, and 29.54 mg/L, respectively, which were determined to be acceptable for quantitative use.

C.2.9 <u>Ninth most chronically sensitive genus – *Gobiocypris*</u>

The chronic toxicity of PFOA (98% purity) on the rare minnow (*Gobiocypris rarus*; not North American resident species) was investigated by **Wei et al. (2007)** using flow-through unmeasured exposure conditions. Two hundred and forty mature male and female rare minnows (about nine months old, 1.4 ± 0.4 g, 47.7 ± 3.6 mm) were obtained from a laboratory hatchery and randomly assigned to eight 20 L glass tanks (30 individuals per tank). Fish were supplied with dechlorinated tap water under continuous flow-through conditions at $25 \pm 2°C$ and a photoperiod of 16-hours:8-hours light:dark. During the 28-day exposure period, fish were fed a commercial granular food (Tetra) at a daily rate of 0.1% body weight. Waste and uneaten food were removed daily. After a one-week acclimation period, 30 randomly selected male and 30 female rare minnows (gender determined by observing the shape of the abdomen and the distance between the abdomen fin and the stern fin) were assigned to one of the four nominal PFOA exposures (0, 3, 10 or 30 mg/L PFOA). Each treatment was performed in duplicate tanks. The flow rate of the test solution was 8 L/hour, and actual PFOA concentrations in the tanks were not verified by chemical analysis. During the exposure period, there were separate inputs for water and PFOA and the mixer helped mix PFOA and water before flowing into the tanks.

The concentration of mixed solution flowing out from the mixer were kept at 3, 10 or 30 mg/L PFOA by adjusting the input flow rate of concentrated PFOA and water, respectively. After 14-day and 28-day exposure periods, fish were anesthetized on ice, and liver samples were taken and immediately frozen in liquid nitrogen and stored at -80°C until analyzed. No mortality was observed in any treatments. The 28-day LOEC (survival) was >30 mg/L PFOA and was acceptable for use as a high-unbounded value from a high-quality study which provides relevant sensitivity information for this fish species.

C.2.10 <u>Tenth most chronically sensitive genus - *Oncorhynchus*</u>

Centre International de Toxicologie (2004) and **Colombo et al. (2008)** evaluated the chronic effects of ammonium perfluorooctanoate (APFO, CAS #3825-26-1, 99.7% purity) to embryos of the rainbow trout, *Oncorhynchus mykiss*. Stock solutions of APFO were prepared by dissolving the test substance directly in the test media or dilution water and then diluting the stock solution to provide a geometric series of test concentrations (nominal concentrations of 3.13, 6.25, 12.5, 25 and 50 mg/L APFO). The early-life-stage (ELS) test was performed under flow-through conditions and in compliance with OECD test guideline 210. Unfertilized trout eggs and sperm were received from a commercial supplier and the eggs were fertilized in the laboratory. One hundred and eighty newly fertilized eggs were randomly selected and allocated, 60 eggs per replicate, to the three replicate test vessels for each control and test concentration. Authors stated that the number of surviving fish was reduced randomly to 30 per replicate just after the end of the hatching period (day 26) in the control. The number of surviving fish was again reduced randomly to 15 per replicate when swim-up and feeding began on day 50. Actively feeding juveniles were fed trout chow two to four times per day, corresponding to approximately 4% of their body weight per day, from day 50 to the end of the 85-day test. Test solutions were continuously renewed during the study by pumping the stock solutions into

flowing dilution water with a peristaltic pump system at a replacement rate of 5.76 times the test vessel volume per day. Dilution water pH was 6.0-8.5, total hardness was 150 mg/L as $CaCO_3$, and water temperature was kept between 11.1 and 12.5°C for embryos and between 11.6 and 14.4°C for larvae and juvenile fish. The dissolved oxygen concentration was greater than 60% air saturation, the light/dark cycle was maintained at constant darkness until seven days after hatching, then 16 hours light and eight hours dark through test end. Observations were made daily as follows: eggs-marked loss of translucency and change in coloration, white opaque appearance; embryos-absence of body movement or heartbeat; larvae and juvenile fish-immobility, absence of respiratory movement or heartbeat, white opaque coloration of the central nervous system, lack of reaction to mechanical stimulus, and abnormalities. The reported 85-day growth and mortality NOEC was 40 mg/L PFO^-; however, the authors' note that the contribution of ammonia from APFO exposure indicates that un-ionized ammonia could be a potential contributor to the observed toxicity of APFO. Although the authors cite U.S. EPA (1999) for un-ionized ammonia toxicity values, that document (and the subsequent U.S. EPA [2013] criteria document) expressed toxicity in terms of a relationship between total ammonia nitrogen and pH and temperature. For rainbow trout, U.S. EPA (1999) declined to specify a chronic value, due to inconsistencies between tests. However, U.S. EPA (2013) set the rainbow trout chronic value at 6.66 mg TAN/L (Total Ammonia Nitrogen/L) at pH = 7. Using the normalization equations in U.S. EPA (2013), the rainbow trout chronic value translated to 3.60 mg N/L at the authors' assumed chronic test pH of 7.8 (see table 7 of Colombo et al. [2008]), which in turn translated to a rainbow trout chronic value as un-ionized ammonia of 0.064 mg un-ionized ammonia/L at pH 7.8 and a reported test of temperature (13°C). Table 7 of Colombo et al. (2008) listed the un-ionized ammonia concentration at their APFO NOEC as 0.013 mg un-ionized ammonia/L, which

is 4.9-fold lower than the EPA's chronic value for rainbow trout re-expressed as an un-ionized ammonia concentration for the test condition. Therefore, the EPA does not believe ammonia was a confounding factor in this test and the study was determined to be quantitatively acceptable for criterion derivation.

C.2.11 Eleventh most chronically sensitive genus – *Pimephales*

Bartlett et al. (2021) also evaluated the chronic effects of PFOA (CAS# 335-67-1, 96% purity, solubility in water at 20,000 mg/L, purchased from Sigma-Aldrich) on fathead minnows (*Pimephales promelas*) via a 21-day early-life stage static-renewal, measured study. The authors followed OECD Test Guideline 210, except that the test ended at 16 days post-hatch (dph) compared to 28 dph for the standard OECD test. Test water (i.e., stock solutions, exposure solutions and controls) was charcoal-filtered UV-sterilized Burlington City water from Lake Ontario (total hardness 120–130 mg/L, alkalinity 89–93 mg/L, Ph 7.4–7.8), and was maintained in a header tank prior to use in testing. Fathead minnow eggs (<18-hour post fertilization) were purchased from Aquatox Labs (Guelph, ON) and exposed to nine nominal PFOA concentrations: 0.01, 0.032, 0.1, 0.32, 1, 3.2, 10, 32 and 100 mg/L. The tests were divided into low concentration (0.01-10 mg/L) and high concentration (32-100 mg/L) tests, with five days in the egg stage and 16 days in the larval fish stage. Tests were initiated with eggs from five to ten egg batches (from different fathead minnow breeding groups) to maximize genetic diversity and variability. There were 20 eggs per beaker, with eight replicates of controls and four replicates of each PFOA concentration in each of the two tests. Embryos and larvae were held in glass, Nitex mesh bottomed (mesh size 500 µm) egg cups within 800-Ml HDPE beakers filled to 700 Ml with test solution. Beakers containing fathead minnow eggs/larvae were aerated, loosely covered, and held in a 25°C incubator with a photoperiod of 16 hours light and 8 hours dark. Larvae were fed 10 µL/fish (0–9 dph) and 20 µL/fish (9–16 dph) of newly hatched brine shrimp slurry per day. The

first feeding (half of the daily aliquot) was two hours prior to the daily solution changeover (to remove excess food and waste), and the second feeding (the other half of the daily aliquot) was after solution changeover, so that food was available at all times during the tests. Endpoints evaluated were survival to hatch, time to hatch, hatching success, deformities at hatch, uninflated swim bladder, survival from the egg until nine and 16 dph, and weight, length, tail length, and condition factor of larvae at nine and 16 dph. The reported 21-day NOEC for mortality, weight, length, and condition factor was 76 mg/L PFOA. The EPA could not independently calculate an EC_{10} value because no effects were observed across the range of concentrations tests. Because the NOEC of 76 mg/L was a relatively tolerant NOEC value it was considered quantitatively acceptable for criteria derivation.

C.2.12 Twelfth most chronically sensitive genus - *Chironomus*

McCarthy et al. (2021) conducted a 19-day "abbreviated full life cycle" PFOA (97% purity, purchased from Sigma-Aldrich) toxicity test on the midge, *Chironomus dilutus*. The PFOA stock solution was dissolved in reconstituted moderately hard water without the use of a solvent and stored in polyethylene at room temperature until use. Authors reported that they followed standard protocols (ASTM 2005; U.S. EPA 2000) with slight modifications. Exposure vessels for both experiments were 1 L high-density polyethylene beakers containing natural field-collected sediment with 60 mL of sediment and 105 mL of test solution. PFOA test solutions were added via pipette to the beakers with the tip just above the sediment substrate. Nominal test concentrations were 0, 26, 87, 149, 210 and 272 mg/L PFOA, respectively. Test concentrations were based on the results of a 10-day range finding test conducted by McCarthy et al. (2021). Egg cases were obtained from Aquatic Biosystems or USGS Columbia Environmental Research Center and held as free-swimming hatched embryos (<24 hour after hatch) before testing. Each beaker held 12 organisms with five replicates per exposure treatment.

Solutions were renewed every 48 hours. PFOA treatment concentrations were measured on days

10, 15 and 20 in the 20-day exposure. Mean measured PFOA concentrations in the 20-day

exposure were 0 (control), 19.9, 59.4, 145, 172 and 227 mg/L PFOA. Percent survival in the

control treatment was 82%. The most sensitive endpoint was survival with an author reported 19-

day EC_{10} of 89.8 mg/L PFOA. The EPA-calculated survival-based EC_{10} was 88.32 mg/L (95%

C.I. = 15.40 – 161.3 mg/L), which was acceptable for quantitative use.

Appendix D   Acceptable Estuarine/Marine Chronic PFOA Toxicity Studies

D.1   Summary Table of Acceptable Quantitative Estuarine/Marine Chronic PFOA 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
Purple sea urchin (adult), *Paracentrotus lividus*	S, M	28 days	PFOA >98%	-	17.00	35	MATC (survival)	31.62	-	**31.62**	31.62	Savoca et al. 2022
Japanese medaka, *Oryzias latipes*	S, U	30 days	Perfluoro-octanoate 96%	7.72	24.82	34.52	NOEC (growth - condition factor)	1.0	-	**>1.0**	>1.0	Oh et al. 2013

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

[b] Values in bold used in the SMCV calculation

D.2 Detailed Study Summaries of Chronic Saltwater PFOA Toxicity Studies Considered for Use in Saltwater Criterion Derivation

The purpose of this section is to present detailed study summaries for chronic estuarine/marine tests that were considered quantitatively acceptable for criterion derivation, with summaries grouped and ordered by genus sensitivity. Unlike Appendix A.2 and Appendix C.2, the EPA-calculated C-R models were not presented below for the four most sensitive estuarine/marine genera because a chronic estuarine/marine criterion or benchmark was not developed.

D.2.1 Most chronically sensitive estuarine/marine genera – *Oryzias*

Oh et al. (2013) evaluated the chronic toxicity of PFOA on the Japanese medaka, *Oryzias latipes*, in a 30-day static exposure. PFOA (96% pure, CAS No. 335-67-1) was dissolved in filtered seawater with a minimal concentration (<0.001%) of DMSO used as a vehicle to prevent cellular damage to the fish. Only nominal concentrations were used throughout the study. Prior to test initiation, the fish were acclimated to a seawater environment. The fish were maintained at 25°C under a constant photoperiod of 16:8 hour (light:dark) and water quality was monitored by measuring the pH, dissolved oxygen, and temperature. Fish from the 3rd generation of *O. latipes* (n = 7/group) that had acclimated to seawater for over one month were used in the exposure experiments. Fish were exposed for 30 days to one PFOA concentration (1.0 mg/L), a 0.22 µm filtered seawater control, and a DMSO carrier solvent control. Test conditions were maintained at an average temperature of 24.82°C, pH of 7.72, dissolved oxygen of 6.04 mg/L, and salinity of 34.52 practical salinity units, with fish fed daily. The 30-day condition factor NOEC of 1.0 mg/L PFOA was selected as the primary endpoint from this study. In the methods section, Oh et al. (2013) also stated, "In our preliminary study, fish mortality was altered 30 days after perfluorinated compound exposure, suggesting that repeated exposure to PFCs for 30 days

at 1 µg/mL [1 mg/L] causes adverse effect on *O. latipes*." The statement about mortality-based effects in their preliminary test is in direct conflict with the condition factor-based results from the primary test described in the publication. Few details are provided about the preliminary test. Results of the final test (i.e., condition factor NOEC of 1.0 mg/L; reported in Table 1 of Oh et al. 2013) were retained as quantitatively acceptable because they provide chronic estuarine/marine data that were otherwise limited.

D.2.2 <u>Second most chronically sensitive estuarine/marine genera – *Paracentrotus*</u>
      Savoca et al. (2022) tested chronic toxicity of perfluorooctanoic acid (PFOA) to sea urchins (*Paracentrotus lividus*) in a 28-day measured, static experiment. Analytical grade PFOA (>98% purity) was obtained from Sigma-Aldrich. Thirty-six adult sea urchins were collected off the coast near Capo Zafferano, Italy, a site where little to no PFOA exposure was expected, and were acclimated in the laboratory for eight days in 200 L of filtered (30 µm) seawater. Four sea urchins were placed into one of nine aquariums containing 15 L of seawater at nominal concentrations (0 (control), 10, and 100 mg/L), with three replicates per concentration treatment. Organisms were kept at 17±1°C with a 35±1% salinity under a 12:12 light:dark cycle. To avoid PFOA uptake, organisms were not fed during the experiment and tanks did not have filtration. Instead, continuous aeration was employed, and PFOA in test water was monitored weekly in each tank. The average recovery of PFOA was stable and close to nominal across all treatments throughout the experiment, and PFOA in control water was less than 10 ng/L. Coelomic fluid was sampled from all individuals weekly to measure PFOA uptake. During the exposure period, sea urchins at the highest test concentration showed sublethal signs of toxicity, including reduced spine mobility, spine loss, and reduced ability to remain anchored to the bottom of the test vessels. Mortality was only observed at the highest test concentration. After the exposure, or in the case of urchins exposed to the highest PFOA concentrations, after the onset of adverse

effects, egg samples were dissected from the gonads of each organism, fertilized, and held at 18±1°C for 48 hours. Adult mortality, adult PFOA accumulation in coelomic fluid, percentage of normal and abnormal embryos, and gene expression in embryos were measured. Adverse effects of PFOA were observed for all endpoints. ANOVA or Kruskal-Wallis tests were used to assess significant differences in PFOA treatments compared to controls. The NOEC and LOEC for adult mortality were 10 mg/L and 100 mg/L, respectively, and the MATC of 31.62 mg/L was determined to be acceptable for quantitative use.

Appendix E   Acceptable Freshwater Plant PFOA Toxicity Studies

E.1   Summary Table of Acceptable Quantitative Freshwater Plant PFOA Toxicity Studies

Species	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Reported Effect Concentration (mg/L)	Reference
Cyanobacteria, *Microcystis aeruginosa*	S, U	12 days	PFOA ≥95%	-	25	MATC (population abundance – cell density)	15.81	Hu et al. 2023
Green alga, *Chlamydomonas reinhardtii*	S, U	96 hours	PFOA >96%	6.8	25	EC_{50} (growth)	51.9	Hu et al. 2014
Green alga, *Chlamydomonas reinhardtii*	S, U	8 days	PFOA >96%	6.8	25	MATC (cell number)	28.28	Hu et al. 2014
Green alga, *Chlamydomonas sorokiniana*	S, U	96 hours	PFOA ≥96%	7.1	25	EC_{50} (population abundance inhibition rate)	140.59	Zhao et al. 2023
Green alga (7.0×10^5 cells/mL), *Chlorella pyrenoidosa*	S, M	96 hours	PFOA >98%	-	25	EC_{50} (growth)	190.99	Xu et al. 2013
Green alga, *Chlorella pyrenoidosa*	S, M	10 days	PFOA >98%	7.1	23	NOEC (biomass)	0.73	Hu et al. 2020
Green alga, *Chlorella pyrenoidosa*	S, M	10 days	PFOA >98%	7.1	23	NOEC (biomass)	0.95	Hu et al. 2020
Green alga, *Chlorella pyrenoidosa*	S, M	10 days	PFOA >98%	7.1	23	NOEC (biomass)	0.95	Hu et al. 2020
Green alga, *Chlorella pyrenoidosa*	S, M	10 days	PFOA >98%	7.1	23	NOEC (biomass)	0.86	Hu et al. 2020
Green alga, *Chlorella pyrenoidosa*	S, M	10 days	PFOA >98%	7.1	23	NOEC (biomass)	0.84	Hu et al. 2020
Green alga, *Chlorella pyrenoidosa*	S, M	10 days	PFOA >98%	7.1	23	NOEC (biomass)	0.97	Hu et al. 2020
Green alga, *Chlorella pyrenoidosa*	S, M	10 days	PFOA >98%	7.1	23	NOEC (biomass)	0.94	Hu et al. 2020
Green alga, *Chlorella pyrenoidosa*	S, M	10 days	PFOA >98%	7.1	23	NOEC (biomass)	0.95	Hu et al. 2020
Green alga, *Chlorella pyrenoidosa*	S, M	10 days	PFOA >98%	7.1	23	NOEC (biomass)	0.83	Hu et al. 2020
Green alga (9×10^5 cells/mL), *Chlorella pyrenoidosa*	S, U	96 hours	PFOA ≥95%	-	25	NOEC (growth)	0.1	Li et al. 2021b

Species	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Reported Effect Concentration (mg/L)	Reference
Green alga (1.5 x 10⁴ cells/mL), *Chlorella vulgaris*	S, U	96 hours	PFOA 95%	-	23	IC_{50} (cell density)	115.5	Boudreau 2002
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, U	14 days	APFO 96.5-100%	-	23	EC_{10} (cell count)	5	3M Co. 2000a; Elnabarawy 1981
Green alga (1.5 x 10⁴ cells/mL), *Raphidocelis subcapitata*	S, U	96 hours	PFOA 95%	-	23	IC_{50} (cell density)	123.4	Boudreau 2002
Green alga (log phase growth), *Raphidocelis subcapitata*	S, M	96 hours	APFO 99.7%	-	21-25	MATC (biomass and growth rate)	16.07	Colombo et al. 2008
Green alga (7.0 x 10⁵ cells/mL), *Raphidocelis subcapitata*	S, M	96 hours	PFOA >98%	-	25	EC_{50} (growth)	207.46	Xu et al. 2013
Green alga, *Scenedesmus obliquus*	S, U	96 hours	PFOA >96%	6.8	25	EC_{50} (growth)	44.0	Hu et al. 2014
Green alga, *Scenedesmus obliquus*	S, U	8 days	PFOA >96%	6.8	25	NOEC (cell number)	40	Hu et al. 2014
Green alga, *Scenedesmus quadricauda*	S, M	96 hours	PFOA 99%	7	22	EC_{50} (growth inhibition rate)	269.63	Yang et al. 2014
Duckweed, *Lemna minor*	S, M	96 hours	PFOA ≥97.6%	6.5	25	NOEC (population growth rate)	9.478	Wu et al. 2023
Water milfoil (4 cm apical shoots), *Myriophyllum sibiricum*	S, M	14 days	PFOA Unreported	8.3–8.7	17.8-22.0	EC_{10} (dry weight)	8.7	Hanson et al. 2005
Water milfoil (4 cm apical shoots), *Myriophyllum sibiricum*	S, M	21 days	PFOA Unreported	8.3–8.7	17.8-22.0	EC_{10} (dry weight)	7.9	Hanson et al. 2005
Water milfoil (4 cm apical shoots), *Myriophyllum sibiricum*	S, M	35 days	PFOA Unreported	8.3–8.7	17.8-22.0	EC_{10} (wet weight)	21.6	Hanson et al. 2005
Water milfoil (4 cm apical shoots), *Myriophyllum spicatum*	S, M	14 days	PFOA Unreported	8.3–8.7	17.8-22.0	EC_{10} (dry weight)	18.1	Hanson et al. 2005
Water milfoil (4 cm apical shoots), *Myriophyllum spicatum*	S, M	21 days	PFOA Unreported	8.3–8.7	17.8-22.0	EC_{10} (plant length)	5.7	Hanson et al. 2005
Water milfoil (4 cm apical shoots), *Myriophyllum spicatum*	S, M	35 days	PFOA Unreported	8.3–8.7	17.8-22.0	EC_{10} (dry weight)	19.7	Hanson et al. 2005

Species	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Reported Effect Concentration (mg/L)	Reference
Lettuce (seed), *Lactuca sativa*	S, U	5 days	PFOA 96%	-	-	EC50 (root elongation)	745.7[b]	Ding et al. 2012b

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, NR=not reported

[b] Reported in moles converted to milligram based on a molecular weight of 414.07 mg/mmol.

E.2   Summary of Quantitatively Acceptable Plant PFOA Toxicity Studies

E.2.1   Cyanobacteria, *Microcystis aeruginosa*

Hu et al. (2023) tested perfluorooctanoic acid (PFOA) on *Microcystis aeruginosa* for 12 days in a static, unmeasured experiment. PFOA (≥95% purity) was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Test organisms were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology in Wuhan, China, and were cultured in BG-11 medium within Erlenmeyer flasks at 25°C and 30µmol m^{-2}s^{-1} photosynthetic photon flux density. *M. aeruginosa* in the exponential growth phase were added to 250 mL Erlenmeyer flasks containing 100 mL of test solution at a density of approximately 1.45x10^6 cells/mL. The experimental design consisted of nominal concentrations of 0 (control), 1, 10, 25, and 50 mg/L PFOA, diluted from a PFOA stock solution of 1,000 mg/L prepared in BG-11 medium. All concentrations were repeated in triplicate, and each Erlenmeyer flask was manually shaken twice daily during the 12-day experiment. One mL of algal-test chemical solution was sampled from each flask daily to measure cell density. Chlorophyll *a*, photosynthesis, respiration, cellular ROS production, growth inhibition, enzyme activity, emission spectra, carbohydrate content, and total RNA were also measured. Statistical differences between treatments and controls were assessed using ANOVA and Dunnett's tests ($P<0.05$). Algal cells began showing signs of growth inhibition after two days of exposure, and decreased cell density in the highest PFOA concentration after four days. Starting on day five, cell density was significantly lower than controls in the 25 and 50 mg/L treatments. The 12-day NOEC and LOEC for cell density were 10 and 25 mg/L, and the MATC of 15.81 was determined to be acceptable for quantitative use.

E.2.2   Green alga, *Chlamydomonas reinhardtii*

Hu et al. (2014) evaluated the growth inhibition of PFOA (>96% purity) with *Chlamydomonas reinhardtii* in 96-hour and eight-day static exposures. Authors stated that the

tests followed OECD test guidance 201 (OECD 2006). *Chlamydomonas reinhardtii* were

supplied by UTEX Culture Collection of Algae, University of Texas at Austin. Dilution medium

was described as modified high-salt media at a pH of 6.8. Algae in exponential growth phase

were exposed to nominal concentrations of 0 (negative control), 1, 3.16, 10, 31.6, 100, 316 and

1,000 mg/L in the 96-hour exposure, and 0, 5, 10, 20 and 40 mg/L in the eight-day exposure.

Experiments were initiated by inoculating equal cell numbers of $1x10^4$ cells/mL in the 96-hour

exposure and $5x10^6$ cells/mL in the eight-day exposure into 250 mL flasks containing a total

volume of 100 mL of algal cell suspension per flask. There were five replicates per treatment in

the 96-hour exposure and three replicates in the eight-day exposure. Algae were incubated at

25°C under cool-white fluorescence lights at 85-90 µmol photons/[m^2 x s] irradiance with a 16-

hour:8-hour light:dark cycle. The 96-hour growth EC_{50} (inhibition based on optical density) was

51.9 mg/L. The 8-day MATC based on cell number was 28.28 mg/L (NOEC and LOEC are 20

and 40 mg/L, respectively). The plant values from the study were acceptable for quantitative use.

E.2.3   Green alga, *Chlamydomonas sorokiniana*

     Zhao et al. (2023) tested perfluorooctanoic acid (PFOA) on *Chlorella sorokiniana* for 96

hours in a 96-hour, static, unmeasured experiment. The study also investigated the effects of

polystyrene microplastics on *C. sorokiniana*, both independently and in combination with PFOA,

but only the independent effects of PFOA are summarized here. PFOA (≥96% purity) was

purchased from Aladdin (Shanghai, China). Algae were purchased from the Freshwater Algae

Culture Collection at the Institute of Hydrobiology in Wuhan, China. Before the experiment, the

algae were cultured in BG-11 media in an illumination incubator at 25±1°C on a 12:12 hour

light-dark cycle at a light intensity of 2,000±50 lux. Algal cultures were shaken three times daily.

C. sorokiniana in the exponential growth phase were added to test vessels containing test

solution at a density of approximately $1.0x10^6$ cells/mL. The experimental design consisted of

nominal concentrations of 0 (control), 0.05, 0.5, and 5.0 mg/L PFOA, diluted from a PFOA stock solution of 500 mg/L prepared in BG-11 medium, with three replicates per treatment. Cell density and chlorophyll *a* were measured every 24 hours, and population growth inhibition rate was calculated after 96 hours. Several additional endpoints related to morphology and oxidative stress were also measured after 96 hours. Statistically significant ($p<0.05$) differences between treatment were assessed using ANOVA and least significant different tests. The EC_{50} for population growth inhibition rate was calculated using SPSS 19 software. The 96-hour EC_{50} value of 140.59 mg/L for population growth inhibition rate was determined to be acceptable for quantitative use.

E.2.4   Green alga, *Chlorella pyrenoidosa*

Xu et al. (2013) performed a 96-hour static, measured algal growth inhibition test on PFOA (>98% purity) with *Chlorella pyrenoidosa*. Algae were obtained from the Aquatic Organism Research Institute of the Chinese Academy of Science and precultured for three generations prior to initiating the test. Dilution medium consisted of number one culture medium supplemented with aquatic number four nutrient solution (Zhou and Zhang 1989). Algae in logarithmic growth phase (7.0×10^5 cells/mL) were inoculated in medium containing PFOA at 0 (negative control), 30, 60, 90, 120, 150, 180, 240, 300 and 360 mg/L. Tests were conducted in 100 mL conical flasks with 50 mL of solution with each concentration replicated three times. Exposure concentrations were verified via UHPLC-MS/MS using the Agilent 1290 Infinity UHPLC system interfaced with an Agilent 646-0 Triple Quadrupole mass. Algae were exposed under a 12-hour:12-hour light:dark cycle at 3,000-4,000 lux and 25°C. Chlorophyll concentration and permeability of cell membranes was determined after 96-hours of exposure. The reported 96-hour growth EC_{50} (inhibition based on optical density) was 190.99 mg/L and was considered to be acceptable for quantitative use.

Hu et al. (2020) also evaluated the toxic effects of PFOA (>98% purity) on the green alga, *Chlorella pyrenoidosa*, in both nutrient rich and nutrient limited media. PFOA was dissolved in deionized water to make stock solutions. Green algae were purchased from the Chinese Academy of Sciences and cultivated in sterile blue-green medium (BG11) with a 14-hour photoperiod and pH of 7.1, at 23 ± 1°C. Algae in the cultures were in the logarithmic growth phase before they were used for testing. Nominal PFOA test concentrations in standard growth media (BG11) were 0.05, 0.2, 0.5 and 1.0 mg/L. In the eight nutrient-limited, -enriched, or -starved tests only one PFOA test concentration (1.0 mg/L) was examined. All experiments included controls, lasted for 10 days and were replicated three times. The initial cell density for each treatment was 1.0×10^6 cells/mL and followed OECD test guidelines (OECD 2011). PFOA was measured in all test treatments at test termination. After exposure day 10, cell density, dry biomass, chlorophyll content, malondialdehyde content, catalase/peroxidase activities, and cell membrane property of algae were measured. In all tests there was no effect on plant biomass compared to the controls at the highest or only test concentration. The 10-day biomass NOECs for the study were 0.73, 0.95, 0.95, 0.86, 0.84, 0.97, 0.94, 0.95 and 0.83 mg/L PFOA for the BG11 media, N,P-limited, N-limited, P-limited, N,P-enriched, N,P-starved, N-starved, P-starved and N,P-enriched tests, respectively. All these NOECs were considered acceptable for quantitative use. Although these NOEC values are relatively low compared to the other plants and animals tested, they represent NOEC values and, therefore, do not suggest effects are expected to occur at these relatively low concentrations.

Li et al. (2021b) conducted a 12-day static, unmeasured toxicity test with PFOA (≥95% purity, purchased from Sigma-Aldrich) on the green alga, *Chlorella pyrenoidosa*. The FACHB-9 strain of the green alga was purchased from the Institute of Hydrobiology, Chinese Academy of

Sciences. The alga was cultured in BG-11 medium at 25°C under a 12-hour:12-hour light:dark photoperiod (2,000 lux) and shaken manually every 12 hours. Two PFOA solutions (0.100 and 100 µg/L) were prepared in sterile water and control solutions were sterile water only. Test solutions were added to flasks containing an initial density of 9 x 10^5 cells/mL growing in BG-11 medium, with three flasks for each treatment. The variation in algal density was observed every day over the 12-day exposure period and chlorophyll pigment content and photosynthetic activity was observed on days 3, 6, 9 and 12. During the first half of the test there was no significant difference in growth of PFOA treatments and the control. On day 12, the growth was reduced by 6.76 and 14.4% relative to the control, in the 0.1 and 100 µg/L PFOA treatments, respectively. Later time points (i.e., > 4 – 12 days of exposure) were not used quantitatively use because the exposure duration was too long (U.S. EPA 2012). The four-day cell density-based endpoint with a NOEC of 0.1 mg/L was acceptable for quantitative use.

E.2.5 Green alga, *Chlorella vulgaris*

Boudreau (2002) performed a 96-hour static algal growth inhibition test on PFOA (acid form, CAS # 335-67-1, 95% purity) with *Chlorella vulgaris* as part of a Master's thesis at the University of Guelph, Ontario, Canada. The author stated that the algal growth inhibition tests followed protocols found in ASTM E 1218-97a (ASTM 1999) and Geis et al. (2000). *Chlorella vulgaris* (UTCC 266 strain) used for testing were obtained as slants from the University of Toronto Culture Collection (UTCC; Toronto, Canada). Stock concentrations were prepared in laboratory-grade distilled water with a maximum concentration that did not exceed the critical micelle concentration for PFOA of 450 mg/L. Dilution medium was Bristol's algal growing media. Toxicity testing consisted of a range-finder test and at least two definitive tests. Nominal test concentrations were 0 (negative control), 6.7, 12.5, 25, 50, 100, 200 and 400 mg/L. Tests were conducted in 60 x 15 mm polyethylene disposable Petri dishes containing 20 mL of test

solution. Each Petri dish was inoculated with 1.5×10^4 cells/mL at initiation and replicated four

times per test concentration. Tests were continuously illuminated with cool-white, fluorescent

light between 3,800 and 4,200 lux and incubated at $23 \pm 1°C$. Replicate Petri dishes were

manually shaken twice a day during testing. Toxicity test endpoints included cell density and

chlorophyll-*a* content. The reported IC_{10}, IC_{25} and IC_{50} based on growth inhibition (measured as

either chlorophyll-*a* or cell density) were 0.014 M (95% Confidence Interval, C.I.: 0.013-0.016),

0.034 M (95% C.I.: 0.032-0.040) and 0.279 M (95% C.I.: 0.249-0.320). Note that the Icx's for

PFOA were reported in molar (M) units, but the EPA judged the units were misreported and

were actually millimolar (mM) units. This judgement was based on the reported test

concentrations in Table 3.1 of the publication and the reported effect concentrations (ICx) would

not fall within this range unless the values were in mM units. Accordingly, the Icx reported as

mM were converted to mg/L by multiplying the mM concentration by a molecular weight of

414.07 g/mol for PFOA. The calculated 96-hour IC_{10}, IC_{25} and IC_{50} expressed as mg/L from the

study were 5.797, 14.07 and 115.5, respectively and acceptable for quantitative use.

E.2.6   Green alga, *Raphidocelis subcapitata*
(formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*)

Elnabarawy (1981) and **3M Company (2000a)** provide the results of four separate

toxicity tests completed with the green alga, *Raphidocelis subcapitata* (formerly *Selenastrum*

capricornutum), and APFO (CAS # 3825-26-1). The toxicant was part of the 3M production lot

number 37 and was characterized as a mixture of APFO (96.5-100% of the compound) and C_6,

C_7 and C_9 perfluoro analogue compounds (0-3.5% of the compound). The toxicity tests followed

a protocol modified from U.S. EPA-600/9-78-018 (1978) and ASTM-E-35.23 (1981). There

were four separate exposure regimes: 1) a four-day exposure + 10-day recovery period, 2) a

seven-day exposure + seven-day recovery period, 3) a 10-day exposure + four-day recovery

period, and 4) a 14-day continuous exposure. Only the results of the continuous exposure are presented here due to the confusion if reported effect concentrations are reported before or after the recovery period. A bacteria-free culture of the alga was obtained from the USEPA (Corvallis, OR) and stored in the dark until testing. Seven-day old stock cultures with an initial density of 1 x 10^4 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 (100, 180, 320, 560, 1000 and 1800 mg/L) and control. Nutrient medium was used as the dilution media for all test treatments and were not renewed during the exposure. Algae were grown at 23°C and continuously shaken at 100 rpm. The author-reported EC_{10}, based on cell counts, was 5 mg/L for the 14-day exposure and is acceptable for quantitative use.

Boudreau (2002) also performed a 96-hour static algal growth inhibition test on PFOA (acid form, CAS # 335-67-1, 95% purity) with *Raphidocelis subcapitata* as part of the Master's thesis at the University of Guelph, Ontario, Canada. The author stated that the algal growth inhibition test with *R. subcapitata* similarly followed protocols found in ASTM E 1218-97a (ASTM 1999) and Geis et al. (2000). *R. subcapitata* (UTCC 37 strain) used for testing were obtained as slants from the University of Toronto Culture Collection (UTCC; Toronto, Canada). Stock concentrations were prepared in laboratory-grade distilled water with a maximum concentration that did not exceed the critical micelle concentration for PFOA of 450 mg/L. Dilution medium was Bristol's algal growing media. Toxicity testing consisted of a range-finder test and at least two definitive tests. Nominal test concentrations were 0 (negative control), 6.7, 12.5, 25, 50, 100, 200 and 400 mg/L. Tests were conducted in 60 x 15 mm polyethylene disposable Petri dishes with 20 mL of test solution. Each Petri dish was inoculated with 1.5 x 10^4 cells/mL at initiation and replicated four times per test concentration. Tests were continuously

illuminated with cool-white, fluorescent light between 3,800 and 4,200 lux and incubated at 23 ±1°C. Replicate Petri dishes were manually shaken twice a day during testing. Toxicity test endpoints included cell density and chlorophyll-*a* content. The reported IC_{10}, IC_{25} and IC_{50} based on growth inhibition (measured as either chlorophyll-*a* or cell density) were 0.130 M (95% C.I.: 0.020-0.162), 0.197 M (95% C.I.: 0.166-0.231) and 0.298 M (95% C.I.: 0.274-0.317). As noted above, although the Icx for PFOA were reported in molar (M) units in the thesis, the EPA judged the units were misreported and were actually millimolar (mM). This judgement was based on the reported test concentrations in Table 3.1 of the publication and the reported effect concentrations (ICx) would not fall within this range unless the values were in mM units. Accordingly, the Icx reported as mM were converted to mg/L by multiplying the mM concentration by a molecular weight of 414.07 g/mol. The calculated 96-hour IC_{10}, IC_{25} and IC_{50} expressed as mg/L from the study were 53.83, 81.57 and 123.4, respectively and acceptable for quantitative use.

More recently, **Xu et al. (2013)** conducted a 96-hour static, measured algal growth inhibition test on PFOA (acid form, >98% purity) with *Raphidocelis subcapitata*. Algae were obtained from the Aquatic Organism Research Institute of the Chinese Academy of Science and precultured for three generations prior to initiating the test. Dilution medium consisted of number one culture medium supplemented with aquatic number four nutrient solution (Zhou and Zhang 1989). Algae in logarithmic growth phase (7.0×10^5 cells/mL) were inoculated in medium containing nominal concentrations of PFOA at 0 (negative control), 30, 60, 90, 120, 150, 180, 240, 300 and 360 mg/L. Tests were conducted in 100 mL conical flasks with 50 mL of solution. Each test concentration and the control were replicated three times. Exposure concentrations were verified via UHPLC-MS/MS using the Agilent 1290 Infinity UHPLC system interfaced with an Agilent 646-0 Triple Quadrupole mass. Algae were exposed under a 12-hour:12-hour

light:dark cycle at 3,000-4,000 lux and 25°C. Chlorophyll concentration and permeability of cell membranes was determined after 96 hours of exposure. The reported 96-hour growth EC_{50} (inhibition based on optical density) was 207.46 mg/L and was considered to be acceptable for quantitative use.

Colombo et al. (2008) evaluated growth inhibition with *Raphidocelis subcapitata* on ammonium perfluorooctanoate (APFO, the ammonium salt of PFOA, CAS # 3825-26-1, 99.7% purity). Authors stated that the 96-hour algal growth inhibition test followed OECD test guidance 201 and European Commission directive 92/69/EEC. The source of *R. subcapitata* used for testing was not reported, but presumably from an in-house culture as the medium reported to be used for both culturing and testing was reconstituted water recommended via the French algae test guideline (AFNOR T 90-304). The media differs slightly from the OECD recommended media with regard to concentrations of P, N, and chelators. Stock solutions of APFO were prepared by dissolving the test substance directly in the test media and diluting to provide a geometric series of test concentrations. A range-finding and two definitive tests were conducted. Definitive tests included six negative control replicates and three replicates at each PFOA concentration. Tests were initiated via inoculation with 1×10^4 cells/mL from an algal culture in log phase growth and carried out under continuous illumination with approximately 2,000 lux and at 21-25°C. Test solutions were agitated to keep algae in suspension during the 96-hour exposure and growth was determined at 24-hour intervals by counting an aliquot of test solution from each replicate test chamber. Test concentrations measured in the second definitive algal test and were 0 (negative control), 5.76, 11.37, 22.70, 46.33, 95.87, 180.67 and 369.67 mg/L. APFO was determined as PFOA from a calibration curve of peak area against APFO concentrations in standard solutions. The limit of quantification (LOQ) of the analytical method was 1 mg/L.

Linearity was checked with a resulting coefficient of determination for the calibration curve of greater than 0.999 in the range of 1-100 mg/L. Accuracy and precision were demonstrated by analyzing six solutions containing nominal concentrations of 2.03 and 50.7 mg/L APFO in Milli-Q water. The mean measured concentrations were 2.02 and 53.7 mg/L, respectively, with calculated precision of 6% and 2% and accuracy of 99% and 106%, respectively. The reported 96-hour NOEC, based on biomass and growth rate, was 11.37 mg/L. The reported 96-hour LOEC was 22.70 mg/L. The calculated MATC was 16.07 mg/L and was considered to be acceptable for quantitative use.

E.2.7   Green alga, *Scenedesmus obliquus*

Hu et al. (2014) evaluated algal growth inhibition of PFOA (>96% purity) with *Scenedesmus obliquus* in both a 96-hour and eight-day static unmeasured exposures. Authors stated that the tests followed OECD test guidance 201 (OECD 2006). *S. obliquus* were supplied by UTEX Culture Collection of Algae, University of Texas at Austin. Dilution medium was HB-4 media adjusted to a pH of 6.8. Algae in exponential growth phase were exposed to nominal concentrations of 0 (negative control), 1, 3.16, 10, 31.6, 100, 316 and 1,000 mg/L in the 96-hour exposure, and 0, 5, 10, 20 and 40 mg/L in the eight-day exposure. Experiments were initiated by inoculating equal cell numbers of 5×10^3 cells/mL in the 96-hour exposure and 5×10^6 cells/mL in the eight-day exposure into 250 mL flasks containing a total volume of 100 mL of algal cell suspension per flask. There were five replicates per treatment in the 96-hour exposure and three replicates in the eight-day exposure. Algae were incubated at 25°C under cool-white fluorescence lights at 85-90 µmol photons/[m² x s] irradiance with a 16-hour:8-hour light:dark cycle. The 96-hour growth EC_{50} (inhibition based on optical density) was 44.0 mg/L. The eight-day NOEC based on cell number was 40 mg/L (the highest test concentration). The plant values from the study were acceptable for quantitative use.

E.2.8   Green alga, *Scenedesmus quadricauda*

Yang et al. (2014) conducted a 96-hour renewal, measured test on the growth effects of PFOA (acid form, CAS #335-67-1, 99% purity) with the green alga, *Scenedesmus quadricauda*. Algae were obtained from in-house cultures originally supplied by the Chinese Research Academy of Environmental Sciences. The algae used for testing were inoculated at a cell density equal to 2.0×10^4 cells/mL in 50 mL beakers. PFOA was dissolved in deionized water and DMSO (amount not provided) and then diluted with M4 medium. Algae in logarithmic growth phase were exposed to 0 (solvent control), 80.00, 128.00, 204.80, 327.68, 524.29 and 838.86 mg/L. Each treatment was replicated three times. Measured concentrations ranged from 75.68 mg/L (before renewal) to 78.8 mg/L (after renewal) in the lowest treatment, and from 764.13 (before renewal) to 831.45 mg/L (after renewal) in the highest treatment. The experiments were conducted at $22 \pm 2°C$ with a 12-hour: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 growth inhibition EC_{50} was reported as 269.63 mg/L and was acceptable for quantitative use.

E.2.9   Duckweed, *Lemna minor*

Wu et al. (2023) tested perfluorooctanoic acid (PFOA) on duckweed, *Lemna minor*, for 96 hours in a static, measured experiment. PFOA ($\geq$97.6% purity) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). DMSO solvent ($\geq$ 99.9% purity) was obtained from Merck (Germany). Duckweed was obtained from in house cultures that had been grown in a modified Swedish Standards Institute (SIS) medium. Duckweed cultures were housed in 150 mm petri dishes with 100 mL SIS medium that was changed every two weeks. SIS medium pH was adjusted to 6.5 by using NaOH or HCl. Plants were cultured at $25\pm1°C$ and 60% humidity under a 12:12 light dark cycle at 2,000 lux. Duckweed was precultured for 1 week in clean SIS media

for seven days prior to testing. Duckweed experiments were conducted in 6-well poly-propylene plates to avoid PFOA sorption to container walls. Each replicate well contained 10 mL of test material and two colonies approximately the same size of a 3-frond *L. minor*. Nominal test concentrations were 0 (control), 0.001, 0.1, and 10 mg/L PFOA. All test chambers included DMSO solvent, and each treatment had three duplicates. PFOA concentrations measured on day 0 were 0.93±0.04, 97.2±0.89, and 9,478±4.74 µg/L. PFOA in solvent controls were not reported for day 0 but was below detection levels when measured after 96 hours. The number of fronds in each treatment well were counted after 48 and 96 hours. In addition, Fourier-transform infrared spectroscopy (FTIR) was performed on a subset of fronds from each treatment at the end of the 96-hour exposure to examine responses to PFOA at the biochemical level. Statistically significant (p<0.05) differences between treatment groups were assessed with one-way ANOVA followed by Dunnett's tests using SPSS Statistics 26. No statistically significant differences in frond number were observed. However, FTIR analysis revealed structural and functional alterations in response to PFOA at the biochemical level. The reported NOEC of 9.478 mg/L for population growth rate was determined to be acceptable for quantitative use.

E.2.10 Watermilfoil, *Myriophyllum sp.*

Hanson et al. (2005) conducted a 35-day microcosm study on PFOA (sodium salt donated by 3M Co., 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 using in-house cultures of *Myriophyllum* spp. 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, 4 cm in length, from in-house cultures using the same sediment were

transferred to each microcosm, with three separate microcosms used for each treatment (nominal concentrations 0, 0.3, 10, 30 and 100 mg/L). Endpoints of toxicity that were monitored on days 14, 21 and 35 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. PFOA treatments were dissolved in the same water (well water) used to supply the microcosms. Results 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. The time-weighted average measured concentrations were 0 (negative control), 0.27, 0.65, 23.9 and 74.1 mg/L. Water quality over the length of the 35-day microcosm experiment was: dissolved oxygen: 7.3-8.5 mg/L; temperature: 17.8-22.0°C; pH: 8.3-8.7; total hardness: 217.5 mg/L as $CaCO_3$. The light:dark cycle was outdoor ambient cycles beginning June 13, 2000 (Guelph, Ontario). The watermilfoil species were equally sensitive to PFOA. The 35-day EC_{10} (based on weight) was 21.6 mg/L for *M. sibiricum* and 19.7 mg/L for *M. spicatum*. The plant values were acceptable for quantitative use.

E.2.11 Lettuce, *Lactuca sativa*

Ding et al. (2012b) conducted a microcosm study where water lettuce, *Lactuca sativa,* was exposed to PFOA (CAS# 335-67-1, 96% purity) for 5 days. Authors stated the test protocols followed U.S. EPA (1996). Test vessels were plastic and test solutions were static and unmeasured. The test employed six exposure concentrations and a negative control, with each treatment being replicated three times. Petri dishes containing lettuce seeds were placed in a plant test chamber with a constant room temperature of 18°C ±2°C and a photoperiod or 16-hours light and 8-hours dark. After 5 days, the number of germinated seeds was counted, and the length of the roots was measured with a ruler to the closest millimeter. The author reported EC_{50}

(endpoint = root elongation) was 1.801 mM, which was converted to 745.7 mg/L and was retained for quantitative use.

Appendix F   Acceptable Estuarine/Marine Plant PFOA Toxicity Studies

Species	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Effect	Reported Effect Concentration (mg/L)	Reference
Green alga, *Chlorella sp.*	S, U	96 hours	PFOA >98%	-	23	30[b]	EC_{50} (population abundance)	127.35	Mao et al. 2023

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, NR=not reported

[b] Salinity of Erdschreiber's medium

F.1 Summary of Quantitatively Acceptable Plant PFOA Toxicity Studies

F.1.1 Green alga, *Chlorella sp.*

Mao et al. (2023) tested perfluorooctanoic acid (PFOA) on an estuarine/marine *Chlorella sp.* For seven days in a static, unmeasured experiment. PFOA (>98% purity) was obtained from Tokyo Chemical Industry Co. Ltd. Algae were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology in Wuhan, China. Algae were obtained from the Algae Culture Collection at the Institute of Hydrobiology in Wuhan, China. Algae were cultured in Erdschreiber medium within a conical flask inside an illumination incubator at $23\pm1°C$ under a 12:12 light:dark cycle at 5,000 lux. Algae were shaken three times per day to prevent sticking to the sides of the flask, and inoculated once every two weeks to maintain optimal growth. *Chlorella sp.* In the exponential growth phase were added to test vessels containing test solution at a density of approximately 5.0×10^4 cells/mL. The experimental design consisted of nominal concentrations of 0 (control), 5, 10, 20, 40, 80, 160, and 320 mg/L PFOA. Testing protocols followed OECD guidelines, and all experiments were performed in triplicate. Statistical analysis included one-way ANOVA, using SPSS version 26 software. Algal cell density and size were measured daily, and algal growth inhibition was calculated using the equation provided in the OECD guidelines. Chlorophyll *a*, maximum quantal yield, cell membrane integrity, esterase activity relative to control, relative electron transfer rate, and reactive oxygen species were reported after 1, 3, 5, and 7 days, respectively. Algae exhibited maximum growth at 20 mg/L, but growth significantly declined at 80 mg/L and higher concentrations. Increasing PFOA concentrations also inhibited chlorophyll *a*, and increased oxidative stress. The 96-hour EC_{50} for algal growth inhibition was 127.35 mg/L, and was determined to be acceptable for quantitative use.

Appendix G   Other Freshwater PFOA Toxicity Studies

G.1   Summary Table of Qualitative Freshwater PFOA 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 hours	PFOA 96%	-	-	EC_{50} (bioluminescence inhibition)	-	19.81	Duration too short for a plant test, missing some exposure details, non-apical endpoint	Rodea-Palomares et al. 2012
Cyanobacteria, *Anabaena sp.*	S, U	24 hours	PFOA 96%	7.8	28	EC_{50} (bioluminescence inhibition)	-	78.88	Duration too short for a plant test, missing some exposure details, non-apical endpoint	Rodea-Palomares et al. 2015
Blue green alga, *Synechocystis sp.*	S, M	4 days	PFOA 96%	7.5	30	NOEC (population abundance)	1->1	1	Duration, focus of study was PFOA removal	Marchetto et al. 2021
Blue green alga, *Synechocystis sp.*	F, M	12-15 days	PFOA 96%	7.5	30	NOEC (biomass)	1->1	1	Duration, focus of study was PFOA removal	Marchetto et al. 2021
Green alga, *Raphidocelis subcapitata* (formerly, *Selenastrum capricornutum*)	S, U	96 hours	PFOA 96.5-100%	2.3-10.3	-	EC_{50} (cell density and growth rate)	-	90	Possible mixture effects of other perfluoro homologue compounds and the amount of isopropanol, wide pH range	3M Company 2000a
Green alga, *Raphidocelis subcapitata*	S, U	96 hours (+ 10-day recovery period)	APFO 96.5-100%	-	23	EC_{10} (cell count)	-	5.3	Unclear if chronic value determined before or after recovery period	3M Company 2000a/Elnabarawy 1981
Green alga, *Raphidocelis subcapitata*	S, U	7 days (+ 7-day recovery period)	APFO 96.5-100%	-	23	EC_{10} (cell count)	-	3.3	Unclear if chronic value determined before or after recovery period	3M Company 2000a/Elnabarawy 1981
Green alga, *Raphidocelis subcapitata*	S, U	10 days (+ 4-day recovery period)	APFO 96.5-100%	-	23	EC_{10} (cell count)	-	2.9	Unclear if chronic value determined before or after recovery period.	3M Company 2000a/ Elnabarawy 1981

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
Green alga, *Raphidocelis subcapitata*	S, U	96 hours	APFO Unknown	-	-	EC$_{50}$ (cell count)	-	1,980	Test substance was not characterized but considered a mixture of APFO and other PFAS impurities; test substance purity unknown and not used quantitatively because other tests from 3M Company (2000a) indicated test purity as low as 78%. 3M Company 2000a also indicated other tests with green alga lacked information on test substance purity.	3M Company. 2000a
Green alga, *Raphidocelis subcapitata*	S, M	72 hours	PFOA 96%	-	21-24	EC$_{50}$ (growth)	-	96.2	Duration too short for a plant test, missing some exposure details	Rosal et al. 2010
Green alga, *Raphidocelis subcapitata*	S, U	4.5 hours	PFOA 96%	-	-	EC$_{50}$ (photosynthetic efficiency)	-	748.2[c]	Duration too short for a plant test, missing some exposure details, non-apical endpoint	Ding et al. 2012b
Green alga (10^4 cells/mL), *Scenedesmus obliquus*	S, U	72 hours	PFOA Unreported	7.5	22	NOEC (growth rate)	-	828.1[c]	Duration too short for a plant test	Liu et al. 2008a
Duckweed, *Lemna gibba*	S, U	7 days	PFOA 95%	-	-	IC$_{50}$ (wet weight)	-	79.92	Culture water not characterized, missing some exposure details	Boudreau 2002
Protozoa, *Tetrahymena pyriformis*	S, U	2 hours	PFOA Unreported	7.2	25	EC50 (population abundance)	-	713.9[c]	Single cell organism	Lim 2022

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Protozoa, *Tetrahymena pyriformis*	S, U	96 hours	PFOA Unreported	7.2	25	EC50 (population abundance)	-	65.1	Single cell organism	Lim 2022
Tubificid worm (0.03 g, 0.8 cm), *Limnodrilus hoffmeisteri*	S, M	96 hours	PFOA 99%	7	22	LC_{50}	-	568.20	Atypical source of organisms	Yang et al. 2014
Planaria (0.9 cm), *Dugesia japonica*	S, U	96 hours	PFOA >98%	-	25	LC_{50}	-	427.7	Poor concentration-response curve	Li 2009
Planaria (10-12 mm), *Dugesia japonica*	R, U	96 hours	PFOA 96%	-	20	LC_{50}	-	39.35	Atypical source of the test organisms	Yuan et al. 2015
Planarian, *Dugesia japonica*	R, U	10 days	PFOA 96%	-	20	LOEC (decrease mRNA expression levels of neural genes DjFoxD, DjotxA and DjotxB)	<0.5-0.5	0.5	Duration too long for an acute test and too short for a chronic test, non-apical endpoint	Yuan et al. 2016b
Planarian, *Dugesia japonica*	S, U	10 days	PFOA 96%	-	20	LOEC (elevated lipid peroxidation; increased mRNA expression levels of HSP 40 and HSP 70)	<0.5-0.5	0.5	Duration too long for an acute test and too short for a chronic test, non-apical endpoint	Yuan et al. 2017
Planarian, *Dugesia japonica*	R, U	10 days	PFOA Unreported	-	25	LOEC (growth, behavioral, genetic and biochemistry changes)	-	15	Duration, effects no longer seen after 8-day recovery period	Zhang et al. 2020
Planarian, *Dugesia japonica*	R, U	10 days	PFOA Unreported	-	-	LOEC (genetic and biochemistry changes)	-	15	Duration too long for an acute test and too short for an acute test, atypical endpoints	Zhang et al. 2022
Chinese pond mussel (1 year), *Sinanodonta woodiana* (formerly, *Anodonta woodiana*)	S, U	48 hours	PFOA Unreported	7.0	24	LC_{50}	-	192.08	Missing some exposure details, duration	Xia et al. 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
Snail (adult, 1 yr), *Sinotaia aeruginosa* (formerly, *Bellamya aeruginosa*)	Sediment	21 days	PFOA >96%	7.98-8.02	24	LOEC (biochemistry and enzyme changes)	-	0.00276 – 0.00286	Sediment exposure, missing exposure details	Xiang et al. 2021
Mud snail (4.0 g, 2.0 cm) *Cipangopaludina cathayensis*	S, M	96 hours	PFOA 99%	7	22	LC$_{50}$	-	740.07	Atypical source of organisms	Yang et al. 2014
Rotifer (<2-hour old neonates), *Brachionus calyciflorus*	R, U[d]	4 days	PFOA 96%	-	20	EC$_{10}$ (resting egg production)	0.125 – 0.25	0.1768 (EPA-Calculated EC$_{10}$: 0.07758)	Only one replicate	Zhang et al. 2014b
Cladoceran, *Daphnia magna*	S, U	48 hours	PFOA 96.5-100%	7.5-8.4	19.4-20.2	EC$_{50}$ (death/immobility)	-	360	Possible mixture effects of other perfluoro homologue compounds and the amount of isopropanol; missing exposure details	3M Company 2000a
Cladoceran, *Daphnia magna*	S, U	48 hours	APFO 96.5-100%	-	-	EC$_{50}$ (death/immobility)	-	>1,000	Possible mixture effects of other perfluoro analogue compounds; <50% effect in highest test concentration; Authors indicate test may have included food	3M Company 2000a
Cladoceran, *Daphnia magna*	S, U	48 hours	APFO 96.5-100%	-	-	EC$_{50}$ (death/immobility)	-	126	Possible mixture effects of other perfluoro analogue compounds; authors indicate test may have included food	3M Company 2000a

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 hours old), *Daphnia magna*	S, U	48 hours	APFO 78-93%	8.0-8.1	21	EC_{50} (death/immobility)	-	221	Possible mixture effects of other perfluoro analogue compounds; low test substance purity (i.e., 78%).	3M Company 2000a
Cladoceran (<24 hours old), *Daphnia magna*	S, U	48 hours	APFO Unreported	8.1-8.3	20.3-20.8	LC_{50}	-	1,200	Test substance is considered a mixture of APFO and other impurities; test substance purity unknown and not used quantitatively because other tests from 3M Company (2000a) indicated test purity as low as 78%.	3M Company 2000a
Cladoceran (<24 hours old), *Daphnia magna*	S, U	48 hours	APFO Unreported	8.1-8.4	19.5-20.1	EC_{50} (death/immobility)	-	584	Possible mixture effects of the inert perfluorinated compounds and other perfluoro analogue compounds; test substance purity unknown and not used quantitatively because other tests from 3M Company (2000a) indicated test purity as low as 78%.	3M Company 2000a
Cladoceran, *Daphnia magna*	R, U	48 hours	APFO 96.5-100%	-	-	EC_{50} (death/immobility)	-	266	Missing test details	3M Company 2000a
Cladoceran, *Daphnia magna*	R, U	21 days	APFO 96.5-100%	-	-	MATC (survival and reproduction)	22-36	28.14	Missing test details	3M Company 2000a

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 hours old), *Daphnia magna*	R, U	21 days	PFOA >99%	7.5	23	LOEC (fecundity)	<0.4141-0.4141	0.4141[c]	Chronic responses in this test did not display concentration-dependent effects beyond the LOEC despite a 25X increase in treatment concentrations	Seyoum et al. 2020
Cladoceran (adult, ~14 days), *Daphnia magna*	S, M	48 hours	APFO 98%	-	21	MATC (arginine, lysine, uridine G-6mountG-6ation)	27.88-46.33	35.94	Non-apical endpoint	Labine et al. 2022
Amphipod (7 days), *Hyalella azteca*	R, M	7 days	PFOA 99.5%	7.31 (6.85-7.76)	22.6 (21.9-23.2)	EC$_{20}$ (growth – biomass)	-	5.0	Duration too short for a chronic exposure	Kadlec et al. 2024
Oriental river prawn (0.30 g, 4.0 cm), *Macrobrachium nipponense*	S, M	96 hours	PFOA 99%	7	22	LC$_{50}$	-	366.66	Atypical source of organisms	Yang et al. 2014
Midge (larva, 10 days old), *Chironomus dilutus*	R, U	10 days	PFOA >97%	-	23	NOEC (survival and growth)	-	100	Lack of effects in all concentrations tested; Range-finding experiment only with few details reported in the publication; Primary focus of the publication was on PFOS	MacDonald et al. 2004
Midge (larva, 10 days old), *Chironomus dilutus*	S, M	10 days	PFOA 97%	-	-	MATC (mortality)	26-272	84.10	Range-finding experiment only; results of the definitive 19-day test by McCarthy et al. 2021 were used preferentially over this test.	McCarthy et al. 2021
Midge (Instar, 3 days), *Chironomus dilutus*	R, M	7 days	PFOA 99.5%	6.96 (6.77-7.19)	22.7 (22.2-23.0)	EC$_{20}$ (growth – biomass)	-	70.6	Duration too short for a chronic exposure	Kadlec et al. 2024

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 (Instar, 3 days), *Chironomus dilutus*	R, M	7 days	PFOA 99.5%	6.96 (6.86-7.11)	23.3 (23.0-23.8)	EC_{20} (growth – biomass)	-	94.0	Duration too short for a chronic exposure	Kadlec et al. 2024
Midge (Instar, 3 days), *Chironomus dilutus*	R, M	7 days	PFOA 99.5%	7.39 (7.26-7.60)	22.9 (20.3-24.0)	EC_{20} (growth – biomass)	-	94.2	Duration too short for a chronic exposure	Kadlec et al. 2024
Midge (multi-generational), *Chironomus riparius*	S, M	~20-38 days / generation	PFOA Pure (unspecified)	7.8-8.2	20	NOEC (emergence, reproduction, sex ratio)	-	0.0089	Only one exposure concentration, static chronic exposure	Stefani et al. 2014
Midge (multi-generational), *Chironomus riparius*	S, M	~20-38 days / generation	PFOA Pure (unspecified)	7.8-8.2	20	NOEC (increased mutation rate)	-	0.0089	Only one exposure concentration, static chronic exposure	Stefani et al. 2014
Midge (larva, 1st instar), *Chironomus riparius*	S, M	~1 year[b]	PFOA Unreported	7.5-8.2	20.1	LOEC (F10 developmental time, adult weight, exuvia length)	<0.0098-0.0098	0.0098	Only one exposure concentration, static chronic exposure, low control survival in 4 of the 10 generations	Marziali et al. 2019
Midge (0.05 g, 1.2 cm), *Chironomus plumosus*	S, M	96 hours	PFOA 99%	7	22	LC_{50}	-	402.24	Atypical source of organisms	Yang et al. 2014
Midge (larva, 3rd-4th instar), *Chironomus plumosus*	S, M	10.33 days	PFOA 96%	-	25	NOEC (mortality)	-	9.8 ng/g (sediment)	10-day subchronic sediment exposure and no effects at the highest concentration tested resulting in a relatively low greater than value that does not inform species sensitivity	Zhai et al. 2016
Rainbow trout (juvenile, 40-50 mm), *Oncorhynchus mykiss*	S, U	96 hours	APFO 99.7%	6.0-8.5	13-17	LC_{50}	-	707[e]	High ammonia concentration in treatment solutions	Colombo 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
Rainbow trout (fry), *Oncorhynchus mykiss*	Diet, U	70 days	PFOA Unreported	-	14	MATC (liver somatic index)	200-1,800 (mg/kg)	600 (mg/kg diet)	Non-apical endpoint	Tilton et al. 2008
Rainbow trout (fry), *Oncorhynchus mykiss*	Diet, U	6 months	PFOA Unreported	-	14	MATC (palmitoyl CoA β-oxidation – liver enzyme)	200-1,800 (mg/kg)	600 (mg/kg diet)	Non-apical endpoint	Tilton et al. 2008
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Diet, U	15 days	PFOA Unreported	-	12	MATC (increase plasma vitellogenin)	5-50 (mg/kg)	15.81 (mg/kg diet)	Test design and lack of exposure details	Benninghoff et al. 2011
Rainbow trout (fry, 10-15 weeks old), *Oncorhynchus mykiss*	Diet, U	6 months	PFOA Unreported	-	12	LOEC (increase tumor multiplicity and size)	<2,000-2,000	2,000 (mg/kg diet)	Test design and lack of exposure details	Benninghoff et al. 2012
Rainbow trout (oocyte, ova), *Oncorhynchus mykiss*	S, M	3 hours	PFOA >97%	-	6	NOEC (accumulation residue)	-	10.58	Duration too short for an acute test	Raine et al. 2021
Rainbow trout (oocyte, ova), *Oncorhynchus mykiss*	S, M	3 hours	PFOA >97%	8.5	6	NOEC (accumulation residue)	-	10.11	Duration too short for an acute test	Raine et al. 2021
Rainbow trout (juvenile, 7 months), *Oncorhynchus mykiss*	R, M	10 days	PFOA Unreported	-	12	LOEC (7-Benzyloxy-4-trifluoromethylcoumarin O-debenzylase levels)	-	0.0005	Test was too long for an acute exposure and too short for a chronic exposure; non-apical endpoint	Solan et al. 2022
Atlantic salmon (embryo-larval), *Salmo salar*	F, U	52 days	PFOA 95%	-	5-7	NOEC-LOEC (growth – weight and length)	0.1->0.1	>0.1	No effects at the highest concentration tested resulting in a relatively low greater than value that does not inform species sensitivity	Spachmo and Arukwe 2012
Atlantic salmon (embryo), *Salmo salar*	R, U	49 days	Perfluoro-n-octanoic acid Unreported	-	5-7	NOEC (growth – weight and length)	0.1->0.1	>0.1	Lack of treatment concentrations and lack of effects observed at a relatively low NOEC	Arukwe 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
Goldfish (6.0 g, 7.0 cm), *Carassius auratus*	S, M	96 hours	PFOA 99%	7	22	LC_{50}	-	606.61	Atypical source of organisms	Yang et al. 2014
Goldfish (juvenile, 27.85 g), *Carassius auratus*	S, M	96 hours	PFOA >98%	7.25	23	Antioxidant enzyme activity	-	>4.931[c]	Only two exposure concentrations, non-apical endpoint	Feng et al. 2015
Goldfish (juvenile, 12 months), *Carassius auratus*	F, M	7 days	PFOA >96%	9	-	LC_{50}	-	>471.5	Test was too long for an acute exposure and too short for a chronic exposure	Dong et al. 2023
Goldfish (juvenile, 12 months), *Carassius auratus*	F, M	7 days	PFOA >96%	9	-	LC_{50}	-	>456.3	Test was too long for an acute exposure and too short for a chronic exposure	Dong et al. 2023
Goldfish (juvenile, 12 months), *Carassius auratus*	F, M	7 days	PFOA >96%	9	-	LC_{50}	-	>411.7	Test was too long for an acute exposure and too short for a chronic exposure	Dong et al. 2023
Goldfish (juvenile, 12 months), *Carassius auratus*	F, M	7 days	PFOA >96%	9	-	LC_{50}	-	>433.2	Test was too long for an acute exposure and too short for a chronic exposure	Dong et al. 2023
Goldfish (juvenile, 12 months), *Carassius auratus*	F, M	7 days	PFOA >96%	9	-	LC_{50}	-	>451.1	Test was too long for an acute exposure and too short for a chronic exposure	Dong et al. 2023
Goldfish (juvenile, 12 months), *Carassius auratus*	F, M	7 days	PFOA >96%	9	-	LC_{50}	-	>440.1	Test was too long for an acute exposure and too short for a chronic exposure	Dong et al. 2023
Common carp (juvenile, ~12 cm, ~20 g), *Cyprinus carpio*	F, M	96 hours	PFOA 99.8%	6.9	23	LOEC (vitellogenin (VTG) activity)	-	6.582	Broad range of test treatments, non-apical endpoint	Kim et al. 2010

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Common carp (adult – 2 years old), *Cyprinus carpio*	F, U (tissue)	56 days	PFOA 96%	6.7-8.0	10-15	LOEC (PCNA-positive hepatocyte abundance)	0.0002-2	2	Poor test design, only two exposure concentrations, non-apical endpoint	Giari et al. 2016
Common carp (adult – 2 years old), *Cyprinus carpio*	F, U (tissue)	56 days	PFOA 96%	6.7-8.0	10-15	LOEC (liver biomarkers)	0.0002-2	2	Only two exposure concentrations, non-apical endpoint	Manera et al. 2017
Common carp (adult – 2 years), *Cyprinus carpio*	F, U	56 days	PFOA Unreported	6.7-8.0	10-15	LOEC (number of rodlet cells)	-	0.0002	Non-apical endpoint	Manera et al. 2022b
Common carp (adult – 2 years), *Cyprinus carpio*	F, U	56 days	PFOA Unreported	-	-	LOEC (histological effects)	-	0.0002	Non-apical endpoint	Manera et al. 2022a
Common carp, *Cyprinus carpio*	R, M	14 weeks	PFOA Unreported	7.71	21.8	NOEC (mortality, length and weight)	-	>0.0921	Greater than low value	Petre et al. 2023
Zebrafish (embryo), *Danio rerio*	R, U	96 hours	APFO 98%	-	26	LC$_{50}$	-	386.3[c]	Inability to verify LC50	Ding et al. 2012c, 2013
Zebrafish (embryo), *Danio rerio*	S, U	72 hours	PFOA 95%	8.3	28.5	LC$_{50}$	-	262	Duration too short for acute test	Zheng et al. 2012
Zebrafish (embryo), *Danio rerio*	S, U	96 hours	PFOA 95%	8.3	28.5	EC$_{50}$ (malformation)	-	198	Non-apical endpoint	Zheng et al. 2012
Zebrafish (embryo, 4 hpf), *Danio rerio*	R, U	120 hours	PFOA Unreported	-	28	NOEC-LOEC (increase relative mRNA expression of hhex and pax 8)	0.1-0.2	-	Duration too short for a chronic test and too long for an acute test, non-apical endpoint, only three exposure concentrations	Du et al. 2013
Zebrafish (adult), *Danio rerio*	R, U (tissue)	28 days	PFOA 96 %	-	26	NOEC (reproduction: fecundity, fertility and hatching)	1->1	1	Not a true ELS test	Hagenaars et al. 2013
Zebrafish (adult), *Danio rerio*	R, U (tissue)	28 days	PFOA 96 %	-	26	LOEC (alterations of gene transcripts)	<0.1-0.1	0.1	Not a true ELS test, non-apical endpoint	Hagenaars 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, 4 cell stage), *Danio rerio*	S, U	Fertilization to 144 hours post-fertilization (6 days)	PFOA Unreported	7.2-7.6	26	EC$_{50}$ (lethal and sublethal endpoint)	-	350	Static chronic exposure	Ulhaq et al. 2013
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hours	APFO Unreported	-	-	LOEC (mortality)	<0.02759-0.02759	0.02759[c]	Duration too long for acute test	Truong et al. 2014
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hours	PFOA Unreported	-	-	NOEC (mortality)	26.50->26.50	26.50[c]	Duration too long for acute test	Truong et al. 2014
Zebrafish (adult), *Danio rerio*	R, U	21 days	PFOA 96 %	-	27	MATC (decrease in inflammatory cytokines (IL-1ß and IL-21) in spleen)	0.05-0.1	0.0707	Duration too short for a chronic test, non-apical endpoint	Zhang et al. 2014a
Zebrafish (embryo, 2 days pf), *Danio rerio*	S, U	72 hours	PFOA 96%	-	28.5	LC$_{50}$	-	157.3[c]	Duration too short for an acute test	Kalasekar et al. 2015
Zebrafish (embryo), *Danio rerio*	S, U	72 hours	PFOA Unreported	-	26	NOEC (embryo toxicity)	-	132.5[c]	Only one exposure concentration and duration too short for an acute test	Bouwmeester et al. 2016
Zebrafish (gastrula stage, 4.5 hpf), *Danio rerio*	R, U	Embryo development to 28 days post-hatch	PFOA Unreported	-	28	LOEC (swim bladder development)	-	4.7	Unconventional test design, diet and water concentrations were not measured	Godfrey et al. 2017b
Zebrafish (3 hpf), *Danio rerio*	S, U	5-day + 9-day observation	PFOA Unreported	7.2-7.7	26-28	MATC (growth – total body length, interocular distance, yolk sac area)	0.2-2	0.6325	Duration too long for an acute test,	Jantzen et al. 2017a
Zebrafish (3 hpf), *Danio rerio*	S, U	5-day + 9-day observation	PFOA Unreported	7.2-7.7	26-28	MATC (swimming activity – distance traveled)	0.02-0.2	0.06325	Duration too long for an acute test, non-apical endpoint	Jantzen et al. 2017a
Zebrafish (embryo, 72 hpf), *Danio rerio*	S, M	48 hours	PFOA Unreported	-	27	LC$_{50}$	-	>500	Duration too short for an acute test	Rainieri et al. 2017
Zebrafish (embryo, ≤2 hpf), *Danio rerio*	S, U	96 hours	PFOA 96%	-	28	LC$_{50}$	-	<50	Results only reported graphically, control mortality not reported	Weiss-Errico et al. 2017

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 3 hpf), *Danio rerio*	S, U	117 hours + 9 days observation	PFOA Unreported	7.2-7.7	27	MATC (morphology)	0.008281-0.08281	0.02619	Duration too long for an acute test	Annunziato 2018
Zebrafish (embryo, 6 hpf), *Danio rerio*	R, M	7 days	PFOA 95%	7.8	25	LOEC (aromatase, CYP19A1 mRNA, estrogen receptor alpha mRNA, 17-beta estradiol, androgen binding protein)	<0.4969-0.4969	0.4969[c]	Duration too long for an acute test and too short for a chronic test, non-apical endpoint	Chen et al. 2018
Zebrafish (embryo), *Danio rerio*	S, U	168 hours	PFOA Unreported	-	-	LC$_{50}$	-	362.5	Duration too long for an acute test	Stinckens et al. 2018
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hours	PFOA Unreported	-	-	Benchmark Dose at 10% extra effect (abnormal development)	-	27.75[c]	Duration too long for an acute test and too short for a chronic test, non-apical endpoint	Thomas et al. 2019
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	18 hours	APFO Unreported	-	-	Benchmark Dose at 10% extra effect (immobility)	-	27.59[c]	Duration too long for an acute test, non-apical endpoint	Thomas et al. 2019
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hours	APFO Unreported	-	-	Benchmark Dose at 10% extra effect (mortality)	-	24.50[c]	Duration too long for an acute test and too short for a chronic test, non-apical endpoint	Thomas et al. 2019
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, M	118 hours	PFOA >96%	-	28	EC$_{50}$	-	210.8[c]	Duration too long for an acute test, no true replication	Vogs et al. 2019
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, M	118 hours	PFOA >96%	-	28	EC$_{20}$ (deformities)	-	147.2[c]	Duration too long for an acute test, no true replication	Vogs et al. 2019
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	66 hours	PFOA 96%	-	28	NOEC (survival and development)	20.70->20.70	20.70[c]	Duration too short for an acute test, only one exposure concentration	Dasgupta et al. 2020
Zebrafish (embryo, 3 dpf), *Danio rerio*	S, U	24 hours	PFOA Unknown	-	28	LC$_{50}$	-	96.06[c]	Duration too short for an acute test, missing exposure details	Gebreab et al. 2020

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 3 dpf), *Danio rerio*	S, U	7 days	PFOA Unknown	-	28	LC_{50}	-	>41.41 to <82.81[c]	Duration too long for an acute test and too short for a chronic test, missing exposure details	Gebreab et al. 2020
Zebrafish (embryo, 4-64 stage), *Danio rerio*	S, M	144 hours	PFOA ≥98%	7.2-7.6	26	NOEC (survival and development)	61->61	61	Duration too long for an acute test and too short for a chronic test	Menger et al. 2020
Zebrafish (embryo, 1 hpf), *Danio rerio*	S, U	48 hours	PFOA Unreported	-	28	LC_{50}	-	300	Duration too short for an acute test	Pecquet et al. 2020
Zebrafish (embryo, 1 hpf), *Danio rerio*	S, M	24 hours	PFOA Unreported	-	28	LOEC (increase neutrophil migration)	<0.685-0.685	0.685	Duration too short for an acute test, atypical endpoint	Pecquet et al. 2020
Zebrafish (embryo, 5-6 hpf), *Danio rerio*	R, U	90-91 hours	PFOA >99%	-	28	LC_{50}	-	57.6	Duration too short for an acute test	Wasel et al. 2020
Zebrafish (embryo, 5-6 hpf), *Danio rerio*	R, U	90-91 hours	PFOA >99%	7	28	LC_{50}	-	487.4	Duration too short for an acute test	Wasel et al. 2020
Zebrafish (embryo), *Danio rerio*	R, M	5 days	APFO >95%	-	25	NOEC (mortality)	-	>1.582[c]	Duration too long for an acute test and too short for a chronic test	Han et al. 2021
Zebrafish (embryo), *Danio rerio*	R, M	5 days	PFOA >95%	-	25	NOEC (mortality)	-	>1.631[c]	Duration too long for an acute test and too short for a chronic test	Han et al. 2021
Zebrafish (embryo, 4 hpf), *Danio rerio*	R, U	5 days	PFOA 96%	7.6-7.8	25.3-25.7	NOEC (abnormal development, growth and survival)	30->30	30	Duration too long for an acute test and too short for a chronic test	Kim et al. 2021
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hours	PFOA >98%	-	28	NOEC (mortality)	-	0.2484[c]	Duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hours	PFOA >98%	-	28	MATC (combined effects)	2.538-6.451[c]	4.047[c]	Duration too long for an acute test and too short for a chronic test	Rericha et al. 2021

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Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, M	166 hours	PFOA Unreported	-	28.5	LOEC (behavior, gene expression)	<0.00882-0.00882	0.00882	Duration too long for an acute test and too short for a chronic test, non-apical endpoints	Yu et al. 2021
Zebrafish, *Danio rerio*	R, U	21 days	PFOA Unreported	-	-	LOEC (mRNA gene expression in kidneys)	0.05-0.1	0.1	Non-apical endpoint	Zhang et al. 2021
Zebrafish (adult, 4-5 months old), *Danio rerio*	R, U	7-day exposure + 1 day of observation	PFOA Unreported	7.2	27	NOEC (immobility)	1.0->1.0	1.0	Duration too short for a chronic exposure, atypical endpoint	Adedara et al. 2022
Zebrafish (embryo, <4 hpf), *Danio rerio*	R, U	5 days	PFOA 95%	-	27	NOEC (mortality, development)	-	>0.0007	Duration too long for an acute test and too short for a chronic test	Haimbaugh et al. 2022
Zebrafish (embryo, <4 hpf), *Danio rerio*	R, U	5 days + 4-6 week observation	PFOA 95%	-	27	NOEC (reproduction, growth)	-	>0.0007	Duration too long for an acute test and too short for a chronic test	Haimbaugh et al. 2022
Zebrafish (embryo, 3 hpf), *Danio rerio*	S, U	117 hours	PFOA Unreported	7.2	28	LC_{50}	-	>20.70[c]	Duration too long for an acute test and too short for a chronic test	Lindqvist and Wincent 2022
Zebrafish (embryo, 5 hpf), *Danio rerio*	S, U	19 hr	PFOA Unreported	7.5	28	LC_{50}	-	33.89[c]	Duration too short	Satbhai et al. 2022
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	PFOA Unreported	-	28	NOEC (mortality, development and morphology)	-	>41.41[c]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2022
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	APFOA Unreported	-	28	NOEC (mortality, development and morphology)	-	>43.11[c]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2022
Zebrafish (embryo 2 hpf), *Danio rerio*	S, M	70 hours	PFOA Unreported	7.2-7.4	-	NOEC (growth-length, mortality, and hatch)	0.1->0.1	0.1	Duration too short for an acute test	Yu et al. 2022
Zebrafish (embryo, 6 hpf), *Danio rerio*	R, U	90 hours	PFOA Unreported	-	28	NOEC (development and growth)	-	>33.13[c]	Duration too short	Phelps et al. 2023
Zebrafish (larva, 72 hpf), *Danio rerio*	R, U	72 hours	PFOA >98%	-	28.5	LC_{50}	-	132.9[c]	Duration too short	Sun et al. 2023a

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, 72 hpf), *Danio rerio*	R, U	72 hours	PFOA >98%	-	28.5	LOEC (growth – length)	-	41.41[c]	Duration too short	Sun et al. 2023a
Zebrafish (embryo, <4 hpf), *Danio rerio*	R, M	116 hours	PFOA Unreported	-	-	LOEC (immunological responses)	0.0001	0.0001	Duration too long for an acute test and too short for a chronic test, atypical endpoint	Tang et al. 2023
Zebrafish (embryo, 2 hpf), *Danio rerio*	R, M	94 hours	PFOA >96%	-	27	NOEC (mortality and development)	-	0.4915	Duration too short	Wang et al. 2023
Zebrafish (embryo, 1 hpf), *Danio rerio*	S, U	119 hours	PFOA 95%	7.2	28	LC_{50}	-	561.01	Duration too long for an acute test and too short for a chronic test	Wasel et al. 2022
Zebrafish (embryo, 5 hpf), *Danio rerio*	R, U	122 hours	PFOA Unreported	6.8-7.0	28	LC_{50}	-	>41.41[c]	Duration too long for an acute test and too short for a chronic test	Hawkey et al. 2023
Zebrafish (embryo, 5 hpf), *Danio rerio*	R, M	91 hours	PFOA 96%	7.0-8.0	28.5	LC_{50}	-	~2	Duration too short for an acute test	Liu et al. 2023b
Zebrafish (2 months), *Danio rerio*	R, U	14 days	APFOA Unreported	7	28	LOEC (biochemical and enzymatic effects)	-	0.03	Non-apical endpoint, duration too long for an acute test and too short for a chronic test	Liu et al. 2023a
Rare minnow (male, 9 months old, 1.4 g, 47.7 cm), *Gobiocypris rarus*	F, U	28 days	PFOA 98%	-	25	MATC (increase relative mRNA expression of AhR in gills)	3.0-10	5.477	Non-apical endpoint	Liu et al. 2008b
Rare minnow (female, 9 months old, 1.4 g, 47.7 cm), *Gobiocypris rarus*	F, U	28 days	PFOA 98%	-	25	MATC (decrease relative mRNA expression of CYP1a and increase relative mRNA expression of PXR in gills)	10.0-30	17.32	Non-apical endpoint	Liu et al. 2008b
Rare minnow (adult, 9 months old), *Gobiocypris rarus*	F, U	28 days	PFOA 98%	-	25	LOEC (polymerase chain reaction (PCR) alterations of genes in liver)	<3-3	3	Non-apical endpoint	Wei et al. 2008b

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
Rare minnow (adult, 9 months old), *Gobiocypris rarus*	F, U	28 days	PFOA 98%	-	-	MATC (change in m-RNA M-H-FABP)	3.0-10	5.477	Non-apical endpoint	Wei et al. 2008a
Rare minnow (adult, 9 months old), *Gobiocypris rarus*	F, U	28 days	PFOA 98%	-	-	LOEC (protein spots identified by MALDI-TOF/TOF)	<3-3	3	Non-apical endpoint	Wei et al. 2008a
Rare minnow (9 months old female, 1.4 g, 47.7 mm), *Gobiocypris rarus*	F, U	28 days	PFOA 98%	-	25	MATC (increase relative mRNA expression of PPARγ in gills)	3.0-10	5.477	Non-apical endpoint	Liu et al. 2009
Rare minnow (9 months old male, 1.4 g, 47.7 mm), *Gobiocypris rarus*	F, U	28 days	PFOA 98%	-	25	MATC (increase relative mRNA expression of PPARγ and PPARα in gills and CYP4T11 in liver)	10.0-30	17.32	Non-apical endpoint	Liu et al. 2009
Rare minnow (9 months old male, 1.3 g), *Gobiocypris rarus*	F, U	14 days	PFOA 98%	-	25	LOEC (apolipoprotein gene expression)	-	3	Duration too short for a chronic test, non-apical endpoint	Fang et al. 2010
Fathead minnow (juvenile), *Pimephales promelas*	S, U	96 hours	APFO Unknown	7.2-7.9	21.8-22.5	LC$_{50}$	-	2,470	Test substance was not characterized but considered a mixture of APFO and other PFAS impurities; test substance purity unknown and not used quantitatively because other tests from 3M Company (2000a) indicated test purity as low as 78%.	3M Company 2000a
Fathead minnow, *Pimephales promelas*	S, U	96 hours	PFOA 96.5-100%	-	-	LC$_{50}$	-	440	Lack of exposure details, possible mixture effects of other perfluoro homologue compounds; only one replicate;	3M Company 2000a

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
Fathead minnow, *Pimephales promelas*	S, U	96 hours	PFOA 96.5-100%	-	-	LC_{50}	-	140	Possible mixture effects of other perfluoro homologue compounds and the amount of isopropanol, low initial pH (3.0-4.3) in highest test concentration	3M Company 2000a
Fathead minnow, *Pimephales promelas*	S, U	96 hours	APFO 96.5-100%	-	-	LC_{50}	-	70	Highest test concentration produced 20% effect, LC_{50} based on extrapolation	3M Company 2000a
Fathead minnow, *Pimephales promelas*	S, U	96 hours	APFO 96.5-100%	7.9-8.0	19	LC_{50}	-	776	Lack of replication	3M Company 2000a/Elnabarawy 1980
Fathead minnow, *Pimephales promelas*	S, U	96 hours	APFO 96.5-100%	7.9-8.0	19	LC_{50}	-	754	Lack of replication	3M Company 2000a/Elnabarawy 1980
Fathead minnow, *Pimephales promelas*	S, U	96 hours	APFO 78-93%	7.7-8.0	20	LC_{50}	-	301	Possible mixture effects of other perfluoro analogue compounds; low test substance purity	3M Company 2000a
Fathead minnow (juvenile), *Pimephales promelas*	S, U	96 hours	APFO Unreported	7.4-8.4	21-22	LC_{50}	-	>1,000	Highest treatment did not produce >50% effect; test substance purity unknown and not used quantitatively because other tests from 3M Company (2000a) indicated test purity as low as 78%.	3M Company 2000a
Fathead minnow (embryo, 48 hpf), *Pimephales promelas*	F, U	30 days post hatch	APFO 96.5-100%	7.0-7.3	25	NOEC (hatch, survival and growth)	100->100	100	Lack of replication for early life stage test	3M Company 2000a

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
Fathead minnow (64 days old), *Pimephales promelas*	S, M	13 days	APFO 96.5-100%	-	-	BCF	-	1.8 (L/Kg)	Steady state not documented, static uptake study; exposure duration is too short for a chronic test	3M Company 2000a
Fathead minnow (adult, 6-8 months), *Pimephales promelas*	F, M	96 hours	PFOA >95%	8.2	25	LC_{50}	-	>16.62	LC50 not reported, inferred, other definitive data available for the species	Villeneuve et al. 2023
Fathead minnow (adults), *Pimephales promelas*	S, M	39 days	PFOA 19.4%[f]	8.5	10.6-26.6	LOEC (mean total egg production)	74.1->74.1	>74.1	Atypical exposure, started with adults, not a true ELS test	Oakes et al. 2004
Topmouth gudgeon (4.0 g, 4.0 cm), *Pseudorasbora parva*	S, M	96 hours	PFOA 99%	7	22	LC_{50}	-	365.02	Atypical source of test organisms	Yang et al. 2014
Topmouth gudgeon (4.0 g, 4.0 cm), *Pseudorasbora parva*	R, M	30 days	PFOA 99%	7	22	EC_{10} (survival)	-	11.78	Not a true ELS test (started with older life stage), atypical source of organisms	Yang et al. 2014
Nile tilapia, *Oreochromis niloticus*	Dietary	6 days	PFOA Unreported	-	22	MATC (survival and growth)	1.0-5.0 (mg/g)	2.236 (mg/g)	Duration too short for a chronic test, dietary exposure, water concentration not reported	Han et al. 2011
Bluegill, *Lepomis macrochirus*	S, U	96 hours	APFO 96.5-100%	7.8-8.0	18-19	LC_{50}	-	569	Only one replicate per treatment	3M Co. 2000a
Mosquitofish, *Gambusia affinis*	S, U	96 hours	PFOA >98%	7.2	25	LOEC (genetics: AKT serine/threonine kinase 3 mRNA)	-	0.50	Non-apical endpoint	Liu et al. 2022

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Murray River rainbowfish (male, adult, 1 year old), *Melanotaenia fluviatilis*	R, M	14 days	PFOA ≥96%	7.1-7.4	23	NOEC (growth and mortality)	9.0->9.0	9.0	Duration too long for acute test and too short for a chronic test, not NA species	Miranda et al. 2020
Medaka (<6 hpf), *Oryzias latipes*	R, U	Embryo development to 48 hours post-hatch	PFOA Unreported	-	25	LOEC (swim bladder development)	-	4.7	Only one exposure concentration	Godfrey 2017
Medaka (adult, male), *Oryzias latipes*	R, U	14 days	PFOA Unreported	-	25	NOEC (adult survival, GSI%, HSI%, K%)	10->10	10	Duration too long for acute test and too short for a chronic test	Ji et al. 2008
Medaka (adult, female), *Oryzias latipes*	R, U	14 days	PFOA Unreported	-	25	NOEC (adult survival, GSI%, HSI%, K%)	10->10	10	Duration too long for acute test and too short for a chronic test	Ji et al. 2008
Medaka (F1 generation, <12 hours old, embryo), *Oryzias latipes*	R, U	7-14 days (assumed)	PFOA Unreported	-	25	NOEC (% hatchability)	10->10	10	Duration too long for acute test and too short for a chronic test	Ji et al. 2008
Medaka (F1 generation, <12 hours old, embryo), *Oryzias latipes*	R, U	7-14 days (assumed)	PFOA Unreported	-	25	MATC (time to hatch)	1.0-10	3.162	Duration too long for acute test and too short for a chronic test	Ji et al. 2008
Medaka (F1 generation, <12 hours old, embryo), *Oryzias latipes*	R, U	28 days post-hatch (assumed)	PFOA Unreported	-	25	NOEC (swim up success)	10->10	10	Pseudoreplication that occurred at hatching stage	Ji et al. 2008
Medaka (F1 generation, <12 hours old, embryo), *Oryzias latipes*	R, U	100 days post-hatch	PFOA Unreported	-	25	NOEC (growth – length and weight)	10->10	10	Pseudoreplication that occurred at hatching stage	Ji et al. 2008
Medaka (F1 generation, <12 hours old, embryo), *Oryzias latipes*	R, U	100 days post-hatch	PFOA Unreported	-	25	NOEC (male/female GSI% and HSI%)	0.1->0.1	0.1	Pseudoreplication that occurred at hatching stage	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 hours old, embryo), *Oryzias latipes*	R, U	28 days post-hatch	PFOA Unreported	-	25	LOEC (larval survival)	<0.1-0.1	0.1	Pseudoreplication that occurred at hatching stage	Ji et al. 2008
Medaka, *Oryzias latipes*	S, U	96 hours	PFOA Unreported	7.0-7.5	25	NOEC (mortality)	-	473	Missing exposure details, only one exposure concentration	Godfrey et al. 2019
Medaka (embryo, <6 hpf), *Oryzias latipes*	R, U	10 days	PFOA Unreported	7.0-7.5	25	NOEC (growth)	-	4.7	Duration too short for a chronic test, only one exposure concentration	Godfrey et al. 2019
Medaka (adult, 16 weeks old, 0.38 g), *Oryzias latipes*	R, U	21 days	PFOA 96%	-	25	LOEC (fecundity)	<10-10	10	Only one exposure concentration, tolerant LOEC value	Kang et al. 2019
Medaka (embryo), *Oryzias latipes*	S, U	3 dph	PFOA >98.0%	7.3	27	LOEC (genetic and hormonal changes)	-	25	Non-apical endpoint	Myosho et al. 2022
Medaka (fry), *Oryzias latipes*	S, U	3 dph	PFOA >98.0%	7.3	27	NOEC (genetic changes)	-	2.5	Non-apical endpoint	Myosho et al. 2022
Northern leopard frog (larva, Gosner 26), *Lithobates pipiens* (formerly, *Rana pipiens*)	R, M	40 days	PFOA 96%	-	20	NOEC (snout-vent length and Gosner stage at 40 d)	1->1	1	No effects at the highest concentration tested resulting in a relatively low greater than value that does not inform species sensitivity	Hoover et al. 2017
Northern leopard frog (larva, Gosner 25), *Lithobates pipiens*	S, M	30 days	PFOA ≥96%	7.8	26.2	NOEC (survival and growth)	0.066->0.066	0.066	Mesocosm exposure	Flynn et al. 2021
Northern leopard frog (larva, Gosner stage 25), *Lithobates pipiens*	R, M	30 days	PFOA ≥96%	-	20	MATC (growth – weight)	0.1251-1.376	0.4149	Highly variable dose-response	Flynn et al. 2022

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Black-spotted pond frog (adult), *Pelophylax nigromaculatus* (formerly, *Rana nigromaculatus*)	S, U	21 days	PFOA >98%	-	-	LOEC (gene expression)	-	0.01	Non-apical endpoint	Lin et al. 2022a
Black-spotted pond frog, *Pelophylax nigromaculatus*	R, U	21 days	PFOA >98%	6.5	20	LOEC (cholesterol and triglyceride levels)	-	0.0025	Non-apical endpoint	Lin et al. 2022b
Black-spotted pond frog (adult), *Pelophylax nigromaculatus*	S, U	21 days	PFOA >98%	6.5	20	LOEC (reduced glutathione)	-	0.91	Non-apical endpoint	Lin et al. 2022c
Black-spotted pond frog, *Pelophylax nigromaculatus*	S, U	21 days	PFOA >98%	6.5	20	MATC (biochemical, genetic and immunological effects)	0.00111-0.01094	0.003485	Non-apical endpoint	Liu et al. 2023c
Gray treefrog (larva, Gosner 40), *Hyla versicolor*	S, U	96 hours	PFOA Unreported	-	21	LC$_{50}$	-	191	Poor control survival, low number of individuals per treatment.	Tornabene et al. 2021
Tiger salamander (larva, Harrison stage 46), *Ambystoma tigrinum*	R, M	30 days	PFOA ≥96%	-	20	NOEC (snout-vent length, weight, and mortality)	0.8677->0.8667	0.8677	Highly variable dose-response	Flynn et al. 2022
American toad (larva, Gosner stage 25), *Anaxyrus americanus*	R, M	26-45 days	PFOA ≥96%	-	20	MATC (days to metamorphosis)	0.09403-0.8728	0.2865	Highly variable dose-response	Flynn et al. 2022
American toad (larva, Gosner stage 25), *Anaxyrus americanus*	R, M	26-45 days	PFOA ≥96%	-	20	NOEC (growth, mortality)	0.8728->0.8728	0.8728	Highly variable dose-response	Flynn et al. 2022
Asiatic toad (tadpole, 1.8 cm, 0.048 g), *Bufo gargarizans*	R, M	96 hours	PFOA 99%	7	22	LC$_{50}$	-	114.74	Atypical source of organisms	Yang et al. 2014

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Asiatic toad (tadpole, 1.8 cm, 0.048 g), *Bufo gargarizans*	R, M	30 days	PFOA 99%	7	22	EC_{10} (survival)	-	5.89	Not a true ELS test, atypical source of organisms	Yang et al. 2014

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

[b] C1 year corresponds to the total duration of the 10-generations study. Most generations did not show statistically significant effects.

[c] Reported in moles converted to milligram based on a molecular weight of 414.07 g/mol PFOA or 431.1 g/mol APFO.

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

[e] Concentration of APFO determined as the anion (PFO-).

Appendix H   Other Estuarine/Marine PFOA Toxicity Studies

H.1   Summary Table of Qualitative Estuarine/Marine PFOA 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 minutes	PFOA 96%	-	18	-	EC_{50} (bioluminescence inhibition)	-	524	Duration too short for a plant test, missing some exposure details, non-apical endpoint	Rosal et al. 2010
Cyanobacterium, *Anabaena sp.*	S, M	24 hours	PFOA 96%	-	28	-	EC_{50} (bioluminescence inhibition)	-	72.3	Duration too short for a plant test, missing some exposure details, non-apical endpoint	Rosal et al. 2010
Cyanobacterium, *Geitlerinema amphibium*	S, U	72 hours	PFOA Unreported	7.6-7.8	20	8	EC_{50} (growth)	-	248.4[b]	Duration too short for a plant test, missing some exposure details	Latala et al. 2009
Dinoflagellate, *Pyrocystis lunula*	S, M	24 hours	PFOA 95%	-	19	-	EC50 (bioluminescence inhibition)	-	18	Duration too short for a plant test, atypical endpoint	Hayman et al. 2021
Golden brown alga, *Isochrysis galbana*	S, U	72 hours	PFOA 96%	-	20	-	EC_{50} (growth inhibition)	-	163.6	Duration too short for a plant test, missing some exposure details	Mhadhbi et al. 2012
Green alga, *Chlorella vulgaris*	S, U	72 hours	PFOA Unreported	7.6-7.8	20	8	EC_{50} (growth)	-	977.2[b]	Duration too short for a plant test, missing some exposure details	Latala et al. 2009
Diatom, *Skeletonema marinoi*	S, U	72 hours	PFOA Unreported	7.6-7.8	20	8	EC_{50} (growth)	-	368.5[b]	Duration too short for a plant test, missing some exposure details	Latala et al. 2009
Purple sea urchin (fertilized eggs), *Paracentrotus lividus*	S, U	48 hours	PFOA 96%	-	20	-	EC_{50} (growth inhibition)	-	110.0	Duration too short for an acute test	Mhadhbi et al. 2012
Blue mussel, *Mytilus edulis*	R, U	21 days	PFOA Unreported	-	16-19	-	LOEC (catalase activity)	<0.2-0.2	0.2	Atypical endpoint	Li et al. 2021a

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
Mediterranean mussel (adult), *Mytilus galloprovincialis*	R, U	16 days	PFOA ≥98%	7.98	15.2	37.9	NOEC (biochemistry, enzymatic changes)	0.100->0.100	0.100	Duration too short for a chronic exposure, non-apical endpoints	Geng et al. 2021
Green mussel (60-65 mm), *Perna viridis*	R, M	7 days	PFOA 96%	-	25	25	MATC (relative condition factor)	0.0114-0.099	0.03359	Exposure duration too short for chronic test and too long for acute test, non-apical endpoint	Liu et al. 2013; 2014c
Green mussel (adult), *Perna viridis*	R, M	7 days + 7 days observation	PFOA 96%	-	25	30	EC$_{50}$ (integrative genotoxicity)	0.093-0.950	0.5940	Exposure duration too short for chronic test and too long for acute test, non-apical endpoint	Liu et al. 2014a
Green mussel (adult), *Perna viridis*	R, M	7 days	PFOA 96%	-	25	25	MATC (CAT and SOD activity)	0.099-1.12	0.3330	Exposure duration too short for chronic test and too long for acute test, non-apical endpoint	Liu et al. 2014b
Green mussel, *Perna viridis*	R, M	7 days + 7 days observation	PFOA 96%	8	25	30	MATC (hemocyte cell viability)	0.0114-0.099	0.03359	Exposure duration too short for chronic test and too long for acute test, non-apical endpoint	Liu and Gin 2018
Manila clam (3.64 cm), *Ruditapes philippinarum*	R, M	21 days	PFOA Unreported	-	12	35	NOEC (mortality)	0.00093->0.00093	0.00093	Only one exposure concentration, apical endpoints are not the focus of study	Bernardini et al. 2021
Manila clam, *Ruditapes philippinarum*	R, M	21 days	APFO >98%	-	12	35	NOEC (biochemistry, cell diameter, cell volume, hematocyte count)	0.00093->0.00093	0.00093	Only one exposure concentration, non-apical endpoints	Fabrello et al. 2021
Dolphinfish (embryo, <8 hr post spawn), *Coryphaena hippurus*	S, M	48 hours	PFOA 96%	-	27	-	LC$_{50}$	-	4	Duration too short for an acute test, high control mortality	Gebreab et al. 2022
Japanese medaka (adult), *Oryzias latipes*	R, U	7 days	PFOA ammonium salt 98%	-	25	-	NOEC (survival, condition factor)	100->100	100	Exposure duration too short for chronic test and too long for acute test	Yang 2010

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Salinity (ppt)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Dusky rockcod (adult), *Trematomus newnesi*	S, U	10 days	PFOA Unreported	-	-1	-	LOEC (gene expression)	-	1.5	Duration too long for an acute test and too short for a chronic test, atypical endpoint	Pacchini et al. 2023
Marbled flounder (adult), *Pseudopleuronectes yokohamae*	R, M	14 days	PFOA Unreported	-	15	37	LOEC (vitellogenin levels)	<0.197-0.197	0.197	Atypical duration, non-apical endpoint	Li et al. 2018b
Turbot (embryo), *Scophthalmus maximus* (formerly, *Psetta maxima*)	R, U	6 days	PFOA 96%	-	18	-	LC$_{50}$	-	11.9	Exposure duration too short for chronic test and too long for 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] Reported in moles converted to milligram based on a molecular weight of 414.07 mg/mmol.

Appendix I   Acute-to-Chronic Ratios

I.1   Acute-to-Chronic Ratios from Quantitatively Acceptable Tests.

Species	Chemical / Purity	Acute Test Duration	Chronic Test Duration	Acute Effect	Chronic Effect	Acute Effect Conc. (mg/L)	Chronic Effect Conc. (mg/L)	ACR	SMACR	Reference
Rotifer, *Brachionus calyciflorus*	PFOA 96%	24 hours	Up to 200 hours	LC_{50}	EC_{10} (intrinsic rate of natural increase)	150	0.5015	299.1	299.1	Zhang et al. 2013a
Water flea, *Daphnia carinata*	PFOA 95%	48 hours	21 days	EC_{50}	MATC (# of average number of offspring per brood and total # of living offspring)	66.8	0.03162	2,113	2,113	Logeshwaran et al. 2021
Cladoceran, *Daphnia magna*	APFO 99.7%	48 hours	21 days	EC_{50}	EC_{10} (average # of live young)	480	20.26	23.69	-	Colombo et al. 2008
Cladoceran, *Daphnia magna*	PFOA Unreported	48 hours	21 days	EC_{50} (immobility)	EC_{10} (# young/starting female)	542.5	7.853	69.08	-	Ji et al. 2008
Cladoceran, *Daphnia magna*	PFOA >98%	48 hours	21 days	LC_{50}	EC_{10} (# young/starting female)	193.3[a]	12.89	15.00	-	Li 2009, 2010
Cladoceran, *Daphnia magna*	PFOA 99%	48 hours	21 days	LC_{50}	EC_{10} (survival)	222.0	5.458	40.67	-	Yang et al. 2014
Cladoceran, *Daphnia magna*	PFOA 98%	48 hours	21 days	EC_{50}	MATC (growth and reproduction)	114.6	0.07155	1,602[b]	-	Lu et al. 2016
Cladoceran, *Daphnia magna*	PFOA Unreported	48 hours	21 days	LC_{50}	EC_{10} (# of offspring)	117.2	8.084	14.50	27.05	Yang et al. 2019

Species	Chemical / Purity	Acute Test Duration	Chronic Test Duration	Acute Effect	Chronic Effect	Acute Effect Conc. (mg/L)	Chronic Effect Conc. (mg/L)	ACR	SMACR	Reference
Cladoceran, *Moina macrocopa*	PFOA Unreported	48 hours	7 days	EC_{50} (immobility)	EC_{10} (mean young/adult)	166.3	2.194	75.80	75.80	Ji et al. 2008
Mayfly, *Neocloeon triangulifer*	PFOA 95%	96 hours	23 days	LC_{50}	NOEC (survival, weight, emergence)	13.05	>3.085	<4.229	<4.229	Soucek et al. 2023
American bullfrog, *Lithobates catesbeiana*	PFOA Unreported	96 hours	72 days	LC_{50}	LOEC (snout vent length)	1,006	0.288	3,493	3,493	Flynn et al. 2019

a Geometric mean of three LC50 values.

b Value not used in the SMACR calculation, because the value is an order of magnitude greater than other ACRs for the species.

Appendix J   Unused PFOA Toxicity Studies

J.1   Summary of Unused PFOA 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
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
Consoer, D.M., A.D. Hoffman, P.N. Fitzsimmons, P.A. Kosian and J.W. Nichols	2014. Toxicokinetics of perfluorooctanoate (PFOA) in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 156: 65-73.	All fish were surgically altered (dorsal aortic cannula, plus a urinary catheter); no controls; non-apical endpoints only
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)
De Silva, A.O., P.J. Tseng and S.A. Mabury	2009. Toxicokinetics of perfluorocarboxylate isomers in rainbow trout. Environ. Toxicol. Chem. 28(2): 330-337.	Study involved a mixture of ECF PFOA, linear PFNA, and isopropyl PFNA added to diet
Dragojevic, J., P. Maric, J. Loncar, M. Popovic, I. Mihaljevic, and T. Smital	2020. Environmental Contaminants Modulate Transport Activity of Zebrafish Organic Anion Transporters Oat1 and Oat3. Comp. Biochem. Physiol. C Toxicol. Pharmacol.231:8 p.	The test duration was extremely brief (~10 mins), the exposure was in vitro, and no apical effects were evaluated.
Elnabarawy, M.T.	1980. Aquatic Toxicity Testing: FC-143 (Lot-37) L.R. 5626 S. Report No. 037; OPPT Administrative Record AR226-0504:3780-3786.	Results reported in another publication
Fernández-Sanjuan, M., M. Faria, S. Lacorte and C. Barata	2013. Bioaccumulation and effects of perfluorinated compounds (PFCs) in zebra mussels (*Dreissena polymorpha*). Environ. Sci. Pollut. Res. 20:2661–2669.	Mixture
Garoche, C., A. Boulahtouf, M. Grimaldi, B. Chiavarina, L. Toporova, M.J. Den Broeder, J. Legler, W. Bourguet and P. Ba.	2021. Interspecies differences in activation of peroxisome proliferator-activated receptor gamma by pharmaceutical and environmental chemicals. Environ. Sci. Technol. 55(24): 16489-16501.	In vitro
Gonzalez-Naranjo, V. and K. Boltes	2014. Toxicity of ibuprofen and perfluorooctanoic acid for risk assessment of mixtures in aquatic and terrestrial environments. Int. J. Environ. Sci. Technol. 11: 1743-1750.	Severe lack of exposure details (cannot judge against data quality objectives)
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
Holth, T.F., M. Yazdani, A. Lenderink and K. Hyllan	2012. Effects of fluoranthene and perfluorooctanoic acid (PFOA) on immune functions in Atlantic cod (*Gadus morhua*). Abstracts Comp. Biochem. Physiol. Part A. 163: S39-S42.	Abstract only; cannot judge against data quality objectives

Author	Citation	Reason Unused
Hoover, G.M.	2018. Effects of Per/Polyfluoroalkyl Substance Exposure on Larval Amphibians. Ph.D.Thesis, Purdue University, West Lafayette, IN	Test is unused because the source material is a PhD Thesis and has not undergone peer review. Only peer-reviewed data are considered in deriving aquatic life criteria
Hoover, G., S. Kar, S. Guffey, J. Leszczynski, and M.S. Sepulveda	2019. In Vitro and In Silico Modeling of Perfluoroalkyl Substances Mixture Toxicity in an Amphibian Fibroblast Cell Line. Chemosphere233:25-33.	In vitro exposure
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 PFOA was of an acute (117-hours) duration but endpoints were measured at 6 months of age
Jantzen, C.E., F. Toor, K.M. Annunziato and K.R. Cooper	2017b. Effects of chronic perfluorooctanoic acid (PFOA) at low concentration on morphometrics, gene expression, and fecundity in zebrafish (*Danio rerio*). Reproduct. Toxicol. 69: 34-42.	Unable to determine dietary exposure concentration
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 pp.	In vitro exposure
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 for PFOA was run two times at separate times (not simultaneously) and the sample size for each treatment group was unclear.
Liang, X. and J. Zha	2016. Toxicogenomic applications of Chinese rare minnow (*Gobiocypris rarus*) in aquatic toxicology. Comp. Biochem. Physiol. Part D 19: 174-180.	Review paper
Liu, C., Y. Du and B. Zhou	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.	In vitro, cultured hepatocytes
Mahapatra, C.T., N.P. Damayanti, S.C. Guffey, J.S. Serafin, J. Irudayaraj and M.S. Sepúlveda	2017. Comparative in vitro toxicity assessment of perfluorinated carboxylic acids. J. Appl. Toxicol. 37: 699-708.	In vitro exposure, zebrafish liver cell cultures
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 not documented); only 12 days
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, xenobiotic biotransformation 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

Author	Citation	Reason Unused
Otero-Sabio, C., M. Giacomello, C. Centelleghe, F. Caicci, M. Bonato, A. Venerando, J.M. Graic, S. Mazzariol, L. Finos	2022. Cell Cycle Alterations Due to Perfluoroalkyl Substances PFOS, PFOA, PFBS, PFBA and the New PFAS C6O4 on Bottlenose Dolphin (Tursiops truncatus) Skin Cell. Ecotoxicol. Environ. Saf.244:10 p.	In vitro
Padilla, S., D. Corum, B. Padros, D.L. Hunter, A. Beam, K.A. Houck, N. Sipes, N. Kleinstreuer, T. Knudsen, D.J. Nix and D.M. Reif	2012. Zebrafish developmental screening of the ToxCastTM Phase I chemical library. Reprod. Toxicol. 33: 174-187.	Severe lack of exposure details, only one exposure concentration
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)
Rotondo, J.C, L. Giari, C. Guerranti, M. Tognon, G. Castaldelli, E.A. Fano and F. Martini	2018. Environmental doses of perfluorooctanoic acid change the expression of genes in target tissues of common carp. Environ. Toxicol. Chem. 37(3): 942-948.	Two exposure concentrations 10,000-fold apart; atypical endpoint
Sanderson, H., T.M. Boudreau, S.A. Mabury and K.R. Solomon	2003. Impact of perfluorooctanoic acid on the structure of the zooplankton community in indoor microcosms. Aquat. Toxicol. 62: 227-234.	Poor experimental design/performance
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 (gills)
Sun, X., Y. Xie, X. Zhang, J. Song, and Y. Wu	2023b. Estimation of Per- and Polyfluorinated Alkyl Substance Induction Equivalency Factors for Humpback Dolphins by Transactivation Potencies of Peroxisome Proliferator-Activated Receptors. Environ. Sci. Technol.57(9): 3713-3721.	In vitro; mammalian study
Tang, J., X. Jia, N. Gao, Y. Wu, Z. Liu, X. Lu, Q. Du, J. He, N. Li, B. Chen, J. Jiang, W. Liu, Y. Ding, W. Zhu and H. Zhang	2018. Role of the Nrf2-ARE pathway in perfluorooctanoic acid (PFOA)-induced hepatotoxicity in *Rana nigromaculata*. Environ. Pollut. 238: 1035-1043.	No apical endpoints were measured; control survival was not reported; test duration of 14 days relatively short for a chronic amphibian study; not NA species
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; no apical endpoints
Ulhaq, M., S. Orn, G. Carlsson, J. Tallkvist and L. Norrgren	2012. Perfluorooctanoic acid toxicity in zebrafish (*Danio rerio*). Abstracts. Toxicol. Letters 211S: S43-S216.	Abstract only, cannot judge against data quality objectives
Williams, T.D., A. Diab, F. Ortega, V.S. Sabine, R.E. Godfrey, F. Falciani, J.K. Chipman and S.G. George	2008. Transcriptomic Responses of European Flounder (*Platichthys flesus*) to Model Toxicants. Aquat. Toxicol. 90(2): 83-91.	Injected toxicant; only one exposure concentration
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.	PFCs mixed in sediment

Author	Citation	Reason Unused
Xia, X., A.H. Rabearisoa, X. Jiang and Z. Dai	2013. Bioaccumulation of perfluoroalkyl substances by *Daphnia magna* in water with different types and concentrations of protein. Environ. Sci. Technol. 47: 10955-10963.	Bioaccumulation (steady state not documented); unmeasured test; only 3 days
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); unmeasured test; only 3 days
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); unmeasured test; only 3 days
Yang, Z., L. Fu, M. Cao, F. Li, J. Li, Z. Chen, A. Guo, H. Zhong, W. Li, Y. Liang, and Q. Luo.	2023. PFAS-Induced Lipidomic Dysregulations and Their Associations with Developmental Toxicity in Zebrafish Embryos. Sci. Total Environ.861:9 p.	Injected toxicant
Yuan, Z. J. Zhang, C. Tu, Z. Wang and W. Xin	2016a. The protective effect of blueberry anthocyanins against perfluorooctanoic acid-induced disturbance in planarian (*Dugesia japonica*). Ecotoxicol. Environ. Saf. 127: 170-174.	Only one exposure concentration; not NA species; non-apical endpoints; static, unmeasured chronic exposure
Zhang, J., B. Wang, B. Zhao, Y. Li, X. Zhao and Z. Yuan	2019a. Blueberry anthocyanin alleviates perfluorooctanoic acid-induced toxicity in planarian (*Dugesia japonica*) by regulating oxidative stress biomarkers, ATP contents, DNA methylation and mRNA expression. Environ. Pollut. 245: 957-964.	Only one exposure concentration; not NA species; non-apical endpoints
Zhang, H., J. He, N. Li, N. Gao, Q. Du, B. Chen, F. Chen, X. Shan, Y. Ding, W. Zhu, Y. Wu, J. Tang and X. Jia	2019b. Lipid accumulation responses in the liver of *Rana nigromaculata* induced by perfluorooctanoic acid (PFOA). Ecotoxicol. Environ. Saf. 167: 29-35.	No apical endpoints were measured; control survival was not reported; test duration of 14 days relatively short for a chronic amphibian study; not NA species

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 2.10.3.3) and when adequate concentrations-response data were published in the study or could be obtained from authors. When concentration-response data were not presented in toxicity studies, concentration-response data were requested from study authors to independently calculate toxicity values. In cases where study authors did not respond to the EPA's request for data or were unable to locate concentration-response data, the toxicity values were not independently calculated by the EPA, and the reported toxicity values were retained for criteria deviation. The EPA also retained author-reported effect concentrations when data availability did not support effect concentration calculation by the EPA. This retention was done to be consistent with use of author-reported toxicity values in previous criteria documents and retain informative toxicity values (that would have otherwise not been used only on the basis of lacking the underlying C-R data). Where concentration-response data were available, they were analyzed using the statistical software program R (version 3.6.2) and the associated dose-response curve (drc) package.

In some cases, the author-reported toxicity values were different than the corresponding effect concentrations calculated by the EPA. Overall, the magnitude of such discrepancies was limited and largely occurred for several potential reasons such as: (1) instances where authors were presumed to calculate effect concentrations using replicate level data, but the EPA only had access to treatment mean data; (2) the model selected to fit a particular set of C-R data, and; (3) the software used to fit a model to C-R data and calculate an effect concentration.

K.1 Fitting Concentration Response Data in R

Concentration-response data were obtained from quantitatively-acceptable toxicity studies when reported data were available. In many scenarios, toxicity studies report treatment-level mean concentrations and mean organismal responses; however, individual-replicate data may also be reported. When fitting C-R curves, replicate-level data were preferred over treatment-level data, if both types of data were available. Within R, the drc package can fit a variety of mathematical models to each set of C-R data.

K.1.1 Fitting Acute Mortality Data

K.1.1.1 Dichotomous Data

Dichotomous data are binary in nature (e.g., live/dead or 0/1) and are typical of survival experiments. They are usually represented as a proportion survived.

K.1.2 Fitting Chronic Growth, Reproduction, and Survival Data

K.1.2.1 Continuous Data

Continuous data take on any value along the real number line (e.g., biomass).

K.1.2.2 Count Data

Count data take on only integer values (e.g., number of eggs hatched).

K.1.2.3 Dichotomous Data

Dichotomous data are binary in nature (e.g., live/dead or 0/1) and are typical of survival experiments. They are usually represented as a proportion survived.

K.2 Determining Most Robust Model Fit for Each C-R curve

The R drc package was used to fit a variety of models to each individual C-R dataset. A single model was then selected from these candidate models to serve as the representative C-R model. The selected model represented the most statistically-robust model available. To

determine the most-statistically-robust model for a C-R dataset, all individual model fits were assessed on a suite of statistical metrics.

K.2.1   Selecting Candidate Models

Initially, models were ranked according to the Akaike information criteria (AIC). The AIC provides a measure of the amount of information lost for a given model by balancing goodness of fit with model parsimony. The models with the lowest AIC, relative to other models based on the same data, tend to be optimal. In some instances, however, the model with the lowest AIC possessed a questionable characteristic that suggested said model was not the most appropriate. Rather than selecting a model based solely on the lowest AIC, the initial ranking step was only used to identify a subset of candidate models that were more closely examined before selecting a model fit for each C-R dataset.

K.2.2   Assessment of Candidate Models to Determine the Most Appropriate Model

Candidate models (i.e., models with low AIC scores relative to other models produced for a particular C-R dataset) were further evaluated based on additional statistical metrics to determine a single, statistically robust curve for each quantitatively-acceptable toxicity test. These additional statistical metrics were evaluated relative to the other candidate curve fits produced for each C-R dataset. Of these statistical metrics, residual standard errors, confidence intervals relative to effects concentration estimates, and confidence bands carried the most weight in determining the most appropriate model to be representative of an individual C-R dataset. These additional statistical metrics included:

K.2.2.1   Comparison of residual standard errors

As with AIC, smaller values were desirable. Residual standard errors were judged relative to other models.

K.2.2.2   Width of confidence intervals for EC estimates

Confidence intervals were assessed on standard error relative to estimate and confirming that the intervals were non-negative. Judged in absolute and relative to other models.

K.2.2.3   Width of confidence bands around the fitted model

A general visual inspection of the confidence bands for the fitted model. Wide bands in the area of interest were undesirable. Judged in absolute and relative to other models.

K.2.2.4   P-values of parameters estimates and goodness of fit tests

Hypothesis tests of parameter values to determine whether an estimate is significantly different from zero. Goodness of fit tests were used to judge the overall performance of the model fit. Typically, the level of significance was set at 0.05. There may have been occasional instances where the 0.05 criterion may not be met, but there was little recourse for choosing another model. Judged in absolute terms.

K.2.2.5   Residual plots

Residuals were examined for homoscedasticity and biasedness. Judged in absolute and relative to other models.

K.2.2.6   Overly influential observations

Observations were judged based on Cook's distance and leverage. When an observation was deemed overly influential, it was not reasonable to refit the model and exclude any overly influential observations given the limited data available with typical C-R curves. Judged in absolute terms.

K.3   Determining Curve Acceptability for use in Criteria Derivation

The final curve fits selected for each of the quantitatively-acceptable toxicity tests were further evaluated and classified to determine whether the curves were: 1) quantitatively-

acceptable for use, 2) qualitatively acceptable for use, or 3) unacceptable. To determine curve acceptability for use in deriving an effect concentration, each individual curve was considered based on the statistical metrics described above and assessed visually to compare how the calculated effect concentration aligned with the underlying raw C-R data. Instead of evaluating curves fits relative to other curve fits for the same data (as was previously described to select the most-robust curve for each test), curve fit metrics were used to assign each curve a score:

- **Quantitatively Acceptable Model**. Model performed well on most/all statistical metrics and resultant effect concentrations were typically used in a quantitative manner.

- **Qualitatively Acceptable Model**. Model generally performed well on statistical metrics; however, the model presented some characteristic(s) that called estimates into question. Such models were considered with caution. These problems may have consisted of any number of issues such as a parameter with a high p-value, poor goodness of fit p-value, wide confidence bands for fit or estimate interval, or residuals that indicate model assumptions are not met. Broadly, effect concentrations from models that were deemed qualitatively acceptable were not used numerically in criteria derivation if quantitatively acceptable models for different endpoints or tests from the same publication were available. If quantitatively acceptable models for different endpoints or tests from the same publication were not available, effect concentrations from the qualitatively acceptable model were used numerically in criteria derivation on a case-by-case basis.

- **Unacceptable Model**. Model poorly fit the data. These models were not used for criteria derivation.

No single statistical metric can determine a given model's validity or appropriateness. Metrics should be considered as a whole. As such, there is a slightly subjective component to these evaluations. That said, this assessment methodology 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 PFOA Benchmarks for Estuarine/Marine Waters through a New Approach Method (NAM)

L.1 Background

The 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (U.S. EPA 1985) recommend that data for a minimum of eight families be available to fulfill taxonomic minimum data requirements (MDRs) to calculate criteria values, including to calculate estuarine/marine aquatic life criteria. Acute estuarine/marine test data are currently available for only three families, a mysid (*Siriella* and *Americamysis*), a sea urchin (*Strongylocentrotus*), and a mussel (*Mytilus*), addressing only three of the eight MDRs; thus, the EPA was not able to derive an acute estuarine/marine criterion element for PFOA based on the 1985 Guidelines MDR specifications (Section 2.10.1). However, the EPA was able to develop an acute PFOA protective benchmark 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 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 help fill missing MDRs and allow the derivation of acute an estuarine/marine benchmark for aquatic life using procedures consistent with those in the 1985 Guidelines. This is important as it provides an approach by which values that are protective of aquatic life communities can be developed, even when MDRs are not fulfilled by direct PFOA test data. This approach is consistent with both the 1985 Guidelines "good science" clause, the EPA's interest in providing useful information to states and Tribes regarding protective values

for aquatic life, and the EPA's intention to reduce the use of animal testing via application of NAMs (https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing).

L.1.1   Introduction to Web-ICE

ICE models, developed by the EPA's Office of Research and Development, are log-linear regressions of the acute toxicity (EC_{50}/LC_{50}) of two species across a range of chemicals, thus representing the relationship of inherent sensitivity between those species (Raimondo et al. 2010). Each model is derived from an extensive, standardized database of acute toxicity values by pairing each species with every other species for which acceptable toxicity data are available. Once developed, ICE models can be used predict the sensitivity of an untested taxon (predicted taxa are represented by the y-axis) from the known, measured sensitivity of a surrogate species (represented by the x-axis; Figure L-1).

ICE models have been developed for a broad range of different chemicals (e.g., metals and other inorganics, pesticides, solvents, and reactive chemicals) and across a wide range of toxicity values. There are approximately 3,400 significant ICE models for aquatic animal and plant species in the most recent version of web-ICE (v3.3, https://www3.epa.gov/webice/, last updated June 2016; Raimondo et al. 2015).

Models were validated using leave-one-out cross validation, which formed the basis for the analyses of uncertainty and prediction robustness. For this process, each datapoint within the model (representing the relative sensitivity of two species for a particular chemical) is systematically removed, one at a time. The model is then redeveloped with the remaining data (following each removal) and the removed value of the surrogate species is entered into the model. The estimated value for the predicted species is then compared to the measured value for that species (Raimondo et al 2010; Willming et al. 2016).

ICE models have high prediction accuracy when values are derived from models with robust parameters (e.g., mean square error, R^2), that fall within a defined range of acceptability, and with close prediction confidence intervals that facilitate evaluating the fit of the underlying data (Brill et al. 2016; Raimondo et al. 2010; Willming et al. 2016). Results of these analyses provide the basis of the user guidance for selecting ICE predicted toxicity with high confidence (Box 1).

ICE models have undergone extensive peer review and their use has been supported for multiple applications, including direct toxicity estimation for endangered species (NRC 2013; Willming et al. 2016) and development of Species Sensitivity Distributions (SSDs) (Awkerman et al. 2014; Bejarano et al. 2017; Dyer et al. 2006, 2008; Raimondo et al. 2010, 2020). The application of ICE-predicted values to develop protective aquatic life values by multiple independent, international groups confirms that values developed from ICE-generated SSDs 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, 2012b; Palumbo et al. 2012; Wu et al. 2015, 2016; Wang et al. 2020; 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).
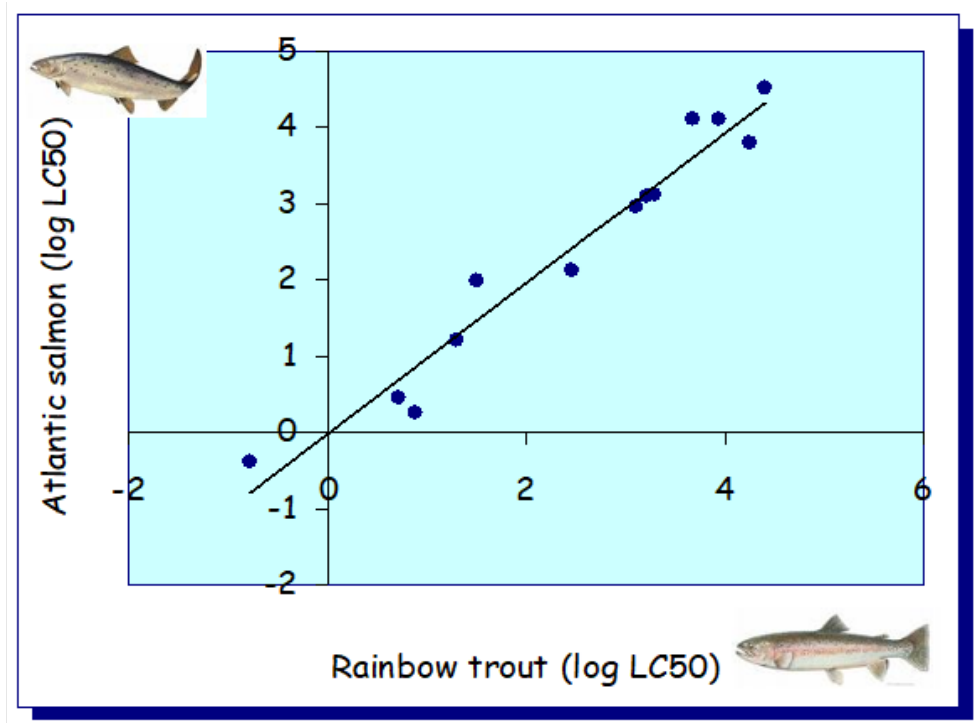
Figure L-1. Example ICE Model for Rainbow Trout (surrogate) and Atlantic Salmon (predicted).
Each model datapoint is a common chemical that was tested in both species to develop a log-linear regression.

Box 1. ICE Model User Guidance Recommended for Listed Species (Willming et al. 2016):

- Close taxonomic distance (within class)

- Low Mean Squared Error (<~ 0.95)

- High R^2 (>~ 0.6)

- High slope (>~ 0.6)

- Prediction confidence intervals should be used to evaluate the prediction using professional judgement for the application (Raimondo et al. 2024).

- For models between vertebrates and invertebrates, using those with lower MSE or MOA-specific models (not available for PFAS) has been recommended for listed species predictions (Willming et al. 2016).

L.1.2   Application of Web-ICE with PFOA

As previously discussed, ICE models have been 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 toxicity data at that time. 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., 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 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 and is described in detail in Raimondo et al. (2024). Briefly, ICE models containing a minimum of 10 datapoints and spanning at least five orders of magnitude were separated into two subsets: 1) a lower subset that contained all paired chemical data corresponding to values below the 75th percentile of surrogate species values and; 2) an

upper subset containing paired chemical data above the 75th percentile of surrogate values. The

lower subset was used to develop "truncated" ICE models. The surrogate species values in the

upper subset were converted to mg/L and entered into the truncated ICE models. The predicted

mg/L value was compared to the respective value of the measured predicted species. Prediction

accuracy was determined as the fold difference (maximum of the predicted/measured and

measured/predicted) between the predicted and the measured value, consistent with previously

published evaluations of ICE models (Raimondo et al. 2010; Willming et al. 2016). Accuracy of

using scaled toxicity as input into ICE models was compared to overall ICE prediction accuracy

as previously reported and prediction accuracy of the respective upper subset data points that

were entered into the models as µg/L (i.e., values beyond the model domain). A total of 3,104

datapoints from 398 models were evaluated. A match-paired comparison showed that the

average fold differences of toxicity values predicted using scaled toxicity was not significantly

different than the respective average fold differences of all cross-validated data points reported in

Willming et al. (2016) (Wilcoxon paired rank sum test, V = 42741, p-value 0.11). Additionally,

Raimondo et al. (2010) and Willming et al (2016) showed a consistent and reproducible

relationship between the taxonomic distance of the predicted and surrogate species, which was

also reproduced using scaled values; the percentage of datapoints predicted using scaled toxicity

was within five-fold of the measured value for over 94% of all validated datapoints for species

pairs within the same order, with a reduction in accuracy coinciding with decreasing taxonomic

relatedness (Raimondo et al. 2024). Comparison of scaled values with those predicted from µg/L

values beyond the model domain showed that predicted values varied by a factor of 10 for

models with slopes ranging from 0.66 – 1.33. Toxicity values predicted from models with slopes

within this range had a median fold difference of 2.4 using mg/L values and 2.8 using µg/L

values (Wilcoxon paired rank sum test, V = 1334749, p-value 0.77). These results and a detailed review of ICE model assumptions are provided in Raimondo et al. (2024)[2].

L.3 Direct Comparison of Web-ICE and Measured Toxicity Values

Since limited PFOA toxicity test data are available for estuarine/marine species, the ability of ICE models to predict PFOA toxicity was evaluated using direct comparisons of freshwater species sensitivity as reported in the criteria document and predicted by Web-ICE. In this comparison, the measured SMAVs for PFOA reported in Appendix A.1 and Appendix B.1 were used as values for surrogate species to predict to all possible species that also had a measured PFOA SMAV reported. The available SMAVs for PFOA that could be used as ICE surrogate values, along with the number of ICE models corresponding to each surrogate, are shown in Table L-1.

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[2] Use of scaled toxicity values and the use of surrogate toxicity values beyond the bounds of the ICE model that are input as µg/L are two approaches that both make extrapolations beyond the bounds of the underlying data. Actual predictions resulting from the two approaches from the same ICE model begin to deviate from one another the further the slope of the ICE model deviates from 1.0 (which is a primary reason why scaled toxicity data were only employed on ICE models with slopes ranging from 0.66 – 1.33). Overall, use of the scaled approach compared to direct extrapolation results a negligible change in the final estuarine/marine benchmark, primarily because the four most sensitive estuarine/marine GMAVs were based on direct toxicity test results, and secondarily, because only a subset of ICE models required use of scaled toxicity data to account for predicting beyond the bounds of the underlying ICE model. For example, the final acute PFOA estuarine/marine benchmark was 7.0 mg/L (see section L.5). Had the values in Table L-4 been predicted using unscaled data that were input as µg/L only (and the model slope requirement of 0.66 -1.33 been retained), the final acute estuarine/marine benchmark would remain unchanged at 7.0 mg/L. Had the values in Table L-4 been predicted using unscaled data input as µg/L only (and the model slope requirement of 0.66 -1.33 was removed), the final acute estuarine/marine benchmark would increase slightly to 7.5 mg/L. While both approaches contain uncertainty, use of the scaled approach resulted in a more protective acute PFOA estuarine/marine benchmark (i.e., CMC = 7.0 mg/L) than an exploratory benchmark that used acute toxicity data estimated through direct extrapolation, with the model slope requirement of 0.66 -1.33 removed (i.e., exploratory CMC = 7.5 mg/L).

Table L-1. Surrogate Species Measured Values for PFOA 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		PFOA SMAV (mg/L)	Number of ICE Models
	Common Name	Scientific		
Amphibian	Bullfrog	*Lithobates catesbeiana* [a]	1,020	9
Amphibian	Clawed frog	*Xenopus sp.* [b]	377	2
Crustacean	Mysid	*Americamysis bahia*	24	28
Crustacean	Water flea	*Daphnia magna*	220[c]	53
Fish	Zebrafish	*Danio rerio*	450.4	2 (juvenile models) 6 (embryo models)
Fish	Bluegill	*Lepomis macrochirus*	664	68
Fish	Rainbow trout	*Oncorhynchus mykiss*	4,001	77
Fish	Fathead minnow	*Pimephales promelas*	593.6	74
Mollusc	Fatmucket	*Lampsilis siliquoidea*	164.4	29
Mollusc	Black sandshell	*Ligumia recta*	161	1

[a] *Lithobates catesbeianus* was used in Web-ICE
[b] *Xenopus laevis* was used in Web-ICE
[c] *D.magna* species mean acute value used in Appendix L is 220.0 mg/L, which differs slightly from the *D. magna* species mean acute value reported in Appendix A.1 (i.e., 213.9 mg/L). This discrepancy was the result of including late-breaking data ahead for the release of the 2024 Final PFOA Aquatic Life Criteria document. The discrepancy in the *D. magna* SMAV used here vs. Appendix A.1 has no influence on the 2024 PFOA acute estuarine/marine benchmark (7.0 mg/L) because the four most sensitive genera used to derive the PFOA acute estuarine/marine are based entirely on empirical, laboratory-based toxicity data.

Table L-2 shows direct comparisons for PFOA measured and ICE-predicted values. The regressions for these comparisons are provided in Section L.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 PFOA 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 PFOA toxicity predicted to this model is then compared to the measured SMAV for the predicted species listed in Appendix A.1, Appendix B.1 and Table L-1. This allows both species of an ICE model to serve as either the predicted or surrogate species. The exception to this was in cases involving zebrafish embryos, as web-ICE v3.3 only included models for which zebrafish embryos were used as surrogates. Accuracy of ICE predictions are presented as the "fold-

difference" between the measured and the predicted species, such that fold difference is the maximum of the ratio of the predicted LC_{50}/measured LC_{50} or measured LC_{50}/predicted LC_{50}. Analyses of ICE prediction accuracy have shown that ICE models over- and under-estimate toxicity values randomly, i.e., there is no systematic bias associated with the models (Table L-2, Raimondo et al. 2010; Raimondo et al. 2024). For accuracy assessments, the fold difference provides a simplified metric to easily see how close predictions are to measured values at a glance. A five-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).

Table L-2. Comparison of ICE-predicted and measured values of PFOA for species using both scaled values (entered as mg/L) and values potentially beyond the model domain (entered as µg/L).

Measured SMAVs are for the predicted species as listed in Appendix A.1, Appendix B.1 and Table L-1. Footnotes indicate where predictions or models do not meet one or more of the user guidance criteria. Footnotes also indicate any differences between species mean acute values here that differ from species mean acute values reported in Appendix A.1.

Predicted Species	Surrogate Species	Toxicity Values Potentially Beyond Model Domain				Scaled Toxicity Values			
		Measured SMAV (µg/L)	Web-ICE Predicted (µg/L)	Confidence Interval (µg/L)	Fold Difference	Measured SMAV (mg/L)	Web-ICE Predicted (mg/L)	Confidence Interval (mg/L)	Fold Difference
Bullfrog (*Lithobates catesbeianus*)	Bluegill (*Lepomis macrochirus*)	1020000	351962.71	47028.33 – 2634108.91	2.9[ab]	1020	1085.27	358.55 – 3284.91	1.06
	Daphnid (*Daphnia magna*)		250407.71	42580.36 – 1472604.12	4.07		265.51	41.71 – 1689.91	3.84
	Fathead minnow (*Pimephales promelas*)		530164.94	178236.61 – 1576976.01	1.92		841.35	368.83 – 1919.18	1.21
	Rainbow trout (*Oncorhynchus mykiss*)		3874927.81	1530344.80 – 9811557.16	3.8		8676.1	4670.29 – 16117.78	8.51
African clawed frog (*Xenopus laevis*)	Fathead minnow (*Pimephales promelas*)	377000	317645.95	5245.20 – 19236405.56	1.19[ab]	377	854.79	96.46 – 7574.84	2.27[a]
Mysid (*Americamysis bahia*)	Bluegill (*Lepomis macrochirus*)	24000	101324.58	39595.31 – 259290.08	4.22[b]	24	88.82	61.09 – 129.12	3.7
	Daphnid (*Daphnia magna*)		30882.91	15730.51 – 60630.86	1.29		95.62	67.73 – 134.98	3.98
	Fathead minnow (*Pimephales promelas*)		25492.89	4248.75 – 152959.48	1.06[c]		34.09	14.21 – 81.79	1.42[c]
	Rainbow trout (*Oncorhynchus mykiss*)		381425.97	119491.45 – 1217541.25	15.89[bc]		655.82	412.11 – 1043.64	27.33[c]
Daphnid[e] (*Daphnia magna*)[e]	Bluegill (*Lepomis macrochirus*)	220000[e]	159433.9	88808.47 – 286224.59	1.38[c]	220[e]	256.05	191.88 – 341.67	1.16[c]
	Bullfrog (*Lithobates catesbeianus*)		482481.09	76477.24 – 3043886.00	2.19		1174.46	256.08 – 5386.36	5.34
	Fathead minnow (*Pimephales promelas*)		94631.7	46980.95 – 190612.55	2.32[c]		183.63	115.53 – 291.86	1.2[c]
	Fatmucket (*Lampsilis siliquoidea*)		331623.04	78520.34 – 1400577.72	1.51		111	31.65 – 389.26	1.98
	Mysid (*Americamysis bahia*)		22344.14	12674.03 – 39392.40	9.85		78.04	54.00 – 112.78	2.82
	Rainbow trout (*Oncorhynchus mykiss*)		516125.21	246588.64 – 1080281.84	2.35[c]		1642.27	1213.56 – 2222.43	7.46[c]
	Zebrafish embryo (*Danio rerio*)		87831.04	13398.62 – 575752.58	2.5[c]		55.49	7.49 – 410.87	3.96[ac]
Bluegill (*Lepomis macrochirus*)	Bullfrog (*Lithobates catesbeianus*)	664000	1501135.53	114395.51 – 19698393.38	2.26[ab]	664	601.13	166.16 – 2174.76	1.1
	Daphnid (*Daphnia magna*)		81671.31	52906.69 – 126074.85	8.13		801.26	627.74 – 1022.74	1.21
	Fathead minnow (*Pimephales promelas*)		218193.44	111739.02 – 426067.60	3.04		357.51	244.06 – 523.68	1.86
	Fatmucket (*Lampsilis siliquoidea*)		408413.42	32355.12 – 5155335.38	1.63[ac]		295.92	21.91 – 3996.50	2.24[ac]

L-10

Predicted Species	Surrogate Species	Toxicity Values Potentially Beyond Model Domain				Scaled Toxicity Values			
		Measured SMAV (μg/L)	Web-ICE Predicted (μg/L)	Confidence Interval (μg/L)	Fold Difference	Measured SMAV (mg/L)	Web-ICE Predicted (mg/L)	Confidence Interval (mg/L)	Fold Difference
	Mysid (*Americamysis bahia*)		23663.56	13808.02 – 40553.53	28.06		266.08	195.07 – 362.94	2.5[d]
	Rainbow trout (*Oncorhynchus mykiss*)		3129709.92	2269044.40 – 4316832.32	4.71		4818.01	4250.43 – 5461.39	7.26
	Zebrafish embryo (*Danio rerio*)		271173.18	34573.71 – 2126901.59	2.45[a]		214.38	35.96 – 1278.11	3.1
Rainbow trout (*Oncorhynchus mykiss*)	Bluegill (*Lepomis macrochirus*)	4001000	353682.57	274243.78 – 456131.98	11.31	4,001	524.72	466.57 – 590.13	7.63
	Bullfrog (*Lithobates catesbeianus*)		781571.06	313951.07 – 1945695.88	5.12		376.93	177.31 – 801.30	10.61
	Daphnid (*Daphnia magna*)		56931.33	35836.37 – 90443.76	70.28[c]		630.92	485.50 – 819.91	6.34[cd]
	Fathead minnow (*Pimephales promelas*)		206200.6	127654.61 – 333075.99	19.4		255.44	192.66 – 338.66	15.66
	Fatmucket (*Lampsilis siliquoidea*)		736514.01	14043.59 – 38626348.23	5.43[abc]		201.6	39.91 – 1018.32	19.85[c]
	Mysid (*Americamysis bahia*)		17330.67	9740.08 – 30836.72	230.86		192.8	137.35 – 270.65	20.75[d]
	Zebrafish embryo (*Danio rerio*)		309598.05	62068.02 – 1544288.83	12.92		91.52	30.44 – 275.13	43.72
Fathead minnow (*Pimephales promelas*)	African clawed frog (*Xenopus laevis*)	593600	524824.76	4876.97 – 56477848.18	1.13[ab]	593.6	257.81	16.14 – 4116.88	2.3[a]
	Bluegill (*Lepomis macrochirus*)		387148.61	201381.26 – 744279.98	1.53		1427.45	1024.71 – 1988.48	2.4
	Bullfrog (*Lithobates catesbeianus*)		1012520.93	299871.50 – 3418793.08	1.71		758.77	320.24 – 1797.81	1.28
	Daphnid (*Daphnia magna*)		111877.81	66019.39 – 189590.43	5.31[c]		1709.52	1209.11 – 2417.01	2.88[cd]
	Fatmucket (*Lampsilis siliquoidea*)		675590	54952.40 – 8305767.53	1.14[ac]		3450.51	576.51 – 20651.62	5.81[c]
	Mysid (*Americamysis bahia*)		34114.93	11148.28 – 104395.31	17.4[c]		635.95	321.17 – 1259.27	1.07[cd]
	Rainbow trout (*Oncorhynchus mykiss*)		3296419.88	1797095.10 – 6046638.29	5.55		8360.63	6502.51 – 10749.71	14.08
	Zebrafish embryo (*Danio rerio*)		400424.37	203694.32 – 787158.28	1.48		724.18	302.07 – 1736.11	1.22
Fatmucket (*Lampsilis siliquoidea*)	Black sandshell (*Ligumia recta*)	164400	153582.08	28669.47 – 822737.61	1.07	164.4	109.67	9.40 – 1278.86	1.5[a]
	Bluegill (*Lepomis macrochirus*)		97837.12	12334.24 – 776059.00	1.68[ac]		733.53	111.00 – 4847.23	4.46[c]
	Daphnid (*Daphnia magna*)		69944.45	23967.21 – 204121.63	2.35		406.14	160.97 – 1024.70	2.47
	Fathead minnow (*Pimephales promelas*)		23355.28	2752.96 – 198138.64	7.04[ac]		104.49	11.80 – 925.23	1.57[ac]

L-11

Predicted Species	Surrogate Species	Toxicity Values Potentially Beyond Model Domain				Scaled Toxicity Values			
		Measured SMAV (µg/L)	Web-ICE Predicted (µg/L)	Confidence Interval (µg/L)	Fold Difference	Measured SMAV (mg/L)	Web-ICE Predicted (mg/L)	Confidence Interval (mg/L)	Fold Difference
	Rainbow trout (*Oncorhynchus mykiss*)		41091.9	3178.59 – 531223.75	4[abc]		1143.35	381.34 – 3428.01	6.95[cd]
Black sandshell (*Ligumia recta*)	Fatmucket (*Lampsilis siliquoidea*)	161000	171536.98	34311.02 – 857594.23	1.07	161	237.72	25.46 – 2219.07	1.48[a]

[a] Confidence interval >1.5 order magnitude.

[b] Input data outside model range.

[c] Guidance for model mean square error, R^2, and/or slope not met.

[d] Does not meet slope criteria for using scaled toxicity (0.66-1.33).

[e] *D.magna* species mean acute value used in Appendix L is 220.0 mg/L, which differs slightly from the *D. magna* species mean acute value reported in Appendix A.1 (i.e., 213.9 mg/L). This discrepancy was the result of including late-breaking data ahead for the release of the 2024 Final PFOA Aquatic Life Criteria document. The discrepancy in the *D. magna* SMAV used here vs. Appendix A.1 has no influence on the 2024 PFOA acute estuarine/marine benchmark (7.0 mg/L) because the four most sensitive genera used to derive the PFOA acute estuarine/marine are based entirely on empirical, laboratory-based toxicity data.

These comparisons are consistent with web-ICE user guidance, previously published reports on ICE model accuracy (Raimondo et al. 2010; Willming et al. 2016), and the above described uncertainty analysis of using scaled toxicity as model input. ICE models predict with acceptable accuracy for PFOA 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 five-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.1 and L.2 demonstrate that the relationship of inherent sensitivity 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).

L.4   Prediction of Estuarine/Marine Species Sensitivity to PFOA

A value of PFOA sensitivity was predicted with web-ICE v3.3 for all possible species using all available surrogate species (Table L-1). Predicted values were obtained by entering all available surrogate species into the web-ICE SSD generator, which predicts to all possible species from all available surrogates simultaneously and exports results into an excel spreadsheet. Web-ICE results were generated using both mg/L and µg/L values to evaluate the full set of possible predictions using both units of measure against the model domain, confidence intervals, and model parameters. First, all available models were evaluated based on the parameter (MSE, R^2, slope) guidance in Box 1, which are the same for an ICE species pair regardless of input value (Table L-3). Models that did not meet the parameter criteria in Box 1

were rejected in this first pass. In the next step, values that were predicted using µg/L were

evaluated against the model domain and selected for the next tier of evaluation when the

surrogate value was within the range of data used to develop the model. If the surrogate value

reported as µg/L was beyond the model domain, the mg/L value was evaluated if it was within

the model domain and if the model slope was between 0.66-1.33 (Raimondo et al. 2024). Cases

in which both units were outside the model domain were not included quantitatively, but the

value with the narrowest confidence intervals was included for qualitative considerations. Values

(using either µg/L or mg/L input value) were excluded quantitatively from the SMAVs but

retained for qualitative consideration if an evaluation of confidence intervals, model parameters,

and the model domain indicated the relationship between surrogate and predicted species was not

informed by robust underlying data. At this stage, specific predictions should be based on

holistic evaluation of all available information provided by the model, confidence interval, and

data used to develop the model. Decisions to exclude a prediction from the SMAV are clarified

in footnotes. Because the sensitivity of a single species can be predicted by multiple surrogates,

we calculated the SMAV where multiple robust models were available for a predicted species.

Each predicted species was then assigned to the appropriate saltwater MDRs as defined in the

1985 Guidelines:

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 PFOA is presented in Table L-4. A total of 48 models representing 21 estuarine/marine species were available in Web-ICE to predict the toxicity of PFOA to saltwater species (Table L-4). Of these, 14 models were initially rejected based on model parameters not meeting the guidance in Box 1, reducing the number of predicted species to 19 represented by 34 models. Further evaluation of ICE predictions resulted in 13 SMAVs representing crustaceans, molluscs, and fish and demonstrated the potential to meet the saltwater MDRs. 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 PFOA.

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	38514.7	50	5	Rejected
Allorchestes compressa	*Daphnia magna*	0.83	1.59	3	0.8	0.039	0.12	5	184.54	100	5	Accepted qualitatively
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	0.93	0.07	840000	64	5	Accepted
Americamysis bahia	*Lepomis macrochirus*	1.01	-0.92	138	0.66	0	0.95	0.13	290000	59	6	Accepted
Americamysis bahia	*Oncorhynchus mykiss*	0.92	-0.5	150	0.6	0	1.08	0.06	1100000	57	6	Rejected
Americamysis bahia	*Pimephales promelas*	0.95	-1.12	46	0.55	0	1.75	2.27	70200000	35	6	Rejected
Chelon labrosus	*Lampsilis siliquoidea*	1.27	1.5	1	0.99	0.0403	0	19.01	281	na	6	Accepted
Chelon macrolepis	*Pimephales promelas*	1.51	-1.04	2	0.97	0.0114	0.05	26	2533.38	100	4	Accepted qualitatively
Crassostrea virginica	*Americamysis bahia*	0.44	1.76	114	0.34	0	0.88	0.003	117648.2	55	6	Rejected
Crassostrea virginica	*Daphnia magna*	0.44	1.54	116	0.28	0	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	22000	100	4	Accepted
Crassostrea virginica	*Lepomis macrochirus*	0.66	0.71	112	0.51	0	0.64	0.36	290000	69	6	Rejected
Crassostrea virginica	*Oncorhynchus mykiss*	0.59	0.97	120	0.5	0	0.68	0.02	570000	68	6	Rejected
Crassostrea virginica	*Pimephales promelas*	0.75	0.44	24	0.61	0	0.68	1.24	206300.75	69	6	Accepted
Cyprinodon bovinus	*Lepomis macrochirus*	0.66	0.7	1	0.99	0.0326	0	7.43	7326.2	na	4	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	0.67	0.0025	182000	64	6	Rejected
Cyprinodon variegatus	*Daphnia magna*	0.53	1.79	84	0.49	0	0.72	0.08	304000	64	6	Rejected
Cyprinodon variegatus	*Lampsilis siliquoidea*	0.72	0.76	1	0.99	0.0392	0	30	22000	na	6	Accepted
Cyprinodon variegatus	*Lepomis macrochirus*	0.74	0.87	82	0.65	0	0.47	0.77	157000	82	4	Accepted
Cyprinodon variegatus	*Oncorhynchus mykiss*	0.75	0.9	87	0.65	0	0.56	0.82	12700000	75	4	Accepted
Cyprinodon variegatus	*Pimephales promelas*	0.69	0.98	24	0.74	0	0.43	2.27	16500000	77	4	Accepted
Farfantepenaeus duorarum	*Americamysis bahia*	1.03	0.06	6	0.81	0.0022	0.55	0.01	720	50	4	Accepted
Farfantepenaeus duorarum	*Daphnia magna*	1.08	0.14	16	0.76	0	1.32	0.04	65686.02	44	5	Rejected
Farfantepenaeus duorarum	*Lepomis macrochirus*	1.16	-1.21	15	0.67	0	1.88	2.32	130000	35	6	Rejected

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
Farfantepenaeus duorarum	*Oncorhynchus mykiss*	1.2	-1.36	15	0.72	0	1.54	0.57	221000	47	6	Rejected
Fenneropenaeus merguiensis	*Daphnia magna*	0.82	1.43	4	0.66	0.0473	0.4	5	1251.41	67	5	Accepted
Gasterosteus aculeatus	*Lepomis macrochirus*	1.15	0	3	0.95	0.0039	0.09	0.36	340	80	4	Accepted qualitatively
Gasterosteus aculeatus	*Oncorhynchus mykiss*	1.05	0.29	4	0.9	0.0038	0.18	0.61	890	83	4	Accepted qualitatively
Hydroides elegans	*Daphnia magna*	0.49	1.59	2	0.96	0.0182	0.01	5	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
Lagodon rhomboides	*Lepomis macrochirus*	1.61	-2.02	1	0.99	0.0301	0	110	760	na	3	Accepted qualitatively
Litopenaeus stylirostris	*Americamysis bahia*	1.04	0.01	5	0.6	0.0401	0.29	0.58	24.09	57	4	Accepted qualitatively
Menidia beryllina	*Lepomis macrochirus*	0.79	0.9	5	0.89	0.0012	0.19	12.3	93800	86	4	Accepted
Menidia menidia	*Lepomis macrochirus*	1.05	-0.35	4	0.96	0.0005	0.14	2.85	97000	83	4	Accepted
Menidia menidia	*Oncorhynchus mykiss*	1.28	-1.4	3	0.94	0.005	0.23	11.24	91000	60	4	Accepted
Menidia peninsulae	*Americamysis bahia*	0.63	0.91	3	0.88	0.0162	0.32	0.01	1160	80	6	Accepted qualitatively
Menidia peninsulae	*Lepomis macrochirus*	0.9	-0.1	3	0.97	0.0012	0.06	0.77	2480	100	4	Accepted
Menidia peninsulae	*Oncorhynchus mykiss*	1.01	-0.36	2	0.91	0.0421	0.35	0.82	1600	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	*Lepomis macrochirus*	1.06	-0.15	3	0.92	0.0093	0.09	0.77	118.76	100	4	Accepted qualitatively
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	*Lepomis macrochirus*	0.6	1.73	3	0.92	0.0095	0.1	1.2	11202.42	80	6	Accepted qualitatively
Tigriopus japonicus	*Pimephales promelas*	0.81	1.12	5	0.76	0.0103	0.11	195.14	27000	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 qualitatively

NA = Not Available

Table L-4. ICE-estimated Species Sensitivity to PFOA.

Values in bold and underlined are used for SMAV.

Common Name	Predicted Species	Surrogate Species	Input Unit	Estimated Toxicity (mg/L)	95% Confidence Intervals (mg/L)	SMAV
Calanoid copepod	*Acartia tonsa*	*Daphnia magna*	µg/L	30.97[abc]	(0.84 – 1138.99)	NA
Amphipod	*Allorchestes compressa*	*Daphnia magna*	mg/L	3608.27[b]	(604.53 – 21536.48)	306.14
		Pimephales promelas	mg/L	**306.14**	(167.3 – 560.19)	
Mysid	*Americamysis bahia*	*Daphnia magna*	µg/L	**30.88**	(15.73 – 60.63)	52.37
		Lepomis macrochirus	mg/L	**88.82**	(61.09 – 129.12)	
		Oncorhynchus mykiss	µg/L	381.43[bc]	(119.49 – 1217.54)	
		Pimephales promelas	µg/L	25.49[c]	(4.25 – 152.96)	
Thicklip mullet	*Chelon labrosus*	*Lampsilis siliquoidea*	mg/L	**21448.8**	(5726.99 – 80330.23)	21448.8
Bigscale mullet	*Chelon macrolepis*	*Pimephales promelas*	mg/L	1475.56[d]	(400.78 – 5432.58)	NA
Eastern oyster	*Crassostrea virginica*	*Americamysis bahia*	µg/L	5.08[c]	(2.55 – 10.1)	111.23
		Daphnia magna	µg/L	8.16[bc]	(3.27 – 20.37)	
		Lampsilis siliquoidea	mg/L	**34.96**	(13.16 – 92.9)	
		Lepomis macrochirus	µg/L	37.78[bc]	(15.7 – 90.87)	
		Oncorhynchus mykiss	µg/L	86.67[bc]	(31.82 – 236.06)	
		Pimephales promelas	mg/L	**353.9**	(161.95 – 773.33)	
Leon springs pupfish	*Cyprinodon bovinus*	*Lepomis macrochirus*	mg/L	**385.84**	(100.03 – 1488.19)	363.56
		Oncorhynchus mykiss	mg/L	2637.1[ab]	(135.14 – 51456.4)	
		Pimephales promelas	mg/L	**342.56**	(204.07 – 575.01)	
Sheepshead minnow	*Cyprinodon variegatus*	*Americamysis bahia*	µg/L	24.42[c]	(12.11 – 49.24)	377.3
		Daphnia magna	µg/L	43.97[c]	(18.29 – 105.67)	
		Lampsilis siliquoidea	mg/L	**236.12**	(42.61 – 1308.27)	
		Lepomis macrochirus	mg/L	**975.2**	(695.11 – 1368.14)	
		Oncorhynchus mykiss	µg/L	**834.95**	(288.9 – 2413.1)	
		Pimephales promelas	µg/L	**105.41**	(29.42 – 377.63)	
Pink shrimp	*Farfantepenaeus duorarum*	*Americamysis bahia*	mg/L	**31.16**	(5.31 – 182.77)	31.16
		Daphnia magna	µg/L	825.2[abc]	(47.76 – 14258.5)	
		Lepomis macrochirus	µg/L	350.34[abc]	(11.8 – 10398.14)	
		Oncorhynchus mykiss	µg/L	4187.17[abc]	(97.76 – 179343.3)	
Banana prawn	*Fenneropenaeus merguiensis*	*Daphnia magna*	mg/L	**2395.84**	(373.14 – 15382.93)	2395.84
Threespine stickleback	*Gasterosteus aculeatus*	*Lepomis macrochirus*	mg/L	1867.23[ab]	(239.79 – 14539.92)	NA
		Oncorhynchus mykiss	mg/L	12299.55[ab]	(605.28 – 249929.11)	

Common Name	Predicted Species	Surrogate Species	Input Unit	Estimated Toxicity (mg/L)	95% Confidence Intervals (mg/L)	SMAV
Polychaete	*Hydroides elegans*	*Daphnia magna*	µg/L	17.26[abc]	(1.79 – 166.56)	NA
		Oncorhynchus mykiss	µg/L	4.67[bc]	(2.22 – 9.84)	
Pinfish	*Lagodon rhomboides*	*Lepomis macrochirus*	mg/L	342.29[d]	(121.48 – 964.44)	NA
Blue shrimp	*Litopenaeus stylirostris*	*Americamysis bahia*	mg/L	28.4[a]	(3.76 – 214.22)	NA
Inland silverside	*Menidia beryllina*	*Lepomis macrochirus*	mg/L	**1373.42**	(509.99 – 3698.68)	1373.42
Atlantic silverside	*Menidia menidia*	*Lepomis macrochirus*	mg/L	**419.03**	(151.91 – 1155.88)	859.43
		Oncorhynchus mykiss	mg/L	**1762.7**	(281.3 – 11045.23)	
Tidewater silverside	*Menidia peninsulae*	*Americamysis bahia*	mg/L	61.53[ad]	(8.51 – 444.5)	279.81
		Lepomis macrochirus	mg/L	**279.81**	(97.91 – 799.61)	
		Oncorhynchus mykiss	mg/L	1974.29[ab]	(15.61 – 249609.1)	
Mysid	*Metamysidopsis insularis*	*Daphnia magna*	mg/L	**894.87**	(209.96 – 3814.04)	853.17
		Lampsilis siliquoidea	mg/L	**813.42**	(361.18 – 1831.9)	
Striped mullet	*Mugil cephalus*	*Lepomis macrochirus*	mg/L	730.34[ab]	(36.69 – 14537.49)	NA
		Oncorhynchus mykiss	mg/L	65159.7[abd]	(132.99 – 31925133.65)	
Harpacticoid copepod	*Tigriopus japonicus*	*Lepomis macrochirus*	mg/L	2812.24[d]	(976.85 – 8096.09)	2432.2
		Pimephales promelas	mg/L	**2432.2**	(825.63 – 7164.93)	
Harpacticoid copepod	*Tisbe battagliai*	*Daphnia magna*	mg/L	1923.22[ab]	(204.62 – 18075.62)	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.5   Derivation of Acute Water Benchmark for Estuarine/Marine Water

The Web-ICE predicted acute data set for PFOA contains 10 genera representing the eight MDR groups that would be necessary for developing an estuarine/marine criterion. However, the EPA supplemented this dataset with acceptable quantitative study data (discussed in Section 3.1.1.2). 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 criterion values for PFOA. GMAVs for the four most sensitive genera were within a factor of 1.5 of each other (Table L-5). The estuarine/marine FAV (the 5th percentile of the genus sensitivity distribution) for PFOA is 14.07 mg/L (Table L-6). The FAV was lower than all of the GMAVs for both the tested species and for values derived using Web-ICE. The FAV is then divided by two to obtain a concentration yielding a minimal effects acute effect value. Based on the above, the FAV/2, which is the estuarine/marine acute water column benchmark magnitude, is 7.0 mg/L PFOA (rounded to two significant figures) and is expected to be protective of 95% of saltwater genera potentially exposed to PFOA 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).

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

Values in bold are derived from empirical PFOA toxicity tests with the species.

MDR Group[1]	Common Name	Species	SMAV (mg/L)	GMAV (mg/L)	Rank	Percentile
C	**Mysid**	*Siriella armata*	**15.5**	**15.5**	1	0.07
D	**Mediterranean mussel**	*Mytilus galloprovincialis*	**17.6**	**17.6**	2	0.14
F	**Purple sea urchin**	*Strongylocentrotus purpuratus*	**20.63**	**20.63**	3	0.21
C	**Mysid**	*Americamysis bahia*	**24**	**24**	4	0.29
F	Pink shrimp	*Farfantepenaeus duorarum*	31.16	31.16	5	0.36
D	Eastern oyster	*Crassostrea virginica*	111.2	111.2	6	0.43
E	Amphipod	*Allorchestes compressa*	306.1	306.1	7	0.50
A	Leon springs pupfish	*Cyprinodon bovinus*	363.6	370.4	8	0.57
A	Sheepshead minnow	*Cyprinodon variegatus*	377.3	370.4	8	0.57
B	Inland silverside	*Menidia beryllina*	1,373	691.2	9	0.64
B	Atlantic silverside	*Menidia menidia*	859.4	691.2	9	0.64
B	Tidewater silverside	*Menidia peninsulae*	279.8	691.2	9	0.64
C	Mysid	*Metamysidopsis insularis*	853.2	853.2	10	0.71
G	Harpacticoid copepod	*Tigriopus japonicus*	2,432	2,432	11	0.79
F	Banana prawn	*Fenneropenaeus merguiensis*	2,396	2,396	12	0.86
H	Thicklip mullet	*Chelon labrosus*	21,449	21,449	13	0.93

1: Estuarine/Marine 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.

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
	Calculated Estuarine/Marine FAV based on 4 lowest values; n=13					
1	*Siriella*	15.5	2.74	7.51	0.071	0.267
2	*Mytilus*	17.6	2.87	8.22	0.143	0.378
3	*Strongylocentrotus*	20.63	3.03	9.16	0.214	0.463
4	*Americamysis*	24	3.18	10.10	0.286	0.535
		Σ (Sum):	**11.81**	**35.00**	**0.71**	**1.64**

$S^2 =$ 2.73 S = slope
$L =$ 2.275 L = X-axis intercept
$A =$ 2.644 A = lnFAV
FAV = 14.07 P = cumulative probability
PVAL= **7.0 mg/L PFOA** (rounded to two significant figures)

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

L.5.1   Estuarine Marine/Benchmark Uncertainty

The 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 from the available and quantitatively acceptable models (see bolded and underlined values in Table L-4; n =21), 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 69.01% to 626.49%. The ICE model with the lowest 95% CI range relative to the LC_{50} estimate (i.e., 69.01%) employed *Lepomis macrochirus* 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., 626.49%) employed *Daphnia magna* as the predictor species and *Fenneropenaeus merguiensis* as the predicted species. Nineteen of the 21 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 PFOA estuarine/marine benchmark.

Four of the 13 GMAVs used to derive the acute PFOA estuarine/marine benchmark were based on empirical toxicity tests. The four GMAVs based on empirical data were also the four most sensitive GMAVs in the GSD (Figure L-2), meaning final estuarine/benchmark magnitude was primarily based on relatively certain empirical toxicity tests and the inherent uncertainty in the ICE models had little influence on the final acute estuarine/marine benchmark magnitude.

The estuarine/marine benchmark appears adequately protective based on the available high quality empirical data (Appendix B.1). The acute PFOA estuarine/marine benchmark (i.e., 7.0) is more than two times lower than the lowest GMAV (i.e., 15.5 mg/L), which was based on

empirical data for *Siriella*. The EPA further evaluated the appropriateness of the

estuarine/marine benchmark by comparing it to empirical, but qualitatively acceptable, data for

estuarine/marine species. The EPA specifically focused on qualitatively-acceptable

estuarine/marine tests reported in Table H.1 that (1) tested an animal species, (2) exposed test

organisms to PFOA for a 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., 14 mg/L). The

EPA identified three individual tests in Table H.1 as meeting the previous criteria:

1. Liu et al. (2013, 2014c) evaluated the chronic effects of PFOA (96% purity,
   purchased from Sigma-Aldrich) on green mussels, *Perna viridis*, via a seven-day
   measured, static-renewal study. A NOEC of 0.0114 mg/L and a LOEC of 0.099
   mg/L was determined for a decrease in the relative condition factor (RCF). The
   study was acceptable for qualitative use only because of the atypical test duration,
   which is too long for an acute test and too short for a chronic test. Additionally,
   the PFOA test displayed a questionable concentration-response patten where there
   was no difference between the RCF at the LOEC (i.e., 0.099 mg/L) and the
   highest test concentration, which contained a PFOA concentration that was more
   than 10X greater (i.e., 1.120 mg/L). The large magnitude between these two
   concentrations in combination with the lack of effects to the RCF observed
   between the LOEC and the highest treatment concentration suggests a true
   concentration-response relationship was not observed for PFOA in this test.

2. Bernardini et al. (2021) reported the results of a 21-day chronic study with the
   Manila clam, *Ruditapes philippinarum*. Subsamples of clams (n=20) were also

collected on test day seven. No significant effects of mortality were observed in the single treatment group throughout the exposure, including at test day seven. The seven-day NOEC, based on mortality, was 0.00093 mg/L PFOA. Although the seven-day NOEC is less than the acute estuarine/marine benchmark, the authors did not report any significant effects to mortality and this study was not useful in understanding the relative protectiveness of the acute PFOA estuarine/marine benchmark.

3. Mhadhbi et al. (2012) conducted a six-day acute test on the turbot, *Scophthalmus maximus* (a non-North American species). Endpoints included dead embryos, malformation, hatch success at 48-hours and larvae survival (missing heartbeat and a non-detached tail) at six days. The reported six-day LC_{50} of 11.9 mg/L PFOA was not used quantitatively because the test duration was longer than the standard 96 hour acute exposure. Nevertheless, this six-day test suggests early life stages of *S. maximus* may be sensitive to acute PFOA exposures. The EPA concluded the acute PFOA estuarine/marine benchmark to be protective on the six-day LC_{50} reported by Mhadhbi et al. (2012) because (1) it was reasonably similar to the most sensitive GMAV used to derive the acute estuarine/marine benchmark (i.e., *Siriella* GMAV = 15.5 mg/L) and (2) the 96-hour LC_{50} that corresponds to the six-day LC_{50} reported by Mhadhbi et al. (2012) was hypothesized to be greater than or equal to the six-day LC_{50} under the premise that acute effect concentrations typically decrease with exposure time (until an incipient lethal concentration is reached).

4. Gebreab et al. (2022) exposed the marine mahi-mahi fish, *Coryphaena hippurus* to PFOA in a 48-hour static acute test with measured PFOA treatments. Authors stated that the test followed a new embryotoxicity assay for mahi mahi that was adapted from assays previously developed and validated for zebrafish embryotoxicity (Berry et al. 2007; Gebreab et al. 2020; Weiss-Errico et al. 2017). The author-reported 48-hour LC_{50} (i.e., 4 mg/L; 95% C.I. = 2-6 mg/L) was less than the acute estuarine/marine FAV of 14 mg/L. Results of this test were not used quantitatively primarily because there was 20-30% mortality in the negative control at the end of the exposure period. The high control mortality suggests the exposed organisms were stressed in the laboratory setting, which compounded any effects of PFOA and likely produced an artificially low LC_{50} value. Therefore, this qualitatively acceptable test did not provide sufficient evidence to conclude the estuarine/marine benchmark was underprotective of *C. hippurus*.

Overall, results of quantitatively- and qualitatively- acceptable empirical toxicity studies with estuarine/marine organisms do not provide any evidence that the aquatic estuarine/marine community will experience unacceptable chronic effects at the acute estuarine/marine PFOA benchmark.

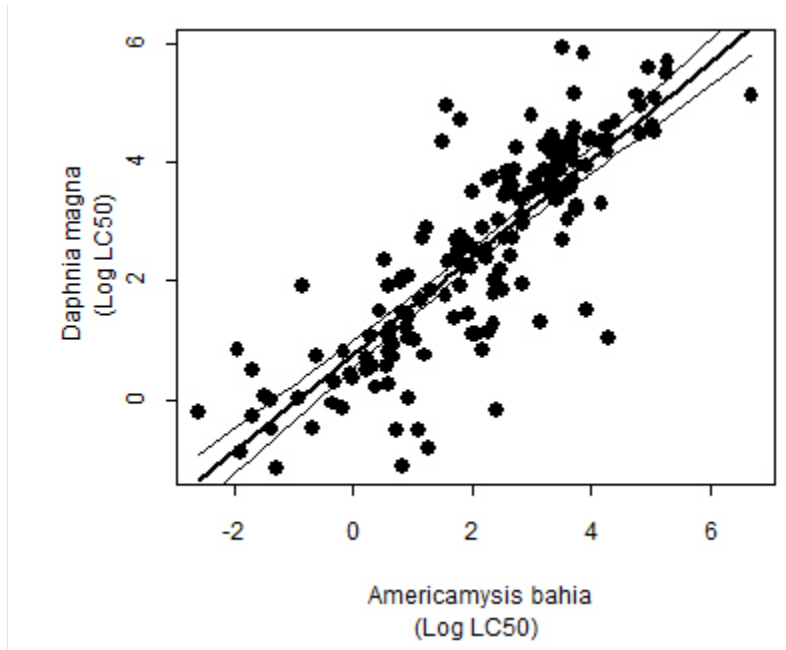## L.6   ICE Regressions Supporting the Acute Estuarine/Marine Benchmark

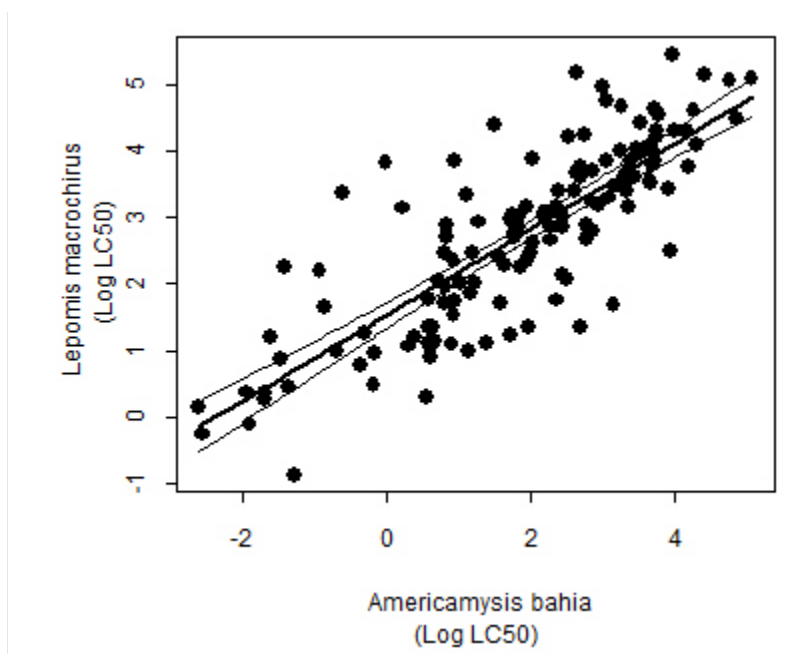**Figure L-3.** *Americamysis bahia* **(X-axis) and** *Daphnia magna* **(Y-axis) regression model used for ICE predicted values.**

Figure L-4. *Americamysis bahia* **(X-axis) and** *Lepomis macrochirus* **(Y-axis) regression model used for ICE predicted values.**
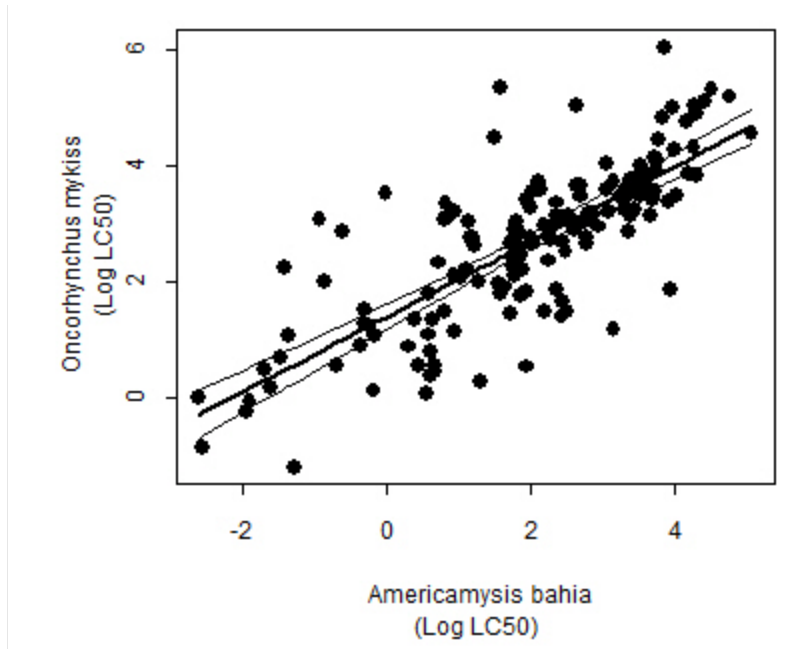
Figure L-5. *Americamysis bahia* **(X-axis) and** *Oncorhynchus mykiss* **(Y-axis) regression model used for ICE predicted values.**
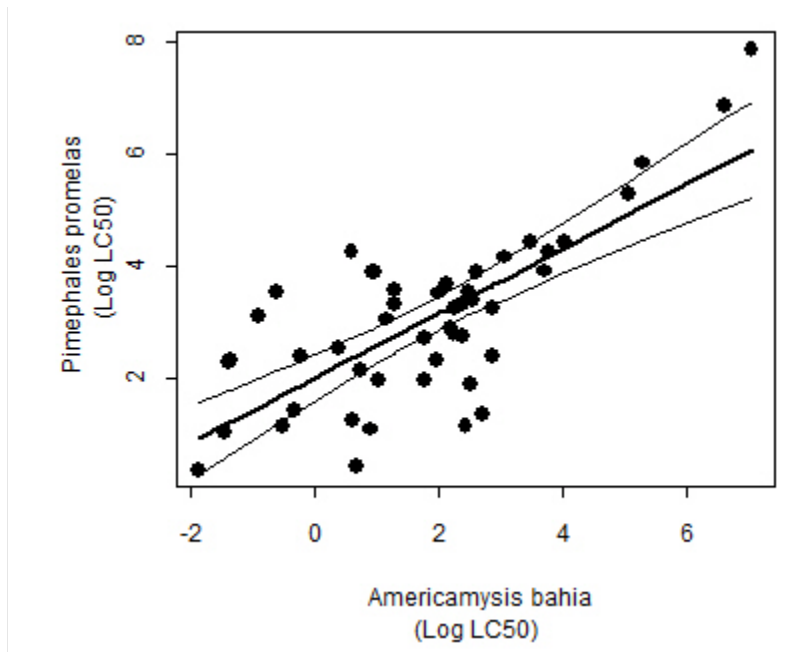

Figure L-6. *Americamysis bahia* **(X-axis) and** *Pimephales promelas* **(Y-axis) regression model used for ICE predicted values.**
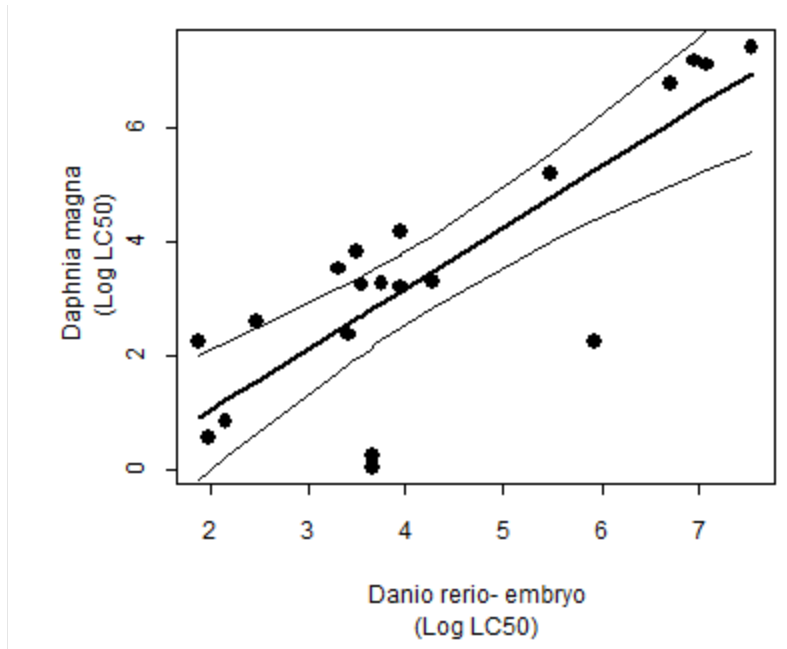
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Figure L-7. *Danio rerio* – embryo (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.
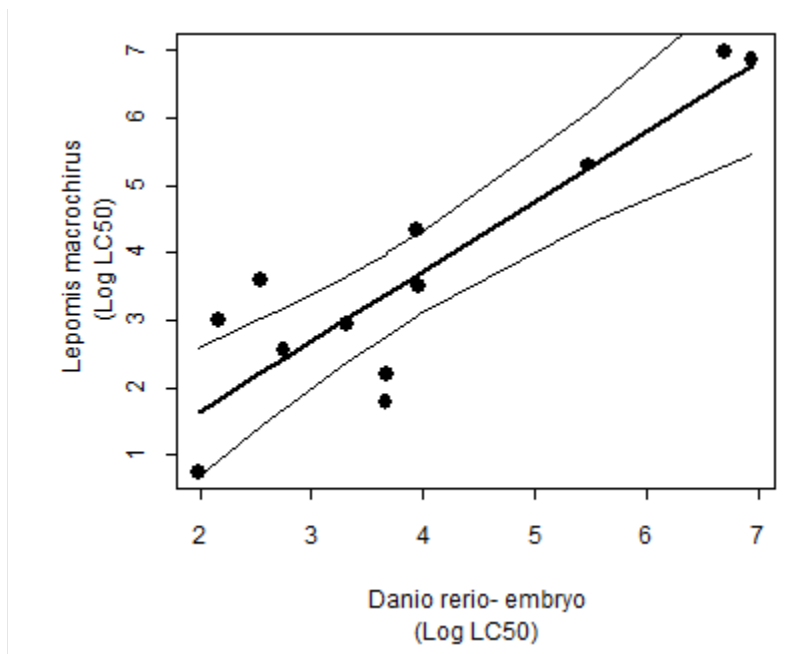

Figure L-8. *Danio rerio* – embryo (X-axis) and *Lepomis macrochirus* (Y-axis) regression model used for ICE predicted values.

Figure L-9. *Danio rerio* – embryo (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

Figure L-10. *Danio rerio* – embryo (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

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

Figure L-12. *Daphnia magna* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.
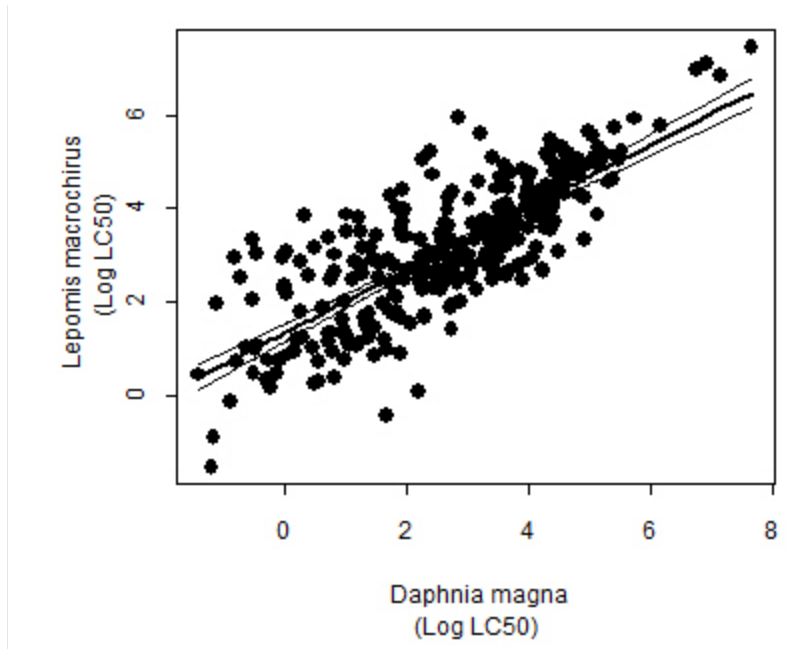
Figure L-13. *Daphnia magna* (X-axis) and *Lepomis macrochirus* (Y-axis) regression model used for ICE predicted values.
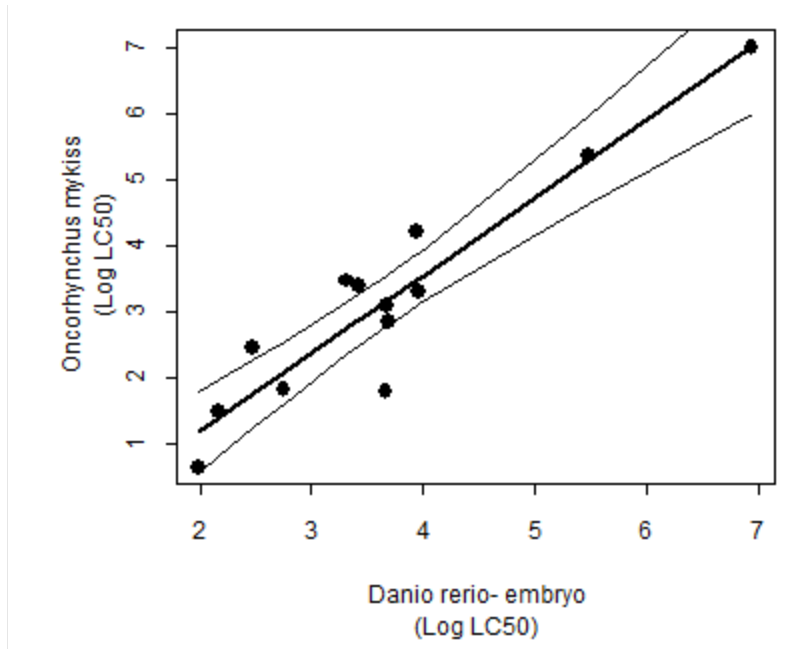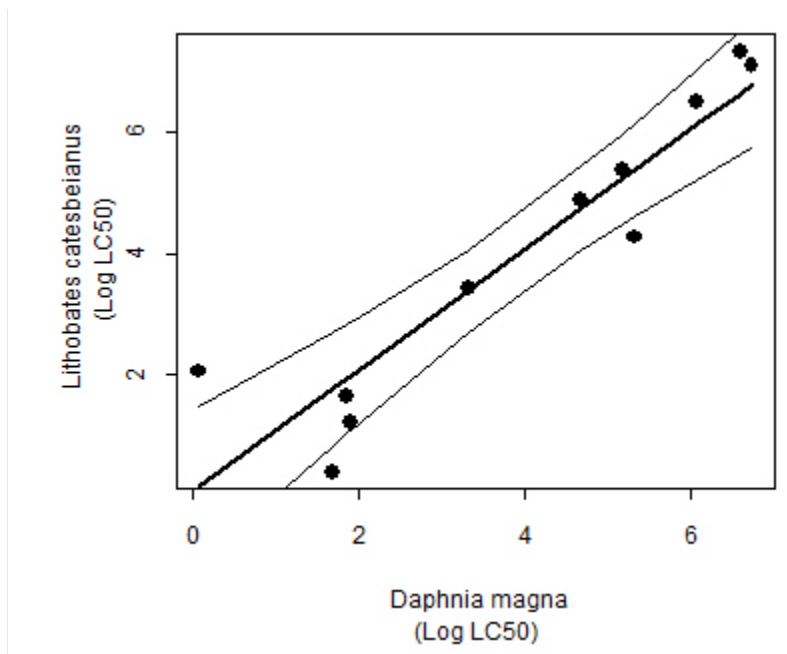

Figure L-14. *Daphnia magna* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.
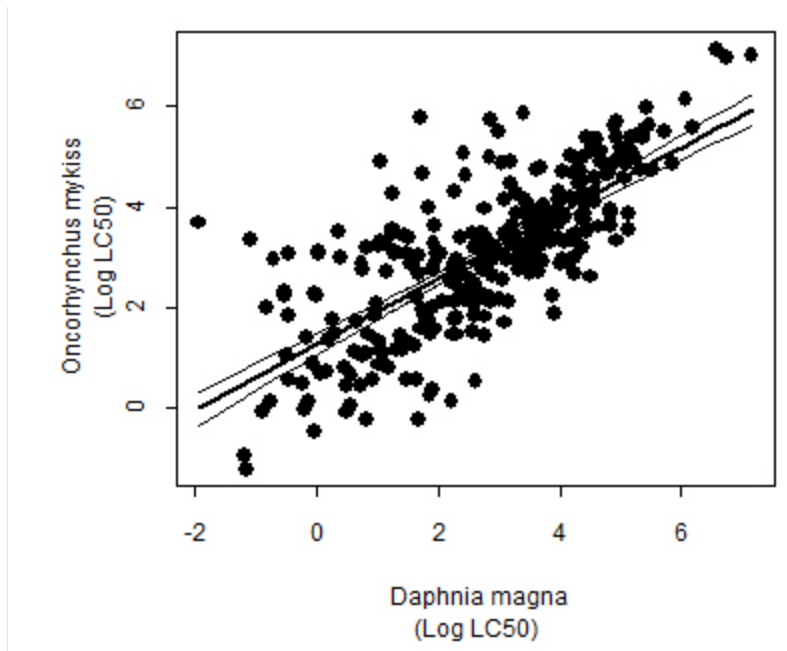
Figure L-15. *Daphnia magna* **(X-axis) and** *Oncorhynchus mykiss* **(Y-axis) regression model used for ICE predicted values.**
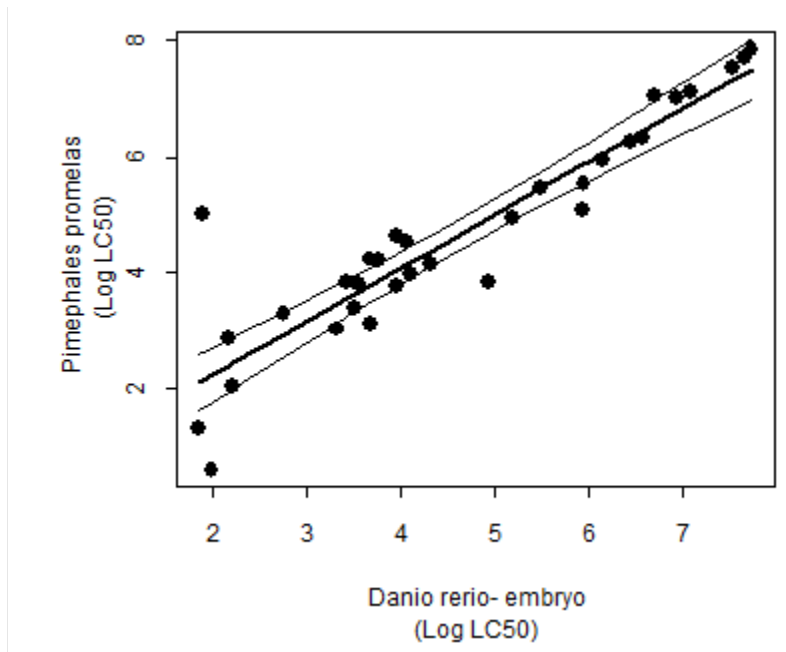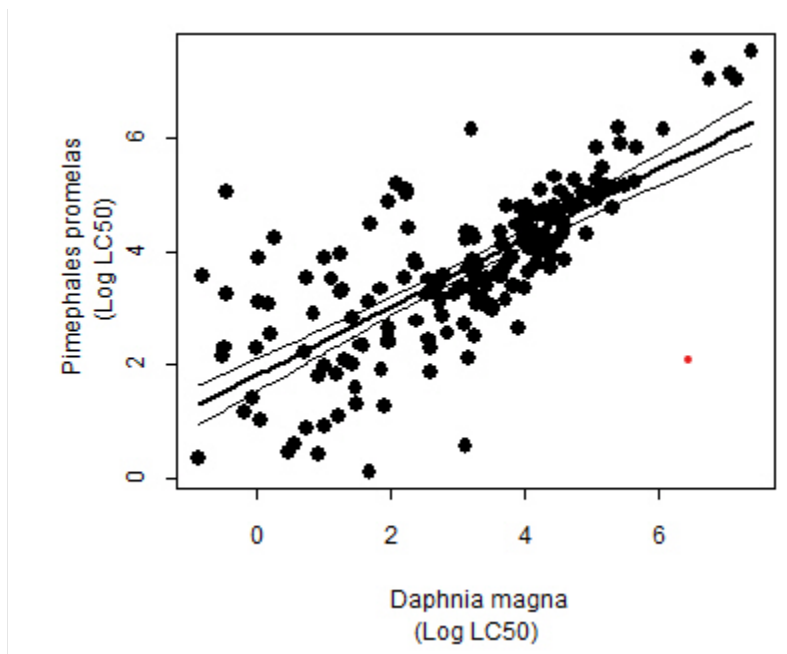

Figure L-16. *Daphnia magna* **embryo (X-axis) and** *Pimephales promelas* **(Y-axis) regression model used for ICE predicted values.**
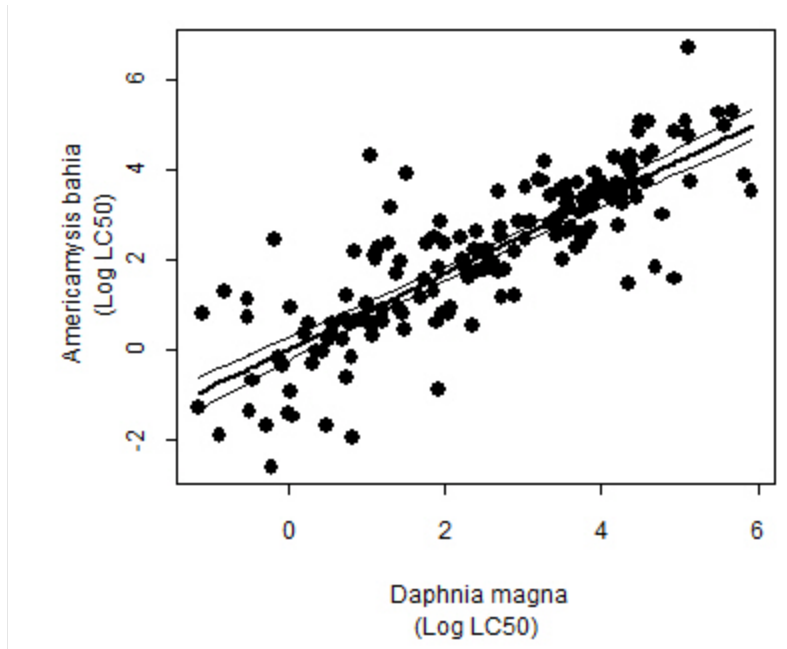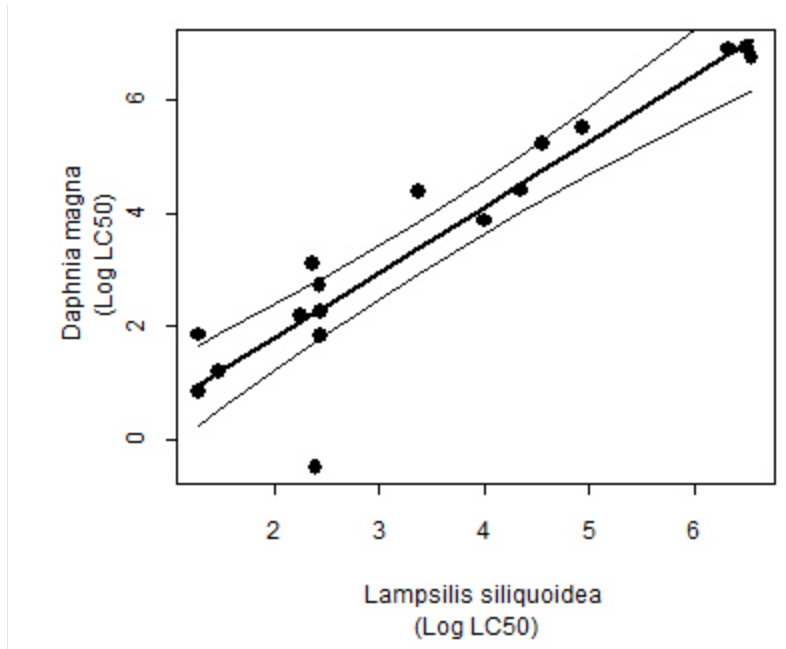
Figure L-17. *Lampsilis siliquoidea* (**X-axis**) **and** *Daphnia magna* (**Y-axis**) **regression model used for ICE predicted values.**
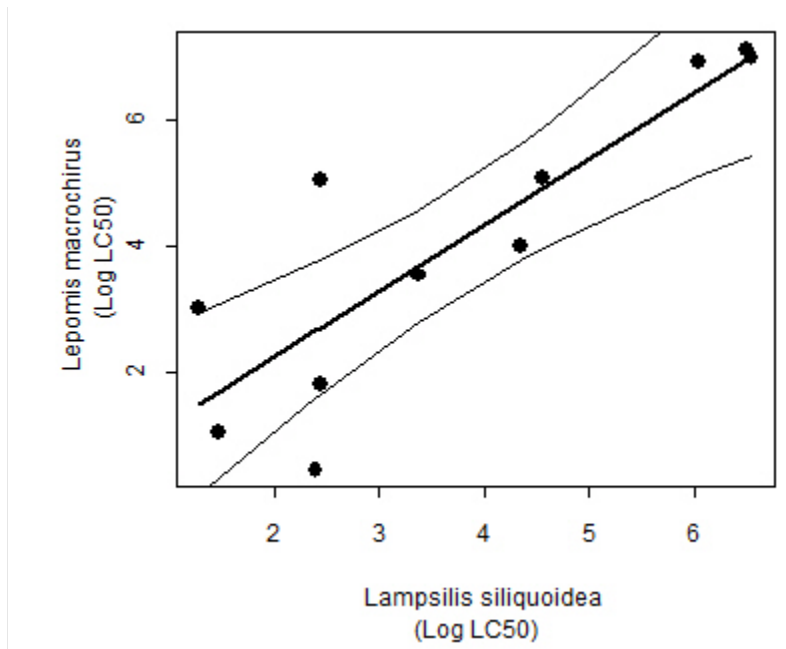
Figure L-18. *Lampsilis siliquoidea* (**X-axis**) **and** *Lepomis macrochirus* (**Y-axis**) **regression model used for ICE predicted values.**
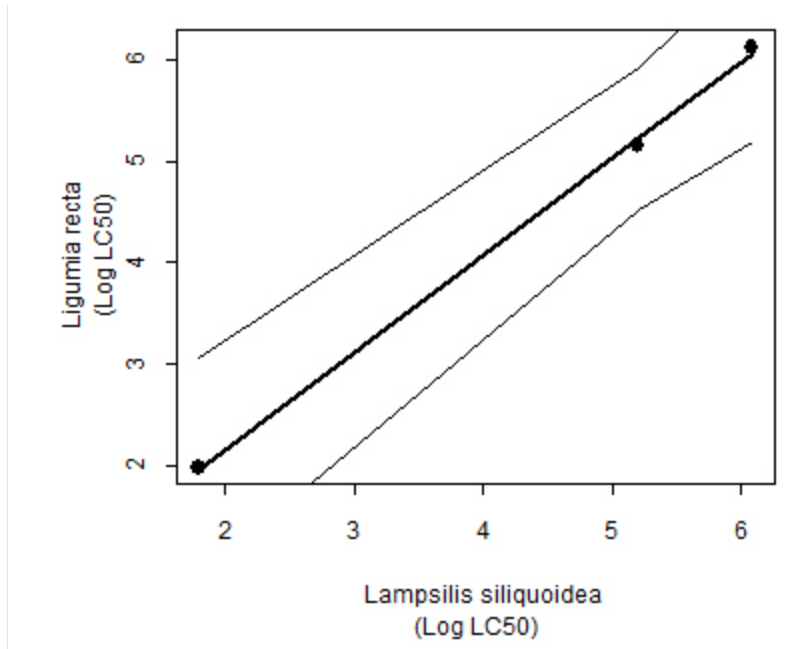
Figure L-19. *Lampsilis siliquoidea* (X-axis) and *Ligumia recta* (Y-axis) regression model used for ICE predicted values.
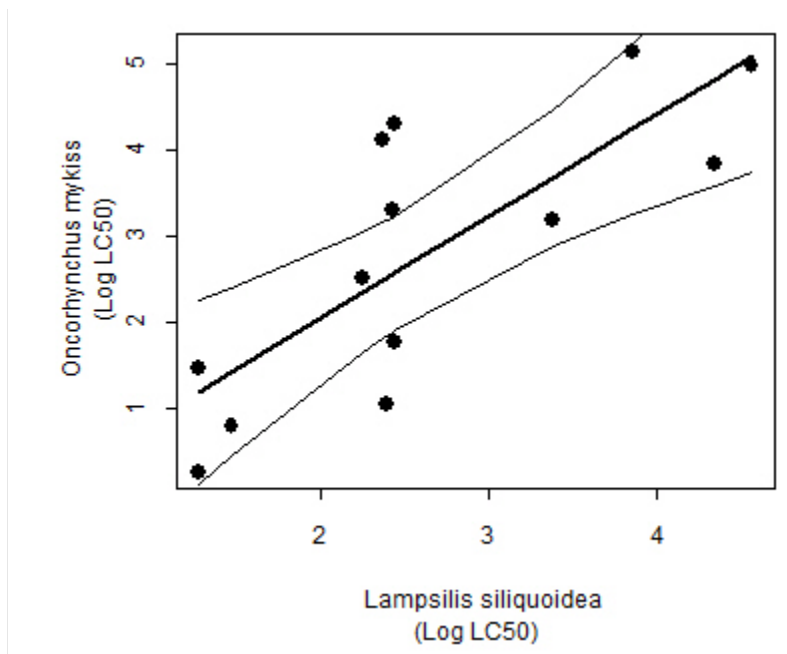
Figure L-20. *Lampsilis siliquoidea* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.
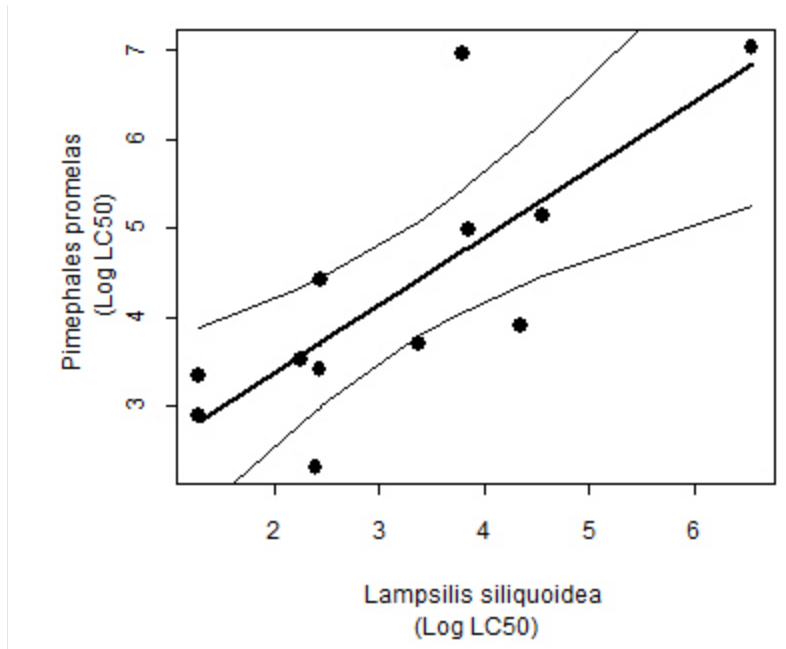
Figure L-21. *Lampsilis siliquoidea* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.
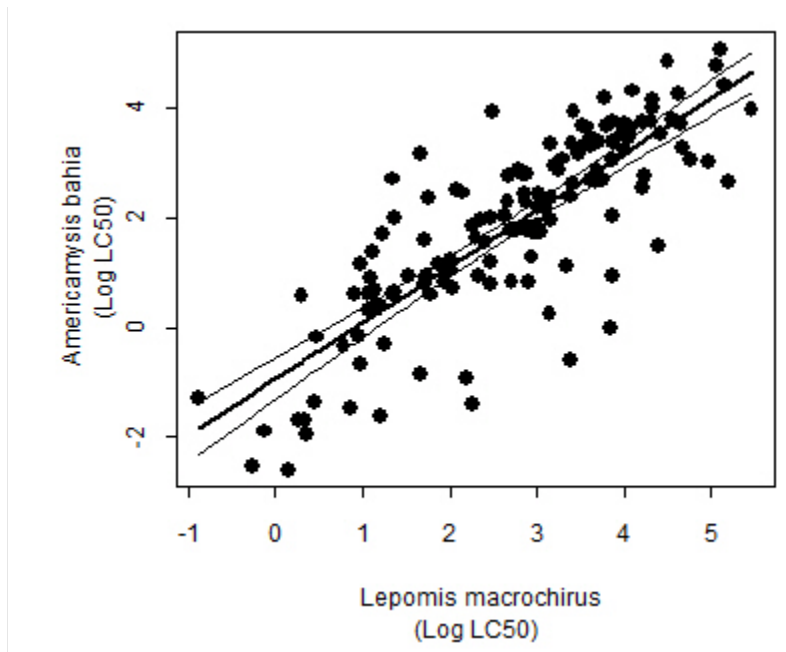
Figure L-22. *Lepomis macrochirus* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.
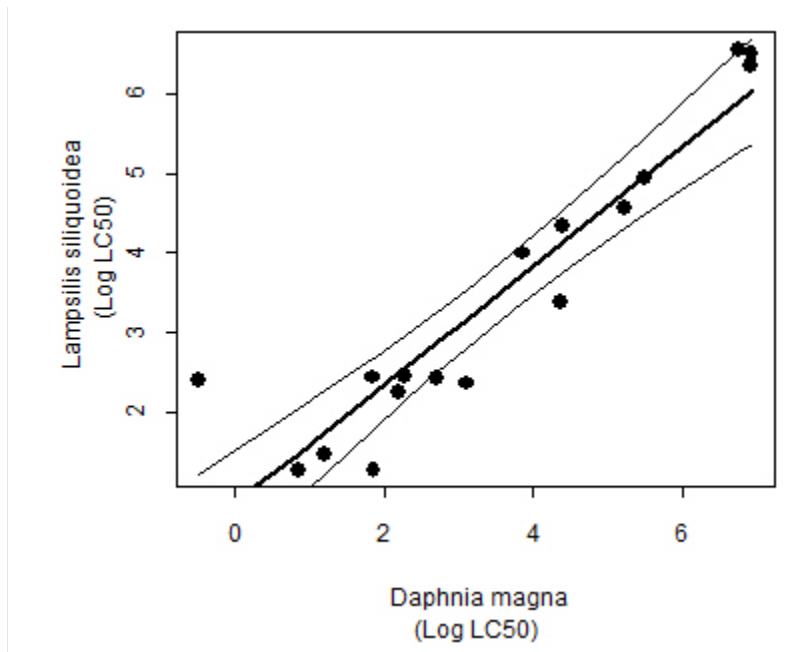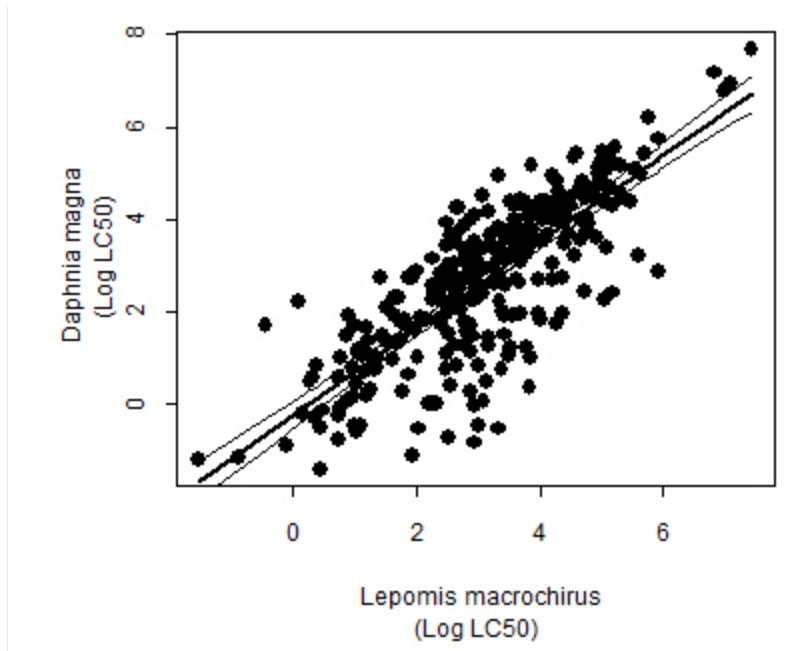
Figure L-23. *Lepomis macrochirus* **(X-axis) and** *Daphnia magna* **(Y-axis) regression model used for ICE predicted values.**
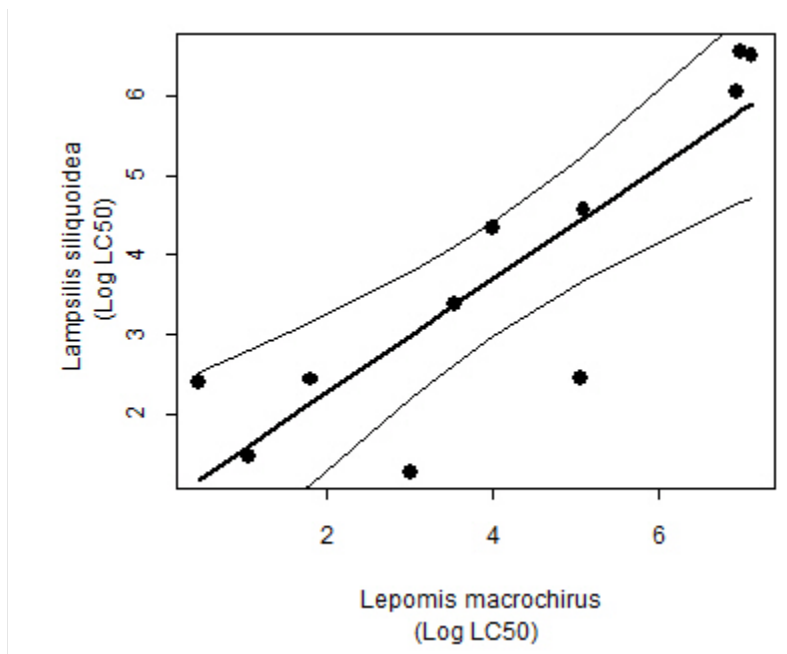
Figure L-24. *Lepomis macrochirus* **(X-axis) and** *Lampsilis siliquoidea* **(Y-axis) regression model used for ICE predicted values.**
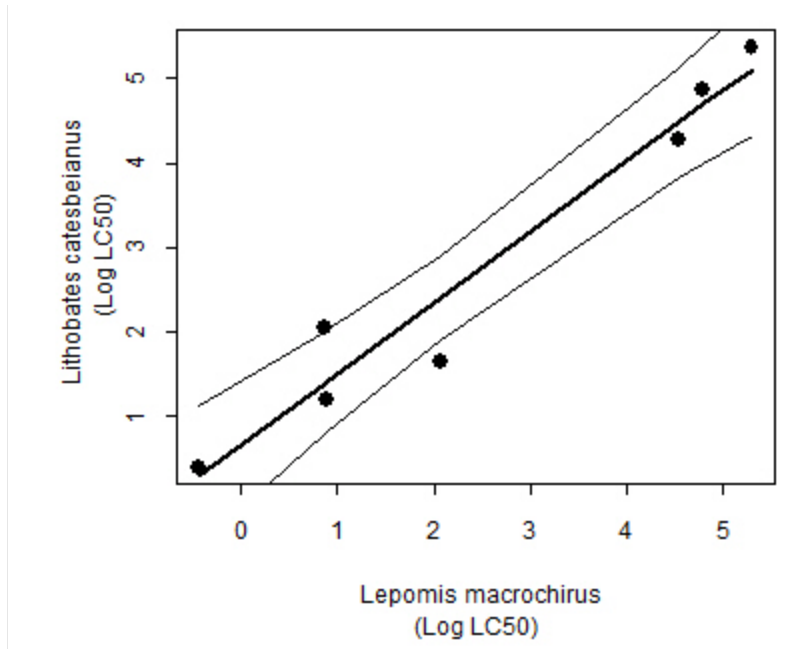
Figure L-25. *Lepomis macrochirus* **(X-axis) and** *Lithobates catesbeianus* **(Y-axis) regression model used for ICE predicted values.**
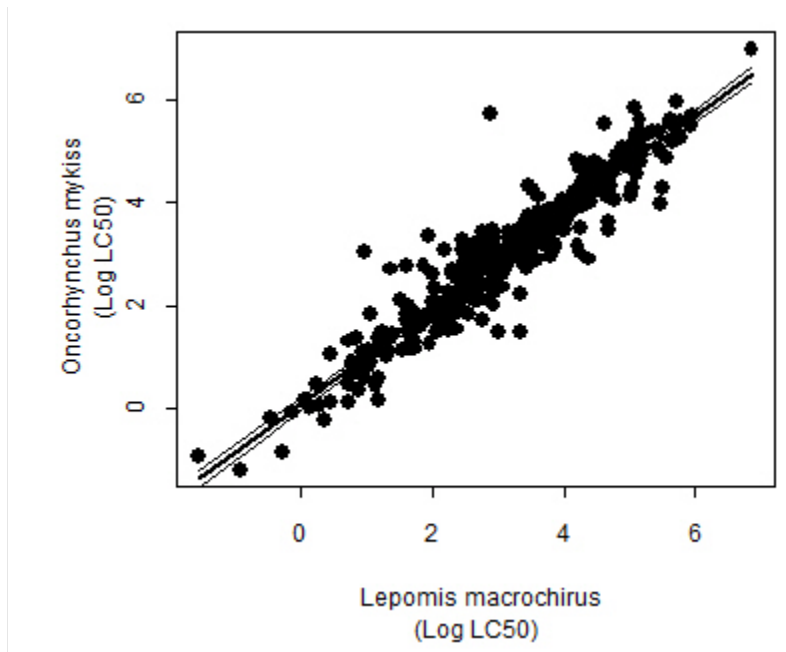
Figure L-26. *Lepomis macrochirus* **(X-axis) and** *Oncorhynchus mykiss* **(Y-axis) regression model used for ICE predicted values.**
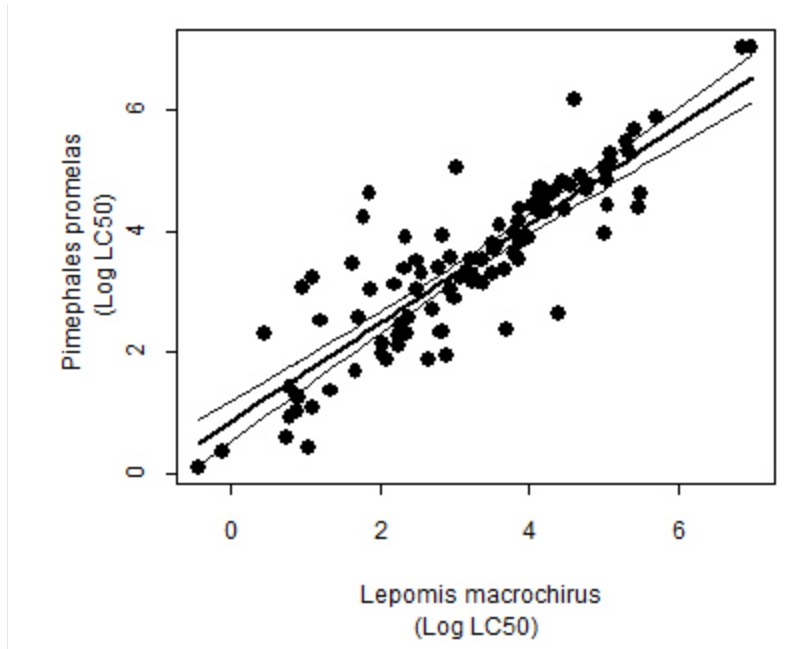
Figure L-27. *Lepomis macrochirus* **embryo (X-axis) and** *Pimephales promelas* **(Y-axis) regression model used for ICE predicted values.**
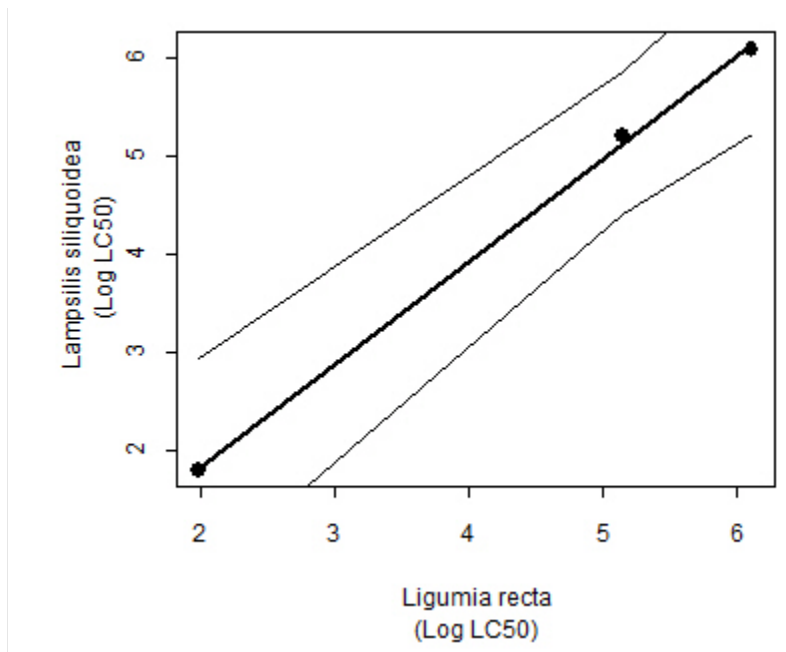
Figure L-28. *Ligumia recta* **(X-axis) and** *Lampsilis siliquoidea* **(Y-axis) regression model used for ICE predicted values.**
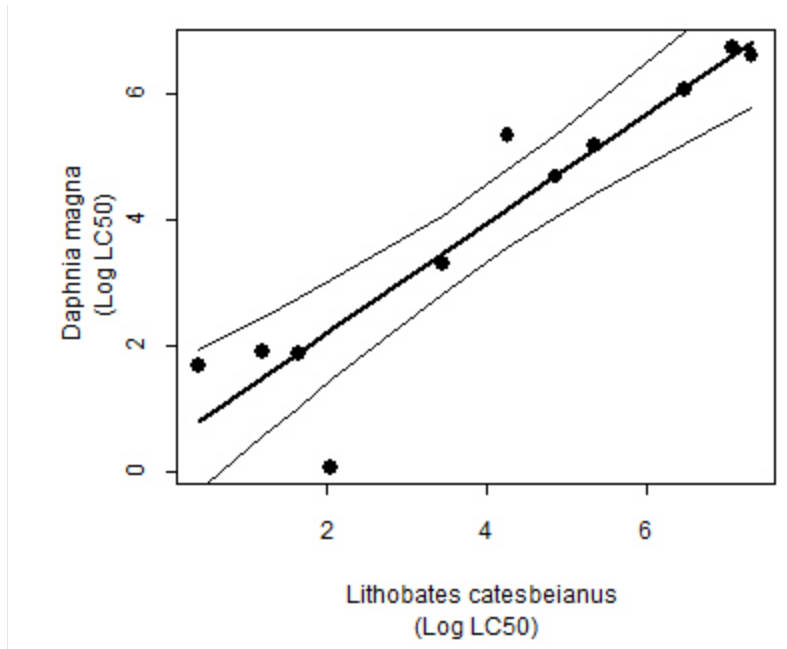
Figure L-29. *Lithobates catesbeianus* **(X-axis) and** *Daphnia magna* **(Y-axis) regression model used for ICE predicted values.**
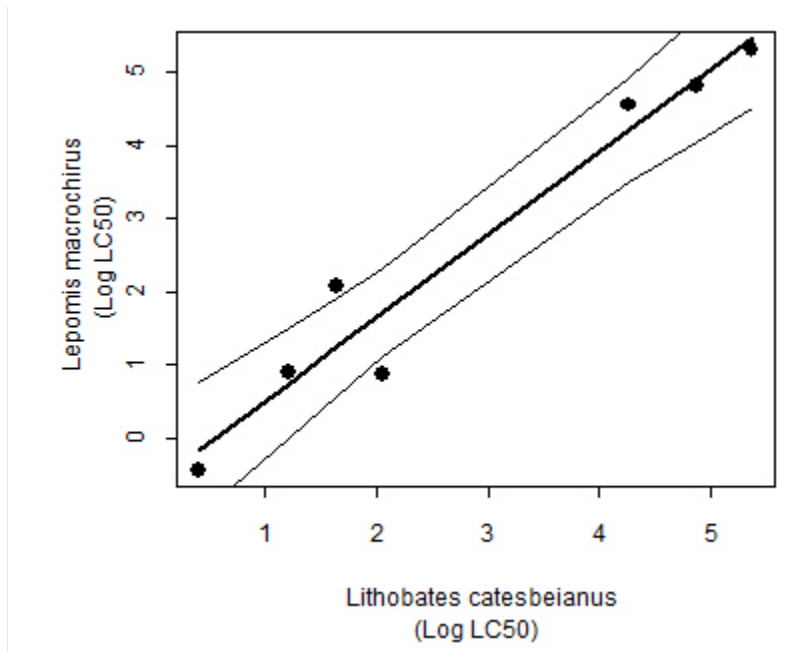
Figure L-30. *Lithobates catesbeianus* **(X-axis) and** *Lepomis macrochirus* **(Y-axis) regression model used for ICE predicted values.**
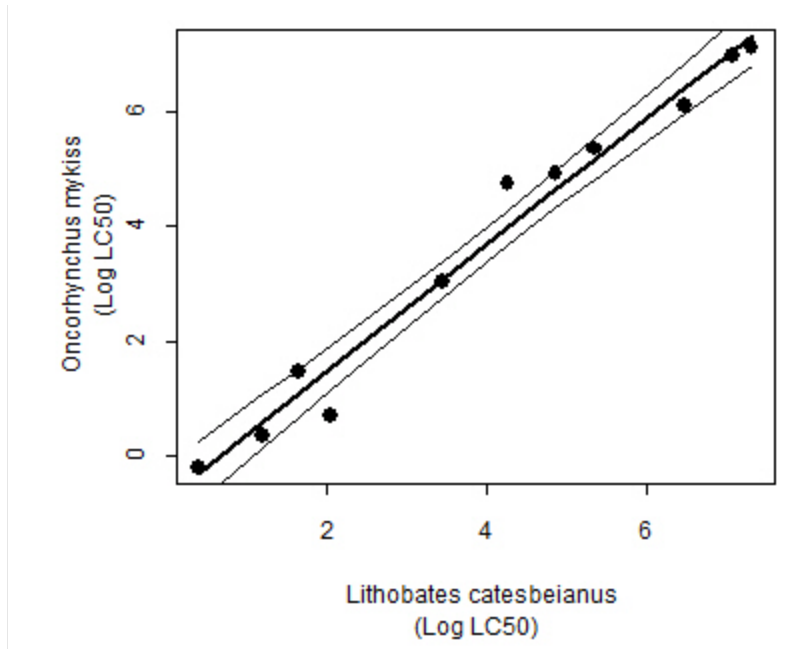
Figure L-31. *Lithobates catesbeianus* **(X-axis) and** *Oncorhynchus mykiss* **(Y-axis) regression model used for ICE predicted values.**
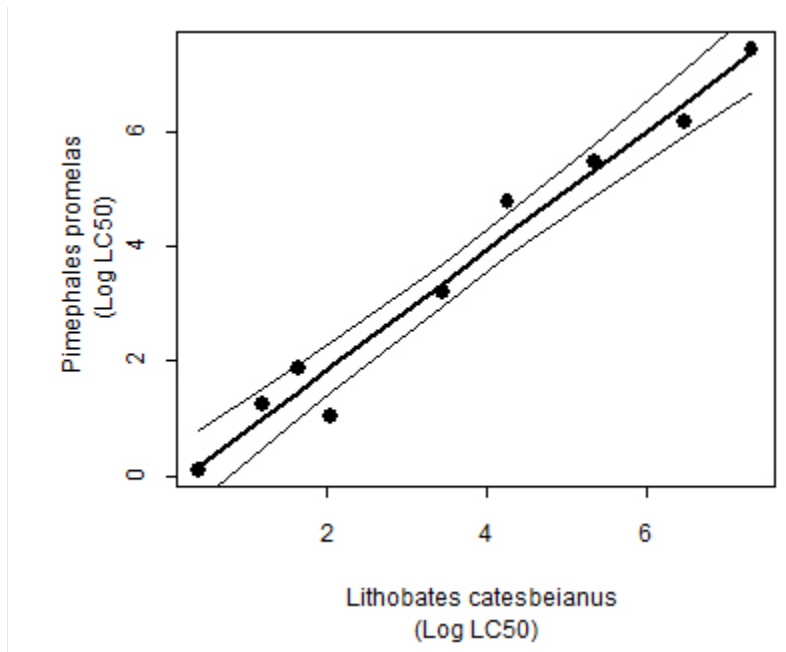
Figure L-32. *Lithobates catesbeianus* **(X-axis) and** *Pimephales promelas* **(Y-axis) regression model used for ICE predicted values.**
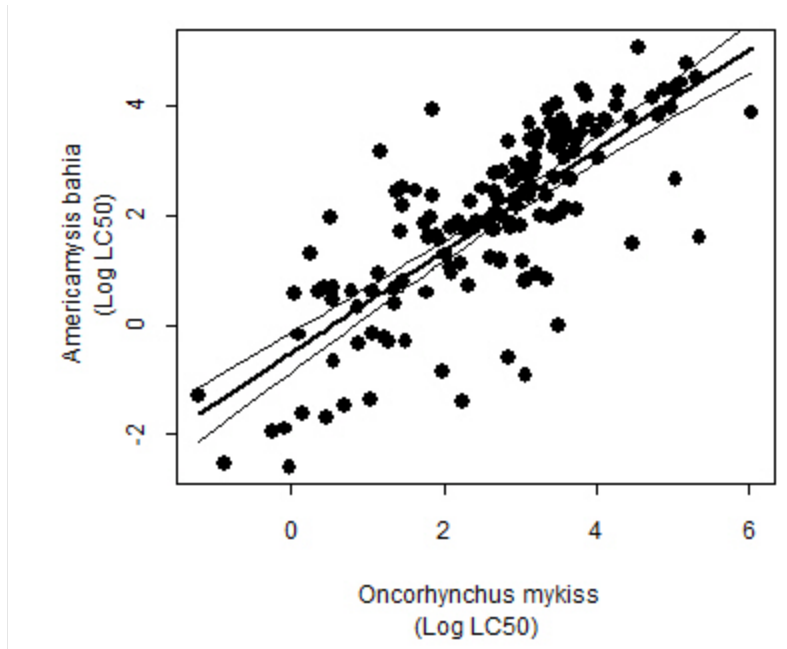
Figure L-33. *Oncorhynchus mykiss* (**X-axis**) **and** *Americamysis bahia* (**Y-axis**) **regression model used for ICE predicted values.**
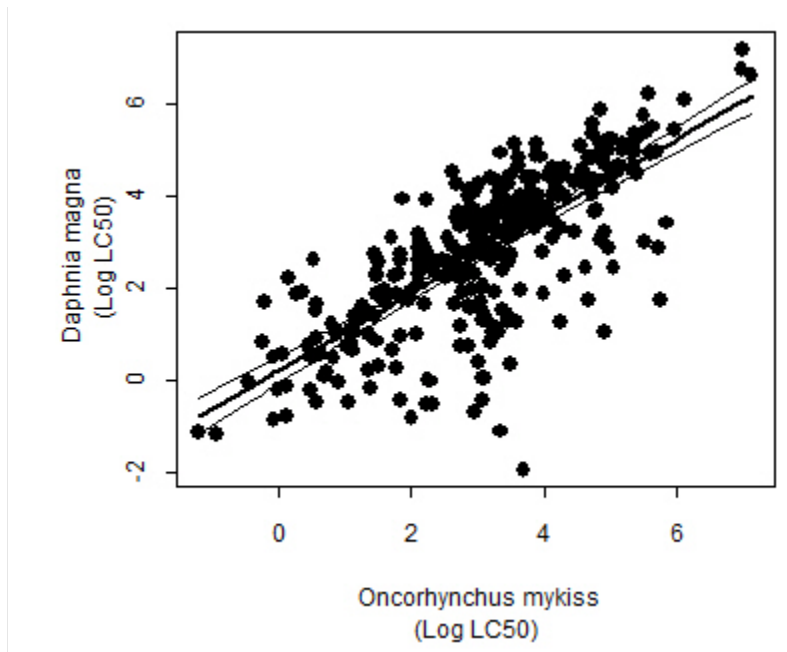
Figure L-34. *Oncorhynchus mykiss* (**X-axis**) **and** *Daphnia magna* (**Y-axis**) **regression model used for ICE predicted values.**
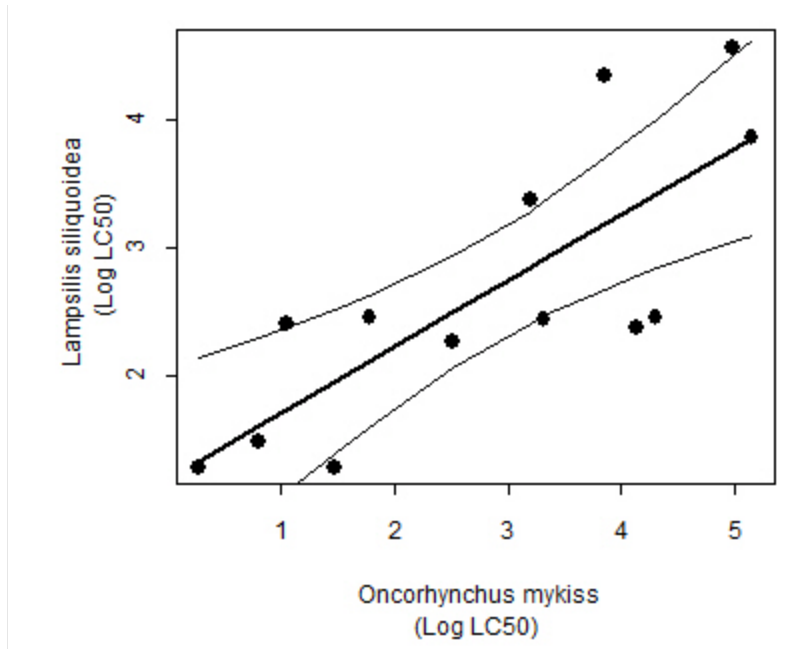
Figure L-35. *Oncorhynchus mykiss* (**X-axis**) **and** *Lampsilis siliquoidea* (**Y-axis**) **regression model used for ICE predicted values.**
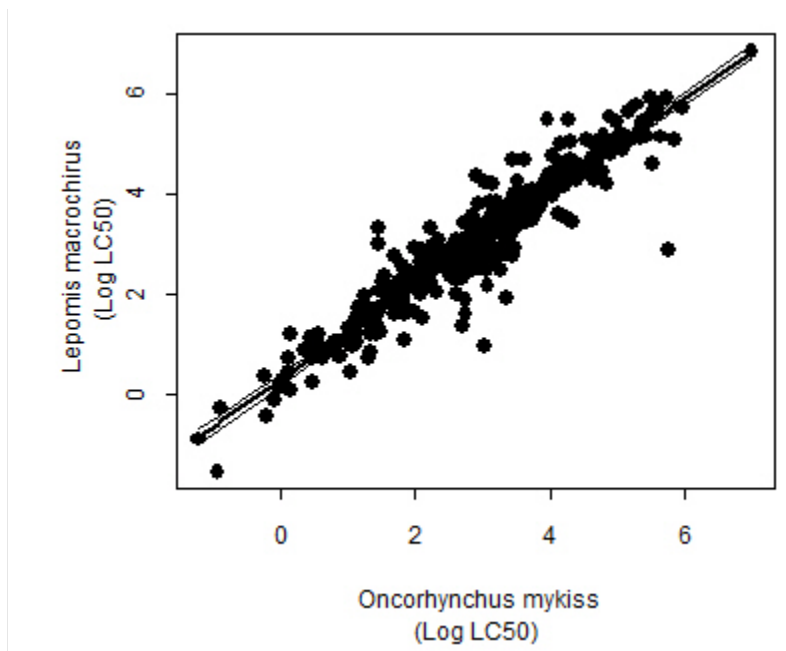
Figure L-36. *Oncorhynchus mykiss* (**X-axis**) **and** *Lepomis macrochirus* (**Y-axis**) **regression model used for ICE predicted values.**
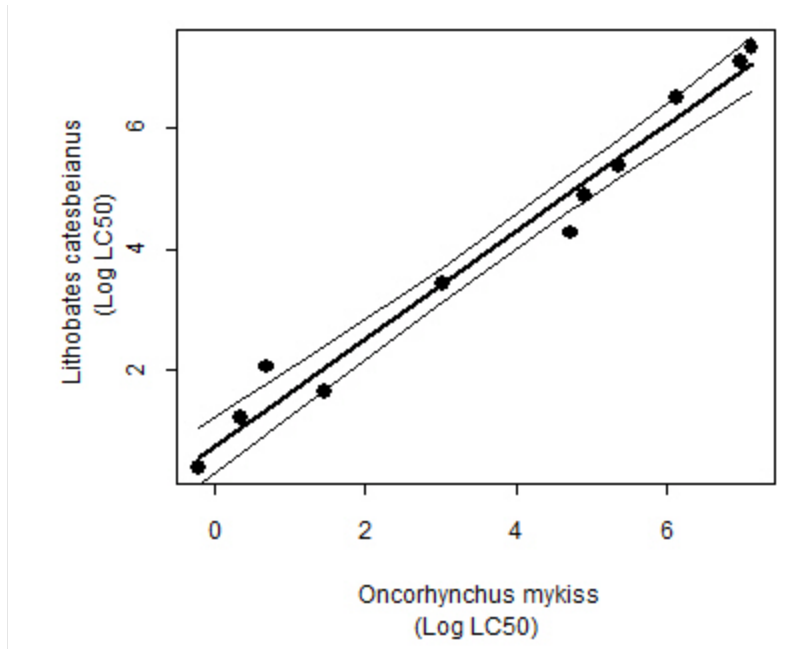
Figure L-37. *Oncorhynchus mykiss* **(X-axis) and** *Lithobates catesbeianus* **(Y-axis) regression model used for ICE predicted values.**
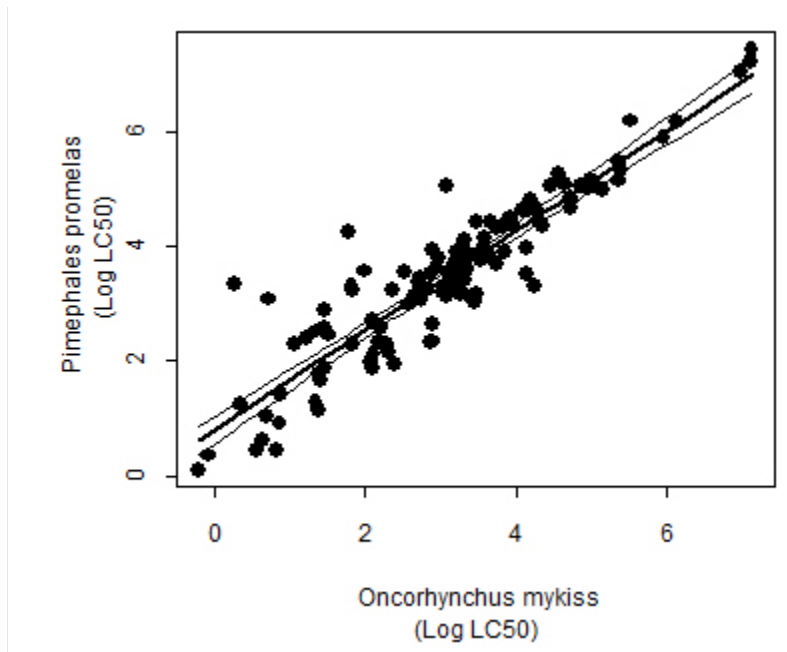
Figure L-38. *Oncorhynchus mykiss* **(X-axis) and** *Pimephales promelas* **(Y-axis) regression model used for ICE predicted values.**
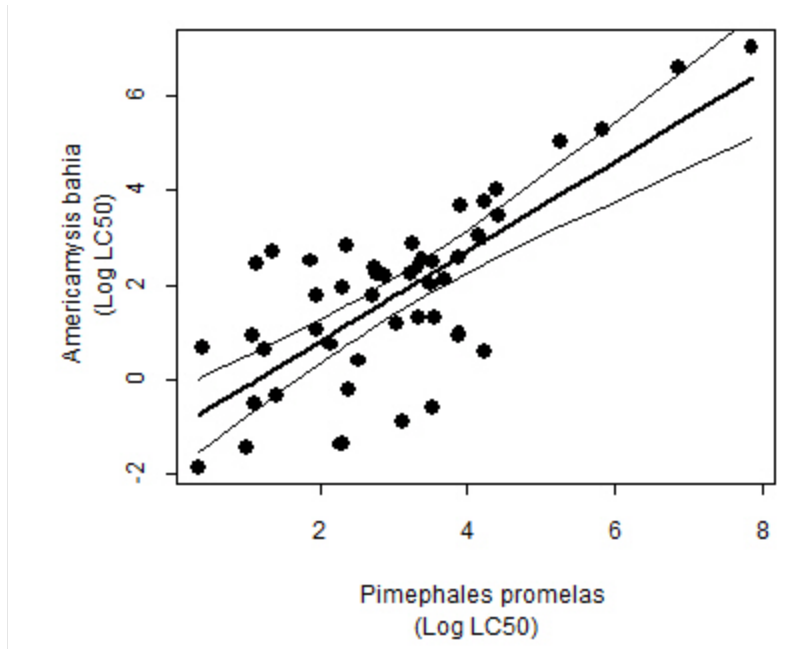
Figure L-39. *Pimephales promelas* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.
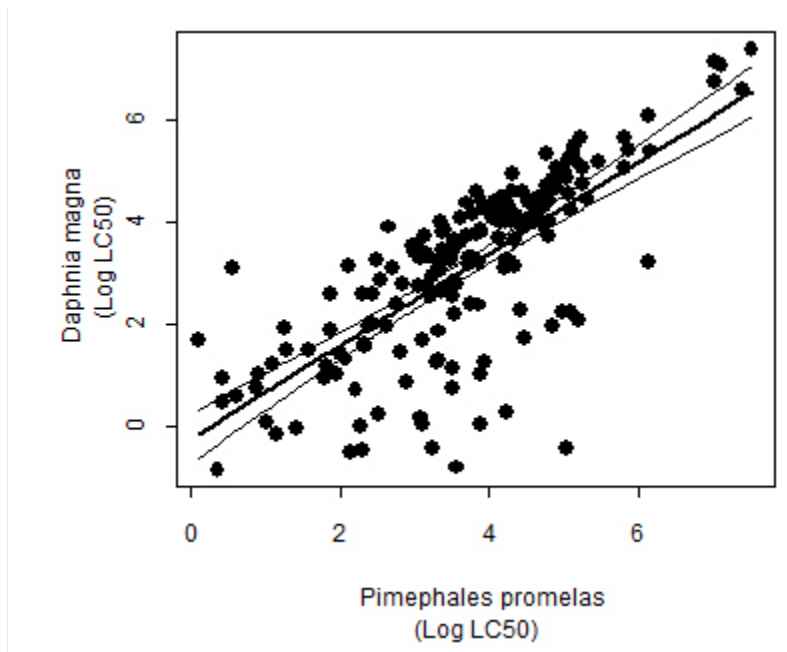
Figure L-40. *Pimephales promelas* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.
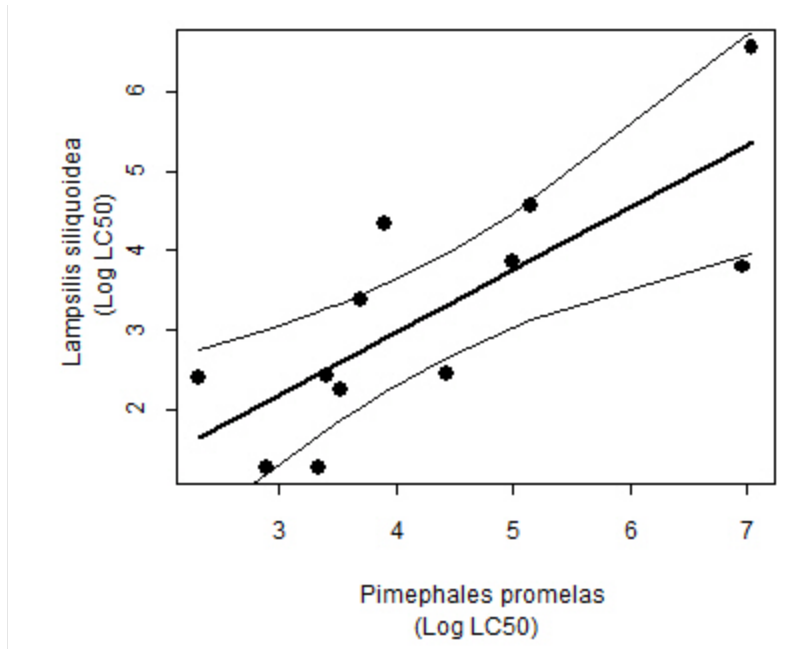
Figure L-41. *Pimephales promelas* **(X-axis) and** *Lampsilis siliquoidea* **(Y-axis) regression model used for ICE predicted values.**
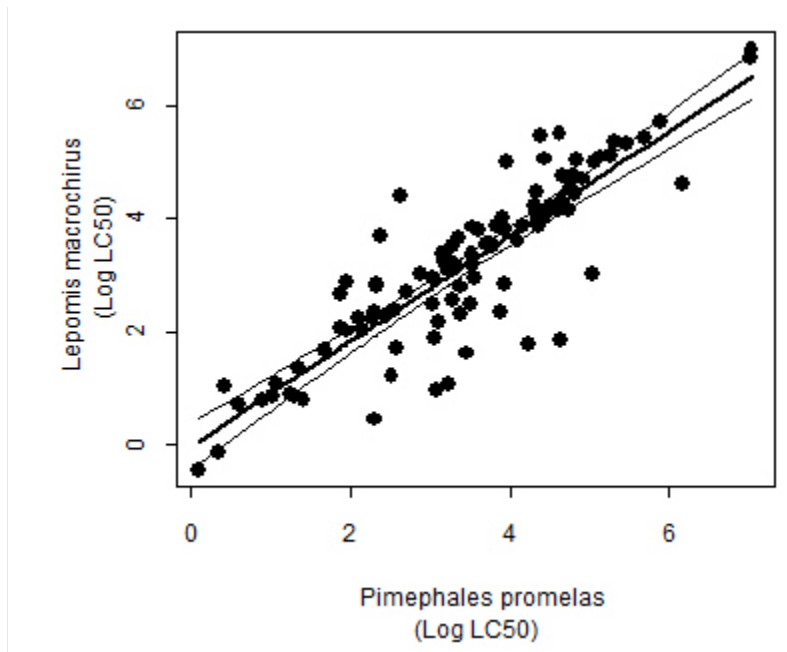
Figure L-42. *Pimephales promelas* **(X-axis) and** *Lepomis macrochirus* **(Y-axis) regression model used for ICE predicted values.**
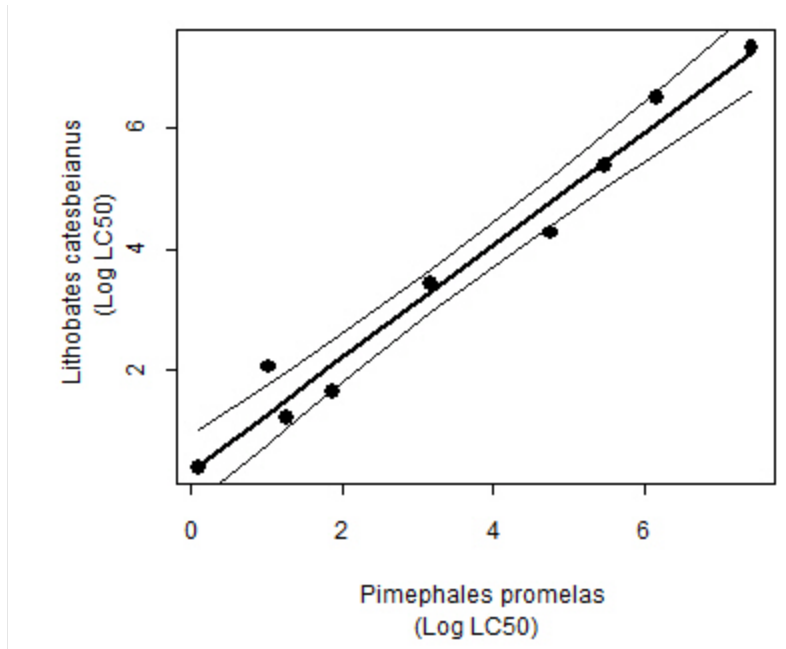
Figure L-43. *Pimephales promelas* **(X-axis) and** *Lithobates catesbeianus* **(Y-axis) regression model used for ICE predicted values.**
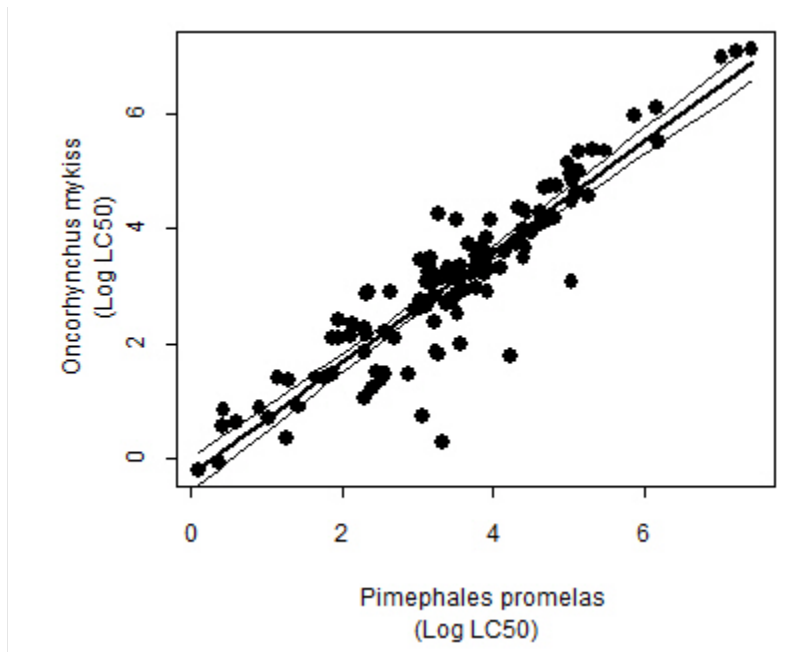
Figure L-44. *Pimephales promelas* **(X-axis) and** *Oncorhynchus mykiss* **(Y-axis) regression model used for ICE predicted values.**
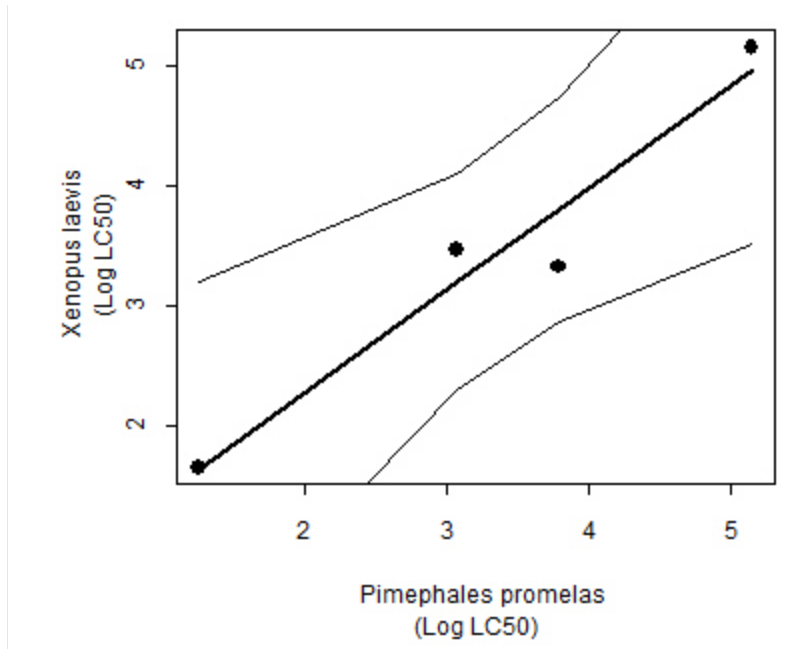
Figure L-45. *Pimephales promelas* **(X-axis) and** *Xenopus laevis* **(Y-axis) regression model used for ICE predicted values.**
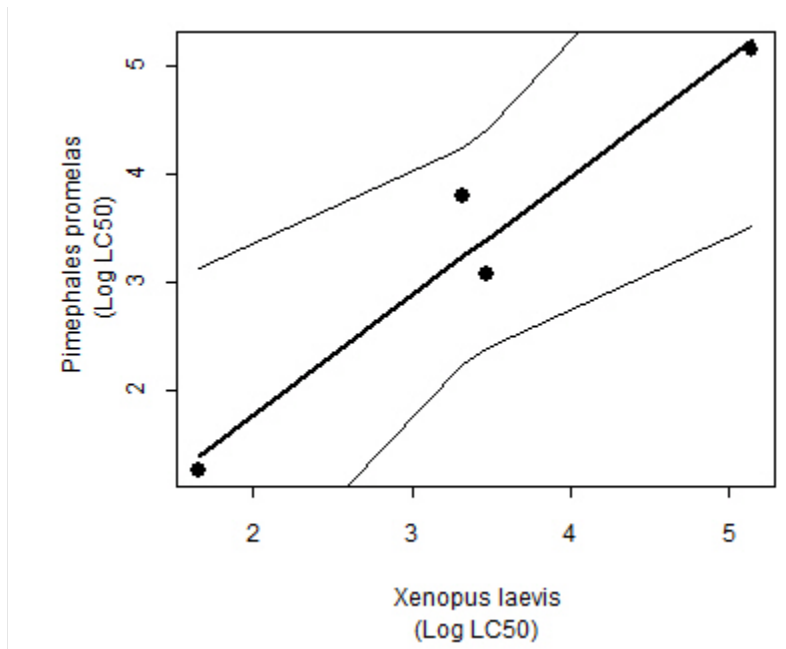
Figure L-46. *Xenopus laevis* **(X-axis) and** *Pimephales promelas* **(Y-axis) regression model used for ICE predicted values.**

Appendix M   Occurrence of PFOA in Abiotic Media

M.1   PFOA Occurrence in Surface Waters

Table M-1. Measured Perfluorooctanoic acid (PFOA) Concentrations in Surface Waters Across the United States.

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
Lake Erie		18.33	15	13-27	Sinclair and Kannan 2006
		35.75	33.5	30-46	Boulanger et al. 2004
		5.460	5.852	3.367-7.16	De Silva et al. 2011
		1.9	1.9	1.6-2.2	Furdui et al. 2008
Lake Huron		3.222	3.475	0.656-4.72	De Silva et al. 2011
		0.592	0.433	0.1-1.1	Furdui et al. 2008
Lake Michigan		1.840	1.840	0.28-3.4	Simcik and Dorweiler 2005
		4.100	3.788	3.579-5.243	De Silva et al. 2011
Lake Ontario		not provided	21	18-34	Sinclair and Kannan 2006
		44.75	48.5	27-55	Boulanger et al. 2004
		4.465	4.150	3.226-6.710	De Silva et al. 2011
		3.773	2.900	1.8-6.7	Furdui 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 in Decatur	7.5<x<25	7.5<x<25	<7.5 – (7.5<x<25)	3M Company 2001
	Pond in Decatur	60	60	60	
	Waterbody in Mobile	55.5	57.0	26.5-83	3M Company 2001
	Pond in Mobile	21.63	21.63	21.63	
	Tennessee River (upstream of Baker's Creek)	<25	<25	<25	Hansen et al. 2002
	Tennessee River (downstream of Baker's Creek)	335.2	355.0	<25-598	
California	Upper Silver Creek	not provided	not provided	10-36	Plumlee et al. 2008
	Coyote Creek	not provided	not provided	<4-13	
Colorado	Animas River	<0.76	<0.76	<0.76	Colorado Department of Public Health and the Environment 2020
	Arkansas River	1.58	0.69	0.36-3.90	
	Arvada Blunn Reservoir	0.80	0.80	0.80	
	Barker Reservoir	<0.78	<0.78	<0.78	
	Bessemer Ditch	2.60	2.60	2.60	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	Big Thompson River	2.90	2.90	2.90	
	Blue River	1.40	1.40	1.40	
	Boulder Feeder Canal	<0.71	<0.71	<0.71	
	Boyd Lake	1.50	1.50	1.50	
	Cache la Poudre River	6.68	6.68	<0.72-13.0	
	Clear Creek	3.05	3.05	3.00-3.10	
	Colorado River	0.77	0.93	<0.77-1.00	
	Coon Creek	<0.76	<0.76	<0.76	
	Eagle River	1.40	1.40	1.40	
	East Plum Creek	<0.67	<0.67	<0.67	
	Erie Lake	0.81	0.81	0.81	
	Fairmount Reservoir	<0.81	<0.81	<0.81	
	Fountain Creek	11.3	13.0	4.30-15.0	
	Fraser River	1.10	1.10	1.10	
	Gore Creek	2.00	2.00	2.00	
	Gunnison River	<0.78	<0.78	<0.78	
	Horsetooth Reservoir	<0.71	<0.71	<0.71	
	Jackson Creek	<0.70	<0.70	<0.70	
	Jerry Creek	<0.77	<0.77	<0.76-<0.78	
	Kannah Creek Flowline	<0.78	<0.78	<0.78	
	Lakewood Reservoir	<0.71	<0.71	<0.71	
	Little Fountain Creek	<0.73	<0.73	<0.73	
	Maple Grove Reservoir	8.50	8.50	8.50	
	Marstron Reservoir	<0.75	<0.75	<0.75	
	McBroom Ditch	3.10	3.10	3.10	
	Mclellen Reservoir	2.20	2.20	2.20	
	Mesa Creek	<0.77	<0.77	<0.77	
	Michigan River	<0.72	<0.72	<0.72	
	Molina Power Plant Tail	<0.78	<0.78	<0.78	
	North Fork Gunnison River	<0.75	<0.75	<0.75	
	Purdy Mesa Flowline	<0.77	<0.77	<0.77	
	Purgatoire River	<0.73	<0.73	<0.73	
	Ralston Reservoir	<0.72	<0.72	<0.72	
	Rio Grande	<0.75	<0.75	<0.75	
	Roaring Fork River	0.88	0.88	0.88	
	San Juan River	<0.69	<0.69	<0.69	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	Sand Creek	14.25	14.25	5.50-23.0	
	Severy Creek	<0.74	<0.74	<0.74	
	Somerville Flowline	<0.75	<0.75	<0.75	
	South Boulder Creek	0.74	0.74	0.74	
	South Platte River	9.68	11.0	4.60-14.0	
	St. Vrain River	5.40	5.40	5.40	
	Strontia Springs	<0.80	<0.80	<0.80	
	Taylor River	<0.70	<0.70	<0.70	
	Uncompahgre River (delta)	0.73	0.73	0.73	
	Welton Reservoir	1.20	1.20	1.20	
	White River	<0.73	<0.73	<0.73	
	Yampa River	<0.74	<0.74	<0.74	
DE, NJ, PA	Delaware River	5.95	5.24	2.12-14.9	Pan et al. 2018
Florida	Waterbody in Pensacola	<7.5	<7.5	<7.5	3M Company 2001
	Pond in Pensacola	<7.5	<7.5	<7.5	
	Waterbody in Port St. Lucie	7.5<x<25	7.5<x<25	7.5<x<25	
	Small pond in Port St. Lucie	422.8	422.8	97-748.5	
	Sarasota Bay	3.6	not provided	not provided	Houde et al. 2006b
Georgia	Waterbody in Columbus	22.92	26.00	<25-26.5	3M Company 2001
	Pond in Columbus	<7.5	<7.5	<7.5	
	Conasauga River	478.9	366.5	32.4-1,150	Konwick et al. 2008
	Altamana River	3.067	3.1	3-3.1	
	Streams and ponds in Dalton	171.7	169.5	51.80-296.0	
	Oostanaula River	115.7	113	100-134	Laiser et al. 2011
	Coosa River	104	104	104	
Louisiana	Waterbodies (locations of concern) near Barksdale A.F.B.	62.67	30.00	<10-370	Cochran 2015
	Reference waterbodies near Barksdale A.F.B.	<10	<10	<10	
Michigan	Raisin River	14.7	14.7	14.7	Kannan et al. 2005
	St Clair River	4.467	4.4	4-5	
	Siskiwit Lake	0.576	0.576	0.558-0.594	Scott et al. 2010
Minnesota	Upper Mississippi River	119.4	2.80	<2-3,600	Newsted et al. 2017; Oliaei et al. 2013
	Lake of the Isles	0.46	0.46	0.46	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	Lake Calhoun	19.44	19.44	19.44	Simcik and Dorweiler 2005
	Lake Harriet	3.38	3.38	3.38	
	Minnesota River	1.2	1.2	1.2	
	Lake Tettegouche	0.47	0.47	0.47	
	Lake Nipisiquit	0.14	0.14	0.14	
	Lake Loiten	0.7	0.7	0.7	
	Little Trout Lake	0.31	0.31	0.31	
New Jersey	Echo Lake Reservoir	4.9	4.9	4.9	NJ DEP 2019
	Passaic River	13.55	13.55	13-14.1	
	Raritan River	8.7	8.7	8.7	
	Metedeconk River	31.1	31.1	28.3-33.9	
	Pine Lake	13.6	13.6	13.6	
	Horicon Lake	1.9	1.9	1.9	
	Little Pine Lake	25.9	25.9	25.9	
	Mirror Lake	13.2	13.2	13.2	
	Woodbury Creek	7.2	7.2	7.2	
	Fenwick Creek	10.5	10.5	10.5	
	Cohansey River	4.6	4.6	4.3-4.9	
	Harbortown Road	3.738	3.738	3.738	Zhang et al. 2016
	Passaic River	18.65	13.24	0.871-47.25	
New Mexico	Alamogordo Domestic Water System	<1	<1	<1	New Mexico Environment Department 2020, 2021
	Animas River	<0.97	<0.95	<0.89-<1.1	
	Canadian River	<0.935	<0.935	<0.89-<1.1	
	Cloud Country Estates WUA	<0.93	<0.93	<0.93	
	Gila River	<0.93	<0.93	<0.93	
	Holloman AFB Golf Course Pond 1	97	97	97	
	Holloman AFB Golf Course Pond 2	117	117	117	
	Holloman AFB Lagoon G	2,450	2,450	2,450	
	Holloman AFB Outfall	74.6	74.6	74.6	
	Holloman AFB Sewage Lagoon	941	941	941	
	Karr Canyon Estates	<0.93	<0.93	<0.93	
	La Luz MDWCA	<1.3	<1.3	<1.3	
	Lake Holloman	1,297	1,300	990-1,600	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	Mountain Orchard MDWCA	<0.89	<0.89	<0.89	
	Pecos River	0.628	<0.96	<0.94-0.936	
	Rio Chama	<0.98	<0.98	<0.96-<1.0	
	Rio Grande	0.791	0.474	<0.86-1.95	
	Rio Puerco	<1.3	<1.3	<1.3	
	San Juan River	<1.06	<0.96	<0.89-<1.9	
	Tularosa Water System	<0.89	<0.89	<0.89	
New York	Washington Park Lake	10.1	10.4	4.83-15.8	Kim and Kannan 2007
	Rensselaer Lake	6.79	7.2	3.27-10.6	
	Iroquois Lake	7.365	not provided	not provided	
	Unnamed lake 1 outside Albany, NY	2.246	not provided	not provided	
	Unnamed lake 2 outside Albany, NY	4.341	not provided	not provided	
	Niagara River	19.67	19	18-22	Sinclair and Kannan 2006
	Finger Lakes	not provided	14	11-20	
	Lake Onondaga	50.67	49.00	39-64	
	Lake Oneida	19	19	19	
	Erie Canal	38.0	30.0	25-59	
	Hudson River	not provided	35	22-173	
	Lake Champlain	not provided	24	10-46	
	Lower NY Harbor	2.02	2.02	2.02	Zhang et al. 2016
	Staten Island	4.049	4.049	4.049	
	Hudson River	7.333	7.333	2.805-11.86	
North Carolina	Cape Fear River	43.4	12.6	<0.2-287	Nakayama et al. 2007
Rhode Island	Narragansett Bay	1.2	1.2	1.2	Benskin et al. 2012
	Allen Cove Inflow	3.784	3.784	3.784	Zhang et al. 2016
	Bristol Harbor	1.168	1.170	1.014-1.320	
	Brook at Mill Cove	36.81	36.81	36.81	
	Buckeye Brook	8.455	8.455	8.455	
	Chickasheen Brook	1.006	1.006	1.006	
	EG Town Dock	1.972	1.972	1.972	
	Fall River	0.64	0.64	0.64	
	Green Falls River	0.6470	0.6470	0.5860-0.7080	
	Hunt River	6.978	6.978	6.978	
	Mill Brook	9.237	9.237	9.237	
	Narrow River	0.9850	0.8985	0.6630-1.480	
	Pawcatuck River	16.98	16.98	14.99-18.97	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	Pawtuxet River	7.546	7.546	7.546	
	Queens River	0.898	0.898	0.898	
	Sand Hill Brook	6.905	6.905	6.905	
	Secret Lake – Oak Hill Brook	0.849	0.849	0.849	
	Slack's Tributary	2.363	2.363	2.363	
	South Ferry Road Pier	0.267	0.267	0.267	
	Southern Creek	10.08	10.08	10.08	
	Woonasquatucket River	7.034	7.034	5.236-8.832	
South Carolina	Charleston Harbor	9.5	not provided	not provided	Houde et al. 2006b
Tennessee	Waterbody near Cleveland	7.5<x<25	7.5<x<25	7.5<x<25	3M Company 2001
	Conasauga River	<0.007[1]	<0.007[1]	<0.007[1]	Laiser et al. 2011
Texas	Rio Grande	3.4	4.3	1.2-4.7	NM Environment Department 2020, 2021
Washington	Puget Sound	2.297	1.680	0.160-8.220	Dinglasan-Panlilio et al. 2014
	Clayoquot Sound	1.180	1.010	0.600-1.770	
	Barkley Sound	1.4	1.4	1.4	
Multiple States (10 Air Force Bases across the continental U.S.)	Surface waters impacted by aqueous film forming foam use	not provided	382	210,000 (maximum)	Anderson et al. 2016

[1] Reported as ng/g by study authors.

M.1.1 PFOA Occurrence and Concentrations in the Great Lakes Region

The Great Lakes are among the most widely studied waterbodies in the U.S. for PFOA occurrence; however, data remain relatively limited in this system. Comparisons across the Great Lakes system indicate PFOA concentrations are higher in Lakes Erie and Ontario than in Lakes Huron, Michigan, and Superior (Figure M-1). For example, mean PFOA concentrations in Lake Erie and Lake Ontario from several studies ranged from 1.9 to 35.75 ng/L and 3.7 to 44.7 ng/L, respectively (Boulanger et al. 2004; Furdui et al. 2008), while the range of reported mean PFOA concentrations in Lake Superior, Lake Huron, and Lake Michigan were comparatively lower. The mean PFOA concentration range was 1.8 to 4.1 ng/L in Lake Michigan and 0.59 to 3.2 ng/L

in Lake Huron (Furdui et al. 2008; De Silva et al. 2011; Simcik et al. 2005). The most western and upstream lake within the great lakes system, Lake Superior, consistently had lower PFOA concentrations than the other Great Lakes, with mean concentrations reported in the literature ranging from 0.23 to 0.25 ng/L (Furdui et al. 2008; Scott et al. 2010; De Silva et al. 2011).

The higher PFOA concentrations in Lakes Erie and Ontario are likely due to higher levels of urbanization around these lakes (Boulanger et al. 2004; Remucal 2019). A mass balance constructed for Lake Ontario by Boulanger et al. (2005) indicated that surface water and wastewater effluent are the major sources of PFOA to the lake. In contrast, inputs from Canadian tributaries and atmospheric deposition of PFAS were the major contributing sources of PFOA to Lake Superior. Inputs from Canadian tributaries and atmospheric deposition were estimated to contribute 59% and 35% of PFOA inputs into Lake Superior, respectively (Scott et al. 2010).

Within the Great Lakes, Remucal (2019) noted there were limited PFOA data to evaluate temporal trends in surface waters. If the dataset was limited to Lake Ontario, which is one of the most well-studied waterbodies for PFOA occurrence in the U.S. (with data from 2002 to 2010) there appears to be a mild decrease in PFOA concentrations over time. This decrease was likely due to the reduction in PFOA manufacturing; however, the downward PFOA trend in Lake Ontario was statistically insignificant, with authors noting additional data over longer time scales were needed to fully inform conclusions (reviewed in Remucal 2019).
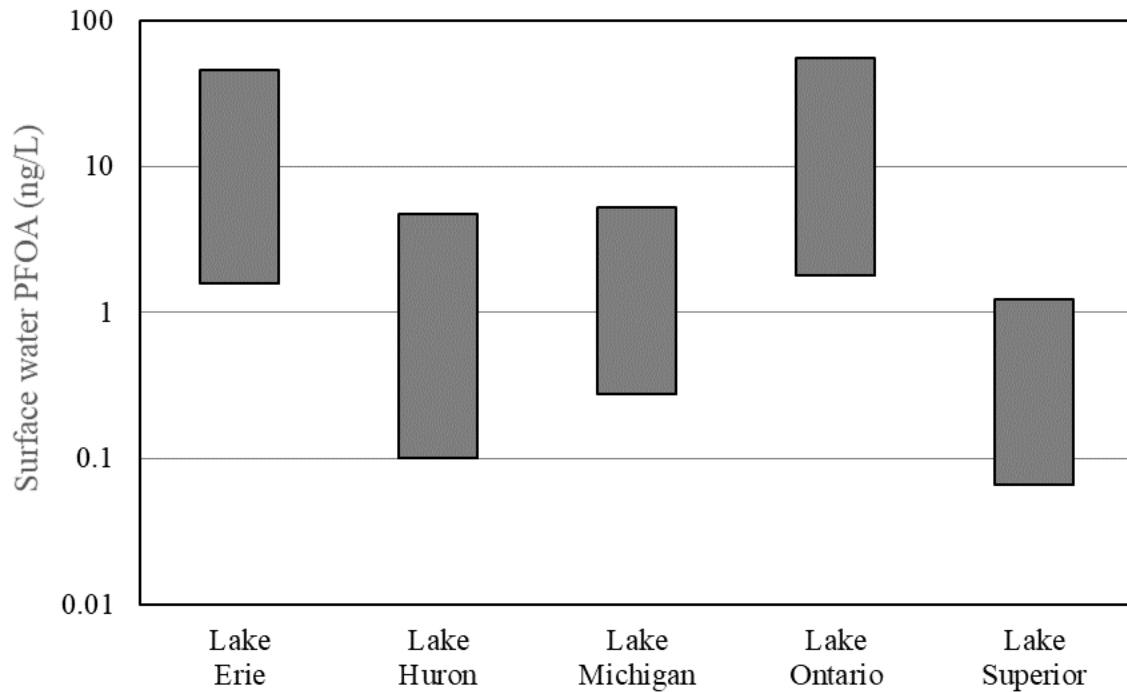
Figure M-1. Distribution of the minimum and maximum concentrations (ng/L) of Perfluorooctanoic acid (PFOA) measured in surface water samples collected from the Great Lakes as reported in the publicly available literature.
This distribution is arranged alphabetically by waterbody.

M.1.2 PFOA Occurrence and Concentrations in the Midwestern U.S.

Similar PFOA concentrations are reported in the publicly available literature for

waterbodies in urban areas across the Midwest and northeastern U.S. along with lower PFOA

concentrations associated with remote areas in the same states (Newsted et al. 2017; NJ DEP

2019; Simcik and Dorweiler 2005; Sinclair et al. 2006). In Minnesota, Simcik and Dorweiler

(2005) observed PFOA concentrations ranging between 0.46 and 19 ng/L in urban areas near

Minneapolis and concentrations ranging between 0.14 to 0.7 ng/L in remote areas in northern

Minnesota.

M.1.3 PFOA Occurrence and Concentrations in the Northeastern U.S.

In New York, Sinclair et al. (2006) quantified (limit of quantification = 2 ng/L) PFOA in

all waters sampled across the state. Unlike PFOS, PFOA was detected at relatively elevated

concentrations across all sites with comparatively little variability (median PFOA concentrations across nine sites ranged from 14 to 49 ng/L) (Sinclair et al. 2006). Additionally, the New Jersey Department of Environmental Protection (NJ DEP) measured PFOA in surface water samples collected from 14 different sites across the state. PFOA concentrations ranged from 1.9 ng/L to a high of 33.9 ng/L, which was quantified in the Metedeconk River downstream of a wastewater treatment plant. NJ DEP (2019) also indicated the Metedeconk River is impacted by PFOA-contaminated groundwater originating from an industrial source. Zhang et al. (2016) reported the surface water median PFOA concentration was 4.05 ng/L (n = 9; Zhang et al. 2016) in 2014 in the New York City Metropolitan area, including sites in New Jersey.

M.1.4   <u>PFOA Occurrence and Concentrations in the Southeastern U.S.</u>

Measured PFOA concentrations in surface waters among southeastern states of the U.S. are highly variable with some of the highest observed concentrations occurring in specific waterbodies near areas with PFOA manufacturing. In 2001 the 3M Company conducted a multi-city study measuring PFOA concentrations across waterbodies with known manufacturing and/or industrial uses of PFOA. In the 3M Company's 2001 report, PFOA concentrations from sites with known PFOA manufacturing uses were compared to PFOA concentrations in waterbodies with no known sources of any PFAS (3M Company 2001). In this comparison study, cities with PFOA manufacturing uses included Mobile and Decatur, Alabama, Columbus, Georgia, and Pensacola, Florida. Measured PFOA concentrations in surface waters, including lentic systems, ranged from not detected (with a detection limit of 7.5 ng/L stated in the report; 3M Company 2001) to 83 ng/L in the cities with known PFOA use in manufacturing processes. These PFOA concentrations were compared to those measured in control cities with no known PFOA applications in manufacturing. These control cities were Cleveland, Tennessee and Port St. Lucie, Florida. PFOA concentrations ranged from not detected to not quantified (limit of

quantification = 25 ng/L; 3M Company 2001) in flowing surface waters. PFOA concentrations, however, ranged from 97 ng/L to 748.5 ng/L in lentic systems (i.e., ponds, lakes, and reservoirs; 3M Company 2001) in St. Lucie, Florida. Lentic water samples were not collected at the other city described as a "control," Cleveland, Tennessee. At the time of the report from the 3M Company, the source of PFOA in lentic waters near Port St. Lucie, Florida was unknown; however, the report noted the presence of visible litter, a greenish film on the water, and contributions from a culvert creating a grayish plume as it entered the waterbody (3M Company 2001). Aside from the samples collected in Port St. Lucie, Florida, this report demonstrated that measured PFOA concentrations in surface waters tend to be higher in areas with PFOA manufacturing and/or industrial use (3M Company 2001).

In separate studies, PFOS and PFOA concentrations were measured in surface waters by Hansen et al. (2002) near Decatur, Alabama and Konwick et al. (2008) in Georgia. Hansen et al. (2002) studied a stretch of the Tennessee river near Decatur, Alabama and Konwick et al. (2008) focused on the Conasauga River in Georgia as areas with known PFOA exposure and use. Hansen et al. (2002) reported discharge from a fluorochemical manufacturing facility entered the Tennessee River towards the middle of the sampling area of the study, allowing for a comparison of PFOA concentrations in relation to the fluorochemical manufacturing facility. In contrast, Konwick et al. (2008) compared the PFOA concentrations measured in the Conasauga River with those from reference sites (i.e., not impacted) along the Altamaha River. In both studies, mean PFOA concentrations were higher in the study areas near manufacturing sources of organic fluorochemicals. Specifically, Hansen et al. (2002) did not detect PFOA above the limit of quantification (i.e., 25 ng/L) upstream of the fluorochemical manufacturing facility. Downstream of the facility, PFOA concentrations ranged from below the limit of quantification at two sites

immediately downstream of the facility to 598 ng/L with a mean concentration of 335.2 ng/L. Similarly, Konwick et al. (2008) observed higher measured PFOA concentrations in the Conasauga River, which ranged from 32.4 to 1,150 ng/L, compared to those in the Altamaha River, ranging between 3.0 and 3.1 ng/L. Consistent with the report from the 3M Company summarized above, the effluent from manufacturing facilities were determined to be the source of increased PFOA concentrations in both the Tennessee and Conasauga rivers (Hansen et al. 2002; Konwick et al. 2008). PFOA concentrations in contaminated areas of the Conasauga River and Altamaha River were relatively consistent with those measured in Alabama and Georgia (3M Company 2001).

Similarly, Nakayama et al. (2007) and Cochran (2015) measured PFAS, including PFOA, 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) reported PFOA exceeded the level of quantification (i.e., 1 ng/L) in 82.3% of samples. PFOA concentrations in the Cape Fear Drainage Basin ranged between < 1 (the lower limit of quantification) and 287 ng/L with a mean concentration of 43.4 ng/L. Cochran (2015) detected PFOA in 64% of all water samples collected with an average concentration of 62.67 ng/L. As in other studies summarized above, lower PFAS concentrations were found in the smallest upland tributaries and highest in the middle reaches of the Cape Fear River. WWTP effluents were identified as the source of PFAS in the study area (Nakayama et al. 2007).

M.1.5   PFOA Occurrence and Concentrations in the Western U.S.

PFOA concentrations in urbanized areas of western U.S. states were consistent with concentrations measured in northeastern states (Sinclair et al. 2006; Zhang et al. 2016) but remained lower than contaminated areas of southeastern states (3M Company 2001). Plumlee et

al. (2008) measured PFOA in surface water samples collected from Coyote Creek and a tributary

of Upper Silver Creek in San Jose, California. PFOA concentrations in Coyote Creek ranged

from below the detection limit (4 ng/L) to 13 ng/L and from 10 ng/L to 36 ng/L in Upper Silver

Creek. Plumlee et al. (2008) postulated a combination of atmospheric deposition of volatile

precursors and surface runoff are likely sources of PFOA to both Coyote and Upper Silver

Creeks.

Dinglasan-Panlilio et al. (2014) measured PFOA concentrations along the Puget Sound in

Washington, as well as Clayoquot and Barkley Sounds in British Columbia, Canada. Broadly,

sampling locations spanned these inland marine systems and included freshwaters and

estuarine/marine waters. Overall, PFOA was detected at all sampling locations (PFOA

concentration range = 0.16 ng/L – 8.2 ng/L), but concentrations were lower than those observed

from sites with known manufacturing and/or industrial PFOA uses. These concentrations were

consistent with those reported in the publicly available literature for remote areas, such as the

northern Great Lakes and rural Minnesota (Simcik and Dorweiler 2005). Dinglasan-Panlilio et al.

(2014) speculate these specific regional sources as well as atmospheric deposition are likely

contributors of PFOA to these remote areas (Dinglasan-Panlilio et al. 2014).

M.1.6   <u>Summary of PFOA Occurrence and Concentrations across the U.S.</u>
Despite the wide use and persistence of PFOA in aquatic ecosystems, current information

on the environmental distribution of PFOA in surface waters across the U.S. remains limited.

Present data are largely focused from freshwater systems collected along the east coast,

southeast, and upper Midwest, focusing primarily on study areas with known manufacturing or

industrial uses of PFAS. Current data indicate that PFOA concentrations measured in U.S.

surface waters vary widely across five orders of magnitude (Figure M-2). Additional data,

particularly in saltwater systems, are needed to better understand PFOA occurrence in aquatic

ecosystems.
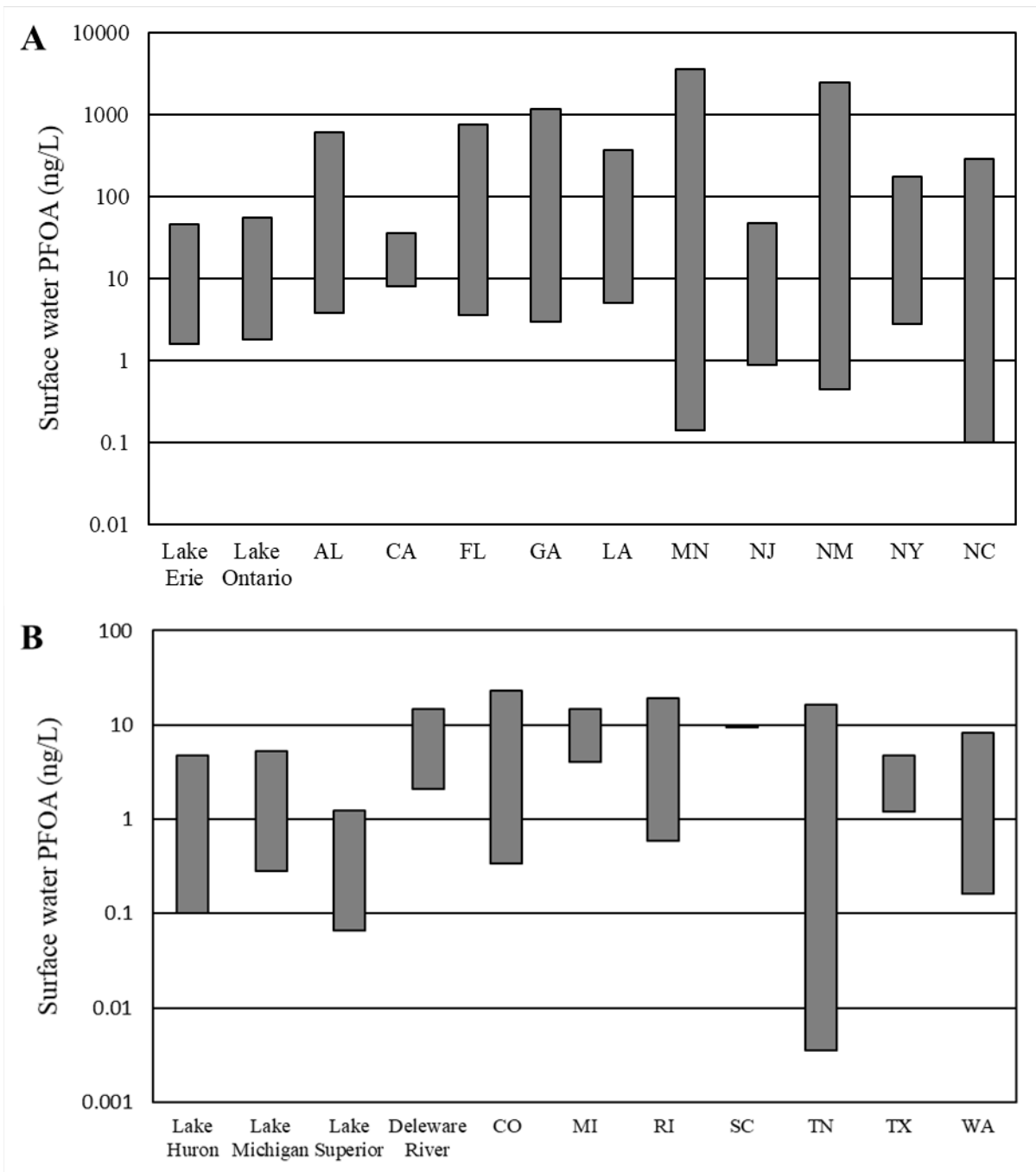
Figure M-2. Comparison of relatively high (A; greater than 30 ng/L) and low (B; less than 30 ng/L) maximum Perfluorooctanoic acid (PFOA) concentrations (ng/L) measured in surface water samples collected across the United States (U.S.) as reported in the publicly available literature.

M.1.7   Comparison to Global Occurrence of PFOA in Surface Waters

Similar to PFAS occurrence in surface waters in the U.S., generally PFOS and PFOA were the dominant PFAS detected in surface waters around the world (Ahrens 2011). On a global scale, concentrations of PFOA measured in surface waters generally range between pg/L and ng/L with some concentrations in the µg/L range. PFOA concentrations measured in the U.S. appear to be similar to those detected in studies with sampling sites in other countries. Global surface water PFOA concentrations reported in the public literature range between not detected and 11,300 ng/L near a PFAS spill site (as summarized below), and Zareitalabad et al. (2013) reported a median PFOA concentration in surface water of 24 ng/L across Canada, Europe, and Asia.

In Canada elevated concentrations of PFOA in surface waters were generally distributed among urbanized areas, suggesting that urban and industrial areas with high population densities contributed to the elevated concentrations of PFOA in surface waters (Gewurtz et al. 2013; Scott et al. 2009). In a systematic, cross-Canada study of PFAS in surface waters, Scott et al. (2009) observed that PFOS and PFOA were the predominant PFAS detected and that generally PFOS concentrations were higher overall, ranging between < 0.02 and 34.6 ng/L, than PFOA concentrations, which ranged between 0.044 and 9.9 ng/L. From the systems sampled in Canada, Scott et al. (2009) indicated that PFOA concentrations measured in Canadian surface waters were lower than those measured in the U.S., Europe, and Asia. However, elevated PFOA concentrations were observed in Etobicoke Creek, a tributary to Lake Ontario, after an accidental spill of a fire-retardant foam containing perfluorinated surfactants at L.B. Pearson International Airport in Toronto, Ontario in June 2000. The measured concentrations of PFOA ranged between not quantified (with a quantification limit of 9 ng/L) to 11,300 ng/L (Moody et al. 2002).

PFOA concentrations measured in surface waters across Europe are similar to those observed in the U.S. Specifically, in a European Union (EU)-wide study of polar organic persistent pollutants, Loos et al. (2009) detected PFOA in 97% of samples with a median concentration of 3 ng/L in surface waters sampled across a wide range of sampling sites (including contaminated and pristine rivers and streams of various sizes). However, relatively high PFOA concentrations of nearly 200 ng/L were detected in the Po River, Italy. Mean PFOA concentrations observed by Pan et al. (2018) were similar to those reported in Loos et al. (2009) and across the U.S. with mean surface water concentrations from waterbodies across western Europe, specifically the Thames River, Mälaren Lake, and Rhine River, ranging between 2.31 ng/L to 8.51 ng/L, with a maximum concentration of 11.7 ng/L detected in the Thames River. Kwadijk et al. (2010) detected PFOA in all surface water samples collected from 20 locations across the Netherlands, with concentrations ranging from 6.5 to 43 ng/L. Huset et al. (2008) measured similar PFOA concentrations in three rivers in the Glatt Valley Watershed, Switzerland, and reported averages from three rivers ranging from 7.0 to 7.6 ng/L. Like in the U.S. and Canada, elevated concentrations of PFOA in surface waters across Europe are higher in urbanized areas and sources have been attributed to waste water treatment plant effluent, AFFF spills, and fluorochemical manufacturing facilities (Ahrens 2011; Huset et al. 2008; Kwadijk et al. 2010; Loos et al. 2007 and 2009).

PFOA concentrations observed in surface waters across eastern Asia were broadly similar to the U.S., Canada, and Europe. In Japan, Saito et al. (2003) observed PFOA concentrations ranging between 0.1 and 456 ng/L in surface waters samples collected from various locations. Similarly, Nguyen et al. (2011) reported PFOA concentrations ranging between 5 and 31 ng/L collected from an urbanized section of the Marina catchment in Singapore. Pan et al. (2018)

reported PFOA concentrations from 112 samples across eastern Asia ranging from 0.15 ng/L to 52.8 ng/L. These 112 samples were collected from eight different water bodies, including; the Yangtze River (median PFOA = 12.2 ng/L; n = 35), Yellow River (median PFOA = 2.45 ng/L; n = 15), Pearl River (median PFOA = 1.82 ng/L; n = 13), Liao River (median PFOA = 9.39 ng/L; n = 6), Huai River, (median PFOA = 6.01 ng/L; n = 9), Han River (median PFOA = 3.69 ng/L; n = 6), Chao Lake (median PFOA = 8.17 ng/L; n = 13) and Tai Lake (median = 17.95 ng/L; n = 15).

Overall, these studies show the widespread distribution and variability of PFOA concentration in surface waters around the world and that surrounding land use and urbanization with high population densities have a large influence on the overall occurrence of PFOA in surface waters (Ahrens 2011; Gewurtz et al. 2013; Loos et al. 2007; Loos et al. 2009; Scott et al. 2009).

M.2   PFOA Occurrence and Detection in Aquatic Sediments

Although aquatic sediments are not anticipated to be a major PFOA sink (Ahrens 2011; Ahrens et al. 2009), PFOA has been detected in aquatic sediments in relatively remote regions. For example, maximum PFOA concentrations of 1.7 µg/kg dry weight (dw) were detected in lake sediments in the Canadian Artic (Butt et al. 2010). Typically, in the U.S., soil and sediment measurements of PFOA near contaminated sites and manufacturing plants occur in the µg/kg dry weight range. For example, PFOA ranged from below the limit of detection (0.017 µg/kg) to 700 µg/kg in sediments near a fluorochemical manufacturer in West Virginia (Lau et al. 2007).

Across ten U.S. Air Force bases where there is a known historic use of AFFF, Anderson et al. (2016) measured sediment (0-1 foot below top of the sediment) samples between March-September of 2014 at the ten locations with PFOA concentrations detected in 67% of samples.

The median concentration of PFOA across all sites was 2.45 µg/kg, with a maximum concentration of 950 µg/kg (Anderson et al. 2016). Lasier et al. (2011) measured PFOA in sediment from the Coosa River, Georgia watershed, upstream and downstream of a land-application site of municipal/industrial wastewater, at concentrations ranging from 0.06-1.97 µg/kg dry weight. Values reported in various locations across San Francisco Bay ranged from below detection to 0.292 µg/kg dry weight (San Francisco Bay RWQCB 2020, Sedlak et al. 2017). Internationally, values ranged from below detection in areas with relatively low population density to µg/kg wet weight in areas of higher human population density, including <LOQ, Gufunes Bay, Iceland (Butt et al. 2010); <LOQ, Faroe Islands (Butt et al. 2010); 0.96 µg/kg wet weight, Tidal Flats of Ariake Sea, Japan (Nakata et al. 2006); 0.29-0.36 µg/kg dry weight, Tokyo Bay, Japan (Ahrens et al. 2010) and <0.05-0.2 µg/kg wet weight, Toronto, Ontario (Vedagiri et al. 2018).

M.3 PFOA Occurrence and Detection in Groundwater

Similar to surface water, PFOS and PFOA are the dominant PFAS detected in groundwater. Subsurface soil samples in Minnesota indicated PFOA concentrations increase with depth, suggesting the migration of PFOA from the surface, through soils, and into groundwaters (Xiao et al. 2015). Generally, PFOA concentrations tend to range in the ng/L range, with some elevated occurrences in µg/L (Ahrens 2011; Xiao 2017). Previous detections of PFOA in groundwater have been associated with the use of AFFF and fire-training locations (Ahrens 2011; Xiao 2017). For example, a maximum PFOA concentration of 105 µg/L was quantified in Michigan groundwater near a site formerly used for military fire-training operations that had been decommissioned for nearly five years prior to sampling (Moody et al. 2003). Similarly, the Minnesota Pollution Control Agency (MPCA) detected PFOA concentrations as

high as ~ 23 µg/L in ground water near a PFAS disposal site, with concentrations decreasing to <

0.1 µg/L 1.4 km from the PFAS disposal site (Xaio et al. 2015). Despite not having been an

active-fire training area, PFOA was still present on various U.S. Air Force Installations where

there is a known history of use of AFFF to extinguish hydrocarbon-based fires. Anderson et al.

(2016) measured groundwater samples between March and September of 2014 at the ten

locations with PFOA concentrations detected in 90% of samples. The median concentration of

PFOA across all sites was 0.41 µg/L, with a maximum concentration of 250 µg/L (Anderson et

al. 2016). These concentrations are consistent with groundwater samples from Holloman Air

Force Base in New Mexico measured in 2017 with PFOA groundwater concentrations in

evaporation ponds and fire training areas ranging from 0.746 -254 µg/L (NMED 2021).

PFOA was detected in groundwater samples across Minnesota in 2006/2007,

approximately five years after the 3M Corporation phased out PFOS production in Minnesota in

2002 (MPCA 2008). Analyses of samples collected from vulnerable, shallow aquifers in both

urban and agricultural areas across Minnesota, with a variety of potential contamination sources

(i.e., industrial and municipal stormwater, pesticides, land application of contaminated biosolids

and atmospheric deposition), indicated that perfluorinated chemicals were present in

concentrations of potential concern in areas beyond the disposal sites and aquifers associated

with them (MPCA 2008). Groundwater samples ranged from <0.001 to 0.0324 µg/L with a

reporting limit of 0.025 µg/L.

M.4 PFOA Occurrence and Detection in Air and Rain

Air concentrations of PFOA in the atmosphere is widely distributed globally. 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). PFOA in the gas phase had a

mean concentration of 3.16 pg/m^3 (range: 1.89-6.53) and in the particulate phase had a mean

concentration of 2.03 pg/m^3 (range: 0.76-4.19) (Kim and Kannan 2007). Kim and Kanaan (2007)

also reported mean PFOA concentrations of 2.53 ng/L and 4.89 ng/L in rain and snow,

respectively. In an urban area in Minneapolis, MN, PFOA was measured in both the particulate

and gas phase. PFOA in the particulate phase ranged from 1.6-5.1 pg/m^3 and from 1.7-16.1

pg/m^3 in the gas phase across the five samples (MPCA 2008). The mean concentration value

reported from a location in Resolute Bay, Nunavut, Canada was 1.4 pg/m^3 (Stock et al. 2007).

These concentrations are greater than PFOA concentrations measured in the particle phase of air

samples measured in Zeppelinstasjonen, Svalbard (Butt et al. 2010). PFOA was measured in

September and December of 2006 and August and December of 2007, with mean concentrations

of 0.44 pg/m^3 (Norwegian Institute for Air Research, 2007a,b).

M.5   PFOA Occurrence and Detection in Ice

Very little information is provided about PFOA concentrations in ice. The PFOA

concentration from a Russian Arctic ice core sampled in 2007 was 131 pg/L (Saez et al. 2008;

Martin et al. 2010). During the spring of 2005 and 2006 surface snow was collected and the

following values were reported for the Canadian Arctic and Greenland, respectively: 13.1-53.7

pg/L and 50.9-520 pg/L (Young et al. 2007).

Appendix N   Translation of The Chronic Water Column Criterion into Other Fish Tissue Types

The PFOA freshwater aquatic life criteria (summarized in Section 3.3) includes chronic tissue criteria for fish whole body, fish muscle, and invertebrate whole-body tissues. Additional values for fish liver, fish blood, and fish reproductive tissues were also calculated by transforming the freshwater chronic water column criterion (i.e., 0.10 mg/L) into representative tissue concentrations using tissue-specific bioaccumulation factors (BAFs). Fish liver, fish blood, and fish reproductive BAFs were identified following the same approaches used to identify fish wholebody, mush muscle, and inverterbrate whole body BAFs, which are described in detial in section 2.11.3.1. Briefly, BAFs were determined from field measurements and calculated using the equation:

$$BAF = \frac{C_{biota}}{C_{water}}$$ (*Equation N-1*)

Where:

C_{biota} = *PFOA concentration in organismal tissue(s)*
C_{water} = *PFOA concentration in water where the organism was collected*

To identify the representative BAFs, a literature search for reporting on PFOA bioaccumulation was implemented by developing a series of chemical-based search terms to identify studies that were reviewed for reported BAFs and/or reported concentrations in which BAFs could be calculated for both freshwater and estuarine/marine species. BAFs from both freshwater and estuarine/marine species were considered because; (1) inclusion of estuarine/marine BAFs expanded the relatively limited PFOA BAF dataset and (2) Burkhard (2021) did not specifically observe notable differences in PFAS BAFs between freshwater and estuarine/marine systems, instead stating additional research is needed to formulate conclusions.

Sources with relevant BAF information were further screened to determine if the reported BAF information from each source was of low, medium, or high quality. Only BAFs of high and medium quality were used to derive the tissue-specific BAFs and corresponding tissue-based values described below.

BAFs based on reproductive tissues identified by Burkhard (2021) were further 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 N-1.

Table N-1. Characteristics of adult fish sampled for the calculation of PFOA 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	7-9	N.R.	N.R.	3.1
Shi et al. 2015, 2018	Crucian carp (*Carassius carassius*)	July 2014	30	24 F 6 M	N.R.	79.4 (F) 60.5 (M)	15.0 (F) 13.7 (M)	6.59
Shi et al. 2015, 2018	Crucian carp (*Carassius carassius*)	July 2014	13	9 F 4 M	N.R.	352.3 (F) 320.7 (M)	24.6 (F) 24.8 (M)	4.64
Wang et al. 2016	Crucian carp (*Carassius carassius*)	April 2014	8	N.R.	N.R.	(16.8-65.1)[1]	(10.0-14.7)[1]	85.4

N.R. = Not Reported
[1] Range.

Additional details on BAF compilation and ranking can be found in Section 2.11.3.1 and Burkhard (2021). 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 3.2.2). Briefly, distributions of BAFs used to derive additional tissue values were based on the lowest species-level BAF reported within a waterbody. 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 N-2) and individual BAFs are provided in Appendix O.

Table N-2. Summary Statistics for PFOA Freshwater 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	13	15.59	10	2.349	0.732	1,109
Blood	5	80.71	34.1	14.90	14	636
Reproductive Tissue	4	9.488	5.62	3.1	3.1	85.41

[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).

Equation N-2 was used to transform the chronic freshwater column criterion (see Section 3.2.1.3) into tissue values using the 20th centile BAFs from the distributions of BAFs described above and summarized in Table N-2:

$$Tissue\ Value = Chronic\ Water\ Column\ Criterion \times 20th\ Centile\ BAF \qquad (Equation\ N\text{-}2)$$

The resulting tissue values that correspond to the 20th centile tissue-specific BAFs used in Equation N-2 are reported in Table N-3. The values reported in Table N-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 N-3 are only presented for comparative purposes and are not recommended criteria.

Table N-3. PFOA Concentrations for Additional Fish Tissue Values.[1, 2]

Category	PFOA Concentration (mg/kg ww)
Liver	0.2349
Blood	1.490
Reproductive Tissue	0.3100

[1] These PFOA concentrations are provided as supplemental information and are not recommended criteria.

[2] Tissue concentrations are expressed as wet weight (ww) concentrations.

Appendix O Bioaccumulation Factors (BAFs) Used to Calculate PFOA Tissue Values

O.1 Summary Table of PFOA BAFs used to calculate tissue criteria and supplemental fish tissue values

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
goldfish	*Carassius auratus*	Blood	611.0	**611.0**	high	17 sites in six major rivers, Korea	Lam et al. 2014
mandarin	*Siniperca scherzeri*	Blood	739.0	739.0	high	17 sites in six major rivers, Korea	Lam et al. 2014
Crucian carp	*Carassius carassius*	Blood	635.6	**635.6**	high	Beijing Airport, China	Wang et al. 2016
European perch	*Perca fluviatilis*	Blood	14.00	**14.00**	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
Cruician carp	*Carassius carassius*	Blood	18.50	**18.50**	high	Tangxun Lake	Shi et al. 2018
Cruician carp	*Carassius carassius*	Blood	34.06	**34.06**	high	Xiaoqing River, China	Shi et al. 2018
common carp	*Cyprinus carpio*	Blood	85.11	85.11	high	Xiaoqing River, China	Pan et al. 2017
Crucian carp	*Carassius carassius*	Gonad	85.41	**85.41**	high	Beijing Airport, China	Wang et al. 2016
European perch	*Perca fluviatilis*	Gonad	3.100	**3.100**	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
Cruician carp	*Carassius carassius*	Gonad	4.641	**4.641**	high	Tangxun Lake	Shi et al. 2018
Cruician carp	*Carassius carassius*	Gonad	6.594	**6.594**	high	Xiaoqing River	Shi et al. 2018
common carp	*Carassius auratus*	Liver	134.0	**134.0**	high	17 sites in six major rivers, Korea	Lam et al. 2014
mandarin	*Siniperca scherzeri*	Liver	601.0	601.0	high	17 sites in six major rivers, Korea	Lam et al. 2014
Mozambique tilapia	*Oreochromis mossambicus*	Liver	7.017	7.017	medium	Matikulu at N2 Bridge	Fauconier et al. 2020
Cape stumpnose	*Rhabdosargus holubi*	Liver	2.714	**2.714**	medium	Matikulu at N2 Bridge	Fauconier et al. 2020
Crucian carp	*Carassius carassius*	Liver	1109	**1109**	high	Beijing Airport, China	Wang et al. 2016
tilapia	tilapia	Liver	67.00	**58.02**	medium	Key River, Taiwan	Lin et al. 2014
tilapia	tilapia	Liver	53.00		medium	Key River, Taiwan	Lin et al. 2014
tilapia	tilapia	Liver	55.00		medium	Key River, Taiwan	Lin et al. 2014
European perch	*Perca fluviatilis*	Liver	7.100	**7.100**	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
European chub	*Leuciscus cephalus*	Liver	10.00	**10.00**	high	Orge River, near Paris, France	Labadie and Chevreuil 2011
goldfish	*Carassius auratus*	Liver	1000	1000	high	Pearl River Delta, China	Pan et al. 2014

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
Mud carp	*Cirrhinus molitorella*	Liver	7943	7943	medium	Pearl River Delta, China	Pan et al. 2014
Leather catfish	*Clarias fuscus*	Liver	158.5	**158.5**	high	Pearl River Delta, China	Pan et al. 2014
grass carp	*Ctenopharyngodon idellus*	Liver	3981	3981	high	Pearl River Delta, China	Pan et al. 2014
Common carp	*Cyprinus carpio*	Liver	2512	2512	high	Pearl River Delta, China	Pan et al. 2014
Chub	*Hypophthalmichthys molitrix*	Liver	398.1	398.1	high	Pearl River Delta, China	Pan et al. 2014
Snakehead	*Ophicephalus argus*	Liver	199.5	199.5	high	Pearl River Delta, China	Pan et al. 2014
Bream	*Parabramis pekinensis*	Liver	794.3	794.3	high	Pearl River Delta, China	Pan et al. 2014
Tilapia	*Tilapia aurea*	Liver	199.5	199.5	high	Pearl River Delta, China	Pan et al. 2014
Silver perch	*Bidyanus bidyanus*	Liver	14.00	**14.00**	high	Shoalhaven region, Australia	Terechovs et al. 2019
common shiner	*Notropis cornutus*	Liver	24.55	**24.55**	high	Spring/Etobicoke Creek, Toronto, Canada	Awad et al. 2011
Cruician carp	*Carassius carassius*	Liver	6.316	**6.316**	high	Tangxun Lake	Shi et al. 2018
Mozambique tilapia	*Oreochromis mossambicus*	Liver	0.7315	**0.7315**	medium	Mvoti at Gledhow	Fauconier et al. 2020
Mozambique tilapia	*Oreochromis mossambicus*	Liver	0.8882	**0.8882**	medium	Mvoti Estuary Mouth	Fauconier et al. 2020
Cruician carp	*Carassius carassius*	Liver	8.654	**8.654**	high	Xiaoqing River, China	Shi et al. 2018
common carp	*Cyprinus carpio*	Liver	17.38	17.38	high	Xiaoqing River, China	Pan et al. 2017
Slender glassy	*Ambassis natalensis*	Muscle	1.809	**1.809**	medium	Matikulu, Estuary Mouth	Fauconier et al. 2020
Mozambique tilapia	*Oreochromis mossambicus*	Muscle	1.434	1.434	medium	Matikulu, N2 Bridge	Fauconier et al. 2020
Cape stumpnose	*Rhabdosargus holubi*	Muscle	1.163	**1.163**	medium	Matikulu, N2 Bridge	Fauconier et al. 2020
Crucian carp	*Carassius cuvieri*	Muscle	1.442	**1.442**	high	Asan Lake, South Korea	Lee et al. 2020
Common seabass	*Dicentrarchus labrax*	Muscle	98.89	98.89	high	Gironde estuary, SW France	Munoz et al. 2017
Spotted seabass	*Dicentrarchus punctatus*	Muscle	190.6	190.6	high	Gironde estuary, SW France	Munoz et al. 2017
Anchovy	*Engraulis encrasicolus*	Muscle	85.00	**85.00**	high	Gironde estuary, SW France	Munoz et al. 2017
tilapia	tilapia	Muscle	51.00	**47.94**	medium	Key River, Taiwan	Lin et al. 2014
tilapia	tilapia	Muscle	45.00		medium	Key River, Taiwan	Lin et al. 2014
tilapia	tilapia	Muscle	48.00		medium	Key River, Taiwan	Lin et al. 2014

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
European perch	*Perca fluviatilis*	Muscle	37.00	**37.00**	high	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
Brown Bullhead	*Ameiurus nebulosus*	Muscle	10.00	10.00	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Common Carp	*Cyprinus carpio*	Muscle	12.59	12.59	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Northern Pike	*Esox lucius*	Muscle	7.943	**7.943[c]**	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Channel Catfish	*Ictalurus punctatus*	Muscle	10.00	10.00	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Pumpkinseed	*Lepomis gibbosus*	Muscle	10.00	10.00	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Smallmouth Bass	*Micropterus dolomieu*	Muscle	7.943	**7.943[c]**	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Largemouth Bass	*Micropterus salmoides*	Muscle	7.943	**7.943[c]**	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Yellow Perch	*Perca flavescens*	Muscle	7.943	**7.943[c]**	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
White Crappie	*Pomoxis annularis*	Muscle	12.59	12.59	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Black Crappie	*Pomoxis nigromaculatus*	Muscle	25.12	25.12	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Adult char	*Salvelinus alpinus*	Muscle	5.882	**5.882**	high	Meretta Lake, Canadian High Arctic	Lescord et al. 2015
eel	*Anguilla anguilla*	Muscle	13.18	**13.18**	high	Netherlands	Kwadijk et al. 2010
Adult char	*Salvelinus alpinus*	Muscle	37.23	**37.23**	high	Resolute Lake, Canadian High Arctic	Lescord et al. 2015
goby	*Gobio gobio*	Muscle	655.6	**655.6**	medium	Roter Main, Upper Franconia, Germany	Becker et al. 2010
Silver perch	*Bidyanus bidyanus*	Muscle	9.000	**9.000**	high	Shoalhaven region, Australia	Terechovs et al. 2019
crucian	*Carassius cuvieri*	Muscle	97.24	97.24	high	Taihu Lake, China	Fang et al. 2014
Lake Saury	*Coilia mystus*	Muscle	313.0	313.0	high	Taihu Lake, China	Fang et al. 2014

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
gobies	*Ctenogobius giurinus*	Muscle	45.28	45.28	high	Taihu Lake, China	Fang et al. 2014
Mongolian culter	*Culter mongolicus*	Muscle	178.7	178.7	high	Taihu Lake, China	Fang et al. 2014
common carp	*Cyprinus carpio*	Muscle	213.0	213.0	high	Taihu Lake, China	Fang et al. 2014
minnow	*Hemiculter leucisculus*	Muscle	135.0	135.0	high	Taihu Lake, China	Fang et al. 2014
silver carp	*Hypophthalmichthys molitrix*	Muscle	14.21	**14.21**	high	Taihu Lake, China	Fang et al. 2014
mudfish	*Misgurnus anguillicaudatus*	Muscle	196.1	196.1	high	Taihu Lake, China	Fang et al. 2014
white bait	*Reganisalanx brachyrostralis*	Muscle	175.6	175.6	high	Taihu Lake, China	Fang et al. 2014
Chinese bitterling	*Rhodeus sinensis Gunther*	Muscle	105.5	105.5	high	Taihu Lake, China	Fang et al. 2014
Cruician carp	*Carassius carassius*	Muscle	1.557	**1.557**	high	Tangxun Lake	Shi et al. 2018
Mozambique tilapia	*Oreochromis mossambicus*	Muscle	0.2918	**0.2918**	medium	Mvoti, Estuary Mouth	Fauconier et al. 2020
Mozambique tilapia	*Oreochromis mossambicus*	Muscle	0.3798	**0.3798**	medium	Mvoti, Gledhow	Fauconier et al. 2020
Cruician carp	*Carassius carassius*	Muscle	2.207	**2.207**	high	Xiaoqing River, China	Shi et al. 2018
common carp	*Cyprinus carpio*	Muscle	2.884	2.884	high	Xiaoqing River, China	Pan et al. 2017
Juvenile char	*Salvelinus alpinus*	WB	434.8	**434.8**	high	9-Mile Lake, Canadian High Arctic	Lescord et al. 2015
grass goby	*Zosterisessor ophiocephalus*	WB	133.4	**133.4**	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. 2013
Common carp	*Cyprinus carpio*	WB	182.0	**182.0**	high	Baiyangdian Lake, China	Zhou et al. 2012
herring	*Clupea harengus membras*	WB	218.8	**218.8**	medium	Baltic Sea	Gebbink et al. 2016
spat	*Sprattus sprattus*	WB	331.1	331.1	medium	Baltic Sea	Gebbink et al. 2016
Grass carp	*Ctenopharyngodon idellus*	WB	8160	**8160**	medium	Bantou Reservoir, Xiamen Sea, China	Dai and Zeng 2019
Crucian carp	*Carassius carassius*	WB	641.9	**641.9**	high	Beijing Airport, China	Wang et al. 2016
grass goby	*Zosterisessor ophiocephalus*	WB	132.7	**132.7**	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. 2013
Spotted seabass	*Dicentrarchus punctatus*	WB	343.0	**343.0**	high	Gironde estuary, SW France	Munoz et al. 2017

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
Chameleon goby	*Tridentiger trigonocephalus*	WB	16273	**16273**	medium	Gulf Park, Xiamen Sea, China	Dai and Zeng 2019
Chinese icefish	*Neosalanx tangkahkeii taihuensis*	WB	61.90	**61.90**	medium	Lake Chaohu, China	Pan et al. 2019
lake trout	*Salvelinus namaycush*	WB	31.00	**156.9**	high	Lake Erie	De Silva et al. 2011
Lake Trout	*Salvelinus namaycush*	WB	794.3		medium	Lake Erie	Furdui et al. 2007
walleye	*Sander vitreus*	WB	266.0	266.0	high	Lake Erie	De Silva et al. 2011
European perch	*Perca fluviatilis*	WB	1.000	**1.000**	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
Lake Trout	*Salvelinus namaycush*	WB	3981	**3981**	medium	Lake Huron	Furdui et al. 2007
Lake Trout	*Salvelinus namaycush*	WB	2512	**2512**	medium	Lake Michigan	Furdui et al. 2007
lake trout	*Salvelinus namaycush*	WB	402.0	**400.0**	high	Lake Ontario	De Silva et al. 2011
Lake Trout	*Salvelinus namaycush*	WB	398.1		medium	Lake Ontario	Furdui et al. 2007
lake trout	*Salvelinus namaycush*	WB	55.00	**331.3**	high	Lake Superior	De Silva et al. 2011
Lake Trout	*Salvelinus namaycush*	WB	1995		medium	Lake Superior	Furdui et al. 2007
Juvenile char	*Salvelinus alpinus*	WB	77.06	**77.06**	high	Meretta Lake, Canadian High Arctic	Lescord et al. 2015
grass goby	*Zosterisessor ophiocephalus*	WB	97.07	**97.07**	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. 2013
Yellowfin goby	*Acanthogobius flavimanus*	WB	2441	2441	medium	Omuta River mouth and estuary, Japan	Kobayashi et al 2018
Sea Bass	*Lateolabrax*	WB	294.1	**294.1**	medium	Omuta River mouth and estuary, Japan	Kobayashi et al 2018
Grey mullet	*Mugil cephalus*	WB	382.4	382.4	medium	Omuta River mouth and estuary, Japan	Kobayashi et al 2018
Juvenile char	*Salvelinus alpinus*	WB	5387	**5387**	high	Resolute Lake, Canadian High Arctic	Lescord et al. 2015
medaka	*Oryzias latipes*	WB	330.0	**330.0**	high	Seven locations across Japan	Iwabuchi et al. 2015

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
common shiner	*Notropis cornutus*	WB	7.586	**7.586**	high	Spring/Etobicoke Creek, Toronto, Canada	Awad et al. 2011
Cruician carp	*Carassius carassius*	WB	2.775	**2.775**	high	Tangxun Lake	Shi et al. 2018
Bleak	*Alburnus alburnus*	WB	199.5	199.5	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Common carp	*Cyprinus carpio*	WB	158.5	158.5	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Mullet	*Liza sp.*	WB	100.0	100.0	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Roach	*Rutilus rutilus*	WB	125.9	125.9	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Rudd	*Scardinius erythrophtalmus*	WB	79.43	**79.43**	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
European catfish	*Silurus glanis*	WB	125.9	125.9	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Ebro chub	*Squalius laietanus*	WB	100.0	100.0	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Cruician carp	*Carassius carassius*	WB	3.713	**3.713**	high	Xiaoqing River, China	Shi et al. 2018
Snail	Gastropoda	Invert	10.58	**10.58**	medium	Matikulu, N2 Bridge	Fauconier et al. 2020
zooplankton	zooplankton	Invert	199.5	**199.5**	medium	Baltic Sea	Gebbink et al. 2016
Ghost Crab	*Ocypode stimpsoni*	Invert	7440	**7440**	medium	Fenglin, Xiamen Sea, China	Dai and Zheng 2019
Copepods	Copepoda	Invert	358.0	29.92	medium	Gironde estuary, SW France	Munoz et al. 2017
Copepods	Copepoda	Invert	2.500		high	Gironde estuary, SW France	Munoz et al. 2019
Brown shrimp	*Crangon crangon*	Invert	458.0	33.94	medium	Gironde estuary, SW France	Munoz et al. 2017
brown shrimp	*Crangon crangon*	Invert	2.500		high	Gironde estuary, SW France	Munoz et al. 2019
Oyster	*Crassostrea gigas*	Invert	20.00	20.00	high	Gironde estuary, SW France	Munoz et al. 2017
Gammarids	Gammarus	Invert	1250	1250	medium	Gironde estuary, SW France	Munoz et al. 2017
Mysids	Mysidacea	Invert	108.0	**16.43**	medium	Gironde estuary, SW France	Munoz et al. 2017
mysids	Mysidacea	Invert	2.500		high	Gironde estuary, SW France	Munoz et al. 2019
White shrimp	*Palaemon longirostris*	Invert	377.0	29.45	medium	Gironde estuary, SW France	Munoz et al. 2017

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
white shrimp	*Palaemon longirostris*	Invert	2.300		high	Gironde estuary, SW France	Munoz et al. 2019
Pacific Oyster	*Crassostrea gigas*	Invert	9680	**9680**	medium	Gulf Park, Xiamen Sea, China	Dai and Zheng 2019
snails	*Bithynia tentaculata*	Invert	47.01	47.01	high	Hogsmill, Chertsey Bourne, Blackwater Rivers	Wilkinson et al. 2018
amphipod	*Gammarus pulex*	Invert	11.16	**11.16**	high	Hogsmill, Chertsey Bourne, Blackwater Rivers	Wilkinson et al. 2018
Orange-striped hermit crab	*Clibanarius infraspinatus*	Invert	6263	**6263**	medium	Jimei Bridge, Xiamen Sea, China	Dai and Zheng 2019
Pacific Oyster	*Crassostrea gigas*	Invert	6410	6410	medium	Jimei Bridge, Xiamen Sea, China	Dai and Zheng 2019
Ghost Crab	*Ocypode stimpsoni*	Invert	6490	6490	medium	Jimei Bridge, Xiamen Sea, China	Dai and Zheng 2019
worms	Sabellidae	Invert	44.21	44.21	medium	Mai Po Marshes, Hong Kong	Loi et al. 2011
zooplankton	zooplankton	Invert	11.70	**11.70**	medium	Mai Po Marshes, Hong Kong	Loi et al. 2011
Snail	*Cerithidea rhizophorarum*	Invert	267.6	**267.6**	medium	Omuta River mouth and estuary, Japan	Kobayashi et al 2018
crab	*Carcinus aestuarii*	Invert	105.6	105.6	medium	Orbetell lagoon, AC Site, Italy	Renzi et al. 2013
bivalve	*Mytilus galloprovincialis*	Invert	128.3	128.3	medium	Orbetell lagoon, AC Site, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	113.8	113.8	medium	Orbetell lagoon, AC Site, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	84.78	**84.78**	medium	Orbetell lagoon, AC Site, Italy	Renzi et al. 2013
crab	*Carcinus aestuarii*	Invert	47.34	47.34	medium	Orbetell lagoon, FC Site, Italy	Renzi et al. 2013
bivalve	*Mytilus galloprovincialis*	Invert	241.8	241.8	medium	Orbetell lagoon, FC Site, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	52.81	52.81	medium	Orbetell lagoon, FC Site, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	38.56	**38.56**	medium	Orbetell lagoon, FC Site, Italy	Renzi et al. 2013
crab	*Carcinus aestuarii*	Invert	234.2	234.2	medium	Orbetell lagoon, LC Site, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	205.6	205.6	medium	Orbetell lagoon, LC Site, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	139.8	**139.8**	medium	Orbetell lagoon, LC Site, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	102.0	**102.0**	medium	Orbetell lagoon, M Site, Italy	Renzi et al. 2013
crab	*Carcinus aestuarii*	Invert	57.55	**57.55**	medium	Orbetell lagoon, NC Site, Italy	Renzi et al. 2013
bivalve	*Mytilus galloprovincialis*	Invert	327.9	327.9	medium	Orbetell lagoon, NC Site, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	65.62	65.62	medium	Orbetell lagoon, NC Site, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	62.24	62.24	medium	Orbetell lagoon, NC Site, Italy	Renzi et al. 2013
crab	*Carcinus aestuarii*	Invert	210.2	210.2	medium	Orbetell lagoon, PC Site, Italy	Renzi et al. 2013

Common Name	Scientific Name	Tissue[a]	BAF (L/kg-ww)	Site Species BAF (L/kg-ww)[b]	Ranking	Location	Reference
bivalve	*Mytilus galloprovincialis*	Invert	11.86	**11.86**	medium	Orbetell lagoon, PC Site, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	186.4	186.4	medium	Orbetell lagoon, PC Site, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	216.4	216.4	medium	Orbetell lagoon, PC Site, Italy	Renzi et al. 2013
waterlouse, water boatmen, amphipods, roundworm	Isopoda, Hemiptera, amphipoda, nematoda	Invert	171.0	**171.0**	medium	Site A Stockholm Arlanda Airport	Koch et al. 2019
Mayflies, Caddisflies, Dragonflies, Water boatmen, Waterlouse, Fresh water amphipods	Ephemeroptera, Trichoptera, Odonata, Hemiptera, Isopoda, Amphipoda	Invert	50.00	**50.00**	medium	Site K the Kvarntorp area	Koch et al. 2019
Fresh water amphipods	Amphipoda	Invert	520.0	**520.0**	medium	Site R Ronneby Airport	Koch et al. 2019
pearl mussel	Unionidae	Invert	47.64	47.64	high	Taihu Lake, China	Fang et al. 2014
freshwater mussel	Unionidae	Invert	15.04	**15.04**	high	Taihu Lake, China	Fang et al. 2014
zooplankton	zooplankton	Invert	55.91	55.91	high	Taihu Lake, China	Fang et al. 2014
zooplankton	zooplankton	Invert	87.10	87.10	high	Taihu Lake, China	Xu et al. 2014
Shrimp	Caridea	Invert	0.9847	**0.9847**	medium	uMvoti, Gledhow	Fauconier et al. 2020
Snail	Gastropoda	Invert	3.840	3.840	medium	uMvoti, Gledhow	Fauconier et al. 2020
amphipod	Gammarus, Hyalella	Invert	2591	**2591**	high	Welland River, Hamilton, Ontario, Canada	De Solla et al. 2012

a – WB (Fish whole body); Invert (Invertebrate whole body)

b – Lowest species level BAF (highlighted in bold) at each site represents the site-level BAF

c – One site level BAF represented by four species tied for lowest species level BAF

O.2 Summary of PFOA BAFs used to calculate tissue criteria and supplemental fish tissue values

Field measured BAFs used to calculate fish and invertebrate PFOA tissue criteria (fish muscle, fish whole body, invertebrate whole body) and supplemental fish tissue values (blood, reproductive tissue, liver) are shown in Appendix O.1. Summary statistics for the BAFs from this table used to derive tissue criteria and additional tissue values (i.e., lowest species-level BAF from each site) are reported in Table 3-11 and Table N-2, respectively. Rankings for individual BAFs were determined by Burkhard (2021), who devised a ranking system based on five characteristics: 1) number of water samples; 2) number of tissue samples; 3) spatial coordination of water and tissue samples; 4) temporal coordination of water and tissue samples; and 5) general experimental design. For the first four characteristics, a score of one to three was assigned, based on number of samples or how closely the water and tissue measurements were paired. For the experimental design characteristic, a default value of zero was assigned; unless the measured tissues were composites of mixed species, in which case it was assigned a three (Burkhard 2021). These sub-scores were then summed and assigned a rank based on the final score. Studies with high quality rankings had scores of four or five, studies with medium quality rankings had scores of five or six, and studies with low quality rankings had scores of seven or higher (Burkhard 2021). Parameters for the scores assigned to the five characteristics are listed in Table 2-2, and additional details can be found in Burkhard (2021). Only BAFs from studies with high or medium quality rankings were included for the final BAF geometric mean calculations used to derive tissue criteria (Table 3-12) and supplemental tissue values (Table N-3).

O.3   PFOA BAFs References

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Appendix P   Example Data Evaluation Records (DERs)

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

1. The toxic effects are related to single chemical exposure (unless the study is being considered as part of a mixture effects assessment);
2. There is a biological effect on live, whole organisms or *in vitro* preparation including gene chips or omics data on adverse outcome pathways potentially of interest;
3. Chemical test concentrations are reported;
4. There is an explicit duration of exposure;
5. Toxicology information that is relevant to OW is reported for the chemical of concern;
6. The paper is published in the English language;
7. The paper is available as a full article (not an abstract);
8. The paper is publicly available;
9. The paper is the primary source of the data;
10. A calculated endpoint is reported or can be calculated using reported or available information;
11. Treatment(s) are compared to an acceptable control;
12. The location of the study (*e.g.*, laboratory vs. field) is reported; and
13. The tested species is reported (with recognized nomenclature).

Following inclusion in the ECOTOX database, toxicity studies are subsequently evaluated by the Office of Water. All studies were evaluated for data quality generally as described by U.S. EPA (1985) in the 1985 Guidelines and in the EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S. EPA 2016b), and 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 PFOA exposure. Office of Water completed a DER for each species by chemical combination from the PFOA studies identified by ECOTOX. Example DERs are presented here to convey the meticulous level of evaluation, review, and documentation each PFOA study identified by ECOTOX was subject to. Appendix P.1 shows an example fish DER and Appendix P.2 shows an example aquatic invertebrate DER.

P.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**   _____ **Field Study/Observation**   (*Place X by One*)
       (*manipulated*)   (*not manipulated*)

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)*

<u>POTENTIAL CHEMICAL MIXTURES</u>: *Describe any potential chemicals mixtures as characterized by study authors (including any confirmation of chemical mixtures).*

<u>DESCRIPTION OF DILUTION WATER</u>: *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

<u>POTENTIAL CHEMICAL MIXTURES PRESENT AT SITE</u>: *Describe any potential chemicals mixtures present at the site as characterized by study authors (including any confirmation of chemicals present at study site).*

<u>EXPOSURE VARIABILITY ACROSS STUDY SITE(S)</u>: *Describe any exposure variability across study site(s) as characterized by study authors (i.e., description of study design with reference and contaminated sites).*

General Notes:

Reviewer's Comments: *Provide additional comments that do not appear under other sections of the DER.*

ABSTRACT: *Copy and paste abstract from publication.*

SUMMARY*: Fill out and modify as needed.*

Acute:

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration (mg/L)	Classification
											Quantitative / Qualitative / Unused

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

Chronic:

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Chronic Limits	Reported Chronic Value (mg/L or µg/g)	Verified Chronic Value (mg/L or µg/g)	Chronic Value Endpoint	Classification
												Quantitative / Qualitative / Unused

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

II. Results *Provide results as reported in the publication (including supplemental materials). Include screen shots of tables and/or figures reporting results from the article <u>following</u> tabulated data table in each associated results section for <u>all studies</u>. Complete tabulated data tables for all studies for studies marked "**Acceptable for Quantitative Use**" and "**Acceptable for Qualitative Use**".*

Water Quality Parameters: *If only general summary data of water quality parameters is provided by study authors (i.e., no specific details of water quality parameters on a treatment level is provided), summarize any information regarding water quality parameters under General Notes below and indicate data not provided in* Table A.II.1.

General Notes: *For aquatic life criteria development, measured water quality parameters in the treatments nearest the toxicity test endpoint(s), e.g., LC$_{50}$, EC$_{20}$, etc., are most relevant.*

-

Table A.II.1. Measured Water Quality Parameters in Test Solutions.
Dissolved oxygen, temperature, pH and [other parameters (hardness, salinity, DOC)] in test solutions during the *[X]*-day exposure of *[test organism]* to *[concentration of treatment(s)]* of *[test substance]* under *[static renewal/flow-through]* conditions.

Parameter	Treatment	Mean	Range
Dissolved Oxygen **(% saturation or mg/L)**	*[1]*		
	[2]		
	j		
	j		
Temperature (°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>

<table>
<tr><td rowspan="7"><i>For Both Controlled Experiments and Field Observations</i></td><td>Parameter</td><td colspan="2">Details</td><td>Remarks</td></tr>
<tr>
<td>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]</td>
<td colspan="2">Control(s):

Treatment(s):</td>
<td></td>
</tr>
<tr>
<td>Number of Organisms per Replicate/ Treatment Group:
• At least 10 organisms/treatment recommended [3]
• At least 7 organisms/treatment acceptable [4]</td>
<td colspan="2">Control(s):
Treatment(s):</td>
<td></td>
</tr>
<tr>
<td>Exposure Pathway:
<i>(i.e., water, sediment, gavage, or diet).</i>
<i>Note: all other pathways (e.g., dermal, single dose via gavage, and injection) are unacceptable.</i></td>
<td colspan="2"></td>
<td></td>
</tr>
<tr>
<td>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]</td>
<td colspan="2">_____ Acute
_____ Partial Life Cycle
_____ Early Life Stage
_____ Full Life Cycle
_____ Other <i>(please remark):</i></td>
<td></td>
</tr>
<tr>
<td>Test Concentrations (remember units):
<i>Recommended test concentrations include at least three concentrations other than the control; four or more will provide a better statistical analysis</i> [3]</td>
<td colspan="2">Nominal:
Measured:
Media measured in:</td>
<td></td>
</tr>
<tr>
<td>Observation Intervals:
• Should be an appropriate number of observations over the study to ensure water quality is being properly maintained [4]</td>
<td colspan="2"></td>
<td></td>
</tr>
</table>

CONTROLLED EXPERIMENT STUDY PARAMETERS: *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for Controlled Experiments only.*

<table>
<tr><td></td><td>**Parameter**</td><td>**Details**</td><td>**Remarks**</td></tr>
<tr><td rowspan="6">*For Controlled Experiments Only*</td><td>**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]</td><td>Duration:
Feeding:
Water type:
Temperature (°C):
Dissolved Oxygen (mg/L):
Health (*any mortality observed?*):</td><td>*Identify number of individuals excluded from testing and/or analysis (if any):*</td></tr>
<tr><td>**Acclimation followed published guidance?**
Describe, if any</td><td>_____ Yes _____ No
If yes, indicate which guidance:</td><td></td></tr>
<tr><td>**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]</td><td>Material:

Size:

Fill Volume:</td><td>*Briefly describe the test vessel:*</td></tr>
<tr><td>**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]</td><td>Test Concentrations Measured
_____ Yes _____ No

Test Solution Delivery System:
_____ Static
_____ Renewal
  Indicate Interval:

_____ Flow-through
  Indicate Type of Diluter:</td><td></td></tr>
<tr><td>**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]</td><td></td><td></td></tr>
<tr><td>**Dilution Series** (*e.g., 0.5x, 0.6x, etc.*):</td><td></td><td></td></tr>
</table>

	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]
		P-16	

	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] o If not available, the 96-hr LC_{50} should be used [2] **Chronic** • Life-cycle/Partial Life-cycle test: o 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: o 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.

P.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**   _____ **Field Study/Observation**   (*Place X by One*)
       (*manipulated*)   (*not manipulated*)

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)	Methodᵃ	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as CaCO₃) 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)	Methodᵃ	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as CaCO₃) 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). <u>For multi-generational studies, copy and paste</u>* Table A.II.5 *<u>below for each generation with reproductive effects data.</u>*

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.

	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 [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.*

	Parameter	Details	Remarks

For Controlled Experiments Only	**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

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	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]		

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	Feeding:		

For Controlled Experiments Only	• Unacceptable for acute tests [2]
	  ○ 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.*

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OBSERVATIONS: *Provide information under Details and any relevant information in Remarks. This information should be consistent with the Results Section in Part A.*

Parameter	Details	Remarks

Parameters measured including sublethal effects/toxicity symptoms:
Common Apical Parameters Include:
Acute
• Daphnids/cladocerans:
  o EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed [2]
• Embryo/larva (bivalve molluscs, sea urchins, lobsters, crabs, shrimp, and abalones):
  o EC_{50} based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed [2]
    ▪ If not available, the lower of the 96 hour EC_{50} based on the percentage of organisms with incompletely developed shells and the 96-hr LC_{50} should be used [2]
• Freshwater mussel (glochidia and juveniles):
  o Glochidia: EC_{50} based on 100 x number closed glochidia after adding NaCl solution - number closed glochidia before adding NaCl solution) / Total number open and closed glochidia after adding NaCl solution [4]
  o Juvenile: EC_{50} based on percentage exhibiting foot movement within a 5-min observation period [4]
• All other species and older life stages:
  o EC_{50} based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed [2]
    ▪ If not available, the 96 hour LC_{50} should be used [2]
Chronic
• Daphnid:
  o Survival and young per female [2]
• Mysids:
  o Survival, growth and young per female [2]
	List parameters:	

Was control survival acceptable?
Acute
• > 90% control survival at test termination [2]
  o Glochidia 90% after 24 hours, or, the next longest duration less than 24 hours that had at least 90% survival [4]
Chronic
• > 80% control survival at test termination [2]
  o 80% in 42 day test with *Hyalella azteca*, slightly lower in tests substantially longer than 42 days [3]
	_____ Yes _____ No
	Control survival (%):	

Parameter	Details	Remarks
Were individuals excluded from the analysis?	_____ Yes _____ No *If yes, describe justification provided:*	
Was water quality in test chambers acceptable? • If appropriate, describe any water quality issues (e.g., dissolved oxygen level below 60% of saturation)	_____ Yes _____ No	
Availability of concentration-response data: • Were treatment level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • Were replicate level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • • If treatment and/or replicate level concentration-response data were included, how was data presented? *(check all that apply)* • Were concentration-response data estimated from graphs study publication or supplemental materials? Should additional concentration-response data be requested from study authors? *If concentration-response data are available, complete* **Verification of Statistical Results (Part C)** *for sensitive species.*	_____ Yes _____ No _____ Yes _____ No _____ Tables _____ Graphs _____ Supplemental Files _____ Yes _____ No *If yes, indicate software used:* _____ Yes _____ No Requested by: Request date: Date additional data received:	

Part C: Statistical Verification of Results

I. Statistical Verification Information: *Report the statistical methods (e.g., EPA TRAP, BMDS, R, other) used to verify the reported study or test results for the five (5) most sensitive genera and sensitive apical endpoints (including for tests where such estimates were not provided). If values for the LC_{50}, LT_{50} and NOEC are greater than the highest test concentration, use the ">" symbol.*

Primary Reviewer: _____ **Date:** _____ _____ **EPA** _____ **Contractor** (*Place X by One*)

Secondary Reviewer: _____ **Date:** _____ _____ **EPA** _____ **Contractor** (*Place X by One*)
(*At least one reviewer should be from EPA for sensitive taxa*)

Endpoint(s) Verified:

Additional Calculated Endpoint(s):

Statistical Method (e.g., TRAP, BMDS, R, other):

II. Toxicity Values: *Include confidence intervals if applicable*

NOEC:
LOEC:
MATC:

EC$_5$:
EC$_{10}$:
EC$_{20}$:
EC$_{50}$ or LC$_{50}$

Dose-Response Curve Classification: *(Place X by One)*
This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A

_____ Dose-Response Curve Acceptable for Quantitative Use
_____ Dose-Response Curve Acceptable for Qualitative Use
_____ Dose-Response Curve Not Acceptable for Use

Summary of Statistical Verification: *Provide summary of methods used in statistical verification.*

Additional Notes:
-

Attachments:
1. *Provide attachments to ensure all data used in Part C is captured, whether from study results reported in the publication and/or from additional data requested from study authors*
   - *Data from study results of the publication should be reported in Results section of Part A*
   - *Additional data provided upon request from study authors should be reported in Table C.II.1 below and original correspondence with study authors should be included as attachments*
2. *Model assessment output (including all model figures, tables, and fit metrics)*
3. *Statistical code used for curve fitting*

III. Attachments: *Include all attachments listed above after the table below.*

Additional Data Used in Response-Curve: *Provide all data used to fit dose-response curve not captured in Results section of DER above in Part A, rows as needed. First row in italicized text is an example.*

Table C.II.1 Additional Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[a] N = number of individuals per treatment; k = number of replicates per treatment level; n = number of individuals per replicate

Part D: References to Test Guidance

6. ASTM Standard E 739, 1980. 2002. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, West Conshohocken, PA.
7. Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses. PB85-227049. National Technical Information Service, Springfield, VA.
8. Mount, D.R. and J.R. Hockett. 2015. Issue summary regarding test conditions and methods for water only toxicity testing with *Hyalella azteca*. Memorandum to Kathryn Gallagher, U.S. EPA Office of Water. U.S. EPA Office of Research and Development. MED. Duluth, MN. 9 pp.
9. Bringolf, R.B., M.C. Barnhart and W.G. Cope. 2013. Determining the appropriate duration of toxicity tests with glochidia of native freshwater mussels. Submitted to Edward Hammer. U.S. EPA. Chicago, IL, May 8, 2013. 39 pp.
10. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.
11. American Public Health Association (APHA). 2012. Standard methods for the examination of water and wastewater. Part 8000 - Toxicity. APHA. Washington, DC.